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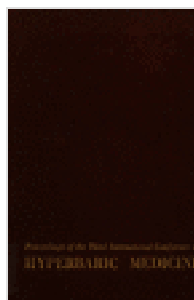

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Proceedings of the
Third International Conference on
HYPERBARIC MEDICINE

Proceedings of the Third International Conference on

HYPERBARIC MEDICINE

DUKE UNIVERSITY, DURHAM, NORTH CAROLINA
NOVEMBER 17-20, 1965

Edited by

IVAN W. BROWN, JR.
and
BARBARA G. COX

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Preface

The first and second International Conferences on Hyperbaric Medicine held in Amsterdam (1963) and Glasgow (1964) brought together investigators interested in the therapeutic possibilities of administering oxygen under increased atmospheric pressure. At the Glasgow conference, it was voted to hold a third conference in the United States. In response to the suggestion of the Committee on Hyperbaric Oxygenation of the Division of Medical Sciences, National Academy of Sciences–National Research Council, Duke University Medical Center agreed to undertake, jointly with the Committee, the organization and conduct of the Third International Conference on Hyperbaric Medicine in Durham, North Carolina, November 17–20, 1965.

We hope that the proceedings of this conference of basic and clinical scientists will further elucidate some of the basic biochemical and physiologic problems associated with therapeutic exposure to increased oxygen tension and will help to define the current status of hyperbaric oxygenation in the treatment of certain disease states.

Ivan W. Brown, Jr.
Barbara G. Cox

Acknowledgments

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The editors are grateful to Dr. Wirt W. Smith for his help in manuscript review, to Jeanne D. Hudson for preparing or redrafting much of the artwork, to Dorothy T. Verwoerdt for editing the discussions, to Harriet M. Gordon and Edith W. Horn for their excellent secretarial assistance, to Norman Grossblatt, Editor of the Division of Medical Sciences, NAS–NRC, for his advice and help in reviewing all of the galley proofs, and to the NAS–NRC Printing and Publishing Office for producing and distributing the volume.

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SESSION I

Oxygen and Cellular Metabolism

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Antioxidant Mechanisms Against Oxygen Toxicity and Their Importance During the Evolution of the Biosphere

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Following are some speculations concerning the place of oxygen in the evolution of the biosphere,[1] the effect of oxygen on the biosphere, and the biological control of some of the oxygen effects.

STAGES IN THE EVOLUTION OF THE ATMOSPHERE

In the initial stages of the earth's development, about 4.5×10^9 years ago,[2] the earth's atmosphere was presumably composed of those light elements most abundant in the cosmos. The five most abundant elements in the universe as a whole are (in decreasing order of quantity) hydrogen, helium, oxygen, carbon, and nitrogen, with hydrogen being by far the most abundant (Table 1).[3] The atoms of hydrogen plus those of helium make up 99.86% of all the cosmic atoms. Oxygen, the third most abundant element in the cosmos, contributes only 0.09% of the total.[4] Essentially, then, the entire universe is composed of these three elements plus carbon and nitrogen.

Excluding the chemically stable helium, the most abundant elements in our present biosphere [5] occur in the same decreasing order as in the cosmos. In contrast, the three most abundant elements in the earth's crust, in decreasing order, are oxygen, hydrogen, and silicon (Table 1). Thus, the atomic composition of living organisms is closely correlated with the atomic composition of the cosmos but is not clearly correlated with the present environment of living organisms on earth.

TABLE 1. Percent Atom Abundance in the Universe

	Percent abundance in:		
Element	Cosmos	Biosphere	Earth's crust
Hydrogen	86.68	62.6	17.70
Helium	13.18	0.0	0.00
Oxygen	0.09	24.9	53.77
Carbon	0.03	10.6	0.13
Nitrogen	0.01	1.1	0.02
Others	0.01	0.8	28.38

We would expect the oxygen, carbon, and nitrogen in the primitive atmosphere to have been bound to the excess hydrogen in the form of water, methane, and

ammonia; hence, the initial atmosphere was probably composed of molecular hydrogen, helium, water, methane, and ammonia.[6] Some hydrogen particles, due to their light mass, would have been able to overcome the gravitational field of the earth and escape continually. This dehydrogenation process has made the earth a pinpoint of oxidation in a reducing universe. Now, an energy source such as the sun can dissociate hydrogen from water, methane, and ammonia, and presumably these reactions occurred in the primitive atmosphere (Table 2). At the same time, there would have been a great tendency for the reverse reactions to occur, with hydrogen again becoming bound. As hydrogen escaped from the earth's gravitational field, the atmospheric concentration would have decreased, and there would then have been less probability of the reverse reactions occurring. The result of this process would have been the net liberation of bound hydrogen from methane, ammonia, and water.

The energy required for the release of hydrogen from methane and ammonia is much less than that for its release from water.[7] Part of the hydrogen liberated from these bound forms would tend to escape continually from the earth's atmosphere. There would have also been a tendency toward continual escape of helium particles from the earth. However, the liberated nitrogen would have remained in the form of molecular nitrogen. The liberated oxygen would have combined with the liberated carbon and other substances, such as silicon and iron, to form oxides. Since molecular oxygen has a high thermodynamic potential, we would not expect it to remain in its free form, and the most stable compounds of carbon and nitrogen in an oxygen atmosphere would be carbon dioxide and molecular nitrogen.

TABLE 2. Reactions Occurring During Atmospheric Evolution [a]

Reaction	$\Delta F°$ (kcal)
$CH_4 \rightsquigarrow C + 2H_2$	12
$2NH_3 \rightsquigarrow N_2 + 3H_2$	8
$2H_2O \rightsquigarrow O_2 + 2H_2$	113
Reduction of O_2	
$C + O_2 \rightarrow CO_2$	−94
$X + O_2 \rightarrow XO_2$	

X, other substances which can be oxidized by O_2.

[a] Standard free energies calculated from Latimer[7] at a temperature of 298°K.

Hence, the terminal result of the escape of free and bound hydrogen from the atmosphere would be the formation of a carbon dioxide and molecular nitrogen atmosphere in which all water would be absent. Finally, even the carbon dioxide and molecular nitrogen would escape. But, during the transition to this terminal phase, there would be expected a production of metastable compounds of carbon and nitrogen, possessing intermediate oxidation states between the reduced forms of methane and ammonia and the oxidized forms of carbon dioxide and molecular nitrogen. Many of these metastable compounds, such as sugars and amino acids, could possibly have interacted with each other to form increasingly complex metastable systems. Eventually, some of these systems could have given rise to life itself. Thus, it is speculated that the "life" atmosphere stage merely represents the transition phase in this evolution of the earth's atmosphere from a "prelife" reducing atmosphere stage to a future "postlife" oxidizing atmosphere stage (Figure 1).[1,3,8,9] Organisms can alter the kinetics of this process but not the net result.

One would expect that these generalizations about the earth's atmosphere would also hold for the other planets. Large cold planets would tend to retain the "prelife" reducing atmosphere. Indeed, the atmospheres of Jupiter and Saturn do contain hydrogen, methane, and ammonia.[8] It seems most likely that the "life" atmosphere stage is ending or has ended on Mars, since its atmosphere contains primarily carbon dioxide,[10] very

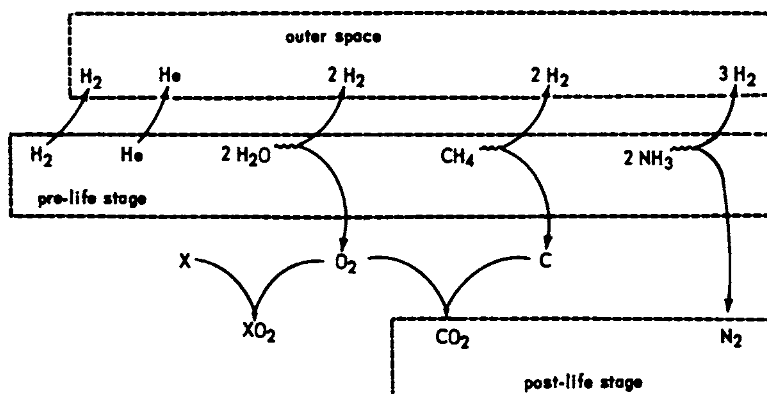


FIGURE 1. Evolution of atmosphere from a "prelife" to a "postlife" stage. X represents other substances oxidized by oxygen. (Reproduced from *Handbook of Physiology* [section 3, vol. 1] American Physiological Society, 1964, p. 153.)

little water,[11] and little or no molecular oxygen.[12] Small hot planets, on the other hand, because of loss of atmospheric constituents, would tend to be in the "postlife" stage. Venus, a very hot planet [13] containing carbon dioxide and perhaps molecular nitrogen, would seem to possess a "postlife" atmosphere.[8] Mercury, relatively close to the sun, has virtually no atmosphere.[8]

PHASES OF BIOLOGICAL EVOLUTION

Now that we have presented the physical processes thought to have taken place during the transition from a "prelife" atmosphere to a "postlife" atmosphere, let us examine the concomitant stages which might have occurred in our biosphere. Many aspects of the origin of a biosphere have been discussed by others.[14] Important steps for the development of the earth's biosphere may have actually preceded the origin of the earth itself.[15] Thus, this development may conceivably have started at the time of the beginning of our galaxy, estimated as about 15×10^9 years ago.[16] However, the oldest known fossil organisms of the biosphere have been estimated to be 1.9×10^9 years old.[17]

Phase 1: Nonbiological Synthesis of Metastable Compounds

During the first phase of evolution (Table 3), nonbiological synthesis of metabolic compounds would have occurred. When the hydrogen concentration in the primitive atmosphere had become sufficiently decreased, an energy source such as the sun could have released hydrogen from methane and thereby pro-

TABLE 3. Phases of Evolution in a Reducing Atmosphere [a]

Phase	$\Delta F°$ (kcal)
1. Nonbiological synthesis of metabolic compounds	
$6H_2O + 6CH_4 \rightsquigarrow C_6H_{12}O_6 + 12H_2$	195
2. Biological release of energy by reduction	
$C_6H_{12}O_6 + 12H_2 \rightarrow 6H_2O + 6CH_4$	− 195
3. Photosynthetic production of hydrogen	
$6H_2O + 6CH_4 \rightarrow C_6H_{12}O_6 + 12H_2$	195

[a] Standard free energies calculated from Latimer[7] at a temperature of 298°K.

duced a substance, such as sugar, representing an intermediate metastable oxidation state of carbon, between reduced methane and oxidized carbon dioxide. Other characteristic metastable compounds of the biosphere could similarly have been produced. Indeed, experimental syntheses of organic compounds under such conditions have been performed.[18,19] The decrease in free atmospheric hydrogen would have lessened the chance of a back reaction reverting the metastable compounds to methane and ammonia. As the probability of a back reaction decreased, the life of these metastable compounds could have progressively lengthened, permitting the development of more complicated metastable compounds. It has been shown, for example, that adenine synthesis in an electron-irradiated atmosphere composed of methane, ammonia, water, and hydrogen increases when the hydrogen concentration is decreased.[19] When these metastable compounds dissolved in the liquid phase of water, opportunity arose for those metastable systems to evolve which eventually resulted in the formation of the primitive biosphere. During this period of time, the entire primitive atmosphere would, for all practical purposes, have been lost.[20] Those atmospheric constituents dissolved in the liquid phase of water would have been retained. This conclusion seems necessary, since the atom abundance of neon in the cosmos is 0.003%,[4] a sizable percent compared to the cosmic nitrogen abundance (Table 1). However, the fact that the earth contains hardly any neon at present implies that neon must have escaped from the earth's atmosphere. Since methane, ammonia, and water have lower molecular weights and consequently larger relative gas velocities than neon, these constituents of the primitive atmosphere must also have escaped. Yet, the initial impact of the primitive atmosphere has not been forgotten, for it is responsible for the presence of the minute biosphere which represents just the infinitesimal skin of the earth.

Phase 2: Biological Release of Energy by Reduction

During the second phase, the primitive biosphere probably utilized the energy released from the reduction of metastable compounds (such as the carbohydrates) by molecular hydrogen, with methane and water as the end products. This energy utilization by the biosphere would have tended to deplete those free metastable constituents present in the biosphere, since nonbiological synthesis (Phase 1) would now be occurring at a slower rate than biological depletion (Phase 2).

Phase 3: Biological Production of Hydrogen

Resynthesis of metastable compounds would, of course, have been essential for the continued existence of the biosphere. During Phase 3, the biosphere would have catalyzed the already-existing synthetic process (Phase 1), resulting in a photosynthetic production of hydrogen gas. Photoproduction of hydrogen gas does occur in some biological systems.[21] Thus, the biosphere, existing in the reducing atmosphere, would be able to photoproduce hydrogen (Phase 3) and at the same time utilize energy (as described in Phase 2). However, the presence of molecular hydrogen would, at the same time, have tended to destroy some of the constituents of the biosphere by reducing them to methane and ammonia, so that the primitive organisms probably had to cope with the problem of hydrogen toxicity. (An example of hydrogen toxicity is the deleterious effect of hydrogen on nitrogen fixation by azotobacter.[22])

Phase 4: Transition from Reducing Atmosphere to Oxidizing Atmosphere

As the hydrogen pressure in the atmosphere decreased, the photodissociation of

water into hydrogen and oxygen became more probable. During this fourth phase (Table 4), a transition from a reducing atmosphere to an oxidizing atmosphere would have occurred. The standard free energy of 680 kcal/12 moles of molecular hydrogen involved in releasing oxygen from water (Phase 4) is much greater than the standard free energy of 195 kcal/12 moles of molecular hydrogen involved in synthesizing sugar from methane (Phase 1).

Phase 5: Biological Release of Energy by Oxidation

During the fifth phase, the biosphere utilized the energy released by the oxidation of metastable compounds (such as the carbohydrates) by molecular oxygen into carbon dioxide and water. Phase 5, occurring in the oxidizing atmosphere, is analogous to Phase 2, occurring in the reducing atmosphere. However, the reduction of 1 mole of sugar results in a decrease of the standard free energy of 195 kcal (Phase 2), whereas the oxidation of 1 mole of sugar results in a decrease of the standard free energy of 688 kcal (Phase 5). Thus, more energy would have been available to the biosphere in the oxidizing atmosphere than in the reducing atmosphere. This energy utilization by the biosphere (*i.e.*, respiration) would then have depleted the metastable constituents of the biosphere, since the photoproduction of oxygen (Phase 4) would be expected to occur at a slower rate than the depletion of oxygen by respiration (Phase 5). Again, a resynthesis of the metastable compounds would have been essential for the continued existence of the biosphere.

Phase 6: Biological Production of Oxygen

During the sixth phase, the biosphere would have catalyzed the already-existing synthetic process (Phase 4), resulting in the photosynthetic production of oxygen gas, as it presently occurs in green plants. (It is to be noted that the free energy involved in the photosynthetic production of oxygen, as depicted in Phase 6, comes almost entirely from the splitting of the water molecule, as depicted in Phase 4; in the photosynthetic process of Phase 6, the carbon dioxide merely acts as a sponge in absorbing the hydrogen.) When Phase 6 was completed, the biosphere was able to use continuously as a source of energy the more efficient oxidative process of respiration (Phase 5), instead of the less efficient primitive reductive process (Phase 3).

Effect of Oxygen on the Biosphere

The sequence of events in the oxidizing atmosphere would have been very similar to that in the reducing atmosphere. However, much more energy was available to the biosphere after the atmosphere had become an oxidizing one. This indicates the importance of oxygen as a biological energy store.

The fact that oxygen provides the bio-

TABLE 4. Phases of Evolution in an Oxidizing Atmosphere [a]

Phase	$\Delta F°$ (kcal)
4. Nonbiological synthesis of oxygen	
$12H_2O \xrightarrow{\sim\sim\sim} 6O_2 + 12H_2$	680
5. Biological release of energy by oxidation	
$C_6H_{12}O_6 + 6O_2 \longrightarrow 6H_2O + 6CO_2$	− 688
6. Photosynthetic production of oxygen	
$6H_2O + 6CO_2 \longrightarrow C_6H_{12}O_6 + 6O_2$	688

[a] Standard free energies calculated from Latimer[7] at a temperature of 298°K.

sphere with a significant biological source of energy does not necessarily mean that oxygen is the element best suited to this purpose. Therefore, let us consider the qualifications of storage energy forms in a biosphere composed of a substantial quantity of hydrogen, since this is the predominant cosmic element. These qualifications are: (1) great abundance, (2) easy accessibility, (3) high thermodynamic potential, and (4) slow reaction rate.

Based upon these criteria, oxygen does indeed appear best qualified as a storage energy form.[1] (1) Oxygen is very abundant, being the third most abundant element in the cosmos (Table 1). (2) Since oxygen occurs in the gaseous state, unless the temperature is exceedingly low it is easily available over the entire surface of a planet. (3) Oxygen in the molecular state possesses a relatively high thermodynamic potential, being surpassed only by the rapidly acting fluorine and chlorine and by other forms of oxygen. (4) Oxygen has a slow reaction rate. However, despite this inherent sluggish behavior, oxygen does react with the metastable substances constituting the biosphere, and this reaction can result in oxygen toxicity.

Oxygen is like Dr. Jekyll and Mr. Hyde: it is a source of energy for the biosphere, but it is also a source of biological destruction. How is the biosphere able to survive at all in an oxygen atmosphere? Gerschman has stated that, "A better understanding of the fundamental mechanism involved inclines us to marvel at the continuous and powerful cellular defenses against oxygen rather than to be surprised at its potential destructive action." [23]

Biological Control of Some Oxygen Effects

Development of Antioxidant Mechanisms Against Oxygen Toxicity

The appearance of molecular oxygen in a previously reducing atmosphere would alter the synthesis of cellular constituents,[24] make available more energy for the biosphere, and confront the biosphere with the problem of oxygen toxicity.[23,25,26] Because of the increased energy made available to most of the biosphere by oxygen, the activity of the biosphere could then be increased. For this reason, it has been speculated that molecular oxygen was required for the origin of the metazoa.[27] Gaffron has pointed out that there are no known multicellular differentiated organisms which are anaerobes.[28]

Various antioxidant mechanisms [23,25,26,29,30–32] were developed by the biosphere to combat oxygen toxicity. If such mechanisms had not developed, the biosphere could not exist. The process by which this natural selection process occurs will not be discussed here.[33] As the partial pressure of the oxygen in the atmosphere increased,[34–36] further antioxidant mechanisms developed. However, just as pH buffers can resist but not completely prevent pH changes, antioxidant mechanisms will inhibit but not completely prevent the toxic effects of oxygen. Consequently (and this point should be emphasized), the toxic action of oxygen exists at even the lowest oxygen pressure. Therefore, possible chronic toxic effects of oxygen should not be ignored. Oxygen may participate in the aging process [26] and can influence the rate of mutations.[26,37] Oxygen toxicity becomes increasingly apparent with both an increased oxygen pressure and a decreased antioxidant defense.

One example of the evolution of an antioxidant defense is the development of the iron enzyme, catalase, which decomposes hydrogen peroxide. Iron by itself acts as a catalyst in the decomposition of hydrogen peroxide, but the catalytic action of catalase is 10^{10} times greater than that of iron.[38] The development of this enzyme required oxidizing conditions.[38]

Another example is the development of antioxidant mechanisms within the photosynthetic apparatus. The presence of intracellular oxygen would tend to produce

photo-oxidative damage by chlorophyll.[39] It appears that the carotenoids intimately associated with the photosynthetic apparatus inhibit this photo-oxidative damage [39] and therefore permit the continuance of photosynthetic production of molecular oxygen. Plants also contain phenolic compounds [40] which are effective antioxidants.[41]

Since oxygen is removed in the chemical reaction responsible for bioluminescence, it has been suggested that bioluminescence was originally used as an effective antioxidant defense when atmospheric oxygen was first produced.[42] Also, since oxygen has been shown to destroy artificial lipid bilayer membranes,[43] it seems probable that development of antioxidant mechanisms was necessary for the maintenance of biological membranes in the presence of an oxygen atmosphere.

Human beings are not naturally exposed to higher oxygen pressures than those near sea level. The highest natural oxygen pressure to be found on the earth's surface should be at the Dead Sea, which is the lowest exposed depression in the world (1286 feet, or 392 meters, below sea level [44]). The total pressure of the atmospheric gases there is about 790 mm Hg,[3] or about 4% greater than the pressure at sea level. With regard to decreased oxygen pressure, individuals living at high altitudes are naturally exposed to significantly lower oxygen tensions than are found at sea level. If these high-altitude individuals are descendants of low-altitude individuals, one might expect the high-altitude individuals to possess antioxidant mechanisms already evolved from their low-altitude ancestors. The result might be a better adaptation of the high-altitude man to his environment than the sea-level man to his. It has been reported that mountain-dwelling natives in Peru can perform physical tasks at high altitude more easily than sea-level individuals can at sea level.[45] Also, the Sherpas in the high Himalayas excel in physical performance over unaccustomed Caucasians venturing into high altitudes.[46]

Significance of Free Radicals to Oxygen Toxicity

Actually, the antioxidant mechanisms which have been developed by the biosphere merely accentuate the inherent sluggish behavior of oxygen. This characteristic has been attributed to the high free energy of activation of oxygen to a free radical state.[47] The Gerschman theory implicates oxidizing free radicals as the basic cause of oxygen toxicity.[48]

Free radicals are usually unstable, requiring energy for their production. A free radical is characterized by an unpaired electron in one of its orbitals. A filled orbital contains two electrons with opposite spins, resulting in a net spin of zero. If an orbital contains only one unpaired electron, the electron spin results in a magnetic moment which gives rise to paramagnetism. Since a strong tendency generally exists to pair electrons in orbitals and eliminate a net electron spin, free radicals are unstable.

Figure 2 illustrates the dissociation of water into ions or radicals. The dissociation constant of H_2O into H^+ and OH^- ions is 1×10^{-14}, whereas the dissociation constant of H_2O into $H^\cdot$ and $OH^\cdot$ radicals is 1×10^{-83}.[7] Hence, the radicals are much more unstable than the ions. It is generally easier to break chemical bonds so that charged species are formed rather

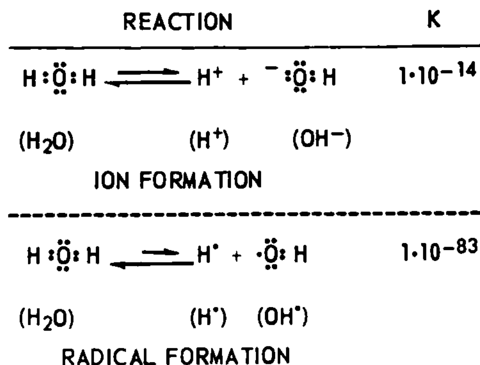


FIGURE 2. Formation of ions and radicals from the dissociation of water. Standard free energies calculated from Latimer[7] at a temperature of 298°K. (Reproduced from Physiologist 8:9, 1965.)

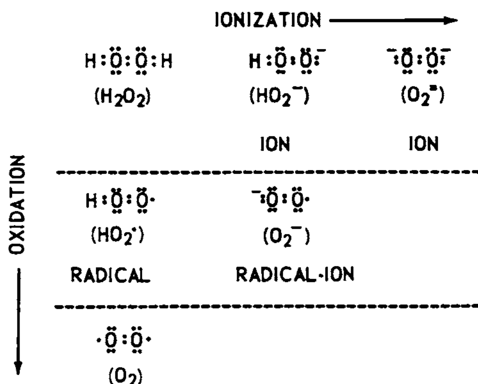


FIGURE 3. Radicals and ions of oxygen. (Reproduced from Physiologist 8:9, 1965.)

than species containing unpaired electrons.

Figure 3 illustrates how a species may possess an electrical charge and also an unpaired electron. If a hydrogen atom is combined with molecular oxygen, the free radical $HO_2^{\cdot}$ (which contains one unpaired electron) is produced. The ionized form of $HO_2^{\cdot}$ is $O_2^{\overline{\cdot}}$, which is a charged free radical.

According to the univalent theory of Michaelis,[49] molecular oxygen can become reduced by accepting only one electron at a time. The free energy changes occurring during the univalent reduction of oxygen by hydrogen are illustrated in Figure 4. Since the free radical states ($HO_2^{\cdot}$, $OH^{\cdot}$, and $H^{\cdot}$) are unstable, the energy released by them is considerable, but they are difficult to produce and thus act as energy barriers for both oxygen and hydrogen peroxide.

Once they are formed, however, they can give rise to nonspecific propagating chain reactions. Indeed, biological systems do exhibit different sensitivities to the nonspecific toxic effects of oxygen. In a chain reaction, the energy required for free radical production is regenerated by the energy liberated in the free radical reaction. One can use as an analogy a water movement from one level down to another by means of a siphon. Energy is required to move the water from the high level to a still higher level in the siphon

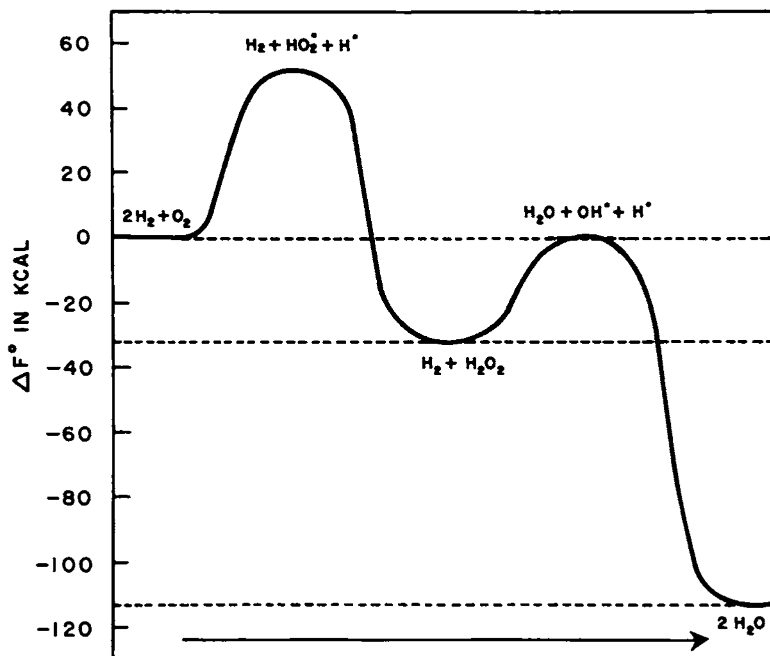


FIGURE 4. Free energy changes occurring during the univalent reduction of oxygen by hydrogen, at a temperature of 298°K, calculated from Latimer.[7] Dotted lines refer to stable or quasistable states. (Reproduced from Radiat. Res. Supplement 3:44, 1963.)

tube. The higher level in the tube corresponds to a free radical or activated state. Once energy has been added to the system to drive the water from the high level to the activated level in the tube, then water will flow from the activated level to the lower level continuously until all the water has been transferred. The energy released in going from the activated level to the low level is used in part to drive the water from the high level to the activated level.

An example of such a chain reaction is shown in Figure 5. The free radicals $R^{\cdot}$ and $RO_2^{\cdot}$ act as chain centers, and an increase in their concentrations can increase the rate of formation of the organic peroxide RO_2H. Biological damage can be caused by the radicals as well as by the peroxide and the radicals derived from the peroxide. These radicals, as well as other similar unstable chemical intermediates, also serve as the catalysts for the inherent biological action of oxygen itself. Increasing the free radical concentration has a pro-oxidant effect, and decreasing the free radical concentration has an antioxidant effect.

The existence of free radicals in biological systems has been demonstrated by the use of electron spin resonance spectroscopy.[50] Some of these biological free radicals may be largely immobile within the cell.[51] It also seems probable that there is a positive correlation between metabolic rate and free radical formation.[26] Thus, it appears that conditions which increase the rate of metabolism increase the organism's sensitivity to oxygen toxicity,[26] and that conditions which decrease metabolism decrease the sensitivity to oxygen toxicity.[26,52]

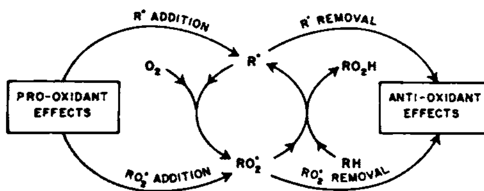
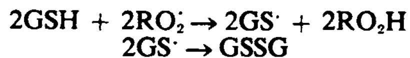


FIGURE 5. Free radical chain reaction. (Reproduced from Radiat. Res. Supplement 3:44, 1963.)

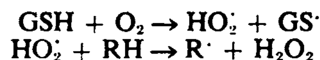
Any biological storage form of energy would eventually tend to be dissipated, resulting in tissue destruction and eventual death of the organism. When the biological antioxidant defenses are damaged, the effects of oxygen toxicity become more apparent. The Gerschman theory of oxygen toxicity points out similarity between the biologically deleterious effects of both x-irradiation and oxygen.[48] X-irradiation increases the free radical concentration, resulting in the release of the stored energy inherent within the system. Thus, an x-ray dose of 500 rads can kill a man; yet the absorbed energy is only 0.0012 kcal/kg of tissue, which is equivalent to a negligible temperature increase of only 0.0012°C. For the primitive biological systems which were predominant when the reducing atmosphere was present, x-irradiation probably would have accelerated hydrogen toxicity. On the other hand, for most of the existing biological systems, x-irradiation merely catalyzes the damaging influence of oxygen by injuring the antioxidant defenses through free radical reactions.

An antioxidant may, under appropriate conditions, exhibit a pro-oxidant effect.[29] For example, a hydrogen donor, such as reduced glutathione, might instead remove free radicals and thus act as an antioxidant, as illustrated by the following reaction:



$$2GSH + 2RO_2^{\cdot} \rightarrow 2GS^{\cdot} + 2RO_2H$$
$$2GS^{\cdot} \rightarrow GSSG$$

It should be pointed out that breaking a given chain reaction by removing the free radical chain centers can possibly produce other free radicals for another chain reaction. A hydrogen donor, such as reduced glutathione, might also activate oxygen to a free radical state ($HO_2^{\cdot}$ or O_2^{-}) and thus now act as a pro-oxidant, as shown below:



$$GSH + O_2 \rightarrow HO_2^{\cdot} + GS^{\cdot}$$
$$HO_2^{\cdot} + RH \rightarrow R^{\cdot} + H_2O_2$$

Thus, glutathione can remove and/or add

free radicals. Experimentally, glutathione has exhibited both antioxidant effects [48,53,54] and pro-oxidant effects.[58]

Cations have also been shown to exert sometimes a pro-oxidant effect,[29,55] presumably by enhancing free radical production.[41] Thus, removal of cations by chelating agents has an antioxidant effect.[29,55]

Therefore, it is apparent that, depending on conditions within the biological system and on the criterion for judging oxidation, the net influence in a given situation may be either pro-oxidant or antioxidant.

SUMMARY

It is speculated that during the time the atmosphere of a planet is changing from one composed of molecular hydrogen, helium, water, methane, and ammonia into one composed of carbon dioxide and molecular nitrogen, a "life" stage is possible, provided an adequate energy source is available and the transition phase lasts long enough to give rise to a biosphere. Although energy can be produced in the biosphere without molecular oxygen, more energy can be produced in a biosphere by its use. A price must be paid for this oxygen utilization, however, since oxygen, acting as a double-edged sword, destroys living material as it supplies the energy for life. The price, then, paid by the biosphere for this convenient source of energy is oxygen toxicity. Antioxidant defense mechanisms were evolved by the biosphere in order to combat the destructive influence of oxygen, which is probably primarily mediated by oxidizing free radicals.

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Control of the Oxidation–Reduction State of Reduced Pyridine Nucleotides *in vivo* and *in vitro* by Hyperbaric Oxygen

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The recent observations that hyperbaric oxygen causes an oxidation of reduced pyridine nucleotide in isolated mitochondria and in tissues of brain, kidney, and liver of the anesthetized rat [1] offer new possibilities to explain the short-term toxic effects of high pressure oxygen. These observations are more relevant to short-term effects than are the slower responses caused by oxygen inactivation of sulfhydryl groups of dehydrogenases[2–4] which have been extended to brain *in vivo*[5,6] and to a wide variety of other materials.[7–10] In the latter studies, sensitive SH groups of particular dehydrogenase systems were pinpointed, especially the α-ketoglutarate system.[11] Detailed studies of the time course of the inactivation of dehydrogenases *in vivo*[12] and *in vitro*[13] emphasize the temporal discrepancies between the rapid effects on the reduced pyridine nucleotide level and the slower effects on the dehydrogenases. These rapid effects appear consistent in their time scale with the rapid incidence of convulsions and may more adequately explain the physiologic effects of high oxygen pressures.[14] The relationship of these observations to those of Wood *et al.*,[15–18] who have observed that γ-aminobutyric acid protects against convulsions, is still obscure.

The experiments described below were performed to extend our fluorometric observations of the oxidation of reduced pyridine nucleotide in liver and kidney, to confirm these observations by analytical measurements, and to enhance our insight on the mechanism of inhibition of reversed electron transfer. A point worth mentioning here is the finding that the influence of the hyperbaric state upon the ATP/ADP ratio *in vivo* is consistent with the inhibition of energy utilization in DPN reduction found *in vitro*.

Materials and Methods

Two general approaches to the measurement of the consequences of high pressure oxygen upon metabolism in tissue, cells, mitochondria, and submitochondrial particles have been employed. The first category includes those methods allowing a "continuous readout" of concentrations. Reduced pyridine nucleotide measured fluorometrically is the most flexible method available, and is applicable to cells and tissues. Flavoprotein can be measured by

fluorescence as well, but cytochromes and quinones up to now have been measured only by their absorption.

The second category of methods involves techniques where a portion of the material under study is rapidly sampled and submitted to detailed chemical analysis. This method, of course, is applicable to all constituents for which analytical methods are available. It does not, however, give continuous readout, and the time resolution is limited by the sampling rapidity.[19]

Animal Chamber

All the continuous readout methods can be used successfully with a small chamber suitable for cell suspensions. Technical difficulties arise in employment of all methods but the fluorometric determination of reduced pyridine nucleotides in the larger animal chamber which is required for the observation of animal tissues under hyperbaric conditions. In this case, observations are made through the window of the pressure tank, and the fluorometer is fitted with telescope optics. These optics allow the imaging of a spot of excitation light on the organ in question and the observation of fluorescent light through a suitable telescope on the photomultiplier, as shown in Figure 1.

FM 21A

FIGURE 1. Fluorometer attached to small animal chamber. Shown left is the compensating photomultiplier, center is the excitation lamp, and right is the measuring photomultiplier.

In testing a small tank (Bethlehem Steel FM-21-A), it could be observed that the pressurization artifact, possibly due to distortion of the glass window, was greater than that in the larger tank used in the experiments reported previously.[1] The cause of this difficulty has not yet been pinpointed, but it was avoided by the use of a fluorescent reference material inside the tank adjacent to the organ being studied. A small portion of the exciting light was reflected onto this fluorochrome and was viewed by a separate photomultiplier and telescope. Since the excitation light for the two fluorescent materials, reference and sample, follows the same path through the glass, and since the two photomultipliers viewed the two separate fluorescences along similar optical paths in and out of the chamber, compensation for the tank artifact was accurate to about 1% for rapid and slow pressurizations that did not cause fogging of the chamber window.

Although negligible artifact was observed with a fixed fluorochrome, persistent artifacts were observed with rat tissues in the tank. Their amplitude depended upon the rate at which the pressure was changed in the tank and were 5–10% of the total fluorescence signal for fast pressurizations. Since the artifact appeared to be zero at constant pressure, changes of fluorescence intensity of the tissue were determined (1) by stepwise changes in pressure, with the plateau value taken about 2 min later, when the artifact had subsided, or (2) by pressurization of the tank slowly through a needle valve at

rates of 3–5 liters/min. The latter method gave more acceptable records, and those included in this report were obtained in this manner. Otherwise, the experimental procedure was similar to that employed in the preliminary series of experiments upon which our earlier report was based.[1] For example, artificial respiration within the chamber was provided for experiments in which amobarbital (Amytal) was used. The tank was opened for injections.

One great advantage of the tank employed in these studies over the larger tank used in the studies reported earlier[1] was the rapidity with which the tank could be depressurized and the door opened (~ 5 sec). This allowed rapid sampling of the tissue under essentially hyperbaric conditions.

Chamber for Cell Suspensions

Measurement of changes in the steady state of pyridine nucleotides in cells and mitochondrial suspensions was generally made fluorometrically with 366-mμ excitation and 450-mμ measurement. However, absorbancy changes were measured spectrophotometrically at 340 mμ, with 375-mμ measurement used as reference. Oxidation and reduction of quinone was also measured with this technique, with 275 mμ used for measurement and 295 mμ as the reference wavelength. In this case, the spectrophotometer illumination was a high intensity deuterium arc, as described elsewhere.[20]

Figure 2 shows the present hyperbaric chamber suitable for the study of cell suspensions. This apparatus has a top window for fluorometric monitoring of oxidation–reduction changes. Shown on the left is a "compensated" type of fluorometer in which the excitation spot is imaged on the surface of the cell suspension; a telescope is provided for the photomultiplier which views the spot. As mentioned in the case of the animal chamber, a portion of the 366-mμ light provides fluorescence excitation for a piece of paper which is viewed by a second photomultiplier. Their

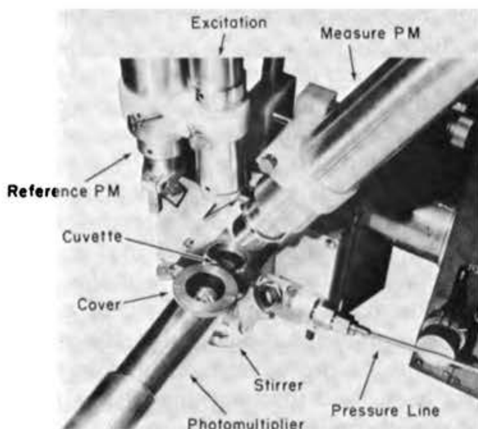

FIGURE 2. Chamber for the study of cell suspensions in mitochondria under hyperbaric conditions. The illustration shows a compensated fluorometer with compensating photomultiplier on the left, excitation source in the center, and measuring photomultiplier on the right. The chamber is also provided for light transmission and measurements with the double-beam spectrophotometer for the purpose of measuring the oxidation-reduction state of cytochromes. The fluorometer for measuring reduced pyridine nucleotide concentration can be replaced by one for measuring flavoprotein only, or flavoprotein and pyridine nucleotide.

outputs are subtracted to provide compensation for fluctuations in the intensity of the excitation light.

Another form of this apparatus not shown employs a special fluorometer which allows nearly simultaneous measurement of reduced pyridine nucleotide (366-mμ excitation, 450-mμ measurement) and flavoprotein (436-mμ excitation, 560-mμ measurement). This fluorometer is also suitable for viewing the pressurized suspension through the top window of the small chamber (Figure 2).

The chamber is also provided with a pair of windows allowing a horizontal light beam to pass through the suspension and thus permits observations of cytochrome changes. This is particularly convenient for ascertaining that the cytochrome chain remains completely reduced under conditions where the high pressure oxygen is causing an oxidation of reduced pyridine nucleotide or flavoprotein, as

observed fluorometrically through the top window.

At present, no provision is available for injecting reagents into the suspension under pressurization; studies have so far been carried out satisfactorily by the addition of various reagents immediately before or after an interval of pressurization.

The apparatus described in Figure 2 contains a rotating magnet which rapidly stirs the contents of the cuvette, a condition essential for rapid equilibrium of the contents of the cuvette with oxygen. It has been used for sampling a suspension of mitochrondria in the course of a change of steady state due to hyperbaric conditions. The suspension is momentarily pressurized, the sample withdrawn, and the experiment continued. Recovery from hyperbaric conditions is slow enough compared to the sampling time to permit this technique to be effective.

Preparation of Animals

Male rats (165–220 gm) were anesthetized by intraperitoneal injection of urethane (1.2 gm/kg body weight) and the required organ was exposed and supported to minimize movement, care being taken not to interfere with blood supply to the organ. Tracheal cannulas were inserted in experiments with amobarbital, where artificial ventilation was sometimes required. All drugs were injected into the cannulated external jugular vein.

The animal was positioned in the pressure chamber so that the light beam, after passing through the glass portal at the top of the chamber (366-mμ excitation), fell onto the exposed organ through a glass plate fitted diagonally above the organ. Part of the light was then reflected onto a paper reference, and recordings were differential between the reference signal and the fluorescence signal from the tissue, as mentioned above.

Drugs and Dosages

Amobarbital was dissolved in saline to give a 25 mg/ml solution. Pentachlorophenol (PCP) was dissolved in NaOH, then titrated with HCl to pH 8.2. 2,4-Dinitrophenol was similarly dissolved, the pH of the final solution being 7.2.

Analytical Methods for Animal Tissues

Exposed liver and kidneys of anesthetized control rats or rats after pressurization were rapidly frozen with tongs precooled in liquid nitrogen. For the experiments in which the organs were frozen for tissue analysis, the chamber was decompressed at the maximum rate (5 sec), and the total time from full oxygen pressure to the freezing of the tissue was between 10 and 13 sec. For the analytical studies in rat brain, unanesthetized rats were subjected to high pressure oxygen treatment and decapitated, and the head was allowed to fall into liquid nitrogen. The freezing time of the brain to below-zero temperature with liquid nitrogen was 22 sec, and was considerably faster than with Freon at −140°C (42 sec). The frozen heads were brought to −20°C in an ice-salt eutectic mixture, at which temperature it was feasible to remove the brain from the skull. The brain was then cooled again to liquid-nitrogen temperature. Tissues were pulverized in a stainless-steel percussion mortar surrounded by dry ice, and aliquots of the powder were taken for acid and alkali extraction and for determination of their water content. Perchloric acid and alcoholic KOH extracts were prepared as described by Williamson,[21] except that the KOH concentration was decreased to 0.5 N since this improved the recovery of the TPNH. Adenine and pyridine nucleotides and glycolytic intermediates were analyzed by fluorometric procedures[22] with the modifications previously described.[21] Recoveries of the reduced pyridine nucleotides were made only with liver extracts where the mean DPNH recovery was 85% and the TPNH recovery 90%. Recoveries of intermediates in the perchloric acid extract were quantitative.

Studies of Rat Organ *in vivo*

Direct Fluorometric Assays

Rat Liver in vivo. As found previously, the fluorescence of reduced pyridine nucleotide observed at the surface of the rat liver decreased at high oxygen pressures. In further support of our previous work,[1] no distinctive plateau in the relationship between fluorescence decrease and oxygen pressure was observed up to oxygen pressures of 10 atm. When pressurization was rapid, the tank artifact mentioned earlier became apparent and was followed by the slower biochemical response of the liver. More satisfactory results were obtained with pressurization through a needle valve, as mentioned above (Figure 3). With this method of pressurization, the oxidation of the nucleotides appeared to "keep pace" with the increase in pressure, so that little further effect was observed after final pressure was reached.

Figure 3 demonstrates that the cycle of oxidation of the pyridine nucleotides is reversible and can be repeated. This pressurization technique was used in later experiments where the effects of uncoupling agents or amobarbital on the hyperbaric response were studied. Depending upon the susceptibility of the animal to irreversible oxygen poisoning and the final pressure of the exposure, two to four such reversible cycles can be obtained. Analyses of results for 14 animals in which such compression cycles were obtained gave decreases of $13 \pm 1\%$ of the initial fluorescence level at pressures of 120–150 psi. The response to anoxia in rat livers, also shown in Figure 3, was transient,[23,24] and the maximum fluorescence increase was approximately 60% of the anoxic response in this preparation.

Amobarbital was more effective in establishing the maximum level of fluorescence, as illustrated by Figure 4, which shows a typical hyperbaric response and reversal on decompression. Amobarbital was then injected, and the fluorescence, which by then had increased quite rapidly, returned to the initial level. This transient effect of amobarbital in the tissue may have reflected (1) initial dilution effects, as the compound was redistributed among various organs, or (2) metabolism of the compound in the liver, such that amobarbital reaching the liver was detoxified at the same rate. In any case, repeating the pressure cycle after amobarbital addi-

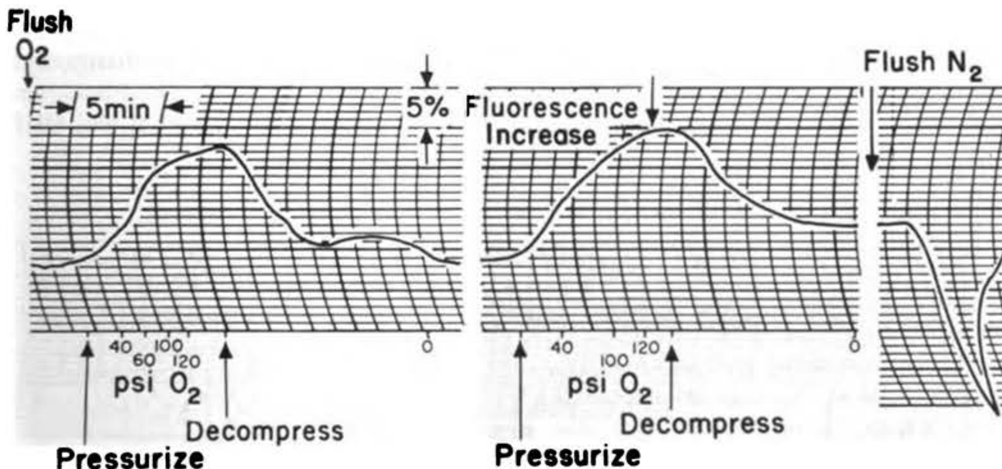

FIGURE 3. The response of rat liver to repetitive pressurization and decompression with oxygen. The values of pressure are included. The sensitivity for measuring fluorescence changes is also indicated.

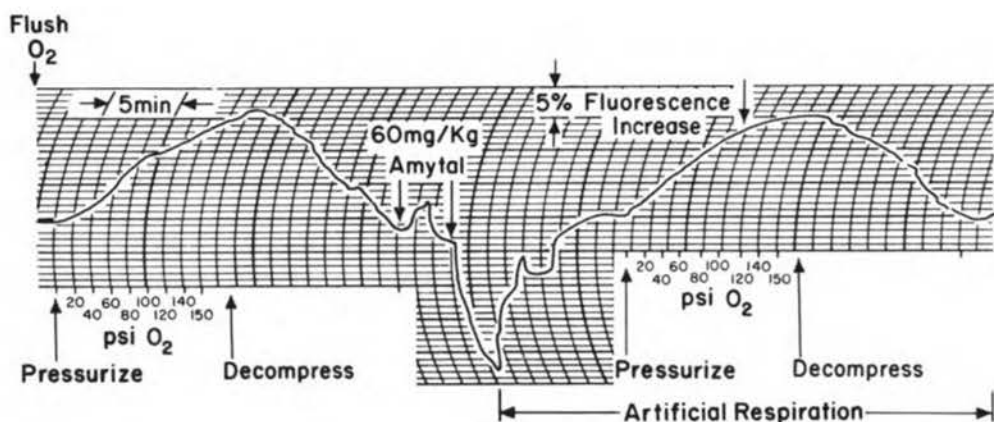

FIGURE 4. Hyperbaric response of rat liver in the presence and absence of amobarbital.

tion in this manner produced the same effect as pressurization before amobarbital. This dose sometimes caused cessation of respiration, and artificial respiration was then employed. Figure 4 also shows that in the liver the hyperbaric oxidative effect was almost as large as the amobarbital reductive action.

In order to compare the hyperbaric response of the liver with that of a known biochemical agent, the effect of uncouplers was tested. In most experiments, PCP was used. In one experiment, 2,4-dinitrophenol gave the same result, but this uncoupler is somewhat less suitable for fluorescence studies due to the possible quenching of DPNH fluorescence. Preliminary experimentation with PCP indicated that a dose of 20 mg/kg increased rat rectal temperature 2–3°C in 30 min and caused an initial stimulation of respiratory rate of 20%. In several experiments, uncoupling agents decreased fluorescence by 7–10% of the initial level of fluorescence within 1–5 min of injection of the uncoupler. (The results of an experiment with PCP are shown in Figure 5.) When the fluorescence had reattained its initial level after a compression–decompression cycle, PCP was slowly injected. A plateau effect of oxidation was achieved with 3 mg PCP (15 mg/kg), 4 mg total PCP having been injected. The compression–decompression cycle was then repeated and obviously elicited a much smaller response than initially, with the final oxidized level reached being the same in both control and PCP-plus-OHP

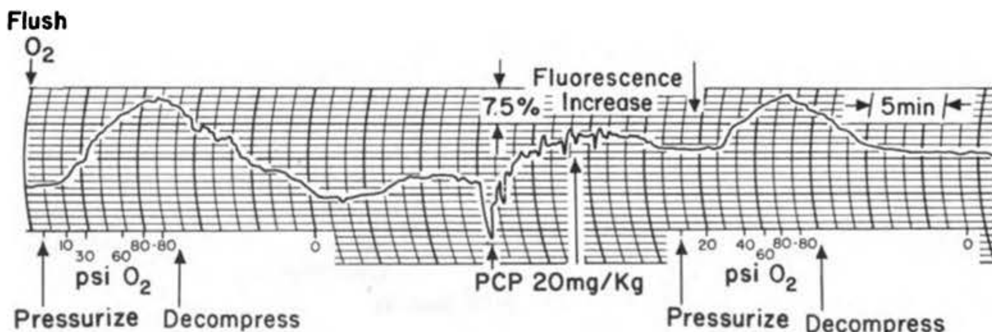

FIGURE 5. Hyperbaric response of rat liver in the presence and absence of an uncoupling agent, pentachlorophenol.

cycles. In other words, the effects of PCP plus OHP equaled the effect of hyperbaric oxygen alone.

Rat Kidney in vivo. Experiments on the kidney under hyperbaric conditions revealed a mean decrease of fluorescence of $10 \pm 1\%$ of the initial level. As previously observed, the hyperbaric effect in the kidney showed a more distinct plateau than that in the liver.[1] The pressure at which a plateau occurred varied from 5 to 9 atm, with half-maximal effects occurring at 2–6 atm. In kidney, as in liver, the rapid oxidation of the pyridine nucleotides under hyperbaric conditions was reversible, but there was often considerable hysteresis in the decompression–compression cycle in the kidney. Figure 6, showing two cycles of compression and decompression, illustrates the types of responses seen in the kidney under conditions of gradual pressurization. Animals were compressed to a final pressure of 120–150 psi in these experiments.

When amobarbital (60 mg/kg) was injected over approximately 2 min, a rapid fluorescence increase occurred as pyridine nucleotides became reduced. However, the large amobarbital effect ($45 \pm 7\%$ of the initial fluorescence level in 10 experiments) was not sustained, and during the 4–5 min after injection the fluorescence returned toward the initial level, then plateaued at different levels of reduction of the pyridine nucleotides in different

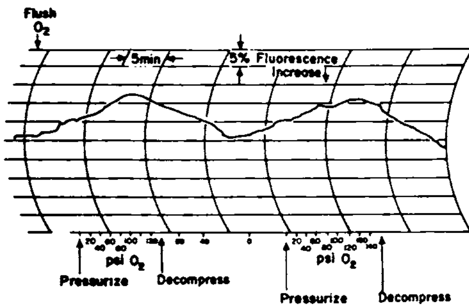

FIGURE 6. The effect of cycles in compression and decompression upon the fluorescence emission of rat kidney.

experiments. Such a level could be maintained for some time in contrast to the effect seen in comparable experiments with liver (range 4–30% increase of fluorescence from the initial level, mean $15 \pm 3\%$). The extent of the hyperbaric response after amobarbital addition depended upon the plateau level of pyridine nucleotide reduction before pressurization. In experiments where only a slightly reduced pyridine nucleotide level was maintained, the decrease in fluorescence due to high pressure oxygen was less than the control compression cycle. In other cases, where a plateau was maintained at considerably more reduced pyridine nucleotide levels, the hyperbaric response appeared greater than the control pre-amobarbital cycle. Whatever the size of the hyperbaric response, the greatest oxidized level reached at 10 atm of oxygen after amobarbital addition was very close to the initial normoxic fluorescence level. The compression cycles after amobarbital addition were also reversible, indicating that simple drift of the reduced pyridine nucleotides to the normal level had not occurred. It appeared in these experiments that high pressure oxygen could reverse the effect of amobarbital in holding pyridine nucleotides reduced, but up to 150 psi oxygen pyridine nucleotides did not appear to be oxidized above the initial normoxic level. This effect in one experiment is illustrated in Figure 7. After a control compression–decompression cycle, amobarbital was injected. When a plateau was reached after amobarbital addition, the hyperbaric cycle was repeated, and the decrease of fluorescence was considerably larger than that in the control cycle, but the final fluorescence level was the same as the initial normoxic level. On decompression, the fluorescence returned almost to the amobarbital plateau level. Nitrogen was then flushed through the chamber, and the animal died in anoxia, with a large reduction of pyridine nucleotides.

The *in vivo* effect of uncouplers on kidney was also studied. Pentachlorophenol

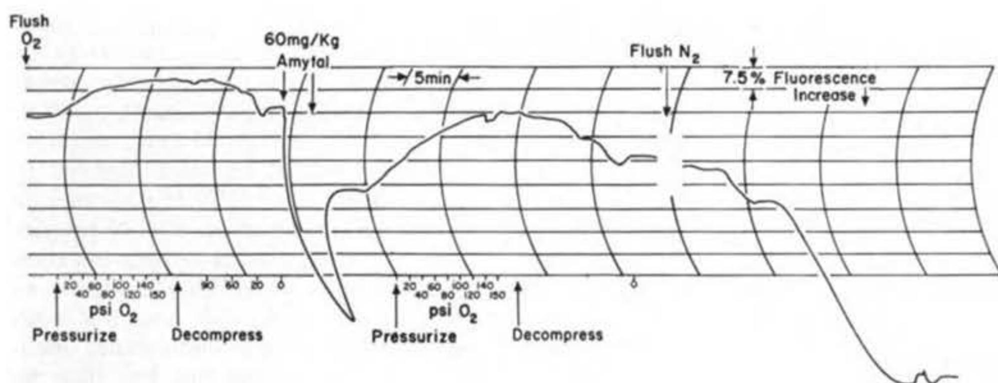

FIGURE 7. Effects of amobarbital upon the fluorescence emission of rat kidney.

was used as the uncoupling agent in experiments where the kidney fluorescence was monitored. As in experiments described for the liver, PCP was titrated into the animal in doses of 0.5 mg, until a plateau level of oxidation of pyridine nucleotides was reached at 1.5–2.0 mg/200 gm body weight. The dose to produce such oxidation of pyridine nucleotides appeared to be more critical in kidney than in liver, with 10 mg/kg producing a maximal effect, while 12 mg/kg caused some reversal of the oxidative action in kidney, possibly due to inhibition of electron transfer by the uncoupling agent. The oxidations produced by PCP in the kidney were small, being $4 \pm 1\%$ of the initial fluorescence level (six trials). In contrast to the liver, the hyperbaric oxygen compression cycle was only slightly reduced after the uncoupler–control hyperbaric cycle showed $9 \pm 1\%$ fluorescence decrease; hyperbaric cycle after PCP showed $7 \pm 1\%$ fluorescence decrease. Thus, a slightly higher oxidative level was reached in the presence of the uncoupler and high pressure oxygen than the level reached with hyperbaric oxygen alone.

Rat Brain in vivo. Results previously obtained with the fluorometric technique[1] showed that the hyperbaric effect on the exposed brain was similar to that on the kidney. Thus, oxidations of 4–10% of the initial fluorescence level were obtained with plateaus at approximately 5 atm. In the present experiments, the chemical assay data were obtained from rats in which the brain had not been exposed, as anesthesia could then be avoided and convulsions observed.

Chemical Determinations by the Sampling Technique

Pyridine nucleotide analyses of several organs of rats exposed to hyperbaric oxygen have confirmed the direct fluorometric method in showing that an oxidation of pyridine nucleotide occurs; in addition, they give a quantitative description of changes in both DPNH and TPNH.

Table 1, outlining results with rat liver, shows that in normal rat liver DPN is the predominant nucleotide. TPNH levels are about one-half those of DPN, while DPNH and TPN are both present in about one-eighth the amount of DPN. After 10-min exposure to oxygen at 9 atm, there is a significant decrease of DPNH. DPN shows no significant change, since the expected change is small compared with the total DPN content. TPN and TPNH, however, change by appropriately equivalent amounts in opposite directions. In liver, unlike kidney and brain, the change of TPNH is greater than that of DPNH.

Most of the DPNH in liver, as with other tissues, is located in the mitochon-

TABLE 1. Effects of Hyperbaric Oxygen on Pyridine Nucleotides in Rat Liver

Condition of rat	No. rats	DPN	DPNH	TPN	TPNH
		(mμmoles/gm fresh weight)			
Normal (controls)	9	862 ± 24	113 ± 6	102 ± 6	440 ± 15
OHP-exposed	8	864 ± 23	59 ± 5	190 ± 10	366 ± 12
OHP change		+ 2	− 54	+ 88	− 74
P value		NS	0.001	0.001	0.005

NS, not significant.

drial compartment of the cell, and the bulk of the DPNH oxidation with hyperbaric oxygen is probably derived from mitochondrial change under these conditions. However, changes in the oxidation–reduction state of the cytoplasmic pyridine nucleotides may be determined qualitatively by measuring the ratio of the reduced to oxidized forms of substrate couples reacting with DPN-linked dehydrogenases in the cytoplasm.[25] Table 2 shows a comparison of the three redox couples, lactate–pyruvate, α-glycerophosphate–dihydroxyacetone phosphate, and malate–oxaloacetate in livers of normal rats and rats exposed to 9 atm oxygen. Under hyperbaric conditions there is a decrease in each one of the substrate couple ratios. The redox potential of the DPN system in the cytoplasmic space may be calculated from these ratios using the Nernst equation:

$$E_h = E_0' - \frac{RT}{nF} \ln \frac{[\text{red}]}{[\text{ox}]}$$

and values of −207, −214, and −185 mv for the mid-potentials of the lactic, α-glycerophosphate, and malate dehydrogenases, respectively. Table 2 shows that the redox potentials calculated from the three substrate couple ratios are in fairly close agreement, and that there is a change of 8 to 13 mv in the positive direction with hyperbaric conditions. These results indicate that the cytoplasmic space, as well as the mitochondrial space, is affected by high pressure oxygen. Analyses of whole-liver extracts for glycolytic intermediates gave no indication of an inhibition at glyceraldehyde phosphate dehydrogenase with hyperbaric conditions; hence it is more probable that the redox change of the DPN system in the cytoplasm is derived from a fundamental change of the redox state in the mitochondria, rather than from a direct inhibition of a cytoplasmic dehydrogenase. We shall return to this point of the compartmentation of the pyridine nucleotide changes later.

TABLE 2. Redox State of Liver Cytoplasmic Pyridine Nucleotides in Rats Exposed to Hyperbaric Oxygen

O_2 pressure (psi)	Lactate / Pyruvate	α-GP / DAP	Malate / OAA
	Substrate couple ratios		
(1 atm)	21.7 ± 1.3	15.1 ± 1.3	156 ± 43
135	12.3 ± 1.1	5.8 ± 0.6	63 ± 5
	Redox potentials (mv)		
(1 atm)	− 248 ± 0.8	− 250 ± 1.1	− 251 ± 3.6
135	− 240 ± 1.2	− 237 ± 1.6	−240 ± 1.0

α-GP, α-glycerophosphate.
DAP, dihydroxyacetone phosphate.
OAA, oxaloacetate.

TABLE 3. Effects of Hyperbaric Oxygen on Pyridine Nucleotides in Rat Kidney

Condition of rat	No. rats	DPN	DPNH	TPN	TPNH
		(mμmoles/gm fresh weight)			
Normal (controls)	5	575 ± 18	44 ± 6	50 ± 2	109 ± 3
OHP-exposed	4	601 ± 28	22 ± 4	63 ± 2	97 ± 8
OHP change		+ 26	− 22	+ 13	− 12
P value		NS	0.05	0.01	0.2

NS, not significant.

Table 3 shows the results of pyridine nucleotide analyses in the kidney. In these experiments, the left kidney of anesthetized rats was exposed, and the experimental rats were subjected to 9 atm oxygen pressure for 10 min. Control rats were left for a similar time at normal atmospheric pressure. As with liver, the DPNH and TPNH levels in kidney decreased in rats exposed to hyperbaric oxygen, and there was an increase of TPN.

Table 4 shows the results of corresponding experiments with brain tissue. The rats were not anesthetized while in the pressure chamber. The oxygen pressure was increased gradually at 20 psi/min to a final pressure of 105 psi. The time of pressurization was 16 min, by the end of which all the rats had exhibited severe convulsions. The DPN levels in normal brain were 40% of those found in liver and 60% of those in kidney. There was a significant increase of DPN in the rats exposed to hyperbaric oxygen and a corresponding decrease of DPNH. Similarly, TPN levels increased and TPNH levels decreased. These changes, although very small, were statistically significant. The total content of TPN + TPNH in brain was 21% of that in kidney and only 6% of that in liver.

These tables and figures make it apparent that in the three organs studied pressurization of the rat caused an oxidation of both DPNH and TPNH. The relative changes of analytically determined DPNH and TPNH have not been followed on a kinetic basis, but the fluorescence traces show that the onset of the effect was extremely rapid. Relative changes of the DPNH and TPNH levels with hyperbaric oxygen and with amobarbital anoxia or nitrogen anoxia in the three organs are summarized in Table 5. There was a 50% oxidation of DPNH with hyperbaric oxygen compared with control levels in all three tissues. The percentage oxidation of TPNH in liver was considerably greater, however, than in kidney or brain. Amobarbital or anoxia, on the other hand, affected mainly the DPN system, as shown in the bottom half of the table, and DPNH increased by 100–200% compared with a TPNH change of only 5–9%.

In addition to changes of the pyridine nucleotides with hyperbaric oxygen, fairly consistent changes of the adenine nucleotides in liver, kidney, and brain were observed. These changes (Table 6) showed that the general change was toward an increase of the ATP/ADP ratio. The

TABLE 4. Effects of Hyperbaric Oxygen on Pyridine Nucleotides in Rat Brain

Condition of rat	No. rats	DPN	DPNH	TPN	TPNH
		(mμmoles/gm fresh weight)			
Normal (controls)	7	344 ± 7	43 ± 4	5.4 ± 0.5	28.3 ± 0.6
OHP-exposed	7	361 ± 4	21 ± 3	9.1 ± 1.0	25.4 ± 0.7
OHP change		+ 17	− 22	+ 3.7	− 2.9
P value		0.05	0.001	0.005	0.01

TABLE 5. Summary of Reduced Pyridine Nucleotide Changes in Rat Tissues

	% Oxidation change [a] (OHP at 135 psi)		
	Liver	Kidney	Brain
DPNH	48	50	51
TPNH	17	11	10

	% Reduction change [a]		
	Liver (Amobarbital)	Kidney (Amobarbital)	Brain (Anoxia)
DPNH	160	204	100
TPNH	5	9	8

[a] Values represent percent of normal control values.

largest and most consistent changes were seen with liver, an organ which lacks a store of creatine phosphate. As seen in the table, the ATP level increased by 11%, while ADP and AMP decreased by 30% and 52%, respectively. With kidney, the only statistically significant change was a 9% decrease of ADP. Brain gave the interesting response of a large increase in the level of creatine phosphate in addition to an increase of ATP, but the latter was not statistically significant, due to the rather large variation among animals. The decrease of AMP but not of ADP was significant in this tissue. We have therefore reached the important conclusion that hyperbaric oxygen causes an increase of high-energy phosphate compounds in addition to an oxidation of the pyridine nucleotides.

A summary of the fluorescence levels reached during hyperbaric oxygenation, anoxia, and in the presence of amobarbital and uncouplers, compared with chemical assays for DPNH and TPNH, is given for liver (Figure 8), kidney (Figure 9), and brain (Figure 10). Fluorescence data include statistics from experiments where final pressures were 120–150 psi oxygen. For the chemical assays of pyridine nucleotides in livers and kidneys of rats injected with amobarbital, the time for tissue freezing was chosen to coincide as closely as possible with the peak of the amobarbital response, as seen in the fluorescence studies. For the reduced state in brain, the rats were made to breathe nitrogen and were decapitated 30 sec after breathing stopped.

In general, the fluorescence data correlated well with the analytical data for the hyperbaric and inhibited states in liver

TABLE 6. Effect of Hyperbaric Oxygen on Adenine Nucleotide Levels [a]

Nucleotide	Liver Control	Liver OHP	Kidney Control	Kidney OHP	Brain Control	Brain OHP
CrP	—	—	387 ± 20	392 ± 31	1250 ± 108	2770 ± 273
ΔCrP	—	—	+ 5		+ 1520 [b]	
ATP	3079 ± 65	3461 ± 56	2160 ± 16	2243 ± 136	1770 ± 202	2036 ± 113
ΔATP	+ 337 [b]		+ 83		+ 266	
ADP	808 ± 36	567 ± 16	372 ± 12	337 ± 11	588 ± 45	553 ± 65
ΔADP	− 241 [b]		− 35 [b]		− 35	
AMP	176 ± 13	86 ± 6	78 ± 6	83 ± 7	229 ± 30	145 ± 31
ΔAMP	− 90 [b]		+ 5		− 84 [b]	

CrP, creatine phosphate.
[a] Values expressed as mμmoles/gm fresh weight.
[b] Significant change ($P = 0.05$ or less).

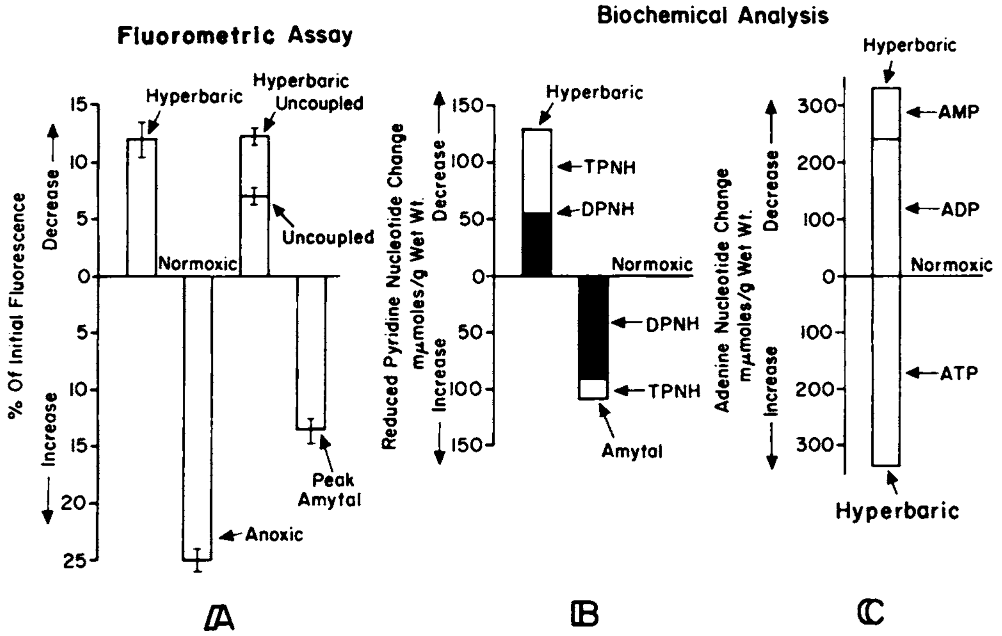

FIGURE 8. Correlation of fluorescence changes in a group of exposed rat livers artificially determined with DPNH and TPNH level. Vertical bars denote two standard errors of the mean.

and kidney. The analytical data for the sum of the reduced pyridine nucleotide increase during anoxia in brain were lower than the corresponding fluorescence change, but the disagreement was not serious in view of the fact that the fluorescence data on brain were the result of a single experiment.

Several points of interest arise from comparison of these data. First, it is apparent that the major part of the fluorescence increase with anoxia or amobarbital in liver, kidney, and brain, as in heart,[26] was due to an increase of DPNH. This increase may be roughly apportioned between the mitochondrial and cytoplasmic spaces. Analyses of pyridine nucleotide changes in isolated liver mitochond-

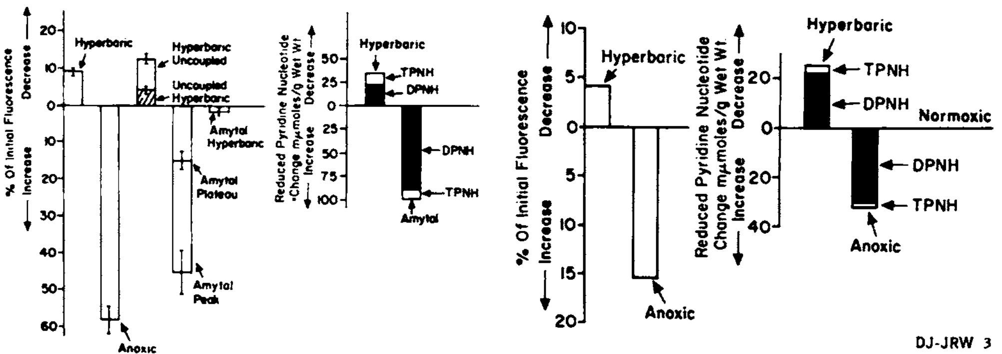

FIGURE 9. Correlation of fluorescence changes in a series of exposed rat kidneys with chemical assays of DPNH and TPNH.

FIGURE 10. Correlation of the fluorescence change in an exposed rat brain with chemical assays of DPNH and TPNH in groups of four to six rats.

ria (not reported in detail here) have shown that the total DPN+DPNH is 2.62 μmoles/gm protein and the total TPN+TPNH is 3.52 μmoles/gm protein. Chemical analyses have shown that the mitochondrial DPN system, in the fully inhibited state (State 5), is 71.5% reduced and the TPN system 95% reduced. Using the value of 60 mg mitochondrial protein per gram of liver (fresh weight, given by Scholz and Bücher [24]), one can calculate that fraction of the total pyridine nucleotide change due to mitochondria. In this manner, the tentative conclusion is reached that in the amobarbital-inhibited liver, 64% of the total DPNH and 43% of the total TPNH is mitochondrial, and during the change from the normoxic to the amobarbital-inhibited state, 70% of the total DPNH change and 45% of the total TPNH change is mitochondrial. This calculation differs from that given by Scholz and Bücher[24] in assigning a smaller proportion of the total TPNH to the mitochondria.

Second, it is clear that in liver, but in neither kidney nor brain, a large part of the total reduced pyridine nucleotide decrease in the hyperbaric state is due to TPNH. By using values of 22% and 80% reduction of the mitochondrial DPN and TPN system in hyperbaric livers, one can calculate that 57% of the total DPNH and 46% of the total TPNH is mitochondrial. On this basis, 68% of the total DPNH change from the normoxic to the hyperbaric state and 28% of the total TPNH change are due to an oxidation of mitochondrial pyridine nucleotides. The significance of these changes to liver function is a subject for future research.

Studies of Cells

One of the ambiguities in the study of intracellular oxidation–reduction levels in solid tissues is the possibility that hyperbaric oxygen provides adequate oxygenation of normally hypoxic tissues, a point particularly relevant to the responses of liver. While the experimental conditions here appeared to be satisfactory in avoiding this ambiguity, the possibility of studying hyperbaric responses in cell suspensions where oxygen diffusibility through the solid tissues is not a factor can present additional evidence on this point.

We have studied suspensions of Ehrlich ascites tumor cells because their relatively low respiration rate (as compared with yeast) makes anoxia unlikely at the concentrations required for our experiment, and in addition these cells have the pathway of energy-linked DPN reduction.*

Hyperbaric Response in Ascites Tumor Cells

When an aerobic suspension of ascites tumor cells was placed in the hyperbaric apparatus with the fluorometer attachment for measuring reduced pyridine nucleotide, as described above, nitrogen flushing gave an increase of fluorescence of approximately 15% over a 30-sec period (Figure 11). When the anaerobic cells were then flushed with oxygen at 1 atm, there was an almost exact return to the initial level, suggesting that the cells were already adequately oxygenated with atmospheric pressures of oxygen. At this point, pressurization at 11 atm led to an

* In an earlier communication [1] we indicated the responses of suspensions of bakers' yeast cells to hyperbaric conditions. While these responses were superficially similar to those observed with other cells and tissues, the presence of a highly active peroxidase in yeast cells which can accept electrons from the cytochrome chain[27] suggests that the hyperbaric response of these cells should be interpreted with caution. This peroxidase could be activated by increased auto-oxidation and enhanced production of H_2O_2 by flavins not associated with the respiratory chain. In addition, various investigators have so far failed to find energy-linked DPN reduction in these cells. Until we have thoroughly investigated the carbon monoxide sensitivity of the hyperbaric response of yeast cells and until the existence of the energy-linked pathway for DPN reduction has been disproved, we will suspend judgment on the interpretation of data obtained from yeast cells and present here our data on ascites tumor cells.

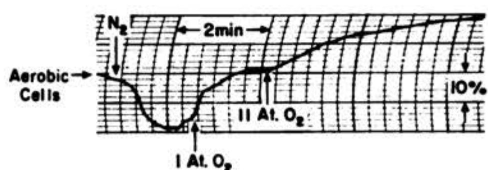

FIGURE 11. Effect of anoxia and high pressure oxygen upon the reduced pyridine nucleotide of ascites tumor cells. Fluorometric measurements: excitation at 366 mμ, measurement at 450 mμ.

oxidation of reduced pyridine nucleotide almost equal in amplitude to the oxygen–nitrogen change. The response was fairly slow; about 4 min elapsed before a plateau was reached.

Figure 12 illustrates the method by which the oxygen concentration required for half-maximal hyperbaric response was obtained. This figure is essentially the same as Figure 11, except that the initial pressurization level was 4 atm, followed by pressurization to 9 atm. A detailed study of this characteristic indicated half-maximal effect at 6 atm with a plateau of 9–10 atm. The oxygen requirements for half-maximal hyperbaric response in ascites tumor cells thus resembled those observed in liver.

Hyperbaric Response of the Inhibited Respiratory Chain

When ascites cells under aerobic conditions were treated with 2 mM sulfide (Figure 13), reduction of pyridine nucleotide occurred in a biphasic reaction, presumably in the mitochondrial compartment first, and then in the cytoplasmic space. When a steady state was reached, the cells were equilibrated with 1 atm of oxygen followed by 9 and then 11 atm. The oxidation of reduced pyridine nucleotide proceeded over the next 10 min to levels determined by the oxygen pressure. This characteristic response of cells was also observed in mitochondrial suspensions (see below).

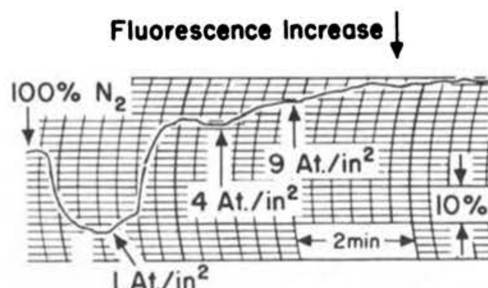

FIGURE 12. The effect of two different oxygen pressures on the response of reduced pyridine nucleotide of a suspension of ascites tumor cells.

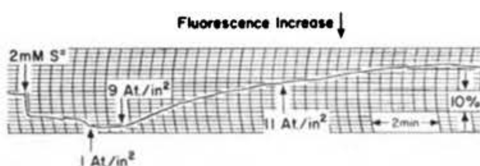

FIGURE 13. The response of reduced pyridine nucleotide of sulfide-inhibited ascites tumor cells to high pressure oxygen.

STUDIES OF MITOCHONDRIA

Rat Liver Mitochondria

Experiments so far have not clearly distinguished the hyperbaric responses of mitochondrial and cytoplasmic spaces. Thus, studies of isolated mitochondria under hyperbaric conditions seem particularly appropriate. Figure 14 indicates fluorometric responses of the reduced pyridine nucleotide component of a suspension of rat liver mitochondria to hyperbaric conditions. The mitochondria,

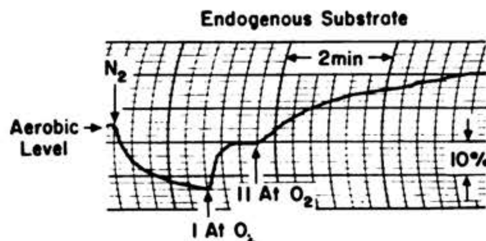

FIGURE 14. The response of the reduced pyridine nucleotide component of rat liver mitochondria to anoxia and to high pressure oxygen.

initially equilibrated with oxygen, were flushed with nitrogen, causing increased fluorescence (a downward deflection). On replacement of the nitrogen with oxygen, the fluorescence returned nearly to the initial level. Thereafter, pressurization with 11 atm oxygen gave, in 4 min, a fluorescence decrease slightly in excess of that observed on adding 1 atm oxygen to the anaerobic system. The reaction was fairly rapid, half-maximal effect being obtained in about 1 min.

Emission Spectra for Hyperbaric Reactions. Using rat liver mitochondria, we examined the emission spectra of the reduced pyridine nucleotide before pressurization, and at 4 and 9 min after 9 atm pressurization. The time course of the reaction is illustrated in Figure 15, and the moments at which spectra A, B, and C were obtained are indicated by the arrows. The wavelength was fixed initially at 480 mμ, and a base line was established. Then a spectrum was recorded by a scanning of the region 400-600 mμ, as indicated in diagram A. The suspension was then pressurized at 9 atm, and the fluorescence diminished according to the indicated time course. When the reaction was approximately half complete, spectrum B was recorded. The reduced pyridine nucleotide fluorescence was considerably less, and the maximum shifted to 495 mμ. When the fluorescence decrease reached a plateau, spectrum C was recorded, and here scarcely any fluorescence in the 480 mμ region remained. Instead, flavin fluorescence at 516 mμ was observed.[28] These data indicate that the component responsible for the fluorescence changes in liver mitochondria has a fluorescence characteristic of reduced pyridine nucleotide.

The Correlation of Fluorescence Changes and Analysis for DPNH and TPNH. Analyses for DPNH and TPNH were carried out before and after pressurization of a sulfide-inhibited system with 16 atm oxygen (Figure 16). The first assay was made immediately after adding sulfide, and two more assays followed at intervals

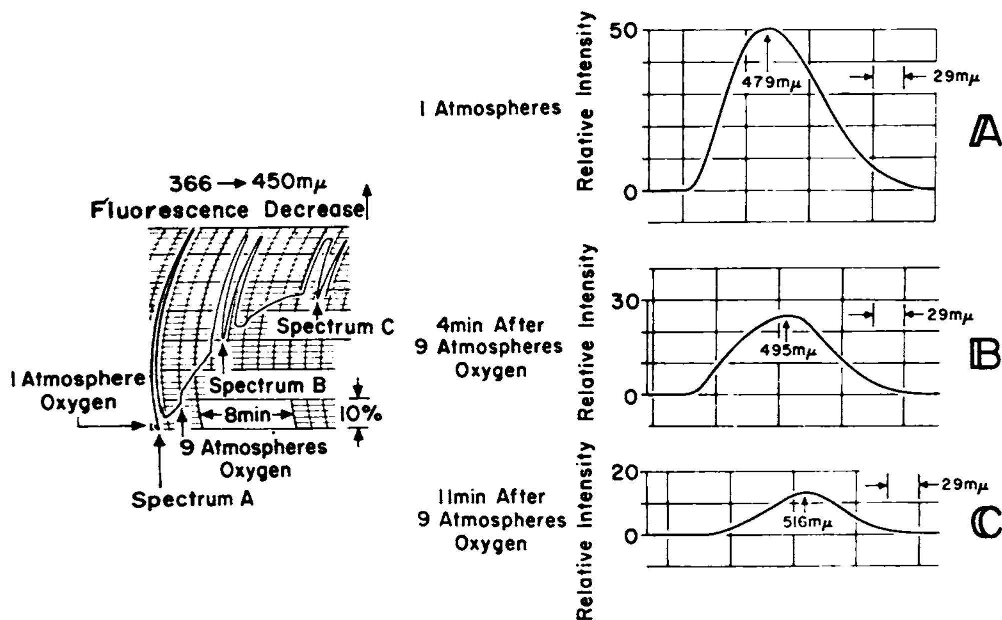

FIGURE 15. Left, the time course of reduced pyridine nucleotide oxidation in rat liver mitochondria with indications A, B, C of the times at which emission spectra are recorded.

of approximately 5 min. The tracings clearly indicate the exhaustion of both DPNH and TPNH as the fluorescence diminished under the hyperbaric condition. Thus, the diagram indicates no particular selective action of hyperbaric oxygen upon DPNH rather than TPNH; presumably, these systems were maintained in appropriate proportions by the energy-linked pathways.[29]

Figure 16 shows the time correlation between the metabolite assays and the fluorescence changes. A more detailed and quantitative analysis appears in Table 7, in which both the reduced and the oxidized forms are evaluated. Conditions in the latter study were similar to those of Figure 16, except that supplements of glutamate and succinate were present. Despite the presence of these substrates, pressurization at 10 atm caused considerable oxidation of DPNH and TPNH; 0.88 mμmoles/mg protein of DPNH and 0.48 mμmoles/mg protein of TPNH were oxidized. These oxidations were less extensive than those observed in the absence of substrate supplement, only one-third of the DPNH and one-sixth of the TPNH having been oxidized.

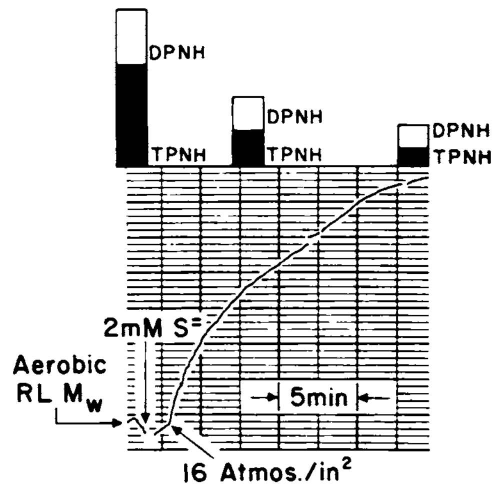

FIGURE 16. A correlation of fluorescence changes of reduced pyridine nucleotide oxidation measured fluorometrically with data obtained by analysis for a suspension of rat liver mitochondria.

Reversibility of the Reaction. Figure 17 illustrates the results of pressurizing the mitochondria at the highest available oxygen pressure, 17 atm, resulting in an abrupt oxidation of reduced pyridine nucleotide. This high pressure was chosen to determine whether the reversibility could occur even under the most unfavorable conditions. Reducing the pressure to 1 atm caused a partial return of the fluorescence intensity. A second pressurization at 17 atm caused a further decrease of fluorescence, and decompression to 1 atm caused a slower and smaller return. It is apparent that reversibility of the reaction decreased with a more prolonged exposure to 17 atm for more than a few minutes.

TABLE 7. Effect of Hyperbaric Oxygen on Pyridine Nucleotides in Rat Liver Mitochondria [a]

Additions	O_2 Pressure	DPN	DPNH	TPN	TPNH	DPN + DPNH	TPN + TPNH
10 μmoles succinate + 10 μmoles glutamate + 1 μmole sulfide	(1 atm)	73	190	21	327	263	348
10 μmoles succinate + 10 μmoles glutamate + 1 μmole sulfide	150	151	102	80	279	253	359

[a] In mμmoles/100 mg protein.

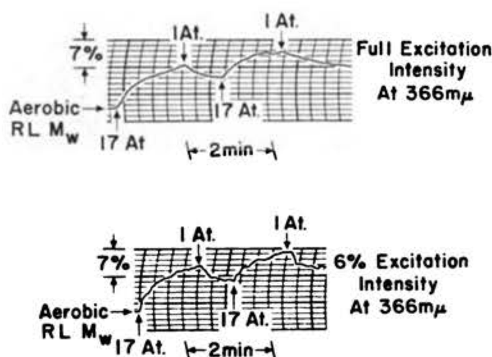

FIGURE 17. Recovery from hyperbaric oxygenation at high (top) and low (bottom) UV excitations. Other conditions similar to those of Figure 14.

Effect of Ultraviolet Excitation Intensity. In order to determine whether the reversibility of the reaction depended upon the intensity of fluorescence excitation, the same experiment was repeated with the fluorescence excitation intensity reduced to 6% of the initial value by means of an appropriate filter (Figure 17). Results showed more fluctuation, since the signal/noise ratio is inferior when at the lower excitation intensity. However, the results appeared to be essentially the same; only a partial return was measurable after pressurization at 17 atm for over 1 min.

Plant Mitochondria

In order to determine whether the phenomenon exhibited here is restricted to animal mitochondria, we obtained a suspension of mitochondria prepared from hypocotyls of the mung bean (through the courtesy of Dr. Walter D. Bonner, Jr.) The technique was essentially the same as that used in the preceding experiments: an aerobic–anaerobic transition, followed by equilibration at 1 atm, and pressurization in this case to 6–9 atm. The phenomenon (Figure 18) was essentially identical to that observed in animal tissues, suggesting a widespread occurrence of the biochemical reaction involved. A graphic presentation of data, similar to

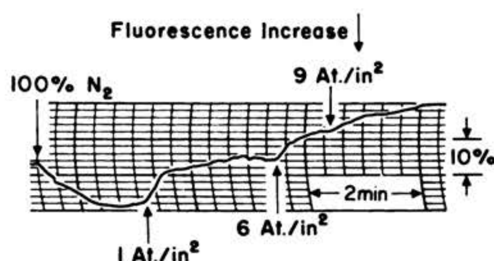

FIGURE 18. Effects of anoxia and hyperbaric oxygenation upon the reduced pyridine nucleotide fluorescence of a suspension of mitochondria prepared from green hypocotyls of the mung bean (courtesy of Walter D. Bonner).

that in this figure, indicated that both the rate and extent of the fluorescence decrease follow linear courses up to 8 atm, half-maximal effects having been observed at 4 atm.

Normoxic, Anoxic, and Hyperbaric States for Pigeon Heart Mitochondria

Figure 19 illustrates a variety of metabolic states for a suspension of pigeon heart mitochondria. Pigeon heart mitochondria at a protein concentration of approximately 20 mg/ml were supplemented with 10 mM glutamate and showed a reduction of DPN over a 1-min period. The transition to the anaerobic state, observed on flushing with nitrogen, more than doubled the fluorescence. As the nitrogen was turned off, a slight oxidation was caused by a low concentration of oxygen diffusing back into the chamber. Pressurization at 1 atm returned the level very nearly to the initial value obtained in the aerobic state. Thereafter, pressurization at 12 atm revealed a hyperbaric effect similar to that observed in a suspension of yeast cells. In order to ensure that a plateau had been reached, the pressure was changed from 12 to 1 atm twice, with very little change in the deflection.

To determine reversibility of the reaction, the oxygen was replaced by nitrogen, and, after a 2-min period of flushing, the system returned to the reduced level—a value very nearly equal to that obtained

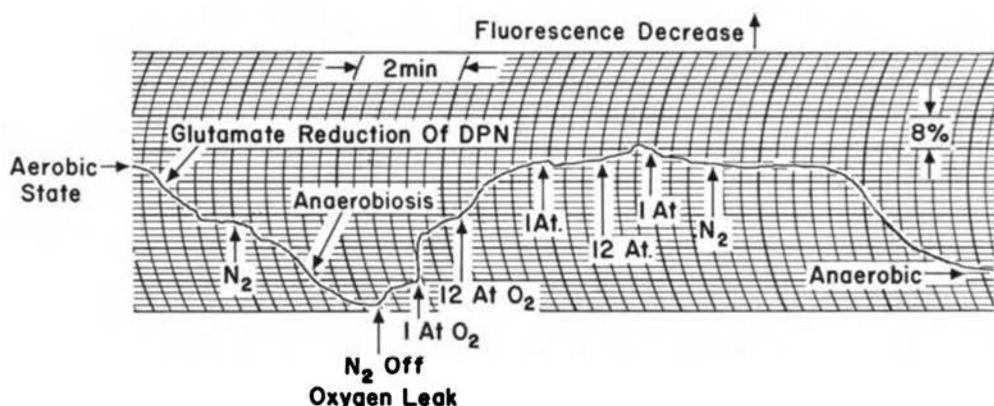

FIGURE 19. Comparison of anoxic and hyperbaric states for a suspension of pigeon heart mitochondria (supplemented with 10 mM glutamate) measured fluorometrically.

in the first nitrogenation. Thus, under these conditions, the hyperbaric effect is reversible in nitrogen.

Response in the Presence of Sulfide. In order to determine whether the response observed in ascites cells in the presence of sulfide could be reproduced with the isolated mitochondria, we performed the experiment illustrated in Figure 20. It was similar to the preceding experiment (Figure 19) in that the mitochondria were supplemented with substrate, in this case succinate and glutamate, to cause DPN reduction under aerobic conditions. Pressurization at 17 atm (diagram A) gave a decrease of fluorescence at the rate of 3.6%/min. The experiment was then repeated (diagram B) except that the addition of substrate was followed by the addition of 1.3 mM sulfide. A slight reoxidation of reduced pyridine nucleotide occurred at this point. After a plateau was established, pressurization at 17 atm gave a rate of decrease of fluorescence of 14%/min—four times as rapid as that obtained in the absence of sulfide.

Since hyperbaric oxygen causes specific effects upon the pathway of reversed electron transport, components in this path-

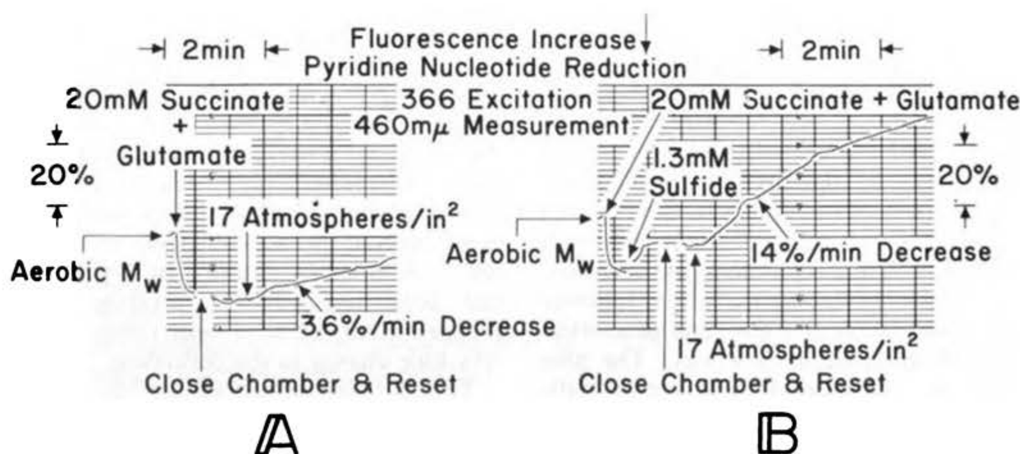

FIGURE 20. Effect of hyperbaric oxygenation upon sulfide-inhibited pigeon heart mitochondria.

way should be more susceptible to hyperbaric effects than the pathway of forward electron transport. We therefore supplemented our observations of reduced pyridine nucleotides with a study of flavin, quinone, and cytochrome components.

Response of Ubiquinone. Using a pressure cell equipped with quartz windows, we measured the effect of hyperbaric conditions upon the steady state of ubiquinone with 275 mμ as the measuring wavelength and 295 mμ as the reference wavelength. This pair was chosen to minimize interference with concomitant changes of DPNH.[30] To avoid further interference with changes of DPNH, the changes of ubiquinone following the addition of sulfide were employed. Under these conditions, most of the quinone can be reduced, but pyridine nucleotide is not reduced.[31,32] Reduction of ubiquinone (Figure 21B), which caused a disappearance of absorption at 275 mμ relative to 295 mμ, gave an upward deflection of the trace, with the reaction occurring several minutes after addition of sulfide. When the trace reached a plateau, pressurization at 200 psi caused a rapid and steady increase of absorption at 275 mμ relative to 295 mμ, indicating an oxidation of reduced ubiquinone. A control experiment with succinate present is indicated in the adjacent panel of the trace, and pressurization here caused only a small change, suggesting that direct reduction of ubiquinone

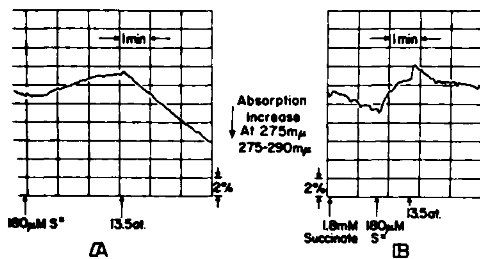

FIGURE 21. Spectrophotometric recordings of the response of ubiquinone component of a pigeon heart mitochondrial suspension under hyperbaric conditions.

through succinate can maintain it in the reduced form. When, however, only endogenous materials were available to reduce it, ubiquinone was oxidized by the hyperbaric conditions. The fact that absorption attributable to the oxidized form of ubiquinone was observed made it unlikely that a ubiquinone radical was formed.

Flavoprotein. Changes in the oxidation–reduction level of flavin were measured fluorometrically with excitation at 436 mμ and measurement at 560 mμ.[28] The fluorometer for this purpose was combined with another fluorometer for measuring DPNH changes, so that both could be measured almost simultaneously.[33] The experimental tracings are shown in Figure 22. It should be remembered that the fluorescence of pyridine nucleotide increases with its reduction while that of flavin increases with its oxidation. On addition of succinate and glutamate, both components were reduced, as indicated by their respective deflections in the opposite direction. On addition of sulfide, a further reduction of flavin occurred, while re-

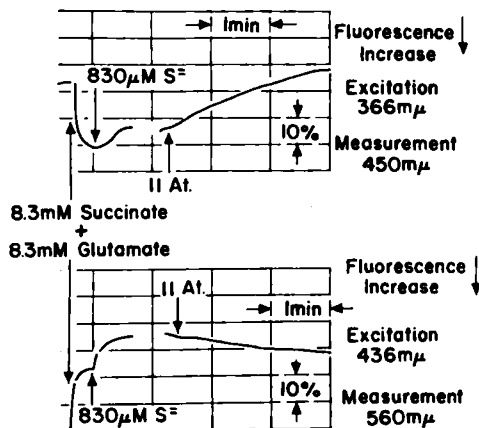

FIGURE 22. A comparison of the response of reduced pyridine nucleotide in flavoprotein to hyperbaric oxygenation. Top tracing shows reduced pyridine nucleotide fluorescence and bottom tracing shows flavin fluorescence. Both components measure fluorometrically the appropriate excitation and emission wavelengths, which are indicated in the figure.

duced pyridine nucleotide became slightly oxidized. On pressurization at 11 atm, the fluorescence of reduced pyridine nucleotide diminished while that of flavoprotein increased. These changes indicate an oxidation of the reduced component in both cases. The extent of the effect was greater for reduced pyridine nucleotide than for flavin, and changes of 10–30% of the oxidized–reduced changes were observed in a series of experiments.

Diphosphopyridine Nucleotide. The results of an experiment designed to illustrate the sensitivity of ATP-driven DPN reduction in a suspension of pigeon heart mitochondria are shown in Figure 23. In a control experiment (left side of figure), the mitochondria were treated with succinate and ATP without pressurization. It is seen that the addition of ATP caused the immediate reduction of DPN, as indicated by the downward deflection of the tracing (in this case, measurements were made by absorbancy at 340 mμ, with 374 mμ as the reference wavelength). When, however, the mitochondria were pressurized at 200 psi for 4 min, addition of succinate followed by ATP caused a slow reduction which proceeded to an extent of only half that observed in the control experiment. The ratio of the initial rates was 8:1.

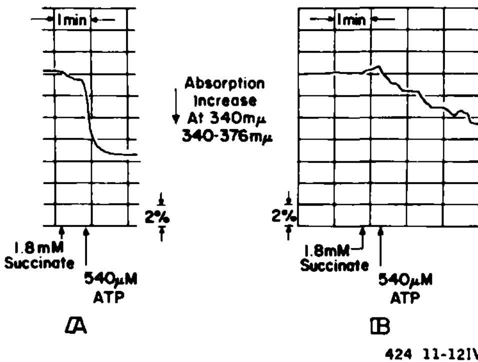

FIGURE 23. Spectrophotometric measurements of the inhibition by hyperbaric oxygenation of the ATP-driven reaction of the reduced bound pyridine nucleotide of a suspension of pigeon heart mitochondria.[1]

Energy-Linked Reduction of DPN in Submitochondrial Particles

In order to further simplify the system and to localize the site of hyperbaric inhibition, we examined the reduction of DPN added to submitochondrial particles. The ATP-driven reduction of DPN is indicated in Figure 24. The fluorometric measurements showed that submitochondrial particles, energized with succinate and ATP, showed immediate formation of DPNH upon addition of 360 μM DPN to a system terminally inhibited by 1.5 mM sulfide. The initial rate was 0.6 μM/sec. When the same particles were treated with oxygen at 16 atm for 80 sec before addition of DPN, the initial rate was only 0.5 μM/sec, a twelvefold inhibition. Other experiments showed that exposure to 12 atm for 30 sec caused 50% inhibition of the rate at 23°C.

The possibility that DPNH oxidation is a factor influencing these results is suggested in Figure 25; the reduction started on the addition of DPN, but at the end of 2.5 min, the DPNH was reoxidized to the initial base line. The rate of oxidation of DPNH was slow (4 mμmoles/mg protein/min) and the rate only tripled at 17 atm pressure. (In Figure 24, the rate of DPN reduction was 36 mμmoles/mg protein/min.) In summary, we found the energy-linked reduction of DPNH in submitochondrial particles to be highly sensitive to hyperbaric inhibition.

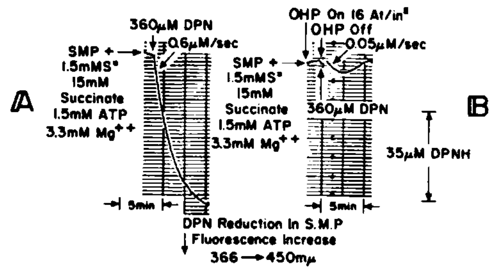

FIGURE 24. Inhibition of the ATP-driven reversed electron transfer reaction with submitochondrial particles under normal (A) and hyperbaric (B) conditions.

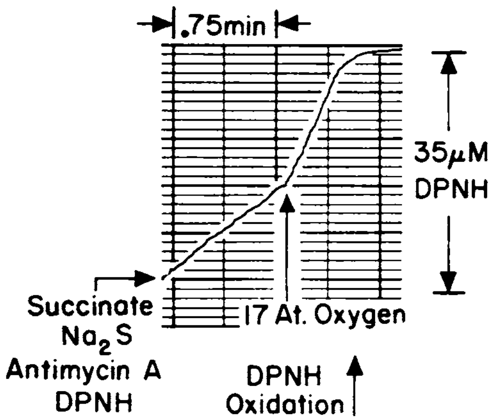

FIGURE 25. An illustration of the relatively small effect of hyperbaric oxygenation at 12 atm upon the rate of oxidation of DPNH by a suspension of submitochondrial particles derived from beef heart.

DISCUSSION

The experimental data are discussed from two standpoints: the site of the short-term hyperbaric response, and the metabolic consequences of the shift of the oxidation–reduction state of pyridine nucleotides. Elucidation of the first point is clearly afforded by observations of the hyperbaric response in isolated mitochondria and the identification of the hyperbaric response with the mitochondrial compartment on the basis of *in vivo* data, particularly the response of the redox couples (see above). We shall now attempt to identify the site of hyperbaric inhibition in the electron transport and energy transfer processes within the mitochondrion.

Site of Hyperbaric Inhibition in Mitochondria and Submitochondrial Particles

A possible site for this inhibitory reaction is indicated by the generalized diagram of the pathways of forward and reversed electron transport shown in Figure 25. There are a number of reactions by which the oxidation state of pyridine nucleotide may be affected under hyperbaric conditions.

The pathways of oxidation which might increase in activity and thereby render

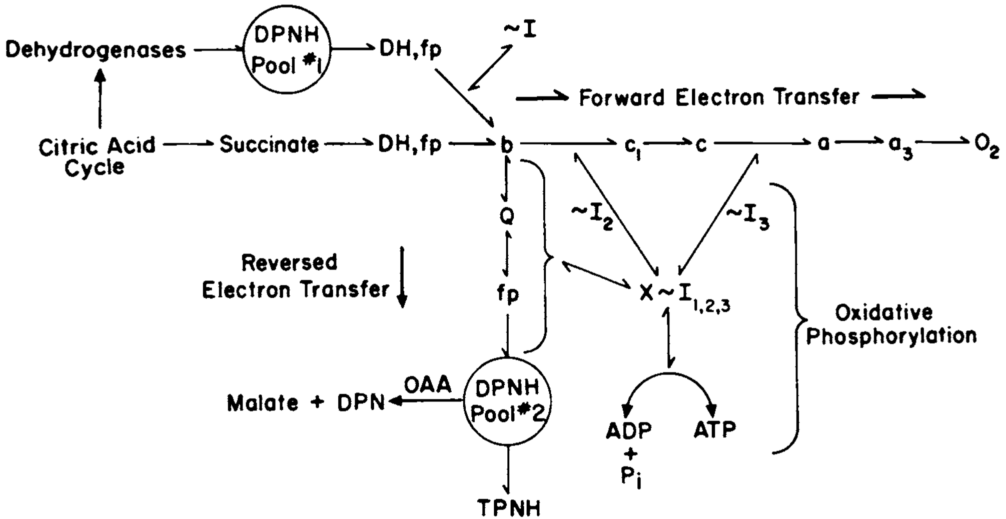

FIGURE 26. A schematic diagram of the respiratory chain including the forward and reversed pathways of electron transfer, with special emphasis on the sites of action of the various inhibitors and the possible site of action of hyperbaric oxygen.

DPNH more oxidized are those involving the chain from Pool 1 of DPNH onwards through the cytochromes to oxygen. Similarly, the decrease of dehydrogenase activity feeding into Pool 1 would cause decreased reduction of DPNH. Similar considerations apply to Pool 2 of DPNH, where any inhibition of the rate of electron flow in reversed electron transfer into this pool would cause diminished DPN reduction. Alternatively, enhanced activity of the hydrogen acceptors which interact with DPNH in Pool 2 or with TPNH would cause increased oxidation of the reduced component. A considerable distinction between DPN reduction in forward electron transport (Pool 1) and DPNH reduction in reversed electron transport is the energy requirement of the latter pathway. For the latter pathway to function, both electron transport and energy supply (in the form of $X \sim I$) is necessary. Thus, we may consider whether the hyperbaric inhibition of DPN reduction described in this paper is due to inhibition of electron transport or of energy transfer into the pathway of reversed electron flow.

Activation of Forward Electron Transfer

Under hyperbaric conditions, activation of forward electron transfer is a reaction that might be expected to occur if the chemistry of cytochromes were unknown. Except for the terminal oxidase, the cytochrome components are highly unreactive toward oxygen—a remarkable characteristic of the respiratory chain. Metals, quinones, flavins, and even DPNH itself do not become auto-oxidizable under hyperbaric conditions. Even in submitochondrial particles, where many of the components are more exposed and possibly even broadened in their specificity, the rate of oxidation of DPNH is merely tripled at pressurization to 17 atm, as shown in Figure 25. It is apparent that the respiratory chain shows very little "oxygen leakage." The terminal oxidase is, of course, completely saturated by oxygen concentrations of a fraction of an atmosphere and does not function any more rapidly at high oxygen pressures.

Formation of free radical forms of the respiratory carriers seems to be unsupported by our observations; flavin is converted to the fluorescent oxidized form and not to the nonfluorescent radical. This is true for ubiquinone as well, which is converted to the fully oxidized form and not to the radical form.[34]

Inhibition of Pyridine Nucleotide Reduction

The inhibition of pyridine nucleotide reduction is much more likely than the activation of its oxidation. The oxidation of sulfhydryl groups to disulfides has been identified in pyruvate oxidase,[2,5,6] α-ketoglutarate oxidase,[11] DPNH dehydrogenase, and lactic dehydrogenase (cytochrome b_2).[35,36]

The measurement of the dehydrogenase activity directly or by oxygen reduction by manometric techniques under conditions of high oxygen pressures is inconvenient and possibly also inaccurate. We have recently measured dehydrogenase activity toward the natural acceptors of the respiratory chain in terms of the steady-state oxidation–reduction level of cytochrome *c*.[13] This method can be directly utilized under high pressure conditions and is a sensitive indicator of changes in the rate of electron flow. The very small effect of high pressure oxygen upon the rate of oxidation of succinate and α-ketoglutarate for the first 10 min of pressurization at 12 atm is illustrated in Figure 27. The data indicate that these two dehydrogenases are not inactivated. Thus, dehydrogenase inactivation in forward electron transfer is not the primary cause of the oxidation of reduced pyridine nucleotide observed in these studies.

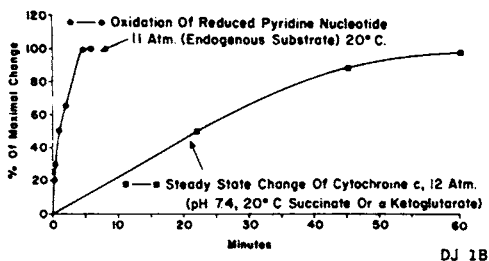

FIGURE 27. A comparison of fast and slow effects of hyperbaric oxygen; the oxidation of reduced pyridine nucleotide in a suspension of mitochondria as compared with the inhibition of electron transfer from succinate or α-ketoglutarate to oxygen.

Inhibition of Electron Transfer in the Reversed Reaction

In view of these results it is appropriate to consider in more detail the possible inhibition of reversed electron transfer in the sequence of reactions leading from succinate through cytochrome *b*, quinones, and flavins, to reduced DPN. Figure 26 also indicates that electrons flow into the respiratory chain past the branch point for reversed electron transfer with hyperbaric inhibition (Figure 25).

The exact point of the branch between forward and reversed electron transfer appears to be different in intact mitochondria from that in submitochondrial particles. In the latter, it is observed that the site sensitive to antimycin is not involved in reversed electron transfer.[37,38] In intact mitochondria, however, it is apparent that not all the properties of the system can be taken into account unless electron flow through the point sensitive to antimycin A is considered. The diagram of Figure 26 indicates an electron transport sequence in the forward and reversed direction that is consistent with observations on mitochondria, namely, that the branching point involves cytochrome *b*. The disruption of the mitochondrial structure to form submitochondrial particles may well have caused the branching point to move up the chain to the level of flavoprotein. Although the reduction of cytochrome *b* shows an energy dependence,[20,39] the fact that hyperbaric oxygen inhibits DPN reduction in submitochondrial particles in which cytochrome *b* is not involved indicates the sensitive site to be in the ubiquinone–flavin region of the reversed electron transfer pathway.

In summary, we find that the state of oxidation of reduced pyridine nucleotide, ubiquinone, and flavin is increased in the hyperbaric state. There is no evidence that a crossover point in reversed electron transport would identify an inhibition at a particular site in the electron carriers which may be participating in this pathway.

Inhibition of Energy Transfer

Two factors compel a serious consideration of the inhibition of energy transfer as the correct explanation for the hyperbaric inhibition. First, we have failed to find a crossover point in the sequence of components believed to participate in reversed electron transfer. Second, the pathway of reversed electron transfer in submitochondrial particles differs from that in intact mitochondria; the cytochrome and points sensitive to antimycin A are bypassed.[37,38] Nevertheless, this modified pathway of the submitochondrial particles is sensitive to inhibition by hyperbaric oxygen. In fact, the inhibition of energy transfer provides a unifying explanation for these three observations. This hypothesis explains hyperbaric sensitivities of reversed electron transport in the different electron flow pathways of the intact mitochondrion and submitochondrial particles and the lack of a crossover point in the electron transfer components. The chemical mechanism of the inhibition of energy utilization in reversed electron transfer requires a more detailed knowledge of the energy of "$\sim I$" used to activate this process. The reaction is so far believed to be essentially a reversal of a process by which the energy captured in oxidation–reduction reactions is used to form high-energy compounds. Despite a number of years of research, the nature of these com-

pounds remains largely unknown. One current hypothesis is that the compounds involve forms of the electron transfer carriers. It is possible, however, that such intermediates involve SH groups which are more labile than those in the electron transfer pathways.

Metabolic Consequences of Inhibition by Hyperbaric Oxygen

The consequences of inhibition of reversed electron transport in isolated mitochondria are increased oxidations of reduced pyridine nucleotide, ubiquinone, and flavin. These changes do not inhibit the ability of the mitochondrion to carry out oxidative phosphorylation, but they do inhibit its ability to transfer hydrogen to various acceptor systems (Figure 26). This metabolic state of the mitochondria differs from that of others which have so far been identified, and it is termed State 7. In State 4, the flow of metabolites blocked by the lack of ADP, phosphate, or both, is essentially a condition of minimal energy expenditure but a state of maximum readiness to phosphorylate ADP or to further reduce DPN. State 6 is a similar inhibited state insofar as the flow of electrons is concerned, but it is one which is caused by the accumulation of cations in the cristal space of the mitochondrion with resulting alkalinity.[40] This state is not reactivated by adenine nucleotides but may be reactivated by the addition of phosphate or anions which permeate the mitochondrial membrane.

State 7 has significant effects on cell metabolism, demonstrated by the metabolite assays. These effects are large enough to cause significant changes in the pyridine nucleotide levels in the tissue of liver, kidney, and brain. A further consequence of State 7 is the rise of the ATP/ADP level in liver, suggesting that the utilization of energy in the reversed electron transfer pathway is great enough to cause a drain on the ATP level *in vivo* which is significant compared with other energy demands. In view of these observations, the viewpoint advanced by Krebs[41,42] appears even less likely, *i.e.*, that our observations of DPN reduction in response to increases in succinate concentration were misinterpreted and were actually due to a competition in the respiratory chain for DPN-linked substrates. It appears that Krebs' hypothesis applies effectively to model systems where succinate added externally can cause the competitive effects which his hypothesis requires. The *in vivo* observation of oxidation of reduced pyridine nucleotides and the shift of the ATP/ADP ratio, combined with observations *in vitro* of constant rates of ketoglutarate and succinate oxidation under equivalent hyperbaric conditions, appear inconsistent with Krebs' hypothesis.

Other metabolic consequences of the hyperbaric response of liver, kidney, and brain have not yet been investigated and are surely worthy of further and detailed study. These observations afford one biochemical explanation for observed disturbances of integrated cell function.

Protection

Two factors appear to be important in maintaining DPN reduction in suspensions of mitochondria: (1) the presence of high concentrations of substrate, particularly succinate, and (2) the maintenance of respiration. Inhibition of respiration by addition of sulfide or cyanide leads to oxidation of reduced pyridine nucleotide. Mitochondria containing only endogenous substrate or low concentrations of glutamate show an oxidation of the reduced pyridine nucleotide even though electron transport is proceeding. The reduction of DPN by ATP in submitochondrial particles is observed to be highly sensitive to oxygen in the absence of DPN. If, however, the reduction of DPN has been initiated before pressurization, the system is much more resistant to OHP. Generally speaking, the reversed electron transfer reaction is protected by maintenance of the reduced state.

Sequence of Events

In attempting to compare the sequence of events which occur in the short-term reversible responses described here and the longer-term irreversible responses,[14] it is important to recognize that two different types of responses are involved in the short-term effects. Each oxygen pressure corresponds to a certain oxidation–reduction level of reduced pyridine nucleotides, and, in the case of kidney and brain, a plateau in this relationship is obtained at pressures readily achieved with the available tank. The pressure for half-maximal displacement of the steady state lies between 2 and 3 atm for kidney and brain, and is somewhat higher for the liver. In the long-term irreversible effects, it appears that high pressure oxygen establishes a rate of change of the state toward irreversible damage, and perceptible rates may be obtained at 1 or 2 atm pressure. Furthermore, since the system is irreversible, accumulated irreversible responses may be obtained at relatively small hyperbaric pressures if sufficiently long exposure times are available. Physiologic responses are probably of a similar nature, *i.e.*, there are pressures at which no, or at most very few, convulsions are observed. These pressures continued over a prolonged interval can lead to irreversible long-term effects such as lung damage.

Thus we may regard the two effects of high pressure oxygen as independent. It is, however, a matter of considerable interest to determine whether the primary biochemical response, the oxidation of reduced pyridine nucleotide, can be also the precursor of the slower secondary effects. It is possible that a mitochondrion in which the reversed electron pathway is inhibited and the DPNH pool oxidized would show a greater sensitivity of its dehydrogenases to high pressure oxygen. On this basis both the short- and long-term toxic effects may well have a common biochemical origin.

ACKNOWLEDGMENTS

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The Oxygen–Hemoglobin Equilibrium

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A subject of considerable current interest and study is the oxygen equilibrium curve of hemoglobin. Although knowledge about this curve has increased steadily in the years which separate us from the work of Bohr [1] and Huefner,[2] the confidence with which the physicochemical models used to represent the data have been regarded has vacillated considerably. Initially, in fact, Huefner regarded it as sufficient to determine a single point on the dissociation curve of a hemoglobin solution, and he was content to calculate the rest of the equilibrium curve by application of the law of mass action to a single reversible reaction. This was short-lived, however, for physiologic work had already indicated the shape of the equilibrium curve not to be hyperbolic, and physiologists lost little time in pointing this out.[3]

Although the physiologists were quick to dispose of Huefner's hypothesis, it was less easy to suggest a satisfactory alternative, and no model became prominent until Hill proposed his well-known equation [4] based on the supposition that a hemoglobin solution contains aggregates of subunits, each containing an iron atom. The mean size of the aggregates was given by the exponent n in the equation $y = Kp^n/(1+Kp^n)$. It was further assumed that such an aggregate might exist either in the ligand-bound or free form but that no intermediate states with partial saturation of a given aggregate were possible. This equation overshadowed its competitor of 1912, put forward by Douglas, Haldane, and Haldane,[5] chiefly because the constants in Hill's equation could be readily determined. The Haldane equation is interesting because it required dissociation of the hemoglobin molecule to a differing extent in the free and ligand-bound states and, in keeping with the cyclical nature of our subject, was duly revived in a more fully worked-out form in 1963.[6] The reign of Hill's equation as a model with physical significance, however, ended abruptly when the molecular weight of hemoglobin in solution was shown by Adair [7] to be 68,000, and the last possible loophole of averaging (which Adair's osmotic pressure measurements left open) was removed when Svedberg and Fåhraeus [8] showed that almost all the molecules in a solution of CO–hemoglobin had a molecular weight corresponding

to a content of four iron atoms per molecule.

Adair gave up the simplifying assumption of infinite heme–heme interaction introduced by Hill and treated the four presumed consecutive reactions of the hemoglobin molecule with ligand by application of the law of mass action to the equilibria between the hypothetical intermediates. The resulting equation contained four constants and was able to fit equilibrium curves of a wide variety of forms. As originally proposed by Adair, the equation required that all four hemes should initially have equal affinity for ligand and that the first product, Hb_4O_2, should have the same affinity for the second molecule of ligand regardless of which heme had combined initially, and so on.

This formulation posed but left unanswered the question of how the reactivity of the heme groups is altered by a combination with a molecule of ligand. The existence of the phenomenon was, of course, recognized and described by the name "heme–heme interaction." There were, however, numerous clues to the nature of the heme–heme interaction, none of which was sufficiently definite to permit detailed specification. Generally, they suggested that the protein molecule might undergo conformational change on binding ligand; the observations were, for example, that the solubility of reduced and ligand-bound hemoglobins differed and that the crystal form of ligand-bound and free hemoglobins was also different. In 1938, Haurowitz[9] showed that, if a crystal of reduced hemoglobin was exposed to oxygen, as the oxygen penetrated the crystal the flat plate of oxyhemoglobin broke up and was reformed into needles of oxyhemoglobin.

It was, perhaps, around 1955 that a high point of confidence in the Adair scheme was reached. Highly accurate determinations of the equilibrium curve by Roughton, Otis, and Lyster[10] and improved kinetic determinations of the carbon monoxide combination reaction had led to results, all of which could be satisfactorily represented mathematically by the Adair equation and its kinetic equivalent. Indeed, in some cases the results were precise enough to admit only one equilibrium and rate constant (or, at most, a very limited choice) in describing the data. It seemed, therefore, that the physicochemical problems were solved and that all that remained was to determine the values of the coefficients describing the equilibria and kinetics of the reactions of hemoglobin with ligands other than oxygen and carbon monoxide.

About this time, however, the hemoglobin molecule was shown to contain two chemically different chains, the alpha and beta chains, so that the first assumption on which the Adair formulation was based could not be exactly true. As a result, all kinds of new and difficult questions arose. It was possible, for example, that one chain alone might possess the power of reacting first with a molecule of ligand. Concurrently with these new findings, doubts also began to be felt about the completeness of the Adair equation, because of its lack both of terms relating to the concentration of hemoglobin (which, as Hill and Woldecamp had shown in 1936, cannot altogether be neglected[11]) and of any expression of conformational change.

Some of these questions can be answered at once. The criticism that the Adair equation does not realistically represent the activity in the hemoglobin molecule because it lacks explicit terms for conformational change is baseless; the introduction of conformational change steps, whether as alternative pathways or as compulsory steps between the acquisition of ligand molecules, has no effect on the form of the equilibrium equations. Rather, it alters the interpretation which should be applied to the coefficients in this equation. The fact is that equilibrium methods are inherently incapable of discriminating between binding mechanisms with and without conformational steps when only overall binding data are available, as for hemoglobin.

More serious and more difficult to answer is the criticism that the Adair equation, by virtue of its four constants, is scarcely amenable to experimental tests. For this reason, attempts have been made to develop models, based at first upon intuition and more recently upon the results of x-ray analysis, in which interaction between hemes is restricted to those occurring, for example, in a square configuration with interaction along the edges of the square, or in a linear conformation with interaction between adjacent members. The effect of such models is to impose restraints upon the values which the coefficients in the successive terms of the Adair equation are able to assume and so to reduce the number of independently variable quantities which must be taken into account in comparing the models with experimental data. Perhaps the earliest of such attempts was that of Pauling,[12] and the most recent has been that of Monod, Wyman, and Changeux.[13] In view of the arbitrary nature of these models, it was scarcely possible to attribute physical significance to any of them.

The reasons for this loss of confidence are the same as those on previous occasions of difficulty and confusion. Experiment has outdistanced theory, and the formulations found adequate to accommodate one set of results have failed to deal with newer experimental findings. The solution for this dilemma must lie in the extension and refinement of experiment, rather than in algebraic speculation. Thus, the question of whether the alpha and beta chains react similarly with ligands is an experimental question which must await the development of new experimental methods capable of yielding the desired information. This is by no means impossible, and evidence might, in principle, be obtained from a study of the genetic variants of hemoglobin in which the individual chains are differentially inactivated. Kinetic and equilibrium studies of these individual chains, when associated with a study of the x-ray structure to determine the conformational state of the genetically varied hemoglobin, might well provide at least a provisional answer. With such an answer in hand, it would once again become reasonable to set about constructing hemoglobin models for the derivation of equilibrium equations.

It is certainly sobering to reflect that some 80 years of study of the ligand-binding behavior of an easily available and relatively stable protein have failed to yield answers to even some of the more elementary questions about the reaction, although on several occasions satisfactory answers appeared already to have been obtained. Even more disturbing is the corollary: Is hemoglobin an isolated example, or will the results of other applications of quantitative methods to biological systems prove similarly ephemeral?

ACKNOWLEDGMENT

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Tissue Oxygen Transport

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A carrier for carbon dioxide in cell walls has recently been postulated by Robin [1] to exist in the gills of fishes, and Fowle and co-workers [2,3] have postulated its presence in the tissue cells of man. This has prompted us to seek a similar mechanism for oxygen. Our approach was similar; we attempted to design an experimental situation in which a carrier mechanism might be limiting tissue respiration. In addition to the carrier mechanism, two other factors can limit tissue respiration—enzyme kinetics and passive diffusion. One would expect both the carrier mechanism and enzyme kinetics to limit tissue respiration in a similar way kinetically, but passive diffusion might show some difference.

Our first step was to derive theoretical equations relating respiration rate to the partial pressure of oxygen in the environment of the tissue sample. In the case of passive diffusion, tissue geometry is quite important. We considered model systems with convergent parallel and divergent diffusion paths for oxygen.

Tissue Model with Converging Diffusion of Oxygen

An attempt to find a theoretical relation between respiration rate and partial pressure of oxygen has been made by Rashevsky [4] for spherical organisms, assuming diffusion alone as being rate-limiting. He assumed that there would be an appreciable barrier to diffusion at a membrane surrounding the sphere, while the diffusion coefficient would be uniform within. It now appears, however, that the cell contains many membranes through which oxygen must diffuse, making it reasonable to use a mean diffusion coefficient. When this is done, Rashevsky's rather cumbersome equation reduces to:

$$v = \frac{\bar{V}\, pO_2}{K_D + pO_2} \quad (1)$$

where v is the respiration rate at the given pO_2, $\bar{V}$ is the maximum respiration rate, and K_D is a function of the diffusion coefficient of oxygen through the tissue and the radius. This has the same form as the Michaelis-Menton equation.

All experimental work on the relationship between the partial pressure of oxygen and the activity of respiratory enzymes shows that it conforms to Michaelis-Menton kinetics,[5-7] *i.e.*, that the rate $x pO_2$ curve has the general shape shown in Figure 1 (curve A).

Thus, with a spherical model one cannot distinguish between kinetic and diffusion limitation of respiration rate. Clearly,

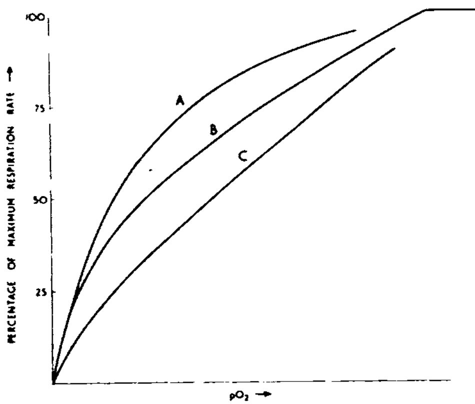

FIGURE 1. Relation of respiration rate to partial pressure of oxygen (A) when enzyme kinetics are limiting, or during diffusion into a sphere, (B) when diffusion of oxygen into a slice is limiting, and (C) when diffusion of oxygen out of a cylinder is limiting. (Reproduced from J. Theor. Biol. 8:124, 1965.)

if enzyme kinetics limit respiration, the relation between rate and pO_2 will have this same form whatever the geometry of the model.

In the spherical model, the diffusion paths are convergent. It seemed reasonable, therefore, to consider models with parallel and divergent paths.

TISSUE MODEL WITH PARALLEL DIFFUSION OF OXYGEN

The simplest model with parallel diffusion is a tissue slice of uniform thickness and large surface area in relation to the thickness. Warburg[8] showed that oxygen would diffuse into such a slice to a depth of $(2DpO_2/a)^{\frac{1}{2}}$, where pO_2 is the partial pressure of oxygen in contact with the slice and a is the respiration rate per unit volume of tissue. Therefore, a slice of area A suspended in a stirred solution would have a respiration rate of:

$$v = 2Aa\left(\frac{2DpO_2}{a}\right)^{\frac{1}{2}} \qquad (2)$$

if enzyme kinetics were not limiting. The respiration rate of such a slice should rise as the square root of the pO_2. With rising pO_2, oxygen would penetrate further into the slice, until the fronts from each side met. Now all the slice would contain oxygen, and, if enzyme kinetics were not limiting, the maximum rate would be achieved. If the slice thickness were H, the two fronts would meet at $H/2$ from the surface. Thus:

$$\frac{H}{2} = \left(\frac{2Dp'O_2}{a}\right)^{\frac{1}{2}} \qquad (3)$$

The partial pressure of oxygen at which this occurs is defined as the critical oxygen tension, $p'O_2$, the lowest oxygen tension at which the maximum respiration rate can be obtained. From equation 3:

$$p'O_2 = \frac{aH^2}{8D} \qquad (4)$$

The form of the relation given by equation 2 is shown in Figure 1 (curve B).

TISSUE MODEL WITH DIVERGENT DIFFUSION OF OXYGEN

Two possible models fulfill the criterion of divergent diffusion: (1) diffusion of oxygen from a point at the center of a respiring sphere, and (2) diffusion of oxygen from the axis of a cylinder. The former model would be difficult to produce, whereas the latter approximates the situation in a tissue supplied with oxygen by capillaries. We thought it profitable to consider only the latter.

In 1919, Krogh[9] developed an equation relating the distance R to which oxygen will diffuse into respiring tissue from a capillary of radius r:

$$pO_2 = \frac{a}{D}\left(1.15R^2\log\frac{R}{r} - \frac{R^2 - r^2}{4}\right) \qquad (5)$$

Applying the same general procedure used before, a curve can be derived wherein respiration rate is plotted against pO_2 for this model (Figure 1, curve C). In this case, however, one cannot construct the complete curve. When the advancing fronts of oxygen from adjacent capillaries meet, the form changes. If the capillaries are uniformly distributed, it can be shown geometrically that the fronts meet when 89.5% of the tissue contains oxygen.

Thus, above 89.5% of the maximum respiration rate, the form of the relationship is altered.

Examination of Experimental Results

The model system with divergent diffusion might seem the best one to examine, since curves A and C differ most. Considerable difficulties are likely to attend attempts to obtain satisfactory experimental results, however, for the reasons given by Brauer.[10]

No such difficulties arise with a system utilizing tissue slices. In this case, diffusion-limited respiration should result in a reasonably well-defined critical oxygen tension—the lowest tension at which the maximum respiration rate is still obtained. This value should rise as the square of the slice thickness. If the rate were kinetically limited, a critical oxygen tension would be hard to discern and virtually independent of slice thickness. Below this critical tension, the curves would have a different form, but one might not see this since comparison of curves is difficult. Curves A and B can be converted to linear plots, however. Lineweaver and Burk [11] showed that Michaelis kinetics give a straight line if the reciprocal of rate is plotted against the reciprocal of substrate concentration, in this case pO_2. Equation 3 shows that curve B would give a straight line of slope one-half if log rate were plotted against log pO_2. Thus, by determining which method gives a straight line when applied to experimental determination of tissue-slice respiration rate as a function of pO_2, it should be possible to determine whether the respiration is diffusion-limited or kinetically limited. An inquiry of this sort seems prerequisite to any experimental approach to the problem of tissue adaptation to hypoxia.

Experimental Materials and Methods

Liver, kidney, heart muscle, and brain were cut in varying thicknesses with the McIlwain slicer. Artificial tissue slices were also prepared by first imbedding heart muscle preparation uniformly in stiff agar and then, when the agar had set, cutting slices in the same way as for the tissues. The artificial slices represent a system in which enzyme kinetics cannot be limiting, and, since the agar has a diffusion coefficient for oxygen lower than that of water, diffusion and not enzyme kinetics must limit respiration.

The polarographic system described by Longmuir and Bourke [12] was used. The cell was filled with a solution 0.05 M with respect to potassium chloride, 0.05 M with respect to potassium phosphate, and 0.12 M with respect to potassium succinate, at a pH of 7.4. The solution was saturated with oxygen at a partial pressure of about 700 mm Hg. The slices were then placed in the cell and the partial pressure of oxygen recorded as a function of time. When all the oxygen had been removed, fresh oxygenated solution was run in, and the experiment was repeated. This was done several times on each sample of slices. Several different thicknesses of each of the tissues and the artificial material were examined in this way.

Because the signal so obtained was too noisy to permit the use of the differential circuit,[13] it was necessary to convert the tracings to respiration rate $x pO_2$ curves graphically. This was done by taking several points on each tracing and measuring the slope of a line just touching the curve at that point. The results were then plotted as double reciprocals and double logs.

Results

When the readings obtained from liver, kidney, and heart slices were plotted as the reciprocals, they lay on a straight line (Figures 2, 3). Only in the case of brain did a log–log plot of the same results give a straight line. Thus, the respiration rate of slices of liver, kidney, and heart appeared to be kinetically limited, rather than diffusion-limited. The reciprocal

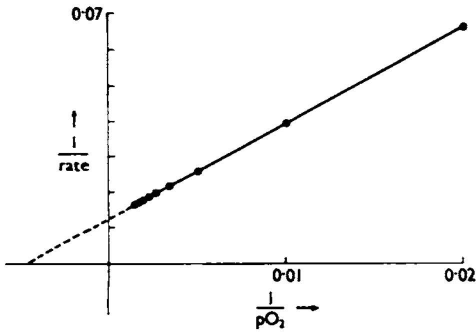

FIGURE 2. Double-reciprocal plot of the respiration rate of liver slices as a function of pO_2 in the suspending fluid. (Reproduced from J. Polarographic Soc. 10:45, 1964.)

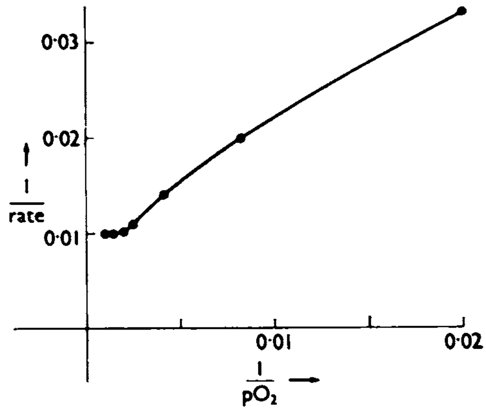

FIGURE 4. Double-reciprocal plot of the respiration rate of artificial slices as a function of pO_2 in the suspending fluid. (Reproduced from J. Polarographic Soc. 10:45, 1964.)

plots made it possible to obtain the Michaelis constant of the tissue, *i.e.*, the pO_2 at which the slice respired at half its maximum rate (a measure of the affinity of some substance in the tissue for oxygen). The values so obtained lay between 80 and 370 mm Hg.

Since this indicated the respiration rate of those slices to be kinetically limited and not diffusion-limited, it was concluded that the limitation should be independent of the geometry of the model. Consequently, some other models of the three tissues, cylinders, and strips were studied. Again, they showed that kinetics and not diffusion was the limiting factor. In the experiments on the artificial tissue, the tracings appeared to resemble those obtained with real tissue. When the results were plotted as before, however, the reciprocal plots did not give a straight line, but the double-log plots did (Figures 4, 5). The double-log plot, although straight in these experiments, with a slope of approximately half (as required by the theory of Longmuir and McCabe [14]), became horizontal at the top. The explanation for this is that as advancing fronts of oxygen penetrate into the slice from each surface they eventually meet. When this happens, each unit volume of the slice contains some oxygen, and therefore respires at the maximum rate; thus no further rise of the external oxygen tension will make the slice respire any faster. The theory of Longmuir and McCabe further

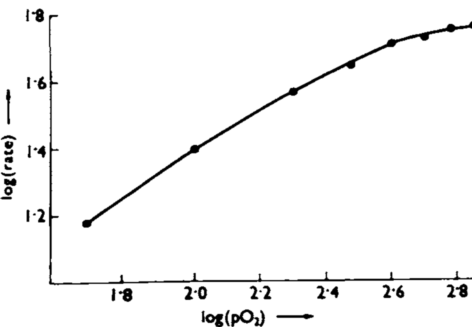

FIGURE 3. Double-log plot of the respiration rate of liver slices as a function of pO_2 in the suspending fluid. (Reproduced from J. Polarographic Soc. 10:45, 1964.)

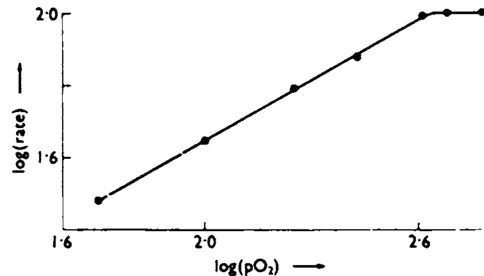

FIGURE 5. Double-log plot of the respiration rate of artificial slices as a function of pO_2 in the suspending fluid. (Reproduced from J. Polarographic Soc. 10:45, 1964.)

requires that the pO_2 of oxygen at the point at which the maximum respiration rate is reached should rise as the square of the slice thickness. This was confirmed experimentally.

There are two difficult questions: (1) How does the brain differ? (2) If, as we know, the percentage of oxygen consumption going through cytochrome oxidase varies from 44% in fresh liver slices to 95% in older ones, why does the relationship between respiration rate and partial pressure have the same form? Both dilemmas could be resolved by postulating an oxygen carrier in liver, heart, and kidney, through which all of the oxygen consumed by these tissues passes. Such a carrier system would give Michaelis kinetics.

Scholander [15] has shown that hemoglobin can facilitate oxygen diffusion through a membrane. The possibility of such a carrier in tissue was investigated by examining the effect of carbon monoxide at a partial pressure of 350 mm Hg on tissue respiration. This gas was found to have no effect on brain respiration; it had a pronounced effect on liver and a smaller one on heart and kidney slices.

When the results from liver were plotted reciprocally, they again gave a straight line, but it lay above the line found in the absence of carbon monoxide. The intercept on the reciprocal-rate axis was the same as before. Classically, this indicates competitive inhibition with an inhibitor constant of 80 mm Hg.

Discussion

If respiration is kinetically limited, the relationship between the respiration rate of a piece of tissue and the external partial pressure of oxygen in the suspending solution is given by:

$$v = \frac{\bar{V} pO_2}{Km + pO_2} \qquad (1)$$

Rashevsky's equation [4] for the relationship between the respiration rate of a sphere of tissue and the external partial pressure of oxygen has exactly the same form. The only difference is that Km is now a function of the diffusion coefficient of oxygen and the radius of the sphere. For models other than a sphere, the form of the equation changes. In the case of a tissue slice, the equation is:

$$v = 2A\alpha\left(\frac{2DpO_2}{\alpha}\right)^{\frac{1}{2}} \qquad (2)$$

It can be seen that equation 1 can be put in a linear form if $1/v$ is plotted against $1/pO_2$. Similarly, in the case of equation 2, a plot of log v against log pO_2 should give a straight line of slope one-half.

That artificial slices and brain behave in the predicted manner when examined in this way is of particular interest. Until recently, all theoretical attempts to relate respiration rate to pO_2 have assumed that tissues conform to the artificial tissue we have prepared. Up to a point, therefore, our findings with this artificial system represent a vindication of these theoretical treatments.

The finding that the respiration rate of real tissues conforms to Michaelis kinetics raises a serious difficulty, however. More than 30 oxidases and oxygenases (the enzymes which react with molecular oxygen) have been described in tissue. Although the activity of all these enzymes conforms to Michaelis kinetics, they have widely varying affinities and the sum of their activities should not approximate Michaelis kinetics. In addition, Longmuir and McCabe [16] have observed that the proportion of oxygen going through cytochrome oxidase (which has the highest affinity for oxygen) varies from 44% to 95%, depending upon the age of the slice; yet no change in kinetics occurs with age.

This anomaly can be resolved by postulating an oxygen carrier in the three tissues, which must behave kinetically in the same way as an enzyme, between the external source of oxygen and all of the terminal oxidases and oxygenases in the tissue. This carrier would have to trans-

port virtually all the oxygen, with little moving by passive diffusion; otherwise the experimental results would lie between the diffusion-limited and kinetically limited pictures.

The nature of such a carrier remains obscure. Scholander [15] has shown that hemoglobin and myoglobin could act in this way, but not under the conditions of the experiments described herein.[17] Such a hypothesis could explain the findings of Longmuir and Bourke,[12] however, without the need for two hypotheses, *i.e.*, a high diffusion coefficient and a low-affinity oxidase through which the bulk of the oxygen passes.

The affinity of the oxygen carrier for oxygen can be determined from the Lineweaver-Burk plot. Values for different tissues lie in the range of 80 to 370 mm Hg. Thus, the carrier cannot be either hemoglobin or myoglobin. The fact that there appears to be no carrier in brain is highly significant. Brain is the only nonpigmented tissue we have studied so far. Clearly, the hypothesis of a carrier system in some tissues does not mean we must discard all our previous concepts of tissue oxygen transport by passive diffusion.

The results obtained by studying the respiration rate of slices of all four tissues in the presence of carbon monoxide seem to confirm that there could be a hemoglobin-like carrier in liver, and possibly in kidney and heart, but not in brain. However, the partial pressure of oxygen for half saturation (80 mm Hg) and the inhibition constant (*ca.* 80 mm Hg) appear to exclude hemoglobin or myoglobin. At the same time, these values and the absence of an effect with brain exclude the possibility that we are looking at kinetics limited by cytochrome oxidase.

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Effect of Hyperbaric Oxygenation *in vitro* on Mammalian Respiratory Chain-Linked Dehydrogenases

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The toxic effect of hyperbaric oxygenation (OHP) on the activity of various dehydrogenases has been studied by a number of investigators,[1-9] whose work has primarily involved enzymatic activity in tissue slices and homogenates. Studies on the stability of several respiratory enzymes during exposure to elevated oxygen tensions have shown cytochrome *c* reductase [8] and succinic dehydrogenase [3-5] to be the most sensitive to oxygen. Based on the work of a number of investigators, a hypothesis has been developed that oxygen toxicity is due to the oxidation of sulfhydryl groups of dehydrogenases,[1-3] and the effects of some protective reagents [4,5,10,11] have been studied. Despite these extensive studies, however, the question remains whether exposure to OHP inactivates soluble sulfhydryl enzymes as well as the particulate enzymes of the respiratory chain found in mitochondria and submitochondrial fractions.

During the past decade, the respiratory chain-linked dehydrogenases have been solubilized and highly purified from a variety of sources by Singer and his coworkers, and the main features of their physicochemical properties have been determined.[12-15] Using the same methods employed by these workers, we have obtained several of these enzyme preparations and have studied the effect of OHP on the enzymatic activities at several stages of purification.

Following are our observations on the effect of OHP on the enzymatic activities of the mammalian respiratory chain-linked dehydrogenases (succinic dehydrogenase, reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase, α-L-glycerophosphate dehydrogenase, and choline dehydrogenase) in multienzyme preparations fractionated to various extents and on a soluble succinic dehydrogenase preparation. In addition, several substances known to stabilize other enzymes (or, for various reasons, expected to prevent oxygen-induced inactivation) were added to soluble succinic dehydrogenase exposed to OHP and the effects evaluated.

Materials and Methods

Reagents

The following reagents were used for the experiments: succinic disodium salt and

2,6-dichlorophenolindophenol sodium salt (Eastman Chemicals); phenazine methosulfate and choline chloride (Sigma Chemical and Merck, respectively); α-L-glycerophosphate (dicyclohexyl ammonium salt), NADH, 2,2′-bipyridine, 1,10-phenanthroline, riboflavin-5′-phosphate, ethylenediamine-tetraacetic acid (tetrasodium dihydrate), and dithiothreitol (Cleveland's reagent, Calbiochem). All other reagents used in the enzyme preparations and enzyme assays were commercial reagents of high purity.

Enzyme Sources

Mitochondria from rat liver and kidney were isolated by the method of Hogeboom[16] modified by adding 5 mmoles $MgCl_2$, 50 mmoles tris buffer, and 25 mmoles KCl per liter to the 0.25 M sucrose solution as the homogenizing medium; the mitochondria were obtained by density gradient centrifugation (Spinco swinging bucket rotor SW-39-L).[17] The Keilin-Hartree heart preparation was made on a small scale exactly as described by King.[18] Beef-heart mitochondria and nonphosphorylating electron transport particles (ETP) were isolated according to the method of Greene and Ziegler[19] with minor modifications.[20]

Soluble succinic dehydrogenase from beef heart was prepared by the method of Bernath and Singer.[12] The enzyme preparations were stored in the frozen state at −20°C in nitrogen and were used at the following intervals after preparation: 2 weeks for the beef-heart mitochondria, Keilin-Hartree heart preparation, and beef-heart soluble succinic dehydrogenase preparation, and 3 weeks for the nonphosphorylating ETP.

OHP Exposure and Assays of Enzymic Activity

Appropriate amounts of the enzyme preparations were transferred to small cups or Warburg vessels, and in each vessel the liquid layer was kept less than 2 mm deep. These were placed, with minimal stirring, in the bottom of a small pressure chamber (Dixie Manufacturing) and exposed to elevated oxygen tensions at room temperature for the time periods and at the pressures described below. Equal aliquots of the preparations served as controls and were exposed to air at 1 atm for the same time periods. The compression and decompression time amounted to less than 2 min. Twenty minutes after the exposure to OHP for the indicated periods, the enzymatic assay procedures were carried out.

In addition, suspensions of the beef-heart mitochondrial preparation were exposed to 100% oxygen by aeration without foaming for 5–15 min at room temperature.

Assays of the succinic dehydrogenase,[12] α-L-glycerophosphate dehydrogenase,[15] and choline dehydrogenase[14] were performed by the phenazine methosulfate method,[21] with 0.5 mg of dye (0.67 mg of dye for succinic dehydrogenase) per milliliter in the presence of 5×10^{-2} M potassium phosphate buffer (pH 7.6), 10^{-3} M cyanide, and either 2×10^{-2} M succinate, 3.3×10^{-2} M choline chloride, or 1.5×10^{-2} M α-L-glycerophosphate for the respective enzyme assays at 37°C.

Concurrently, several experiments were performed by spectrophotometric assay,[22] with both phenazine methosulfate as the immediate electron acceptors and 2,6-dichlorophenolindophenol as the terminal acceptor, to test the advantage of adding a second dye in this assay. The assay for NADH dehydrogenase was performed by the ferricyanide method[23] in the presence of 120 μmoles phosphate buffer (pH 7.4), 1.8 μmoles NADH, ETP, and increasing quantities of 0.01 M ferricyanide.

Protein determinations were carried out with Folin phenol reagents.[24] Absorption spectra of the soluble succinic dehydrogenase preparations were obtained with a Zeiss spectrophotometer.

Results and Discussion

Beef heart was selected as an enzyme source for our studies so that the effect of OHP on the enzymatic activity at several levels of purification, particulate to soluble preparations of the same organ, could be observed. Mitochondria from frozen beef hearts were used even though the succinate oxidase system of this particulate preparation is largely impaired. The advantage of studying these mitochondria is that phenazine methosulfate reacts directly and solely with the flavoprotein enzyme in them. It has been suggested that there are two reaction sites for phenazine methosulfate in mitochondrial preparations from *fresh* beef heart, both of which are close to or on the dehydrogenase itself.[25] Therefore, the limited reactivity of the enzymes in the preparation from frozen beef hearts simplifies interpretation of the results of assay of succinate–phenazine methosulfate reductase. The succinate–phenazine methosulfate reductase system has been selected because several other oxidants (*e.g.*, methylene blue, ferricyanide, coenzyme Q, and cytochrome *c*) react poorly or not at all with the succinic dehydrogenase.[26]

Table 1 shows the effect of OHP (assayed after decompression, of course) on the enzyme activity of succinic dehydrogenase in beef-heart mitochondria and in the Keilin-Hartree heart preparation. The reaction rate was examined over 10-, 30-, and 60-min periods. OHP exposure ranged from 1 to 5 hours at 3 atm of oxygen; exposure time was 1 hour at 5 atm of oxygen. At 3 atm of oxygen for 1–3 hours, as well as at 5 atm of oxygen for 1 hour, the succinic dehydrogenase activity in beef-heart mitochondria showed no significant decrease, compared to exposure to air for the indicated periods. When very careful measurements were made on the initial reaction rates, a slight relative decrease of succinic dehydrogenase activity appeared at 3 atm of oxygen for 5 hours in the case of beef-heart mitochondria and at 3 atm of oxygen for 3 hours and 5 atm of oxygen for 1 hour in the Keilin-Hartree heart preparation. This was an unexpected finding.

Stadie *et al.*,[5] who used a succinate–methylene blue reductase method to study pigeon breast muscle in 8 atm of oxygen and rat-brain homogenates in 7 atm of oxygen, found no decrease of activity in the first 20 min or in 60-min reaction periods. The results of Dickens,[4] ob-

Table 1. Effect of OHP on Succinic Dehydrogenase Activity in Beef-Heart Mitochondria and Keilin-Hartree Heart Preparations [a]

	Reaction time: initial rate (min)	Activity (% of control)[b]			
		3 atm O_2			5 atm O_2
		1 hr	3 hr	5 hr	1 hr
Beef-heart mitochondria	10	91	102	82	95
	30	103	96	85	95
	60	111	94	83	91
Keilin-Hartree preparations	10	—	83	—	85

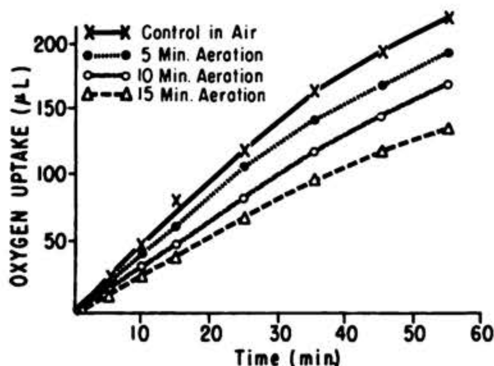
[a] The assays were performed manometrically by the rate of oxygen uptake at 37°C during the indicated period in a system composed of the final concentration of 0.05 M phosphate buffer (pH 7.6), 1×10^{-3} M KCN, 0.02 M succinate, and either 0.58 mg protein of mitochondria or 0.74 mg protein of Keilin-Hartree preparations in a total volume of 3.0 ml. After temperature equilibration, 2.0 mg of phenazine methosulfate and succinate was tipped into the main compartment. The specific activities of succinic dehydrogenase in aged beef-heart mitochondria and Keilin-Hartree preparations were 0.82 μmoles and 1.03 μmoles (respectively) of succinate oxidized per minute for each milligram of protein.

[b] Control values at 1 atm in air taken as 100%.

tained by the succinate–methylene blue reductase method with pig heart muscle, indicated decreases of 22% and 45% during the first 10 min of the assay period after exposure of this particulate preparation for 3 hours to oxygen at 1 and 4.4 atm, respectively. However, when the long-term oxidation of succinate was measured, both investigators demonstrated a significant decrease of the succinate oxidase activity of various tissue slices or cell-free preparations under several pressures of OHP. Recently, Thomas *et al.*[7] reported that the oxidation of ^{14}C-labeled succinate, in cell-free rat homogenate at 37°C exposed to 5 atm of oxygen for 30 min, was not significantly impaired.

These conflicting results on the effect of OHP on the initial rate of enzyme activity prompted us to study the effect of direct aeration with oxygen on the particulate preparations and to compare these results with the effect of OHP on particulate and soluble enzyme preparations.

Figure 1 presents the results of the effect of oxygen aeration on succinic dehydrogenase activity in beef-heart mitochondria. The initial rate of activity, observed after 10–15 min of oxygen aeration, was found to decrease significantly from 66% to 56%, as compared to controls. This suggested that the slight decrease of this enzymatic activity occurring as a result of OHP exposure of the preparations, compared to the aeration experiment, might be partly due to less complete exposure of the enzyme to oxygen.



% of Control	Air	OXYGEN AERATION 5 Min.	10 Min.	15 Min.
Initial Rate 0-10 Min.	100	90	66	56

FIGURE 1. Effect of oxygen aeration on succinic dehydrogenase activity in beef-heart mitochondrial preparations.

In order to observe the effect of OHP on other respiratory chain-linked dehydrogenases in particulate preparations, the effects of OHP at 5 atm of oxygen for 2 hours were compared for succinic dehydrogenase in beef-heart mitochondria, α-L-glycerophosphate dehydrogenase in fresh rat-kidney mitochondria, and choline dehydrogenase in fresh rat-liver mitochondria (Figure 2). Compared to the results from controls in air, no significant change occurred in initial reaction rates among these preparations. Despite the differences of the tissues from which the

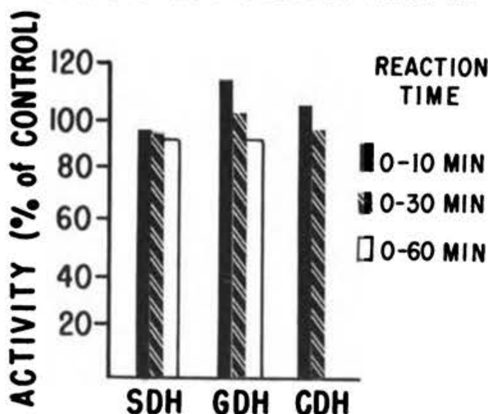


FIGURE 2. Comparison of the effect of OHP on the activity of succinic dehydrogenase, α-L-glycerophosphate dehydrogenase, and choline dehydrogenase at the mitochondrial level at 5 atm of oxygen for 2 hours. The amounts of enzyme preparations used were 5 mg protein of rat-kidney mitochondria for α-L-glycerophosphate dehydrogenase and 4.3 mg protein of rat-liver mitochondria for succinic dehydrogenase. The specific activity of the former in rat-kidney mitochondria incubated in air was 0.041 µmole of α-L-glycerophosphate oxidized per minute for each milligram of protein; the activity of the latter in rat-liver mitochondria was 0.072 µmole of choline chloride oxidized per minute.

enzymes were obtained, the susceptibility to OHP under the conditions described was the same.

The enzymes in these tissues were chosen for study because of the relatively high activity of α-L-glycerophosphate dehydrogenase bound to mitochondria in pig brain,[15] skeletal muscle,[15] rat kidney, liver, and brain,[27,28] and choline dehydrogenase in mitochondria from rat liver.[14] Succinic dehydrogenase activity is relatively high in most animal tissues.

The effect of OHP on NADH dehydrogenase activity of nonphosphorylating electron transport particles is presented in Figure 3. Since serious complications inherent in this assay, when conducted at fixed ferricyanide concentrations, have been emphasized,[23] various concentrations of ferricyanide were used and the results were extrapolated to the maximum reaction velocity with respect to ferricyanide

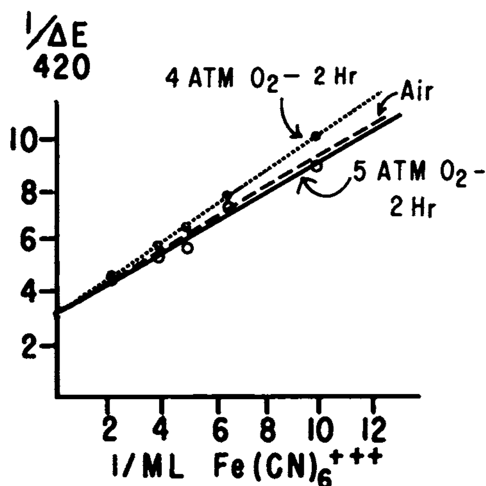


FIGURE 3. Effect of OHP on NADH dehydrogenase activity of nonphosphorylating ETP. The ferricyanide assays were performed at 25°C with a Zeiss spectrophotometer in a system composed of a final concentration of 0.04 M phosphate buffer (pH 7.4), 0.15 mM NADH, 0.09 mg protein of ETP, and 0.0125 M ferricyanide, as indicated above, in a total volume of 3.0 ml. The specific activity of NADH dehydrogenase in nonphosphorylating ETP used was 17.8 μmoles of NADH oxidized per minute for each milligram of protein, at maximum reaction velocity with respect to ferricyanide.

concentration, plotted by the double reciprocal method. Comparison of the enzyme activity between the control in air and the OHP sample at 4 and 5 atm for 2 hours showed no differences. Dickens [4] reported that pig-heart diaphorase, when measured by the cozymase–methylene blue method at a fixed dye concentration, was not at all inactivated by a 2.5-hour exposure to 4.4 atm of oxygen at 38°C. A similar lack of effect of elevated tensions of oxygen has been obtained for the flavoprotein diaphorase by Dixon *et al.*,[8] using the indophenol method of assay on preparations exposed to air for 18 hours. From these results, we might suggest that NADH dehydrogenase in the two types of flavoprotein preparations is not inactivated by OHP under these conditions.

On the whole, the experimental evidence of these four enzyme activities tested at the particulate level showed no great susceptibility to oxygen under high pressure, especially in terms of the initial reaction rates of activity. Consequently, experiments on soluble succinic dehydrogenase preparations isolated from beef heart were designed. Throughout these experiments, essentially the same soluble enzyme preparations isolated from the same beef heart were used, the only difference being the period of storage at −20°C in nitrogen. However, a preliminary experiment revealed that several days of storage under these conditions did not significantly alter the results. The OHP exposure conditions were the same, without shaking or stirring, as described for the experiments with the particulate preparations. Enzyme activity was measured for the first 10 min. Figure 4A shows a series of inactivation curves with different oxygen tensions at 23°C for soluble succinic dehydrogenase preparations; these make it clear that the rate of inactivation is proportional to the oxygen pressure (Figure 4B). The half-life of soluble succinic dehydrogenase under the conditions described was 5.5 hours at 1 atm of oxygen, 3.5 hours at 3 atm

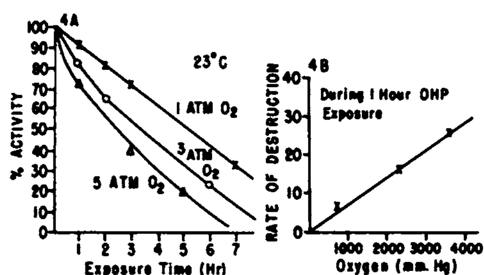


FIGURE 4. Effect of OHP on rate of inactivation of soluble succinic dehydrogenase preparations. Succinate–phenazine–reductase specific activity measured in aged soluble succinic dehydrogenase preparation (gel eluate stage) was 3.4 μmoles of succinate oxidized per minute for each milligram of protein, at maximum reaction velocity with respect to phenazine methosulfate.

of oxygen, and 2.5 hours at 5 atm of oxygen (23°C).

These results on succinic dehydrogenase activity in both the particulate and soluble preparations make it apparent that the soluble preparation is far more susceptible to OHP than the particulate preparation, a conclusion which is in accord with the one noted previously—that most enzymes, when present in tissue slices or in the intact animal, are less susceptible to oxygen poisoning than when they are in cell-free preparations.[6] This may indicate that protective mechanisms in the cell or particulate preparations counteract the oxidizing potential of molecular oxygen, or it may simply indicate the accessibility of molecular oxygen to the enzymes.

Because of the numerous reports [4,5,10,11,29] on the mechanism of oxygen toxicity and the protective action of various substances, it was of interest to us to test the effects of adding a number of these substances to the soluble succinic dehydrogenase. Hopkins and Morgan reported that substrate and competitive inhibitors protected the succinic dehydrogenase from the action of agents which attack sulfhydryl groups.[30] The protection against oxidizing agents afforded by succinate and malonate has been amply confirmed with highly purified, soluble preparations,[31] as well as with particulate preparations.[32–34] Also, the protection by succinate for tissue slices of rat liver and heart against the inactivating effect of OHP has been reported.[4,5]

Thus, we included a study of the effect of OHP on the enzyme activity of soluble succinic dehydrogenase preparations preincubated with succinate at 5 atm of oxygen for 1 hour. One hour after the OHP exposure, the succinic dehydrogenase activity was assayed, the amount of substrate being adjusted just before performance of the assay (Figure 5). Surprisingly, there was marked inhibition of the activity as compared to that of controls exposed to air, for both the initial rate and the 60-min reaction rate. Three soluble enzymes isolated at different times from different individual beef hearts showed the same results. Although no explanation for this marked inhibition can be made on the basis of this preliminary experiment, it suggests that the mode of action of OHP on the soluble enzyme preparation is entirely different from that of oxidizing agents or oxygen at ambient pressure.

After completion of this experiment, the effects of several chelating agents, SH-protecting reagents, and riboflavin-5′-phosphate were tested on the soluble succinic dehydrogenase exposed to 5 atm of oxygen for 2 hours (Table 2). It was found that a trace amount of EDTA preincubated with the soluble enzyme under OHP produced a detrimental effect, while iron-chelating agents (α,α'-dipyridyl and

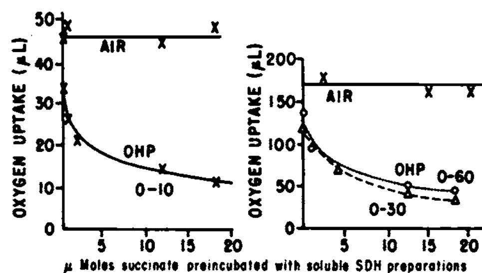


FIGURE 5. Effect of OHP on the activity of soluble succinic dehydrogenase preparations preincubated with succinate at 5 atm of oxygen for 1 hour.

TABLE 2. Effect of Addition of Various Substances on Enzymatic Activity of Soluble Succinic Dehydrogenase Preparations Exposed to 5 atm of Oxygen for 2 Hours

	Activity (% of control)[a]			
		OHP with additive (moles)		
Substance added	OHP alone	0.4×10^{-6}	1×10^{-6}	2×10^{-6}
EDTA	69	50	53	45
α,α′-Dipyridyl	56	85	83	85
o-Phenanthroline	60	91	81	—
Reduced glutathione	52	27	33	39
Dithiothreitol	82	65	63	62
Riboflavin-5′-phosphate	61	46	56	61

[a] Control values at 1 atm in air taken as 100%.

o-phenanthroline) increased the activity. Iron-chelating agents have been reported to inactivate the enzyme by initiating structural changes in the protein.[26] The question of catalytic participation of the iron in this enzyme has been discussed but left open by Singer and Massey,[35] although agreement is general that intact iron–protein bonds are essential for the full activity of succinic dehydrogenase. From these considerations, the results obtained from the chelating agents definitely merit further investigation.

The SH-protecting agents, reduced glutathione and dithiothreitol, showed detrimental rather than protective effects at 5 atm of oxygen for 2 hours; this was also true for a trace amount of riboflavin-5′-phosphate.

The effects of the agents examined for their protective action on the soluble enzyme preparations indicate that the action of OHP on the soluble enzymes must be different from its action on the particulate preparations, because the same protective agent is not equally protective for the same enzymatic activity in different forms, soluble and particulate.

SUMMARY

The effect of OHP on the enzymatic activity (especially initial reaction rates) of the mammalian respiratory chain-linked dehydrogenases (succinic dehydrogenase, α-L-glycerophosphate dehydrogenase, NADH dehydrogenase, and choline dehydrogenase) in both particulate and soluble preparations has been studied. Oxygen at a pressure of 3 atm for 1–3 hours and 5 atm for 1–2 hours had no significant effect on the particulate preparations, while in soluble succinic dehydrogenase preparations the rate of inactivation was proportional to the oxygen pressure under the same exposure conditions. The protection by succinate reported for the particulate preparations under OHP was not found in soluble succinic dehydrogenase preparations. The iron-chelating agents (α,α′-dipyridyl and *o*-phenanthroline) partially protected against inactivation of the enzyme by OHP, while SH-protecting agents (reduced glutathione and dithiothreitol), riboflavin-5′-phosphate, and EDTA had rather detrimental effects.

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Antioxidant Mechanisms and Hemolysis Associated with Lipid Peroxidation

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That oxygen poisoning and, in particular, erythrocyte hemolysis induced by oxygen under pressure may be associated with the peroxidation of endogenous cellular lipids is a most attractive concept. One may well posit the disruption of lipid membrane systems and the inactivation of lipoprotein and SH-dependent enzymes taking place as a result of the formation and presence of peroxidized lipid within the cell. No biochemical constituents are potentially more sensitive to oxidation, when removed from their cellular milieu, than certain of the unsaturated fatty acids. This potential is not ordinarily realized because of the presence of antioxidant mechanisms which are presumably linked to basic cellular metabolic processes and which cease to operate entirely only with the death of the cell.

Central among the known lipid antioxidants, or free radical chain-breakers, is vitamin E. It follows, then, that erythrocytes of animals deficient in vitamin E might be expected to be more sensitive to the stresses of hyperbaric oxygenation. In fact, Taylor[1] has reported erythrocyte hemolysis in such animals exposed to oxygen at 5 atm. This observation has been confirmed and elaborated upon by Kann *et al.*[2]

This paper is a report of experiments which indicate that, in addition to vitamin E, the intracellular GSH (reduced glutathione) of the erythrocyte, as well as SH groups present in the erythrocyte membrane, act to protect these cells against the lytic action of lipid peroxides. In these experiments, we made use of the fact that when rat liver microsomes are incubated with TPNH and ATP a peroxidation of endogenous unsaturated lipids is induced.[3,4] Once initiated in the microsomes, the peroxidizing chain reaction may be propagated to the lipids of other admixed subcellular organelles and, in fact, to the lipid membranes of intact cells such as those of the erythrocytes. For example, as shown in Table 1, when microsomes are mixed with normal human erythrocytes (sufficient in vitamin E) and incubated with TPNH and ATP, lysis of the erythrocytes follows and is virtually complete after 90 min (Experiment 3). If either TPNH, ATP, or microsomes are omitted from the reaction medium, no lysis greater than that of the control erythrocytes is observed (Experiments 4, 5). However, it should be noted that no lysis ensues if the erythrocytes are not first

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TABLE 1. Microsomal-Induced Lysis of Normal Human Erythrocytes

Exper. no.	Erythrocyte pretreatment [a]	Additions [b]	% lysis [c]			
			30 min	60 min	90 min	120 min
1	None	None	1	3	5	7
2	None	Complete system	1	4	6	9
3	NEM	Complete system	17	35	86	100
4	NEM	Complete system minus TPNH	1	4	6	8
5	NEM	Complete system minus microsomes	1	4	7	9
6	NEM	Complete system, microsomes added at 30 min	1	5	8	9
7	PCMBS	None	2	3	8	10
8	PCMBS	Complete system	15	28	75	100
9	PCMBS + NEM	Complete system	52	94	100	—
10	PCMBS + NEM	Complete system minus TPNH	1	3	7	10

[a] Freshly drawn blood with heparin was centrifuged at 1500 rpm. The plasma and buffy coat was removed and the erythrocytes washed three times with isotonic saline buffered at pH 7.4. Aliquots of washed cells were then resuspended in either buffered saline, buffered saline containing 2.0 mM NEM, or buffered saline containing 2.0 mM PCMBS. The cells were allowed to stand at room temperature for 5 min, after which they were washed four times with buffered saline. GSH was determined in aliquots of cells so treated.[5] Erythrocytes treated only with buffered saline contained 1.1 mM GSH, NEM-treated cells contained no detectable GSH, and PCMBS-treated cells contained 1.1 mM GSH.

[b] Incubations were carried out in tubes shaken vigorously at 37°C. The volume of the reaction mixture was 5.0 ml. The complete system contained: 2.5 mM tris-HCl at pH 7.4, 150 mM KCl, 4 mM ATP, 0.5 mM TPNH, and erythrocytes to make about a 5% hematocrit. Reactions were initiated by adding microsomes containing 2.0 mg protein. Microsomes were prepared by the method of Ernster *et al.*[6]

[c] Aliquots of the reaction medium were removed at the indicated time intervals and the percentage lysis determined after removing the unlysed cells by centrifugation and converting the released hemoglobin to cyanmethemogloblin for determination at 540 mμ.

treated with NEM (*N*-ethylmaleimide) (compare Experiments 2 and 3). NEM penetrates into the erythrocyte and reacts with free GSH to reduce its concentration to negligible amounts (Table 1, footnote *a*).

If one allows the peroxidation of endogenous microsomal lipids to proceed to completion (about 30 min) before adding the microsomes to the erythrocyte suspension, then no hemolysis is observed in NEM-treated cells despite the fact that GSH is absent (Experiment 6). This suggests that lysis results not from the mere presence and toxic effects of lipid peroxides, but from the active propagation of a peroxidizing chain reaction to the lipids of the erythrocyte membrane, which results in its disruption.

PCMBS (*p*-chloromercuriphenylsulfonic acid), unlike NEM, does not penetrate into the erythrocyte. It does react with SH groups on the erythrocyte membrane, however, leaving the intracellular GSH of the cell unaltered. Pretreatment of erythrocytes with PCMBS also predisposes the cells to lysis in the presence of the microsomal system despite the fact that the internal GSH is unchanged (Experiment 8). Pretreatment of erythrocytes with both NEM and PCMBS results in even more rapid rates of lysis than observed with either agent alone (Experiment 9). Again, cells treated in such a fashion do not exhibit marked lysis in the absence of TPNH, which is required for initiation of the peroxidizing chain in the microsomes (Experiment 10).

In experiments not described in this communication,[7] the addition of large amounts of vitamin E to the reaction prevented lysis of both NEM- and PCMBS-

pretreated cells in the complete system. In addition, erythrocytes from rats made partially deficient in vitamin E underwent accelerated lysis in the presence of the microsomal peroxidizing system without pretreatment with either NEM or PCMBS.

These various experiments may be interpreted to indicate that there are at least three components which make up the "antioxidant system" of the erythrocyte: (1) the free GSH of the cell (NEM-sensitive), (2) an SH moiety of the cell membrane (PCMBS-sensitive), and (3) vitamin E. Possibly each of these three components represents a distinct, separately acting antioxidant pool for the erythrocyte. More likely, however, they represent an interdependent antioxidant mechanism. For example, one may imagine vitamin E as the terminal antioxidant in the cell, with its effectiveness dependent on its continual regeneration to an active form. Such regeneration might well be dependent upon the interaction of the PCMBS-sensitive component of the membrane with intracellular GSH (Figure 1).

It is clear from these experiments that the endogenous vitamin E of the erythrocyte does not protect these cells from peroxidation in the absence of either membrane SH groups or cellular GSH. Conversely, cells deficient in vitamin E undergo lysis despite the presence of intact membrane SH groups and GSH. Thus, one may conjecture that the need for membrane SH groups and GSH reflects the requirement that endogenous vitamin E be maintained in some reduced and, hence, active form. The need for this mechanism would obviously be circumvented in the presence of large amounts

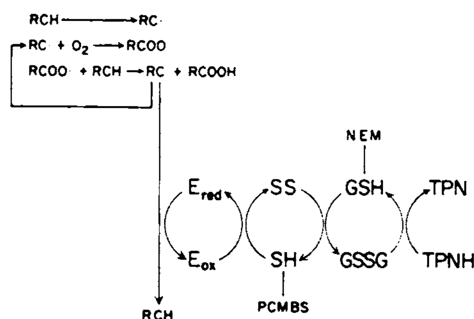

FIGURE 1. A hypothetical scheme for the termination of the chain reaction involved in the peroxidation of unsaturated fatty acids (RCH) of the erythrocyte membrane. The free radical intermediate (RC·) is reduced by vitamin E, which, in turn, is regenerated through the interaction of membrane SH groups (PCMBS-sensitive) and intracellular GSH.

of vitamin E. Experiments to clarify these interactions are currently in progress in our laboratory.

Finally, it should be noted that although we have used this curious microsomal system as a means of peroxidizing erythrocyte lipids and inducing hemolysis, the hemoglobin of the erythrocyte itself, particularly under conditions of hyperbaric oxygenation, may be an equally effective initiator of peroxidation and hemolysis. Thus, hemolysis and anemia associated with oxygen at high pressures may well result from membrane damage following peroxidation of membrane lipids. The intracellular GSH of the erythrocyte appears to be stable, or at least readily regenerated, under such conditions. It would, therefore, seem that in the organism sufficient in vitamin E, if peroxidation occurs, the SH component of the membrane represents the oxygen-sensitive portion of the antioxidant complex.

ACKNOWLEDGMENT

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Mechanisms of Erythrocyte Damage During *in vivo* Hyperoxia

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The increased use of oxygen under high pressure for medical purposes [1-6] has prompted reinvestigation of the biochemical basis of oxygen toxicity. The possibility occurred to us that oxygen toxicity might occur when certain compounds were oxidized in excess of that which would occur under normal conditions. A specific consideration was the peroxidation of unsaturated fatty acids in lipid.

Unsaturated fatty acids readily autoxidize *in vitro* to form lipid peroxides.[7-10] The reaction takes place nonenzymatically in the presence of oxygen and ferrous ions. It is inhibited by alpha-tocopherol. *In vitro,* peroxidation of erythrocyte (RBC) lipid has been shown to cause hemolysis.[11] The biologic relevance of this observation has been uncertain because lipid peroxidation has never been shown to occur *in vivo.*

Taylor has shown that during exposure to OHP hemolytic anemia occurs in tocopherol-deficient rats but not in tocopherol-supplemented rats.[12] Since tocopherol's only known biochemical effect is inhibition of lipid peroxidation,[13] this observation suggested that RBC lipid peroxidation could occur during OHP and cause hemolytic anemia. This conclusion could not be reached directly, however, since in this study lipid peroxides were not measured.

The present investigation was designed to establish the presence or absence of *in vivo* lipid peroxide formation during OHP and to explore the possible relationship between RBC lipid peroxidation and hemolysis during OHP.

Methods

DBA/2 mice, male and female (6–9 months old, average weight 25 gm), were divided into two groups differing only in their tocopherol status. One group of mice was fed a standard Chow diet, then given a supplement of 0.5 mg alpha-tocopherol (Aquasol E, U.S. Vitamin and Pharmaceutical Corp.) by intraperitoneal injection either 0.5, 3, or 18 hours prior to study. Tocopherol deficiency was induced in the other group of mice by feeding them a tocopherol-free test diet (General Biochemicals) for a minimum period of 6 weeks prior to study. Proof of tocopherol status was obtained by determining lytic sensitivity of RBCs from mice in each group to hydrogen peroxide by a method which we have described previously.[14]

All food, water, and combustible ma-

terial were removed from metal animal cages which had been coated with a solution of equal parts saline and glycerine as a fire safety precaution. The animals to be exposed to OHP were placed in the cages and put into the hyperbaric chamber (volume 12 ft^3). Constant circulation within the gaseous environment was achieved by continual flushing with medicinally pure oxygen from a cylinder. Expired CO_2 was absorbed, and several determinations performed during the exposure period revealed no CO_2 in the gaseous environment, as measured in a micro-Scholander gas analyzer. A pressure of 60 psia was reached over a period of 30 min and maintained for 90 min. During this time, ventilation remained constant at a rate of 10 liters/min. Slow stepwise decompression was carried out over a 30-min period, so that total exposure time to 100% oxygen was 2.5 hours. In each study, 40 tocopherol-supplemented and 40 tocopherol-deficient mice of comparable age and sex were used. Half of the mice from each group were exposed to OHP and half remained at normal atmospheric conditions.

Blood was obtained from mice by severing axillary vessels surgically exposed under ether anesthesia. It was collected in heparinized pipettes or microhematocrit tubes. Blood from the mice exposed to OHP was compared to blood from mice left at normal atmospheric conditions.

Microhematocrits, reticulocyte counts, and Heinz-body preparations were performed.[15] Whole-blood methemoglobin levels were determined spectrophotometrically.[16] Reduced glutathione (GSH) content of RBCs was determined by the method of Beutler *et al.*[17] RBC glucose-6-phosphate dehydrogenase activity was determined as described by Zinkham *et al.*,[18] except that a change of absorbance of 0.001 was taken as one enzyme unit and activities were expressed as enzyme units/μl packed cells/min. RBC catalase activity was determined by the method described by Feinstein.[19]

Lipid peroxides were determined by measuring the chromagen formed by the reaction of 2-thiobarbituric acid with malonylaldehyde.[20] The term "lipid peroxide" has been used in this report with the understanding that products of lipid peroxidation and not lipid peroxide themselves were measured. RBCs were washed twice in physiologic saline, and 0.2 ml of RBCs from each study group was mixed with 1.5 ml of 10% trichloroacetic acid and filtered through Whatman #1 filter paper. One milliliter of the filtrate was added to 1.2 ml of 0.67% 2-thiobarbituric acid, thoroughly agitated, then heated in a boiling water bath for 15 min. After the mixture was cooled to room temperature, spectra were obtained on all samples and final readings of absorbance taken at 535 mμ against a blank in which 0.2 ml saline was used in place of 0.2 ml RBCs.

RESULTS

The *in vitro* lytic sensitivity of mouse RBCs to hydrogen peroxide is shown in Figure 1. RBCs from tocopherol-deficient mice lysed much more than RBCs from tocopherol-supplemented mice.

The effect of *in vivo* OHP on hematocrit, reticulocyte count, and plasma hemoglobin is shown in Table 1. Before exposure to OHP, no significant differences in these parameters were noted between

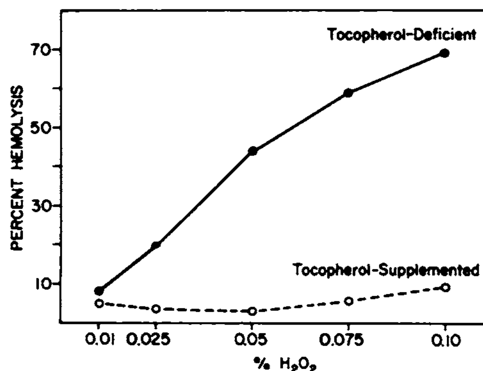

FIGURE 1. *In vitro* lytic sensitivity of mouse erythrocytes to hydrogen peroxide.

TABLE 1. Hematologic Effects of *in vivo* Hyperoxia on Mice

Study group	Hematocrit (%)	Reticulocytes (%)	Appearance of plasma
Tocopherol-supplemented	45	0.8	Normal
Tocopherol-deficient	45	1.0	Normal
Tocopherol-supplemented, OHP	50	1.2	Normal
Tocopherol-deficient, OHP	28	14.0	Bright red

tocopherol-deficient and tocopherol-supplemented mice. During OHP, hemolysis occurred in tocopherol-deficient mice as indicated by the fall in hematocrit and by the appearance of hemoglobin in plasma. No evidence of hemolysis was noted in tocopherol-supplemented mice exposed to OHP.

No Heinz-body or methemoglobin formation occurred during OHP. RBC GSH content and glucose-6-phosphate dehydrogenase activity rose in tocopherol-deficient mice exposed to OHP, but remained constant in all other mice. Catalase activity was unaffected by OHP.

Lipid peroxide content of mouse RBCs before and after exposure to OHP is shown in Table 2. Lipid peroxides were present in RBCs obtained from tocopherol-deficient mice immediately after exposure to OHP. No lipid peroxides were present in RBCs of tocopherol-supplemented mice after exposure to OHP or in RBCs from either group of mice before exposure to OHP.

Further studies were performed to determine whether the lipid peroxides present in RBCs of tocopherol-deficient mice exposed to OHP had been formed *in vivo*

TABLE 2. Lipid Peroxide Levels in Erythrocytes from Mice

Study group	Lipid peroxides (mμmoles malonylaldehyde/ml RBCs)
Tocopherol-supplemented	0
Tocopherol-deficient	0
Tocopherol-supplemented, OHP	0
Tocopherol-deficient, OHP	45

or *in vitro*. To prevent *in vitro* lipid peroxidation, tocopherol was injected into tocopherol-deficient mice after their exposure to OHP. One hour later they were exsanguinated. Blood was collected in pipettes rinsed with physiologic saline containing alpha-tocopherol (0.5 mg/ml), and all subsequent steps of the thiobarbituric acid test were performed with solutions containing alpha-tocopherol (0.5 mg/ml). Results of previous experiments (Table 3) had shown that tocopherol used in this fashion prevented *in vitro* peroxidation of lipid in erythrocytes of mice fed the tocopherol-deficient diet. As seen in Table 4, lipid peroxide levels in RBCs of tocopherol-deficient mice exposed to OHP were not decreased when *in vitro* lipid peroxidation was prevented.

Subsequent studies have further clarified the chronological relationship between RBC lipid peroxidation and lysis. These studies differed from those already described in three important respects. First, mice used in these studies were maintained on the tocopherol-free diet for 9 weeks prior to study instead of the customary 6 weeks. Second, these studies were carried out in a different hyperbaric chamber, which was cylindrical in shape with a diameter of 6 in., a length of 16 in., and a total volume of approximately 450 $in.^3$ The continual circulation of the gaseous environment was maintained by a constant influx of medicinally pure oxygen from a cylinder and by a constant exhaust at a rate of 10 liters/min. The mice (no more than eight in any given exposure) were placed directly in the tank. Third, exposure times were shorter in these studies. Mice were exposed to 100% oxy-

TABLE 3. Effect of *in vitro* Pro-oxidants on Lipid Peroxide Content of Tocopherol-Deficient Mouse Erythrocytes [a]

Study group	mμmoles malonylaldehyde/ml RBCs			
	Bubbled O_2	OHP	H_2O_2	UV radiation
Tocopherol-deficient	84	80	120	98
Tocopherol-deficient, supplemented with tocopherol 1 hour before exsanguination [b]	0	0	0	0

[a] Values given are those obtained from pooled blood of 10 mice in a single experiment.
[b] Blood was collected and processed in solutions containing tocopherol.

gen at 60 psia for 60 min, with 15 min allowed for compression and 15 min for decompression. Hence, total exposure time to 100% oxygen was only 90 min.

As seen in Figure 2, before and immediately after exposure to OHP there was no evidence of hemolysis. Ten minutes after exposure, while the mice were maintained at normal atmospheric conditions, hemolysis began, first indicated by the appearance of visible hemoglobin in

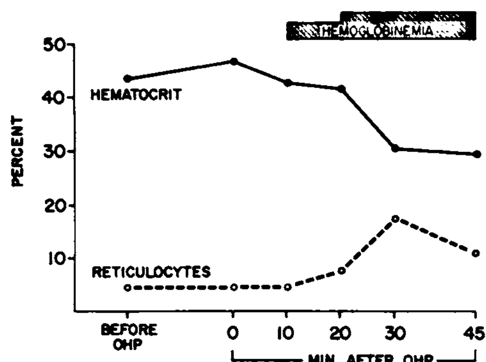

FIGURE 2. Hematologic effects of hyperbaric oxygen on tocopherol-deficient mice. Note that hemolysis began after exposure while the mice remained at normal atmospheric conditions.

plasma. Thirty minutes after exposure, the hematocrit had fallen to 30%, the plasma was bright red, and hemoglobinuria was observed. During this time reticulocytes increased to a high of 17%.

The relationship between RBC lipid peroxide content and lysis is shown in Figure 3. Immediately after exposure, before hemolysis began, RBCs contained high levels of lipid peroxides. These levels decreased as hemolysis progressed.

The relationship between RBC and plasma lipid peroxide content is shown in Figure 4. Immediately after exposure, plasma contained no lipid peroxides, while RBCs contained high levels of lipid peroxides. Plasma lipid peroxides appeared for the first time 10 min after exposure, when hemolysis began. The appearance of plasma lipid peroxides coincided with the first decrease in RBC lipid peroxides.

Five hours after exposure, neither RBCs nor plasma contained lipid peroxides. Urine examined 1 hour after exposure contained a substance which, on acid hydrolysis and heating with 2-thiobarbituric acid, formed a pink pigment which ab-

TABLE 4. Lipid Peroxide Levels in Tocopherol-Deficient Mouse Erythrocytes After Exposure to Oxygen at High Pressure

Study group	Lipid peroxides (mμmoles malonylaldehyde/ml RBCs)
Tocopherol-deficient, OHP	36
Tocopherol-deficient, OHP, supplemented with tocopherol after OHP 1 hour before exsanguination [a]	36

[a] Blood was collected and processed in solutions containing tocopherol.

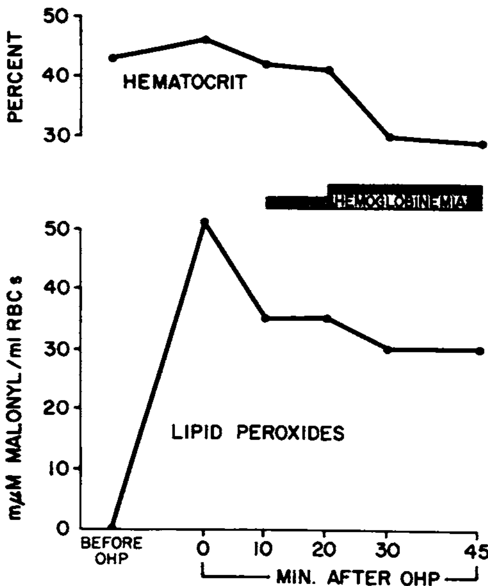

FIGURE 3. Relationship between erythrocyte lipid peroxide levels and lysis. Note that erythrocytes contained high quantities of lipid peroxides immediately after exposure, before hemolysis began.

sorbed maximally at 535 mμ and was, presumably, malonylaldehyde. Subsequent *in vitro* studies have shown that formed lipid peroxides disappear during incubation at 37°C in plasma but not during similar incubation in saline.

DISCUSSION

Peroxidation of lipid *in vitro* is a well-known reaction. The possibility that this reaction might occur *in vivo* has been suggested previously[21] but never proven. Theoretically, if peroxidation of lipid does take place *in vivo,* it should occur most easily in tissues which contain either unusually high concentrations of unsaturated fatty acids, high quantities of lipid pro-oxidants, or some combination of these factors. Because the levels of oxygen reached in tissues *in vivo* during OHP are far greater than those which can be achieved by any other means, we hypothesized that peroxidation of lipid *in vivo* might occur during OHP.

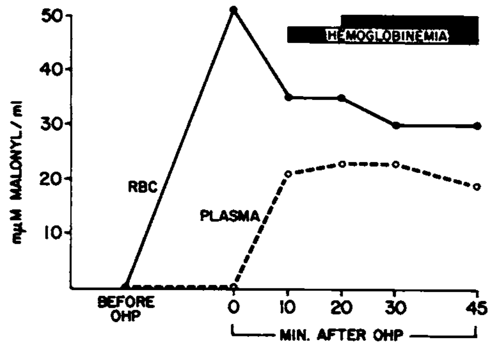

FIGURE 4. Relationship between erythrocyte and plasma lipid peroxide levels. Note that immediately after exposure plasma contained no lipid peroxides and that lipid peroxides appeared in plasma when hemolysis began.

The results of this investigation have established the fact that peroxidation of lipid can occur *in vivo*. The observation that RBCs from tocopherol-deficient mice contained lipid peroxides after OHP but not before OHP strongly suggested that RBC lipid had been peroxidized *in vivo* during OHP. However, this observation alone was not conclusive, since lipid can be peroxidized by exposure to atmospheric oxygen, and in our study RBC lipid peroxide content was measured after RBCs had been exposed to oxygen in room air during the collection of blood and washing of RBCs. Thus, the lipid peroxides detected in RBCs could have been formed *in vitro*. This interpretation could not in itself explain the presence of lipid peroxides in RBCs of tocopherol-deficient mice only when they had previously been exposed to OHP. However, it could be argued that the effect of OHP had been merely to initiate processes leading to lipid peroxidation, and that subsequent peroxidation of lipid by atmospheric oxygen *in vitro* had therefore proceeded more rapidly than usual. This question was answered by designing an experiment which permitted lipid peroxidation to occur *in vivo* but not *in vitro*. When we employed the technique which we showed prevented peroxidation of lipid *in vitro,* RBCs from tocopherol-deficient mice exposed to OHP still contained the same high quantities of

lipid peroxides. This proved that the lipid peroxides present had been formed *in vivo*.

Having established the fact that lipid peroxides did form *in vivo* during OHP, our next objective was to determine the relationship between peroxidation of lipid in RBCs and hemolysis. With regard to this question, the following observations were made. (1) During OHP, hemolysis occurred in tocopherol-deficient mice but never in tocopherol-supplemented mice. This observation is compatible with the hypothesis that *in vivo* RBC lipid peroxidation can cause hemolysis, since the only known biochemical effect of tocopherol is inhibition of lipid peroxidation. (2) Hemolysis never occurred during OHP in the absence of RBC lipid peroxidation. Conversely, whenever RBCs contained lipid peroxides hemolysis occurred. This observation established a direct association between RBC lipid peroxidation and lysis. (3) Peroxidation of RBC lipid occurred well before hemolysis began. This observation proved that RBC lipid peroxides did not form as a result of hemolysis. (4) As hemolysis progressed after OHP, RBC lipid peroxide levels decreased. This observation suggested that hemolysis occurred in those RBCs containing the highest quantities of lipid peroxides. (5) Evidence of other possible causes of hemolysis during OHP, such as oxidation of hemoglobin or glutathione, or inhibition of enzymes such as glucose-6-phosphate dehydrogenase or glutathione peroxidase, never was observed after OHP. These data taken together permit the conclusion that hemolytic anemia during OHP is caused by peroxidation of RBC lipid.

The exact mechanism by which RBC lipid peroxidation causes hemolysis is not known. Two possible mechanisms will be considered. First, unsaturated fatty acids are present in phospholipid, which is a structural component of the RBC membrane. Their peroxidation might cause rupture of double bonds in the fatty acid chain with a resultant anatomic defect in the cell membrane. An alternative mechanism of hemolysis was suggested by studies in our laboratory of dogs exposed to OHP.[22] In these studies, we found increased osmotic fragility after OHP, in association with decreased RBC acetylcholinesterase activity. A cause-and-effect relationship between these two changes has not yet been established. However, the inhibition of acetylcholinesterase activity was proven to be an effect of lipid peroxides, a finding which aroused speculation that lipid peroxides might also inhibit other sulfhydryl enzymes. Glyceraldehyde-3-phosphate dehydrogenase is a key sulfhydryl enzyme involved directly in ATP formation in the RBC. Its inhibition by lipid peroxides might well lead to hemolysis by decreasing ATP formation with resultant failure of cation transport culminating in osmotic lysis. We have previously reported[23] an alteration in the pattern of RBC glycolytic intermediates after OHP, which might be attributable to glyceraldehyde-3-phosphate dehydrogenase inhibition. We have not yet demonstrated by direct enzymatic assay inhibition of the activity of this enzyme.

The relevance of these data to humans was established in one patient we studied who developed hemolytic anemia after OHP.[24] *In vitro,* his RBCs, both before and after OHP, showed unusually high lipid peroxide formation and lysis during incubation with hydrogen peroxide. Although he showed no evidence of tocopherol deficiency, his RBCs behaved *in vitro* like those of tocopherol-deficient mice. On the basis of his unusual RBC lytic sensitivity to hydrogen peroxide, we were able to correctly predict his hemolytic response to OHP.

This patient demonstrated another interesting similarity to tocopherol-deficient mice in that his central nervous system response to OHP was abnormal. As reported previously,[25] we have consistently observed an increased incidence of seizures and mortality during OHP in tocopherol-deficient mice compared to tocopherol-supplemented mice. This observation, together with our observations in RBCs, is

consistent with the hypothesis that *in vivo* peroxidation of lipid during OHP is an underlying biochemical abnormality responsible for oxygen toxicity.

The observation of Helvey[26] that hemolytic anemia occurs in normal humans exposed to 100% oxygen at reduced atmospheric pressure in simulated space capsule environments led us to speculate that peroxidation of RBC lipid could also be responsible for hemolysis under these circumstances. If so, the information obtained during this investigation will be relevant to pathologic change that occurs during exposure of animals and humans to high oxygen environments, not only at increased atmospheric pressure, but also at normal or reduced atmospheric pressure.

SUMMARY

Studies of the effect of *in vivo* OHP on RBCs were carried out in tocopherol-deficient and tocopherol-supplemented mice. Hemolysis occurred only in tocopherol-deficient mice exposed to OHP. *In vitro* lytic sensitivity of RBCs to hydrogen peroxide paralleled their *in vivo* lytic sensitivity to OHP. These studies established the *in vivo* formation of lipid peroxides in RBCs of tocopherol-deficient mice during OHP and demonstrated the causal role of RBC lipid peroxidation in hemolysis during OHP.

The relationship between RBC lipid peroxidation and hemolysis was discussed. The relevance of these observations to pathologic events that occur in animals and humans exposed to high oxygen environments at normal or reduced atmospheric pressure was suggested. The data presented were consistent with the hypothesis that peroxidation of lipid during OHP is a basic underlying biochemical abnormality responsible for oxygen toxicity.

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In vivo Effects of Hyperbaric Oxygen Toxicity and Succinate as a Preventative

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EFFECTS OF HIGH PRESSURE OXYGEN *in vivo*

The general effects of high pressure oxygen toxicity have been described in several excellent review articles.[1-5] The depression of tissue respiration associated with OHP and the observation that the exposure of brain tissue slices to OHP resulted in decreased brain potassium, increased brain sodium, and a decrease in the ability to accumulate glutamate,[6] has led to the hypothesis that OHP exerts its toxic effect by reducing the energy available to the cell.

To test this hypothesis, we measured the concentrations of adenosine triphosphate (ATP) in brain, liver, and kidney in control rats and in rats surviving exposure to 100% oxygen at 5 ata for 30 min and 90 min. When it was found that OHP decreased the ATP concentration in these tissues, we attempted to ascertain whether irreversible damage had occurred to the oxidative phosphorylation system during the *in vivo* exposures which caused depressed ATP concentrations by performing respiration and oxidative phosphorylation studies on these tissues using succinate and alpha-ketoglutarate as substrates. Succinic dehydrogenase activity was determined in brain, liver, and kidney of control rats and rats exposed to 100% oxygen at 5 ata for 1.5 hours, to see if a correlation existed between succinic dehydrogenase activity and alterations in respiration and oxidative phosphorylation with succinate.

With depressed ATP concentrations resulting from OHP exposure, the possibility of the release of lysosomal enzymes and subsequent cellular catabolism was investigated by determining the percent of free cathepsin activity and the percent of acid-soluble nitrogen in brain, liver, and kidney of control rats and rats exposed to 100% oxygen at 5 ata for 1.5 hours.

Methods

Fasted (16–20 hours) male Sprague-Dawley rats (150–225 gm) were used in all studies. Control animals were exposed to air at 1 ata. OHP-exposed animals were subjected to 100% oxygen at 5 ata for 30 min or 90 min. All other experimental procedures were the same for controls and OHP-exposed rats.

To determine ATP concentration, tissue was prepared by a technique which prevented rapid ATP loss.[7] The ATP concentration in the tissue preparations was determined by the firefly luminescence

method[8,9] and results expressed as a percentage of control values.

Respiration and oxidative phosphorylation and succinic dehydrogenase activity were determined in the following manner. The cerebral hemisphere, kidney cortex, and liver of the rat were rapidly removed at the end of the exposure period and homogenized as previously described.[9,10] The polarographic method was used to determine respiration and oxidative phosphorylation of the homogenates at 25°C.[9,11,12] The respiration rate stimulated by adenosine diphosphate (ADP qO_2), the basal respiration rate (basal qO_2) and the ADP–oxygen (ADP/O) ratio were determined for each tissue homogenate with succinate and with alpha-ketoglutarate. The method of Cooperstein, Lazarow, and Kurfess[13] as modified by Hall[14] was used to determine succinic dehydrogenase activity of similarly prepared homogenates.

The free and total cathepsin activity was determined by the method of Anson as modified by Hall.[14] Total and acid-soluble nitrogen was determined by micro-Kjeldahl digestion coupled with Nessler reagent.

Results

Figure 1 shows ATP concentration and succinic dehydrogenase activity as a percent of control values, percent of free

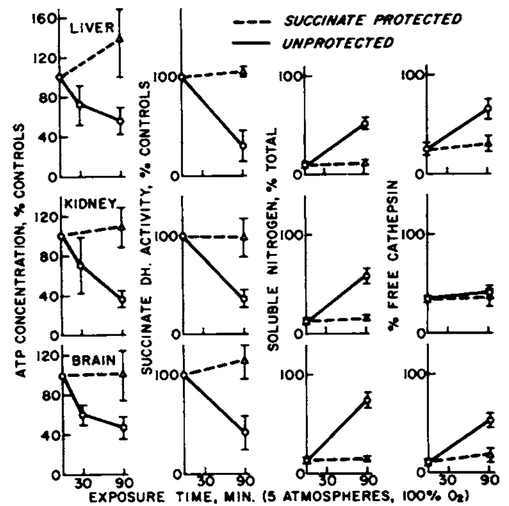

FIGURE 1. ATP concentration and succinic dehydrogenase activity in brain, liver, and kidney of rats exposed to 100% oxygen at 5 ata. Values expressed as percent of control values, percent of free cathepsin activity, and amount of acid-soluble nitrogen (in percent of total nitrogen). Each point represents minimum of six animals.

cathepsin activity, and acid-soluble nitrogen as a percent of total nitrogen, for brain, liver, and kidney of rats that were exposed to 5 ata of 100% oxygen. Table 1 shows the results of the respiration and oxidative phosphorylation studies on brain, liver, and kidney for succinate and for alpha-ketoglutarate.

At 5 ata of 100% oxygen, the ATP concentration in all three tissues was re-

TABLE 1. Effects of Hyperbaric Oxygen on Tissue Respiration and Oxidative Phosphorylation

	N*	Succinate			Alpha-ketoglutarate		
		ADP/O	ADP qO_2	Basal qO_2	ADP/O	ADP qO_2	Basal qO_2
Normal liver	7	2.05±0.12	7.96±0.98	3.12±0.03	3.05±0.22	3.26±0.59	1.68±0.22
1.5 hr at	8	1.93±0.09	13.06±0.73	4.57±0.38	2.94±0.15	5.56±0.88	2.78±0.31
3 ata		$P = 0.04$	$P < 0.01$	$P < 0.01$	$P > 0.5$	$P > 0.05$	$P < 0.01$
Normal kidney	8	2.05±0.06	14.2±0.72	6.59±0.20	2.97±0.15	6.96±0.25	2.72±0.13
1.5 hr at	7	1.97±0.15	11.60±1.81	7.18±1.58	2.96±0.16	6.00±0.98	3.68±0.70
3 ata		$P > 0.5$	$P < 0.01$	$P = 0.3$	$P > 0.5$	$P < 0.01$	$P < 0.01$
Normal brain	6	1.96±0.17	4.33±0.10	2.10±0.51	2.71±0.23	2.18±0.17	1.07±0.18
1.5 hr at	6	1.81±0.11	5.87±0.03	3.29±0.23	2.80±0.17	3.36±0.13	2.05±0.10
3 ata		$P = 0.10$	$P < 0.01$	$P < 0.01$	$P > 0.5$	$P < 0.01$	$P < 0.01$

* N, number of animals from which organs were removed for preparation of homogenate.

duced significantly (29–39%) within 30 min, approximately 20–30 min before the animals began to exhibit symptoms of oxygen toxicity. After 90 min of exposure, all tissues showed marked reduction in ATP concentration (brain 53% decrease, liver 44% decrease, kidney 63% decrease). Thus, high pressure oxygen caused a decrease in tissue energy stores which, theoretically, could result in disturbances in metabolic functions of the tissues.

When returned to normal oxygen tension, tissue homogenates obtained from the OHP-exposed rats maintained their efficiency for oxidative phosphorylation as shown by the ADP/O ratio for succinate and for alpha-ketoglutarate. The ADP-stimulated respiration rate, which is a reflection of the ATP production capacity, increased with liver (up 64% with succinate, up 70% with alpha-ketoglutarate) and with brain (up 35% with succinate, up 54% with alpha-ketoglutarate). Kidney cortex had decreased ADP-stimulated respiration rate (down 20%) after OHP exposure. Basal respiration rates for all three tissues were greater after rats were exposed to OHP. It is interesting to note that even though the maximum respiration rate in brain and liver increased 35% and 54%, respectively, and decreased only 20% in kidney, the succinic dehydrogenase activity was reduced 57% in brain and 69% in liver and kidney homogenates from the OHP-exposed rats. Thus, the succinic dehydrogenase activity level did not reflect the capacity of the tissue to utilize succinate when returned to normal oxygen tensions.

All three tissues from the OHP-exposed animals were found to have markedly increased levels of acid-soluble nitrogen (74%, 52%, and 59% of total nitrogen for brain, liver, and kidney, respectively, as compared with normal values of 13%, 10%, and 13%). Total nitrogen concentrations were not significantly different from normal.

The percent of free cathepsin activity in all three tissues from the OHP-exposed animals was significantly elevated (brain 450%, liver 640%, and kidney 119%). The value for kidney is misleading since the total cathepsin activity at this exposure was significantly reduced only in the kidney. (Kidney homogenates from rats exposed to 100% oxygen at 3 ata for 2 hours had 88% free cathepsin activity, total activity being unchanged).

Rapid inhibition of alpha-ketoglutarate dehydrogenase activity and rapid depression of pyruvate oxidase activity has been demonstrated in rat brain mitochondria exposed to OHP.[15] Such an inhibition would result in the inhibition of three of the four Krebs cycle intermediates that result in ATP production when oxidized (alpha-ketoglutarate, succinate, and malate). Thus, a reduction in tissue ATP concentration would be expected. Our results indicate that rats exposed to OHP show a definite decrease in tissue ATP concentration, which, if it is due to inhibition of alpha-ketoglutarate and succinate oxidation, should be rapidly reversible on restoration of normal oxygen tensions. That this is indeed the case is evidenced by the near-normal or elevated respiration rates in all three tissues with these substrates.

Hall[16] has shown that the percent of free cathepsin activity in brain, liver, and kidney from OHP-exposed rats increases with increasing pressure of 100% oxygen. Soluble nitrogen increases significantly in brain, liver, and kidney only after the percent of free cathepsin activity has been increased.[16] The increased soluble nitrogen is indicative of catabolism within the tissues and is similar to the situation reported in anoxic liver where a marked increase in free nitrogen occurs after prolonged periods of anoxia[17] which have been shown to significantly decrease ATP concentration in tissues.[9]

Succinate as a Preventative of Oxygen Toxicity

The decrease in ATP concentration of tissues from animals exposed to OHP

suggested that efforts to protect against oxygen toxicity should be directed toward maintaining normal tissue ATP concentration and consequently metabolic functions. Succinate has the highest ATP production capacity (among succinate, alpha-ketoglutarate, and glutamate) in rat brain, liver, and kidney.[9] When succinate is the substrate, 50% more oxygen is utilized per ATP molecule formed than when substrates linked with nicotinamide adenine dinucleotide (NAD) are being oxidized. Thus, succinate uses more oxygen and causes a higher ATP production than other oxidative phosphorylation substrates. Both factors counteract local effects of OHP. It has previously been shown in minced rat brain that succinate oxidation is more resistant to oxygen exposure than pyruvate or glucose oxidation.[18] Dickens[1] observed that succinate oxidation continued in brain slices exposed to 4.6 ata and, to a lesser degree, L-glutamate oxidation continued. This latter observation is particularly pertinent in view of the postulation of the existence of a glutamate → gamma amino butyric acid (GABA) → succinate shunt.[19] This shunt operates around the alpha-ketoglutarate system by withdrawal of alpha-ketoglutarate through transamination with GABA, and re-entry of the carbon chain into the Krebs cycle at the succinic acid level. The inhibition of alpha-ketoglutarate oxidation was mentioned above.[15]

These observations and the observation that GABA offers some degree of protection to rats[19] further suggest that succinate might serve as a protective agent against OHP toxicity. Chance *et al.*[20] have observed that one effect of OHP is the rapid inhibition of the reduction of NAD, and that succinate slows the rate of inhibition of the reduction of NAD significantly.[20] The reduction of NAD is essential for ATP production with all oxidative phosphorylative substrates except succinate. Thus, succinate would not be affected by such OHP inhibitory action on NAD reduction. Chance *et al.*[20] further observed that there is minimal inhibition of electron flow from succinate into the electron transport chain at 12 ata of oxygen pressure. Succinate has been reported to monopolize the respiratory chain and inhibit the oxidation of reduced NAD.[21]

All of the above observations strongly support the hypothesis that succinate should be an effective protective agent against the toxic effects of OHP. To test this hypothesis, two sets of experiments were performed. In the first experiments,[22] four groups of fasted (16–20 hours) male Sprague-Dawley rats (160–225 gm) were exposed to 100% oxygen at 5 ata for 1.5 hours. Groups 1, 2, and 3 were injected intraperitoneally with 7.5 ml of isotonic saline, 0.4 M dextrose (at pH 7.4), and 0.4 M sodium succinate (at pH 7.4), respectively, 1 hour before the OHP exposure. Group 4 consisted of succinate-injected control rats which were killed 2.5 hours after succinate injection, at which time the ATP concentration was determined. Tissue ATP concentrations were similarly determined in brain, liver, and kidney in all rats which survived the OHP exposure.

Ninety percent of the saline-injected rats died during the exposure period. Surviving animals had a significant decrease in ATP concentration in brain (53% decrease), liver (49% decrease), and kidney (63% decrease), and all survivors died within 15 min of the end of the exposure period.

Fifty percent of the 10 dextrose-treated animals died during the exposure. Thirty percent had symptoms of oxygen toxicity (convulsions, loss of consciousness) and significantly lowered ATP concentration in all three tissues. Two of the 10 animals appeared normal and had tissue ATP concentrations within the range of normal.

All of the succinate-treated animals[22] survived the exposure period and appeared normal and alert. Brain, liver, and kidney ATP concentrations from these

animals were normal or above normal (Figure 1).

Subsequently, animals of another group were injected intraperitoneally with sodium succinate (10 mmoles/kg of a 0.4 M solution) 1 hour preceding the exposure to 100% oxygen given at 5 ata for 1.5 hours. At the end of the exposure period, succinic dehydrogenase activity, percent of free cathepsin activity, and percent of acid-soluble nitrogen were determined on brain, liver, and kidney of these animals by the methods described above.

The succinate-treated OHP-exposed rats were able to maintain normal levels of ATP concentration, succinic dehydrogenase activity, percent of free cathepsin activity, and percent of acid-soluble nitrogen in brain, liver, and kidney (Figure 1). Unprotected rats surviving the same OHP exposure showed marked alterations in ATP concentration, succinic dehydrogenase activity, percent of free cathepsin activity, and percent of acid-soluble nitrogen in brain, liver, and kidney when compared with normal values.

These observations strongly support the hypothesis that succinate is an effective protective agent against hyperbaric oxygen toxicity. Certain problems, however, attend the effective use of succinate as a protective agent against OHP toxicity. Intraperitoneal injections did not, for example, protect a fed rat. Animals fasted more than 24 hours and animals that were given intraperitoneal injections containing 15 mmoles of sodium succinate/kg body weight frequently died within 1 hour of the time of injection. Thus further work is needed to determine a more effective route of administration and the proper dosage schedule to provide adequate protection against OHP toxicity without having a sodium succinate toxicity problem. Should the pharmacologic problems be resolved, succinate has one distinct attribute for OHP work: it should offer no protection to anaerobic systems, which have been shown to be particularly susceptible to OHP therapy.

ACKNOWLEDGMENTS

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DISCUSSION

Discussion of papers by Gilbert (pp. 3–14), Chance et al. *(pp. 15–41), Gibson (pp. 42–45), Longmuir (pp. 46–51), Hashimoto* et al., *(pp. 52–60), Hochstein (pp. 61–64), Kann and Mengel (pp. 65–72), and Sanders* et al. *(pp. 73–78)*

DR. F. DICKENS, *Session Chairman (London, England)*: We have been listening to a fundamental contribution to this problem. The application of these extremely elegant methods to the actual changes in nucleotide level in the tissues is something quite new. Before throwing open the discussion, I should like to present three figures pertinent to the topic at hand.

Figure 1 shows the time of onset of toxic symptoms or signs in animals and the pressure in atmospheres absolute of oxygen plotted against the log time in minutes. Each of these groups is a series of observations by different authors with different species, using different indicators of onset of oxygen toxicity; therefore, it is not surprising that the spread of the results is quite broad. The solid line on the right is based on the most extensive lethal results on animals, done on mice by Dr. Rebeca Gerschman. The time scale, being logarithmic, is about six times that of the average first onset of symptoms in these animals. For example,

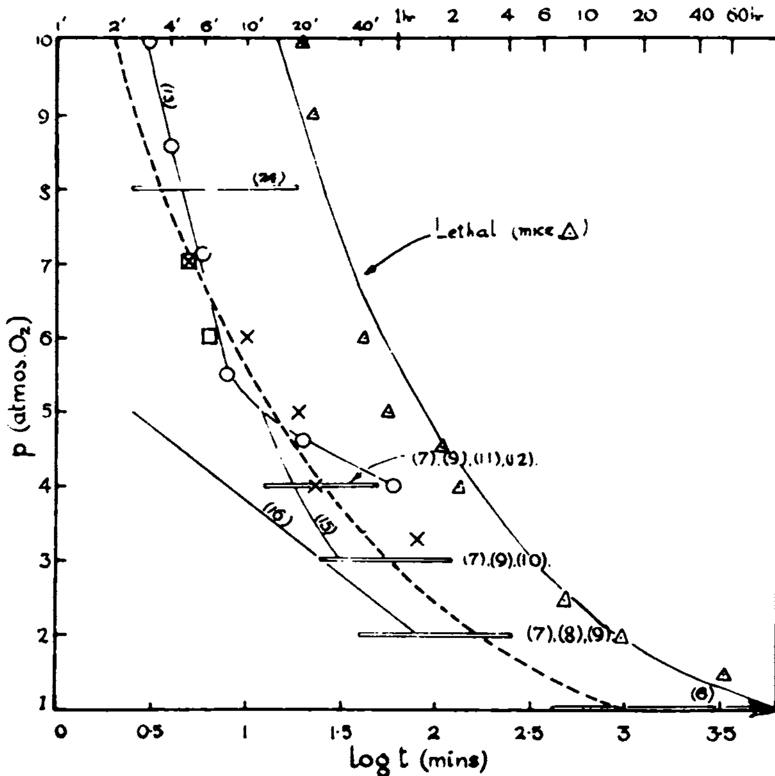

FIGURE 1. Times of onset of toxic signs (broken curve) and of death (upper continuous curve) at various pressures of oxygen. The combined data of many authors and for various species are represented. (Reproduced from *Neurochemistry*, 2nd ed., Charles C Thomas, 1962, p. 851.)

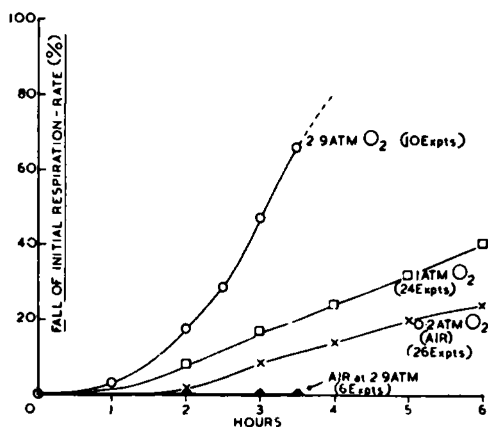

FIGURE 2. Mean fall of respiration rate in slices of rat brain cortex. (Reproduced from Biochem. J. 40:151, 1946.)

at 3 atmospheres you will notice that about 45 minutes are required on an average for onset of symptoms, and a longer period (about 180 minutes) for the average period of LD_{50} in mice.

Figure 2 compares these results with some early measurements which I did on the effect *in vitro* on brain cortex respiration (Q values) of rats exposed to 2.9 atmospheres oxygen (Biochem. J. 40:145, 1946). You will see that in oxygen there was a marked fall of approximately 50% in 180 minutes at 2.9 atmospheres. This represents the time required to kill 50% of the mice and is roughly six times the time required for onset of symptoms. Nevertheless, at this pressure, there is a marked fall of respiration in the brain. This was also shown independently by W. C. Stadie, B. C. Riggs, and N. Haugaard in Philadelphia (J. Biol. Chem. 160:191, 1945). You will note that 1 atmosphere of oxygen was also toxic, though much less so, and in air at 0.2 atmospheres there was a fall in respiration which, curiously enough, was avoided in these experiments by using air at 2.9 atmospheres.

Figure 3 shows an effect of metals on respiration. This study was done with slices of brain cortex exposed to 4.4 atmospheres for 100 minutes. Respiration rate is represented by the columns. The air control produces a Q value of about 9; however, after exposure of the slices to 4.4 atmospheres of oxygen, it falls to 2 or 3. The succeeding plain columns indicate the results produced by the addition of various amounts of cobalt ions, and the protection was quite definite under these *in vitro* conditions. Dr. H. P. Marks, who was working with us at the time, repeated some of these metal experiments in animals (*Report to the Royal Naval Personnel Research Committee*, Medical Research Council Publication RNP/44/101, England, 1944) and achieved some protection with cobalt and zinc, and also with nickel which we did not test. We did not find any protection with zinc, but the fact that the cobalt did afford protection not only for glucose oxidation but also for oxidation of lactate and pyruvate, and also protected in the whole animal, would perhaps fit in with some of the observations about antioxidants, the action of which could very well be influenced by cobalt.

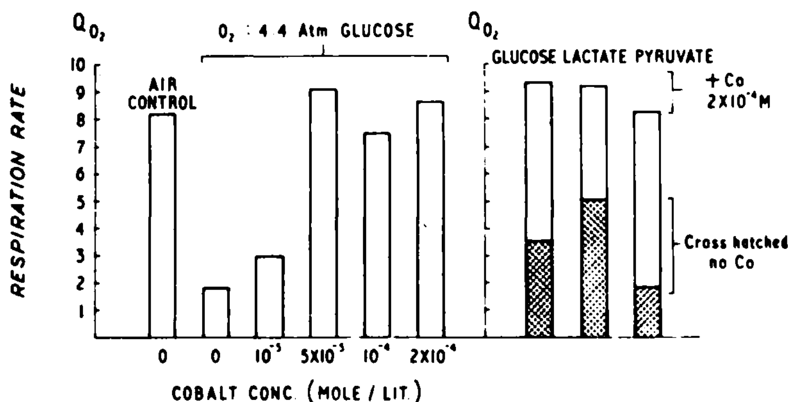

FIGURE 3. Effect of some metal ions as protectors of brain cortex respiration against high oxygen pressures. (Reproduced from *Oxygen in the Animal Organism*, Pergamon Press, 1964, p. 506.)

DR. B. CHANCE: I would like to emphasize the difference in the experimental conditions employed by Jamieson, Williamson, and me as compared with those employed by Sanders and Hashimoto. This is best illustrated by the figure (Figure 27, p. 37) in which we compared the rapidity of inhibition of the pathway for energy-linked NAD reduction with the rapidity of the inhibition of electron transport in the respiratory chain. It is apparent that the inhibition of the former reaction is complete after 4 minutes of pressurization at 11 atmospheres. At this time, reversed electron transport is completely inhibited, whereas forward electron transport is scarcely affected. The latter process is inhibited in 60 minutes. A working hypothesis is that the short-term process is related to the short-term physiological effects of high oxygen pressures. The longer-term effect is probably the cause of lung damage in chronically exposed animals.

In the short-term effect, we find a rise of ATP due to the blockage of energy utilization in reversed electron transport. In the long-term effect, there must be a fall of ATP due to the inhibition of electron transport.

Since we identify your studies with the long-term inactivation of electron transport, it is difficult to find a biochemical reason for the alleviation of high pressure oxygen effects by succinate.

My position can be made clearer by looking again at Figure 26 (p. 35), which indicates the two pools of reduced pyridine nucleotide, one directly reduced by dehydrogenases, the other reduced by succinate through a pathway of reversed electron transport (*b-Q-fp*) which requires the mediation of a high-energy intermediate ($X \sim I$). This diagram illustrates that the reduction of NAD in the energy-requiring pathway can be inhibited without inhibiting the reduction and oxidation of NAD in the pathway of forward electron transfer.

This distinction is elaborated in a more complex diagram (Figure 1). This diagram represents a current hypothesis of the sequence of action of the electron transport and energy transfer components of the respiratory chain. The complete functional unit of oxidative phosphorylation and reversed electron transport is called the oxysome (Science 140:379, 1963). The pathway of forward electron transfer begins with malate or succinate, which interacts respectively

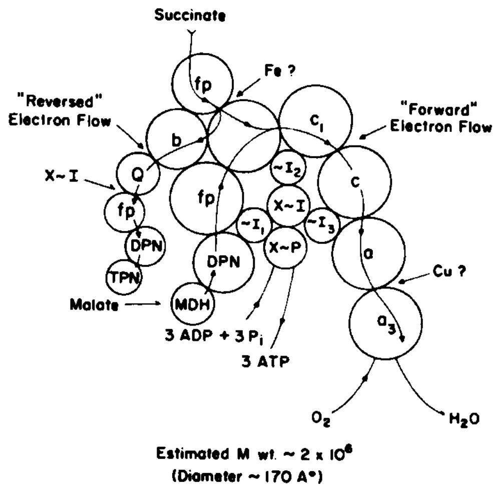

FIGURE 1. Electron and energy flow in the oxysome.

with the appropriate flavoprotein (FP) or dehydrogenase (MDH). In the latter case, DPN is reduced and activates the DPNH dehydrogenase flavoprotein (FP). The component immediately adjacent to the flavoproteins can be nonheme iron or ferridoxin-like compounds, and a part of cytochrome *b* as well. Electron transfer from the two pathways joins and flows through the respiratory cytochrome c_1, c_a, and a_3, reducing oxygen to water. This pathway is designated the pathway of forward electron flow.

Energy is tapped off from this electron flow at three sites designated by the "$\sim$" intermediates, $\sim I_1$, $\sim I_2$, $\sim I_3$, which interact further to form a common intermediate $X \sim I$ and a phosphorylated intermediated $X \sim P$, which in turn phosphorylate ADP to ATP. The sum total of the reaction for the three sites leads to the formation of three molecules of ATP.

Electrons can flow in a "reversed" pathway, that is to say, against the apparent thermodynamic gradient from succinate at zero volts to DPN or TPN at approximately −320 millivolts. The system of carriers is now completely identified, but, oddly enough, most of the cytochrome *b* and quinone are active in this pathway. Flavoprotein is indicated to transport electrons to bring about reduction of TPN, and with the aid of a transhydrogenase to reduce TPN as well. Energy is required for this pathway in order to drive this reaction against the thermo-

dynamic gradient of 320 millivolts, and a possible point for the utilization of the high-energy intermediate ($X \sim I$) to activate this pathway is indicated.

This diagram emphasizes the two functions of DPN, one in forward electron transfer to feed electrons from substrates into the respiratory chain, the other in reversed electron transfer toward reduction of DPN. The data above indicate that it is either the transport of electrons through the reversed pathway or the utilization of $X \sim I$ in this pathway that is rapidly inhibited under hyperbaric conditions.

Independent experiments indicate that reduction of the electron transfer carriers protects this pathway to some extent against hyperbaric inhibition. It would seem reasonable that any method of increasing the intracellular succinate concentration might protect *in vitro,* provided the succinate penetrates the cell membrane.

DR. DICKENS: Dr. Chance, do you feel that, in Dr. Sanders' experiments, succinate has traversed this reversed electron flow pathway, thereby keeping the NAD reduced?

DR. CHANCE: This is a provocative question. First, we have not determined whether or not succinate, administered in the way Dr. Sanders recommends, will maintain the NAD level reduced under hyperbaric oxygen conditions.* It would seem that an increase in the intracellular succinate concentration would tend to a more reduced state in the mitochondria and thereby protect against the high pressure oxygen conditions.

DR. A. P. SANDERS: We have not determined succinate levels. Obviously, this is the step to be taken. My personal feeling is that our data actually support your hypothesis. If it does not revert, it must be that the succinate has, within itself, the capability of providing sufficient ATP. This seems somewhat questionable. Let me make one additional comment. Dr. Dickens' observation that glutamate maintained to a lesser degree in oxygen, and also the observation by Dr. Wood that gamma-aminobutyric acid gives protection, fit beautifully with the succinate findings. The glutamate–gamma-aminobutyric acid shunt into succinate indicates that succinate is the actual final substrate being fed in.

DR. DICKENS: Perhaps Dr. Wood will comment on the effects of gamma-aminobutyric acid. As he believes that these substances are a source of succinate in the tissue, and that the latter protects against high oxygen pressures, his observations would be highly pertinent to the topic now under discussion.

DR. J. D. WOOD (*Toronto, Canada*): Gamma-aminobutyric acid has, of course, two actions. One is its role in oxidative metabolism, and the other its role in the modulation of nerve transmission. Until today, I was leaning more and more toward the theory that its protective effect against oxygen poisoning is due to its modulating action, because we found that the specific molecular structure required for a compound to afford protection against oxygen poisoning was the same as that required for it to have a depressant action on nerve transmission. However, in view of what the speakers have said today, I may have to revise my thinking and consider also the possibility that the protective action of GABA is mediated by the increased supply of succinate via the GABA shunt pathway.

DR. G. G. NAHAS (*New York, N. Y.*): How important is it, Dr. Sanders, that your animals be fasted 72 hours before treatment with succinate? It is known that starvation itself protects against oxygen toxicity, and, through activation of adenylcyclase and cyclic 3′,5′-AMP, induces sympathoadrenal stimulation and mobilization of fat stores as well.

DR. SANDERS: I am afraid there has been a misunderstanding. The fasting period involved was 16 to 20 hours, not 72 hours. Rats fed by the intraperitoneal injection are not protected by this. It is probably a competitive phenomenon involved with the absorption across the intestines. However, in

* Shortly after the conference, several experiments were performed by Dr. Chance according to the procedures described herein, except that the rats were given massive injections of succinate (according to the understanding of Dr. Sanders' experimental procedure). Nevertheless, the NADH oxidation in high pressure oxygen was observed to persist. The explanation for this result was that the succinate did not reach the cells due to permeability barriers.

another species, we found that protection was afforded under this condition, so we cannot clearly define it at this time. As all of this work has taken place only within the last 6 months, there are still many problems to be worked out.

DR. NAHAS: The question arises whether peroxidation plays as large a role in oxygen toxicity as Dr. Kann indicated, and I would like to ask him if animals which are not tocopherol-deficient present increased peroxidation under conditions of high oxygen pressure?

DR. H. E. KANN: There is good evidence that lipid peroxidation does occur in tissues of normal animals and humans exposed to hyperbaric oxygen. For instance, our patient who developed hemolytic anemia after exposure to hyperbaric oxygen was almost certainly not tocopherol-deficient. Because lipid peroxides are highly reactive, they are often difficult to detect directly even when indirect evidence strongly indicates that they have been formed.

DR. DICKENS: Dr. Kann, you mentioned one case of hemolysis in a human patient. Has this been observed frequently in man?

DR. KANN: We have not found any other patients who developed frank hemolytic anemia after exposure to hyperbaric oxygen. However, erythrocyte damage, indicated by an increase in autohemolysis, regularly occurs in humans exposed to hyperbaric oxygen.

DR. C. J. LAMBERTSEN (*Philadelphia, Pa.*): I am impressed by the development of an ability to put together some of the biochemical aspects of oxygen poisoning and the effects of oxygen on the whole animal. The most striking observations are those dealing with the apparent multiplicity of sites of oxygen action at the biochemical level, and the multiplicity of forms of gross oxygen poisoning. I would like to suggest that these different sites may have extremely different rates of development of toxicity. For example, one may see a particular kind of toxicity in the brain, which is different from that in the lung. It is important to determine whether these various rates of development are entirely biochemical or are, in addition, related to the particular nature of the organ which is being poisoned. In other words, can one really compare the length of time it takes the brain to be demonstrably poisoned with the length of time it takes an entirely different kind of organ, such as the lung, to demonstrate a toxic effect? It is also important to consider the recovery rate of the diverse chemical systems that are involved in oxygen poisoning. Moreover, once particular chemical sites have been poisoned, can we expect reversibility of each of these, or are some of these going to be irreversibly damaged by oxygen?

DR. DICKENS: This brings up the question whether the change in the ATP or adenosine nucleotide level is different in the various tissues.

DR. SANDERS: We have observed 1, 3, and 5 atmospheres and have seen a depression in brain ATP preceding that of liver and kidney. However, the really striking thing to us has been that the animal does not really develop acute trouble until the liver and kidney also decline very drastically. Thus, the question arises: is this a pure phenomenon, or is there an interrelationship here? Our slides show that at the time the surviving animals are removed from the chamber, the lowest percent of ATP concentration is found in the kidney. In addition, the percentage of free cathepsins seems to affect the kidney very rapidly. I am not making any proposals as to which mechanisms are involved here, but I am saying that even though we saw depressions in ATP in the brain preceding the onset of convulsions and the onset of trouble in the animal, these depressions did not occur until we actually observed these other phenomena. The other very impressive thing, in comparing the percentage of free cathepsins, such as acid-soluble nitrogen, is the extreme rapidity with which this takes place. Once it moves, it starts into place much faster than under conditions of anoxia or autolysis.

DR. DICKENS: Just for the record, I might mention that the order of sensitivity to oxygen of various tissues found *in vitro* by the Philadelphia workers and by ourselves was: brain (most sensitive), spinal cord, liver, testes, kidney, lung, and muscle. However, the percentage fall may not necessarily

be an obvious indication of sensitivity, since there may be an excess of ATP in some tissues and only a barely adequate amount in others.

DR. CHANCE: Dr. Lambertsen's question a few moments ago probes at the limits of our knowledge of the relationship between enzymatic activity and physiological function. We may consider this relationship at a number of levels. First, we may consider that there is a direct relationship between oxidation–reduction levels in mitochondria and electrical activity of membranes. This would appear to be an unlikely relationship, but one that is certainly not disproved. For example, it may be that the mitochondria in the nerve endings serve a special role in the excitability of the cortex and may thereby provide the link between oxidation–reduction states and excitability. On the other hand, the oxygen-sensitive reaction which we study in mitochondria may simply be an example of a similar reaction, concerned with the excitability of membranes, which has not yet been discovered. In any case, there may indeed be multiple targets of high pressure oxygen, such as the short-term and long-term effects which the discussion above has already indicated.

DR. DICKENS: This re-emphasizes Dr. Chance's own observation that the effect is not only very rapid but is reversible. This, however, does not apply, as far as I know, to poisoning of sulfhydryl groups, whether in enzymes or other systems.

DR. G. S. BALLA (*Dallas, Texas*): In our study involving the use of intra-arterial hydrogen peroxide, we observed the development of acute hemolysis in two patients with carcinoma of the cervix, within the first week of the peroxide infusions. The hematocrit levels dropped from 35 to as low as 15. They responded to transfusions, and when the hematocrit levels rose to 35 again, we would resume the peroxide infusions and radiation therapy without further hemolysis. I do not know how to explain this phenomenon except by what Dr. Kann brought out this morning.

DR. R. ROSENBAUM (*New York, N. Y.*): I have a comment and a question. The use of the term "lipid" can be rather confusing to a morphologist. In the first place, there are lipids such as the lipofuscins (so-called "tissue linoleums"), which, when they are oxidized, do not break down as does the lipid of the red-cell membrane. Hence, certain oxidative states on certain kinds of lipids can produce stabilization. Our own results with lysosomes and lipid coats surrounding the lysosome suggest that there is a stabilization effect. This would somewhat disagree with Dr. Sanders' data on cathepsin. At this point I would like to ask him why he used cathepsin as a lysosomal membrane. It is a sulfhydryl-dependent enzyme, whereas acid phosphatase, which is an equally reliable index of lysosomal presence or absence, is not. Dr. Sanders, did you select cathepsin specifically because it is a sulfhydryl-dependent enzyme? Did you get a release?

DR. SANDERS: Both free cathepsin and acid phosphatase levels have been studied by our group, and the cathepsin activities were observed to be very sensitive to hyperoxia. This dramatic increase in free cathepsin activity (followed by increased soluble nitrogen) due to high pressure oxygen more readily illustrated the severity of the effects of OHP than did the observed changes in free acid phosphatase. The free cathepsin activity, the acid-soluble nitrogen, and the free acid phosphatase activity were all maintained at normal levels during those OHP exposures when succinate was used as indicated.

DR. F. BERNHEIM (*Durham, N. C.*): One of the attractions of the lipid peroxide theory is that lipid peroxidation rates are a function of oxygen tension. Under normal conditions, the tissue oxygen tension is quite low, but under hyperbaric oxygen, if the animal is not completely protected by antioxidants, lipid peroxidation can occur. As Dr. Kann has pointed out, you cannot find these peroxides, but you can show a greater rate of lipid peroxidation in the isolated tissue. In the brain, Wolman (*Selective Vulnerability of the Brain in Hypoxaemia*, Blackwell, 1963, pp. 349–356) and Becker and Galvin (Aerospace Med. 33:985, 1962) have shown that the brains of animals exposed to high oxygen tensions show lipid peroxidation rates twice that of normal. The degree of respiratory inhibition which Dickens

finds in the various tissues parallels the rate of lipid peroxidation in the tissues when they are incubated *in vitro*. The fact that cobalt inhibits peroxidation could explain its protective action. Also, lipid peroxides inhibit a large number of enzymes, and this could account for many of these effects *in vitro*.

DR. DICKENS: I am glad to have your comments, but at the same time it should be emphasized that the toxic effects on tissue respiration do not coincide on the time scale with what we believe to be the pharmacologic effects of oxygen.

DR. E. A. STEAD (*Durham, N. C.*): It is possible to think of a situation in which the circulatory failure would be a protection against the toxic effects of hyperoxia. Was there evidence of circulatory failure which might have allowed the enzyme systems to remain relatively unharmed?

DR. SANDERS: In these experiments we did not do any cardiovascular or circulatory studies.

DR. Q. GIBSON: A number of years ago we did some experiments to see whether malonate would influence tissue metabolism. We injected mice intraperitoneally with enormous doses of malonate which served to cut down metabolism, measured simply by gaseous exchange, quite markedly. When we did the controls, however, we observed that succinate reproduced this effect exactly. I wonder if some of these effects are due to the cause which has just been suggested.

UNIDENTIFIED SPEAKER: Dr. Longmuir, how did you measure the amount of carrier?

DR. I. S. LONGMUIR: We used our polarographic technique for measuring oxygen tension. We placed in the cell a very large load of liver slices, waited half an hour to allow them to become fully cyanide-sensitive, and then abolished their respiration with cyanide or azide. At this point we generated oxygen in the cell electrolytically at a constant rate. In the absence of any carrier, we expected the oxygen tension to rise linearly. There was, in fact, a little peak in the curve, and from that peak, we could calculate the capacity of the carrier.

DR. DICKENS: In closing, I would like to paraphrase a comment which Wallace Fenn made at the symposium on *Oxygen in the Animal Organism* (*I. U. B. Symposium*, vol. 31, Pergamon Press, 1962). He was pointing out, in relation to the cosmic development, that oxygen produced by living plants was accumulating, and that living animals somehow appeared to make use of it. At the same time, the cells, in order to survive, must have built up antioxidant properties. These, however, have their limitations, and when unphysiological oxygen tensions are reached, the phenomenon which we heard described this morning results. Perhaps, these defenses are always rather insufficient, and it has been suggested that the basis of aging may be a slow oxidation of all our tissues due to the fact that we have to breathe this terrible stuff oxygen to the extent of 20% of the atmosphere, whether we like it or not!

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SESSION II

Oxygen Toxicity

Chairmen: JOHN W. BEAN
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University of Michigan School of Medicine
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PHILLIP PRATT
Ohio Tuberculosis Hospital and
Department of Pathology
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Role of Central Nervous System and Pulmonary Damage as Cause of Respiratory Failure in Rats Exposed to Hyperbaric Oxygen

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Damage to the central nervous system and lungs is recognized as the principal manifestation of oxygen toxicity in mammals under hyperbaric conditions, but the interrelationship between these effects remains obscure.[1-3] We have attempted to analyze the sequence of events leading to death of rats exposed to high pressure oxygen by monitoring the electroencephalogram (EEG), the electromyogram (EMG) from the diaphragm, respiratory excursions, and cardiac rate.

Methods

Rats were exposed to pressures of 4, 5, and 7 ata of oxygen. During compression, the EEG was recorded from bipolar electrodes applied to the outer table of the skull or beneath the inner dura. For the EMG, bipolar electrodes were sewn into the ventral abdominal surface of the origin of the exposed right diaphragm to provide a bioelectric signal of the motor activity of the respiratory pathway. A thermistor in the tracheal cannula monitored the respiratory flow excursions. The electrocardiogram (ECG) was almost invariably seen superimposed on the EMG tracing.

Results

The principal changes with time of exposure to OHP recorded during these experiments in urethane-anesthetized rats were (1) the appearance of bursts of high-amplitude activity on the EEG (termed "spikes"), followed by initial depression of EEG amplitude, followed finally by electrical silence, (2) slight increase in amplitude of respirations on pressurization, followed by a gradual decrease of respiratory rate and later by the onset of a slow gasping which gradually diminished in amplitude and frequency, (3) on compression, a slight increase in EMG amplitude maintained until gasping commenced, and the accompaniment of each gasp by large and prolonged bursts of EMG activity, and (4) progressive decrease in heart rate throughout each experiment.

The sequence of events is illustrated by sections of the tracings obtained in a typical experiment. Figure 1 shows responses of a rat anesthetized with urethane (1.2 gm/kg body weight) and compressed to 7 ata in oxygen. Control responses are shown in Figure 1A. Immediately following compression, a slight rise in amplitude

and decrease in frequency of respirations occurred, accompanied by a slight rise in amplitude of the EMG. Within 27 min (Figure 1C), spikes appeared on the EEG. Respiration changed dramatically 3–5 min later, with the onset of gasping and prolonged bursts of high amplitude potentials appearing on the EMG (Figure 1D). In the period from 27 to 32 min after compression, EEG activity decreased, and it continued to decrease further over the next 30 min. Figure 1E shows the final signs of EEG activity recorded in the experiment; 60 min after compression, gas flux in the trachea had decreased, but the EMG showed a continued electrical activity of considerable magnitude. During the next 6 min, respiration virtually ceased (Figure 1F) in spite of continued bioelectric activity from the diaphragm, suggesting that passage of gas to the lungs was impeded by broncho-alveolar changes such as spasm, collapse, or consolidation. At necropsy the lungs of this animal were grossly congested, liverlike in appearance, and weighed 1.8 gm (normal lung weight for the experimental animals used being approximately 0.9 gm[4]). Progressive bradycardia, as described by Whitehorn and Bean[5] and

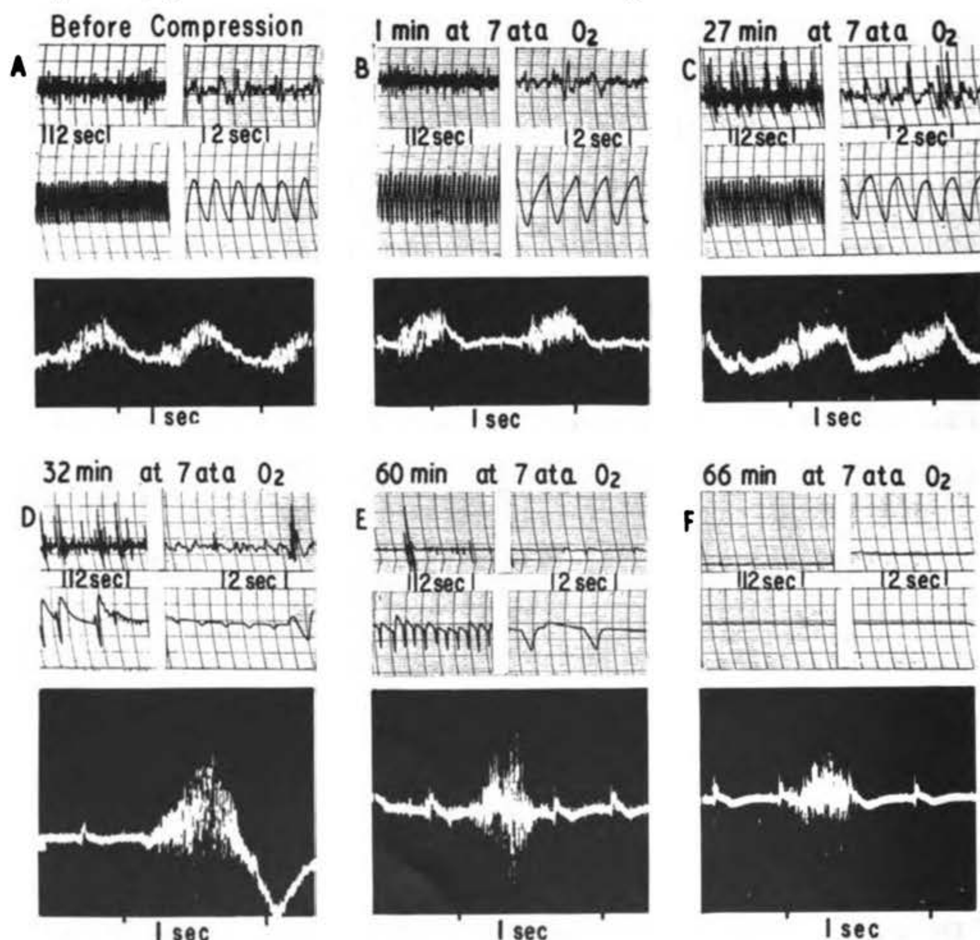

FIGURE 1. Sections of the tracings obtained from a urethane-anesthetized rat before and during exposure to 7 ata of oxygen. The top tracing in each block shows the EEG, the middle tracing the respirations, and the lower tracing the EMG. Note the large increases in EMG amplitude in sections D and E, although respiratory air movement has decreased.

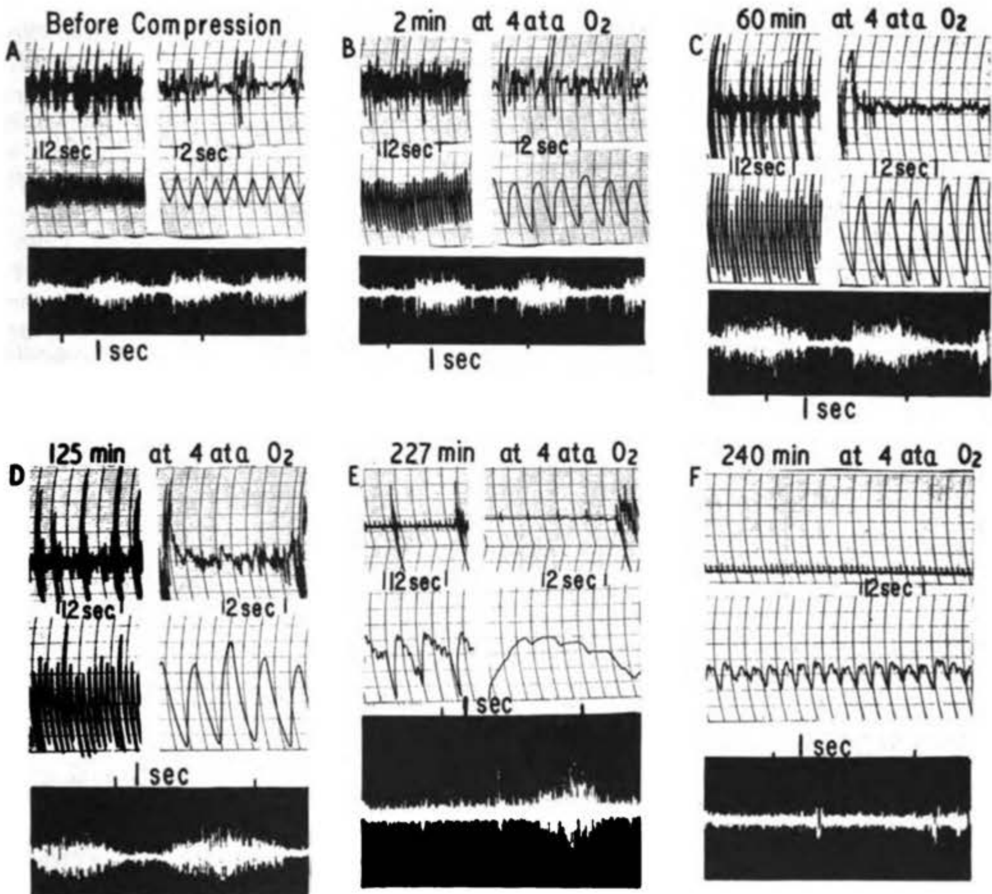

FIGURE 2. Selected portions of the recordings taken from a urethane-anesthetized rat before and during exposure to oxygen at 4 ata. Top tracings in each block show the EEG, middle sections show the respirations recorded from the tracheal thermistor, and the lower section shows the EMG. The ECG can be clearly seen on the myogram record. Recordings at two different time scales are presented for the EEG and respirations.

Taylor[6], occurred throughout the exposure to OHP (Figure 1), but the heart continued to beat for 10 min after all the evidence of respiratory activity had disappeared.

Similar results were found for all other rats compressed to 5 or 7 ata in oxygen. At 4 ata, two of four rats died with only partial lung damage, and in these two cases, very large bursts of activity failed to appear in the EMG, which showed only progressively reduced activity consistent with diminution of respiratory excursions. (Records from one such rat compressed to 4 ata are shown in Figure 2.) As before, EEG spikes appeared before respiration showed marked change (Figure 2C, 2D). At 227 min, respirations became deep and slow and EMG activity also increased. On the other hand, by this time EEG activity had decreased except for a continuation of some spike activity (Figure 2E). Toward termination of this experiment (at 240 min, Figure 1F), the EEG signal had disappeared (only the ECG is seen on the tracing), and respiratory amplitude had also decreased. The EMG had also decreased in amplitude and continued to decline at equal pace with progressive respiratory failure, respiration ceasing 24

min later. At necropsy the lungs of this rat showed partial consolidation and damage, weighing 1.2 gm. Slowing of heart rate occurred during this experiment, but slow beating continued for an hour after breathing ceased. Apparently, in such partly damaged lungs, sufficient oxygen remains in patent alveoli and dissolved in exudates to maintain cardiac action for considerable periods during exposure to OHP, provided the pulmonary circulation is sufficiently intact for gas transport to take place.

Similar experiments were performed in anesthetized rats to examine the effects of adding carbon dioxide to OHP on the physiologic parameters recorded for the previous experiment. Two percent CO_2+ 98% O_2 compressed to 4 or 7 ata and 5% CO_2+95% O_2 compressed to 4 ata were used. At 4 ata, 2% CO_2+98% O_2 produced EEG spikes in two of four rats, the remaining two animals showing no spike activity. Depressions in amplitude of the EEG, without spikes, occurred immediately on compression with 2% CO_2+ 98% O_2 to 7 ata and with 5% CO_2+ 95% O_2 to 4 ata and also at an early stage in the experiments with 2% CO_2+98% O_2 at 4 ata. The depression progressed until EEG activity disappeared, and the time required for this was relatively shorter than in rats similarly compressed in oxygen alone; such disappearance of EEG activity always occurred before respiration ceased.

A major difference in response was also seen in the EMG pattern. Immediately upon compression in the CO_2+O_2 gas mixtures, respiratory amplitude increased by threefold to fourfold, the respiratory rate decreasing slightly while the EMG amplitude approximately doubled. Bradycardia accompanied these changes. Compared to animals compressed in oxygen alone, the animals compressed in the CO_2+O_2 mixture usually showed little further change in the EMG, until terminal depression of both respiration and EMG activity took place. Occasionally a short transition period intervened, where moderately increased EMG amplitude was recorded. Regardless of the latter, however, in the terminal phase respiratory failure was accompanied by decrease in EMG activity. In many of these rats compressed in CO_2+O_2 gas mixtures, lung damage (based on macroscopic evidence at necropsy) was absent or slight, with patent alveoli. In rats which showed some increase in EMG activity, lung damage appeared significantly more marked.

Tracings from a rat compressed to 7 ata in 2% CO_2+98% O_2 are shown in Figure 3. EEG amplitude decreased while depth of respirations and EMG amplitude rose considerably (Figure 3B), presumably due largely to stimulation by CO_2; 20 min after compression (Figure 3C) a further depression of EEG activity took place, with slowing of respiration. After 8 min more, EEG activity had almost dis-

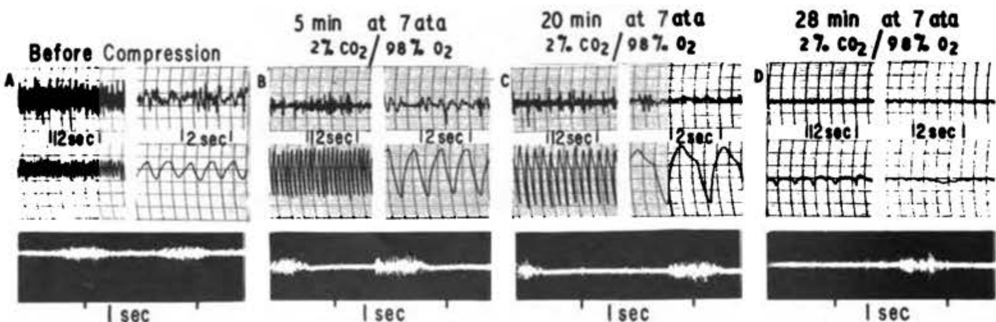

FIGURE 3. Records from a urethane-anesthetized rat before and during compression to 7 ata in 2% CO_2+98% O_2. The top tracing in each block shows the EEG, the middle tracing the respirations, and the lower tracing the EMG.

appeared, respirations were very shallow, and only small EMG potentials were recorded (Figure 3D). Over the next 7 min, respirations and EMG activity diminished further to eventual disappearance. The heart continued to beat for 45 min longer. At necropsy, the lungs showed slight reddening and their weight was little in excess of normal values.

Discussion

These data show that in most anesthetized rats exposed to OHP until cessation of breathing, motor impulses continued to be transmitted down the respiratory pathway from the respiratory center to the diaphragm, but pulmonary damage was sufficiently severe to prevent significant gas displacement. However, in most anesthetized rats compressed in oxygen supplemented with high concentrations of CO_2, a block in the respiratory pathway appeared to occur before severe lung damage and alveolar consolidation developed.

Since large increases in EMG amplitude appeared to correlate well with the degree of lung damage, this point was tested further by compressing four rats in oxygen (without added CO_2) to 5 ata. Five minutes after the high-amplitude EMG change was registered, the rats were decompressed at a rate of 1 ata per minute and the lungs were immediately removed and examined. All four rats showed considerable lung damage both macroscopically and microscopically, similar to that previously described,[4] with hemorrhagic exudates and edematous changes. The lungs weighed 1.4–2.0 gm. Three additional animals were similarly treated but allowed to remain in air after decompression. In these animals, breathing ceased 1–2 hours after decompression and the lungs showed similar damage at necropsy. In this latter group, the EMG showed high amplitude activity for 15–45 min after decompression, succeeded by a gradual decline in activity. In comparison, rats that had been pressurized in oxygen for time periods insufficient to cause increase in EMG amplitude showed little lung damage when sacrificed and examined after decompression.

Anesthesia has been shown to modify central nervous system and pulmonary damage due to high pressure oxygen [3,7–9] and would also contribute to the results obtained in these experiments. All the rats were anesthetized with urethane and survived considerably longer than similarly pressurized unanesthetized animals, the latter all showing very severe lung damage at necropsy when pressurized in oxygen, with or without the addition of CO_2.

The manifestation and severity of convulsions in unanesthetized rats or the occurrence of EEG spikes in anesthetized animals did not affect the eventual appearance of pulmonary damage. Also, changes in the EEG correlated poorly with survival times and with the onset of EMG changes.

These results and those reported previously[10] do not support the suggestion that pulmonary damage is primarily dependent on neurogenic factors.[8] Nevertheless, anesthetic agents, including urethane, are potent in protecting against pulmonary damage, as shown previously.[3, 8]

Indeed, the data suggest that in acute oxygen poisoning a "race for death" occurs between primary organic lung damage and neurologic damage causing a block in the respiratory pathway, with death from pulmonary damage predominating in the unanesthetized rat. In the anesthetized rat, however, a preferential protection of lung tissues sometimes allows central nervous system effects to predominate and cause a progressive respiratory depression through neuronal and motor failure. The addition of CO_2 to OHP exposures has been shown to potentiate central nervous system damage in both unanesthetized and anesthetized rats.[11] The present results in anesthetized rats further support this previous result in that neurogenic respiratory motor fail-

ure appears to predominate under such conditions of hypercapnia.

The location of this neurogenic block was not ascertained in the present experiments in which the diaphragmatic EMG was monitored. Some further preliminary experiments have been performed, however, in which the thoracic phrenic nerve was stimulated to determine the threshold for contraction of the diaphragm. Such thresholds were measured in anesthetized control rats and in rats decompressed from $5\% CO_2 + 95\% O_2$ at 4 ata immediately after cessation of respiration. The thresholds registered were significantly different: 129 ± 12 mv for control animals and 193 ± 12 mv for treated rats. This preliminary result may indicate that inhibition of excitability or conduction in the phrenic nerve, or inhibition of neuromuscular transmission plays a part in the respiratory depression. Direct stimulation of the diaphragm muscle in curarized artificially respired rats may also provide information in this respect but has not yet been attempted.

SUMMARY

1. In rats exposed to high pressure oxygen, cerebral cortical damage registered by the EEG appeared much earlier than damage to medullary centers.

2. In rats compressed in oxygen without added CO_2, the respiratory pathway usually remained intact and active at a time when breathing stopped, the latter appearing to be due predominantly to mechanical factors resulting from lung damage and impeding the excursions of gas in the broncho-alveolar tract during respiration.

3. In anesthetized rats exposed to hyperbaric oxygen supplemented by raised CO_2 concentrations, respiratory depression and cessation often occurred before the lung tissue was significantly damaged and such cessation appeared primarily due to central nervous system damage predominating and causing a depression of centrifugal impulses from medullary centers. However, such respiratory pathway block may also have been partly due to changes in the peripheral neuromuscular structures and their connections.

4. General anesthesia preferentially protected rats against pulmonary damage and may have allowed central nervous system damage to predominate and cause changes in respiration which dominate the clinical picture.

5. Central nervous system effects and lung damage both contribute in causing respiratory failure in rats exposed to OHP, but the two effects appear to be at least partly independent.

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DISCUSSION

Dr. G. G. Nahas (*New York, N. Y.*): With 2% carbon dioxide at 7 atmospheres, the partial pressure of the pCO_2 in the expired air is about 100 mm Hg, and pH is very low. This, in turn, should produce sympathoadrenal stimulation and catecholamine output. I wonder to what extent this stimulation can account for the difference in your two groups.

Dr. Jamieson: I do not know the mechanism of the difference between the groups, so I cannot answer your question fully. Our own studies and much of Dr. Bean's work do indicate this sympathetic hormone release seems to occur with oxygen anyway. How much more occurs with carbon dioxide plus high pressure oxygen I do not know. Perhaps you have some comments on this, Dr. Bean.

Dr. J. W. Bean, *Session Chairman* (*Ann Arbor, Mich.*): I would like to ask you, Dr. Jamieson, whether you tried different concentrations of carbon dioxide.

Dr. Jamieson: No, but it certainly would be worth doing, particularly since we can still get such respiratory stimulation even at 7 atmospheres of oxygen with carbon dioxide. It would be helpful to see just where you can stop this reaction and how much carbon dioxide you need to get respiratory stimulation at different oxygen pressures—but I am sure Dr. Lambertsen's group will be doing that sort of work.

Dr. Bean: I am interested because we have experimented on unanesthetized rats and found that carbon dioxide, up to about 2%, increases the lung damage, but with an increase equivalent to about 300 mm partial pressure, you get no lung damage. The question is whether this is a depression of the CNS because of the excessive carbon dioxide which approximates an anesthetized state; this, of course, does protect against convulsions and the lung damage. I am not sure whether Dr. Jamieson would agree, but there is probably a sympathetic involvement associated with convulsions—not necessarily by direct sympathetic action on the lungs to give local vascular changes and neurogenic pulmonary edema, but possibly by its influence on cardiovascular hemodynamics. You cannot deny, when you look at these lungs, that a circulatory response is causally involved in some of this lung damage rather than simply a toxic effect of oxygen directly either on the lung tissue itself or on the CNS.

Dr. Jamieson: I agree. The reason for saying that pulmonary damage and CNS effects are partly independent can be seen in the example of anesthesia. A group of anesthetized animals after 1 hour, for example, have neither convulsed nor developed lung damage—but leave them for 2 hours, and they all die of lung damage. So, you have protected them by prolonging the time for development of lung damage, but you have not stopped the lung damage by stopping the convulsions, and this is also true of the carbon dioxide. However, there may also be partial linking of central and pulmonary effects.

Dr. Bean: It is true that you cannot separate them entirely. The first effects are really

not very evident sometimes, but they are there.

DR. D. G. McDOWALL (*Glasgow, Scotland*): I have anesthetized dogs at 2 atmospheres for 11 hours, and these dogs were protected against pulmonary oxygen damage the same way as your anesthetized rats. However, the dogs that were breathing spontaneously developed respiratory arrhythmia going on to respiratory arrest, and when artificial ventilation of these dogs was done with air and they were decompressed, spontaneous regular respiration returned. I wonder if you have any experience on the reversibility of this process.

DR. JAMIESON: No. Artificial ventilation is something we have planned to do for some time but have not gotten around to yet. I would very much like to look at this.

DR. E. L. NAGEL (*Miami, Fla.*): Was there an effort to keep the alveolar ventilation comparable between the two groups of animals?

DR. JAMIESON: No, none at all.

The Independent Effects of Restraint and Ammonium Salts on Susceptibility of Mice to Oxygen Toxicity .

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In oxygen toxicity experiments on mice, some type of cage is usually used to permit simultaneous observation of many separate animals. This involves restraint of the animal, which is a stressor [1] and results in stimulation of the sympathetic nervous system. Physiologic responses of restrained animals to experimental procedures may be drastically altered from responses of the same animals when they are unrestrained.[2,3] The study of this factor, therefore, is a prerequisite for defining standard environmental conditions for oxygen toxicity experimentation.

Acidosis is known to decrease the incidence of epileptogenic seizures in man.[4] Studying the effects of acidifying agents under hyperbaric oxygen conditions may provide insight into the biochemical mechanism of oxygen toxicity. Ammonium chloride was selected as the principal drug for our studies because: (1) it is an excellent acidifying agent, and (2) cerebral ammonia detoxification mechanisms may sufficiently modify central nervous system (CNS) biochemistry to result in an alteration of the animals' responses to the CNS manifestations of oxygen toxicity. (The term "ammonia," as used herein, signifies the sum of the ammonium ion and the free base.)

METHODS

Restraint of male Swiss mice (ICR strain) weighing 20–35 gm was accomplished by placing the animals in individual compartments of a rectangular plexiglass holding chamber containing enough holes to permit gas circulation. The space within the compartments was varied with plexiglass inserts, also containing holes for gas circulation.

In separate experiments, drugs were administered intraperitoneally, the animals were immediately placed in individual compartments of a rectangular plexiglass chamber of which they occupied no more than 10% in volume, and a 10-min absorption period was permitted before pressurization. Another 5 min of absorption was allowed in a small-animal hyperbaric chamber (Bethlehem) while it was being flushed with 100% oxygen (8

liters/min) to denitrogenate both the chamber and the animals.

Compression to 5 ata was accomplished at a uniform rate of 1.2 ata/min, the temperature change from adiabatic compression being less than 3°C. During exposure to 5 ata of oxygen, the chamber was flushed at a rate of 1 liter/min to prevent carbon dioxide accumulation. Animals were observed continually during the 40-min exposure. Onset of toxicity was recorded as the time of the first detectable sign of seizure activity, as measured from the time full pressure was attained.[5] Companion experiments were run simultaneously on unpressurized animals outside the chamber to determine possible direct effects of the test substances. All experiments were paired so that control and experimental animals were observed simultaneously in the chamber. Animals were decompressed at a constant rate of 0.6 ata/min.

Results

Effect of Restraint

In four separate series of experiments (Table 1), marked restraint decreased the mean preconvulsive latency period by approximately 50%. The restrained animals occupied approximately 40% of the cage volume, whereas the controls occupied approximately 10%.

Effect of Ammonium Salts

NH_4Cl up to 350 mg/kg did not significantly alter the susceptibility of mice to oxygen-induced convulsions (Figure 1). The degree of protection against convulsions was highly significant with NH_4Cl and $(NH_4)_2SO_4$ above 400 mg/kg. Since the question arose whether the protection against oxygen-induced convulsions was due to a possible acidosis or to the ammonia,[2] included in the experiments was a series of animals given injections of NH_4Cl neutralized to pH 7.2 with NaOH and exposed to OHP simultaneously with the NaCl-treated and unneutralized NH_4Cl-treated animals. The degree of protection from oxygen toxicity with unneutralized NH_4Cl doses above 400 mg/kg was highly significant, as was that which occurred with the neutralized NH_4Cl-treated animals. Although there was no significant difference in the degrees of protection between unneutralized and neutralized NH_4Cl in the same dose range, animals treated with neutralized NH_4Cl tended to convulse sooner than the corresponding unneutralized NH_4Cl-treated animals.

The data also revealed that high concentrations of NaCl had no significant

TABLE 1. Effect of Restraint on Susceptibility of Mice to Oxygen Toxicity[a]

Expt. no.	Treatment	Cage area (cm^2)	Cage vol. (cm^3)	Av. mouse wt. (gm)	Av. mouse vol. (cm^3)	No. mice	Convulsion time (sec)	Probability
1	Restrained	15.7	79.2	29.6	33.2	18	519 ± 249	<0.001
	Control	41	266	28.5	32.3	16	1174 ± 612	
2	Restrained	11.3	73.5	26.7	30	4	320 ± 15	<0.05
	Control	40–87	266–568	27.3	30	4	555 ± 127	
3	Restrained	11.3	73.5	26.5	29.5	8	160 ± 59	<0.005
	Control	40–87	266–568	25.6	29.5	8	360 ± 105	
4	Restrained	11.3	73.5	24.2	27.5	4	187 ± 3	<0.01
	Control	40–87	266–568	27.5	32	4	390 ± 65	

[a] Exposure conditions: 5 ata of oxygen.

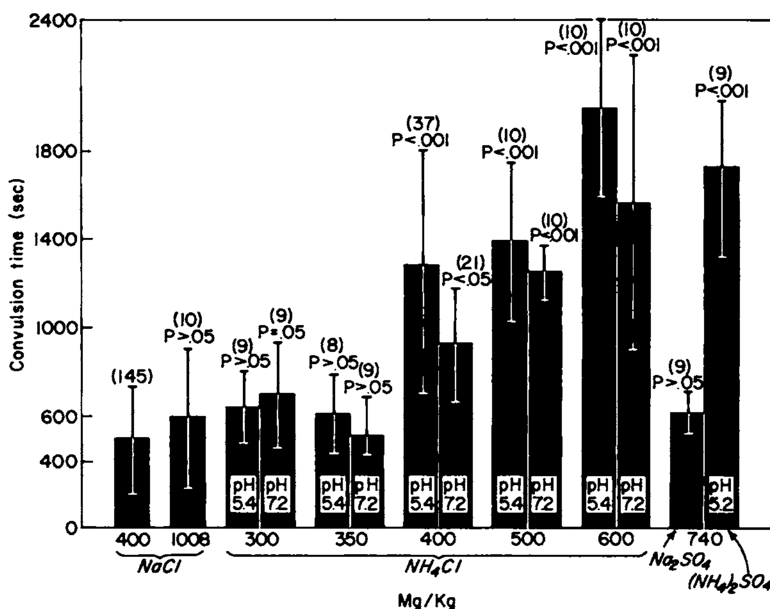

FIGURE 1. Effect of ammonium salts on the susceptibility of mice to oxygen toxicity, under exposure conditions of 5 ata of oxygen. Numbers in parentheses represent numbers of animals used. Vertical line in center of each bar represents one standard deviation from the mean. *P* represents significance from the control. Na_2SO_4 and $(NH_4)_2SO_4$ were equimolar in concentration. The 740-mg/kg dose of $(NH_4)_2SO_4$ and the 600-mg/kg dose of NH_4Cl contained the same concentration of ammonia.

physiologic effects on the CNS symptoms of oxygen toxicity. The concentration of chloride in the high-NaCl test solution was equivalent to the chloride concentration given to mice in a 600 mg/kg dose of NH_4Cl. Thus, neither the sodium nor the chloride ions exerted any measurable effect on the susceptibility of mice to oxygen poisoning. The large number of animals in the control group represent the pooled controls from all experiments.

The above experiments, however, did not eliminate the possibility that a protective effect may have resulted from acidosis associated with unneutralized NH_4Cl or $(NH_4)_2SO_4$ administration. To ascertain whether the hydrogen ion has a beneficial effect in protecting against oxygen toxicity and simultaneously to eliminate the possibility of ammonium ion effects, HCl was administered to the mice in molar concentrations equal to the molar concentration of 400 mg/kg NH_4Cl. The data indicated that treatment with HCl gave significant protection against oxygen-induced convulsions (Figure 2). Unneutralized NH_4Cl gave a significantly greater degree of protection than HCl.

In contrast to their protective effects on the CNS manifiestations of oxygen toxicity, neither neutralized or unneutralized NH_4Cl nor HCl displayed any protective effect against the pulmonary aspects of oxygen poisoning (Figure 3). These substances had no appreciable effect on the ratio of lung weight to body weight of unexposed animals. It should be noted that all oxygen-exposed animals had significantly higher mean lung-weight:body-weight ratios than exposed animals. These data indicate a direct effect of oxygen on the lungs.

DISCUSSION

The data on restraint indicate that environmental conditions may markedly

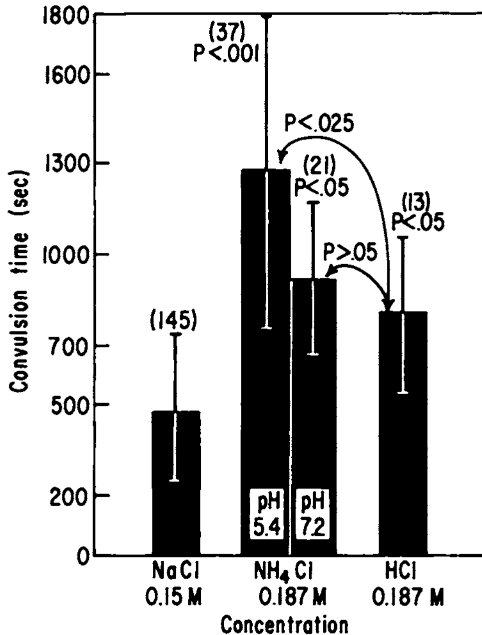

FIGURE 2. Comparison of NH_4Cl and HCl on the susceptibility of mice to oxygen toxicity, under exposure conditions of 5 ata of oxygen. Numbers in parentheses represent total number of animals. Vertical line in center of each bar is the standard deviation. *P* values represent significance from control unless otherwise indicated.

alter physiologic responsiveness of animals and that caution should be exercised in the use of restraint for convenience in OHP experimentation. In these experiments, the decrease in the preconvulsive latency period was probably due to stimulation of the sympathetic nervous system.[8] Bean[6] reported that epinephrine increased the sensitivity of animals to oxygen toxicity. We suggest that careful consideration be given to environmental conditions under which OHP experiments are performed. Additional significance of these data may become evident when the results obtained by different investigators are compared.

The drug data presented herein suggest a specific protective effect for ammonia against the CNS manifestations of oxygen toxicity. This conclusion is based on four observations: (1) at equimolar concentrations, unneutralized NH_4Cl gave significantly more protection than HCl, (2) NH_4Cl neutralized to pH 7.2 in order to preclude acidotic effects also afforded highly significant protection, (3) the protective effect was not due to the chloride anions, since NaCl containing a chloride concentration equivalent to that in a 600-mg/kg dose of NH_4Cl exerted no measurable protective effects, and (4) $(NH_4)_2SO_4$ also gave significant protection against oxygen toxicity.

It is interesting that ammonia, which

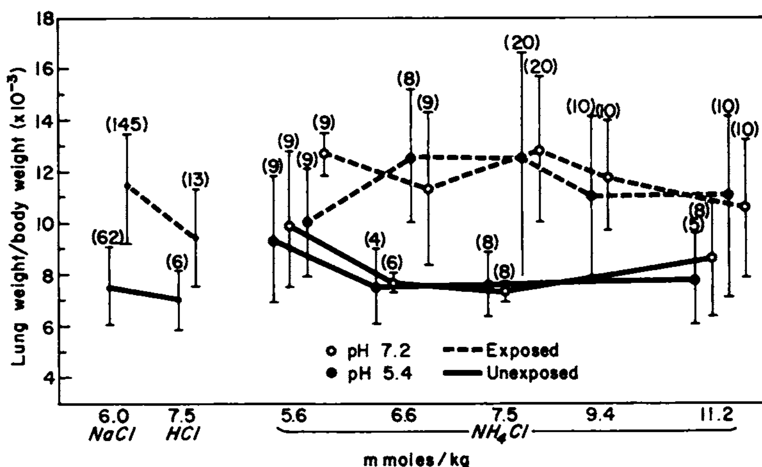

FIGURE 3. Effect of ammonium salts, HCl, and NaCl on the lung-weight:body-weight ratio of mice exposed to 5 ata of oxygen.

in high concentrations is a convulsant,[7] failed to potentiate the oxygen and thereby decrease the preconvulsive latency time. The absence of potentiation may be due to differences in the site of action of these two substances, however. Ammonia convulsions are thought to originate in the spinal cord,[8] whereas oxygen is thought to exert its effects primarily in the brain.

Ammonia-induced acute pulmonary edema, as reported by Koenig and Koenig,[9] was observed only rarely because toxic doses of NH_4Cl were not used in these experiments. In view of the work of these investigators, it is noteworthy that there was no evidence that oxygen and NH_4Cl potentiated each other to produce a greater degree of lung damage than would have been produced by either agent alone.

The protection against oxygen toxicity by acidifying agents may illuminate the mechanism whereby starvation increases resistance to oxygen toxicity.[10,11] Since starvation also results in metabolic acidosis, and our data indicate that acidifying substances increase resistance to oxygen toxicity, one might postulate that: starvation→acidosis→increased resistance to OHP. Of course, there is the second possibility that starvation-induced metabolic changes occur that also increase resistance to oxygen convulsions.

Several mechanisms could explain the data presented in this paper. The first is based on physiologic effects resulting from the acidifying agents; the rest have a more biochemical basis, involving ammonia metabolism.

NH_4Cl- and HCl-treated animals showed a marked hyperpnea before pressurization. In addition, the NH_4Cl-treated animals had a conspicuous behavioral depression. If the observed hyperpnea resulted in hyperventilation (reliable blood pH and gas measurements on unanesthetized mice were not obtained), then the concomitant elimination of carbon dioxide would result in a cerebral vasoconstriction and thereby decrease cerebral blood flow, with a consequent decrease in mean cerebral pO_2.[12] Animals treated with neutralized NH_4Cl also exhibited increased ventilatory responses, but these responses appeared less marked than those seen in animals treated with unneutralized NH_4Cl or HCl. This observation implies that there may be a direct respiratory-stimulating effect of ammonia. Brassfield *et al.*[13] and Koenig and Koenig[9] also reported respiratory-stimulating effects of ammonia.

The observation that unneutralized NH_4Cl gave more protection than equimolar concentrations of HCl, coupled with the observation that neutralized NH_4Cl also produced marked protective effects and that both NH_4Cl solutions tended to depress animal behavior, implies that more may be involved in protecting mice against the CNS manifestations of oxygen toxicity than just hyperventilation. Cerebral ammonia detoxification mechanisms are important for the removal of endogenous and exogenous sources of ammonia. These detoxification reactions may provide clues to the molecular mechanism for the defense against oxygen-induced convulsions, as well as to the defense against convulsive disorders in general.

A key reaction in ammonia removal is the reductive amination of alpha-ketoglutarate (α-KGA) to glutamate. Glutamate can be readily decarboxylated[14-16] to form gamma aminobutyrate (GABA), which has been shown to protect against oxygen-induced convulsions.[17] Roberts *et al.*[14] have shown that the pH optimum of the glutamic decarboxylase is on the acid side; thus, an acidosis might be expected to favor the formation of GABA. Increased GABA could play an important role in the GABA metabolic shunt to bypass an oxygen-inhibited alpha-ketoglutaric dehydrogenase.

The reductive amination of α-KGA to glutamate results in the removal of α-KGA, thereby decreasing the amount of dicarboxylic acid available for functioning of the tricarboxylic acid (TCA) cycle. Any decreased functioning of the TCA

cycle may result in a protection against oxygen toxicity or may bring into play alternate metabolic pathways which may result in protection against oxygen toxicity.

The major mechanism for the fixation of free ammonia in the CNS is the ATP-dependent formation of glutamine from glutamate and ammonia.[18] Increases of glutamine concentration were found in rat, dog, and cat brains after ammonia infusion.[19] Glutamine formation involves the consumption of ATP, resulting in a decrease of the total available supply of ATP. This decrease in available ATP may be the explanation for the inhibition of various reactions, including synthesis of acetylcholine, and the inhibition of responses to electrical stimuli.[20-24]

The above three biochemical explanations for the protective effect of NH_4Cl against oxygen toxicity are based on the stimulation or activation of various enzyme systems. Another explanation for ammonia protection is based on enzyme inhibition. It is well known that ammonia production by nerve increases with increased neural activity. It is conceivable that exogenously supplied ammonia could depress biochemical reactions leading to ammonia formation and thereby depress nervous system activity. Such a mechanism implies that the addition of ammonia should induce a behavioral depression or a sleeplike state. Ammonia production is decreased during anesthesia and sleep. Indeed, ammonia-treated animals did reveal a marked behavioral depression. It is doubtful whether exogenously supplied ammonia could induce narcosis or sleep since higher concentrations of ammonia cause convulsions. It would be of interest to understand the biochemical relationship between ammonia-induced depression and ammonia-induced convulsions. The data presented herein and available information of the physiology and biochemistry of ammonia suggest that ammonia may be an important regulatory substance for nerve activity.

Although drug administration may alter overt responses to oxygen toxicity to give the impression of protection, there is no assurance of true protection on the cellular and subcellular levels of organization, as opposed to a masking effect.[25] Only a detailed study of structure and function will show us whether true cellular protection against oxygen toxicity occurs. Studies such as the one reported in this paper serve to delineate physiologic and biochemical systems that may be worthy of more intensive study.

ACKNOWLEDGMENT

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DISCUSSION

Dr. J. Sendroy (*Bethesda, Md.*): What were your criteria for establishing that you had acidosis?

Dr. Gottlieb: Unfortunately, we had no real criteria since we were unable to obtain reliable blood gas measurements on these animals. We were guided by the respiratory and behavioral responses of the animals to the test solutions, as well as by the fact that the solution of ammonium chloride had a pH of approximately 5.4, and that the conversion of ammonia to urea results in the production of hydrogen ions.

Dr. Sendroy: But in spite of what you said about the role of ammonia in the biochemical discussion, injected ammonia is cleared almost instantly as urea. The BUN goes up immediately, and therefore protects the organism from acidosis. Did you measure BUN?

Dr. Gottlieb: We did not measure BUN. I doubt if the ammonia is cleared instantly

as urea. This is supported by the observation that mice treated with 500 and 600 mg/kg ammonium chloride manifested a prolonged behavioral depression. In addition, I should like to point out that BUN does not protect against acidosis but against ammonia intoxication. The metabolism of ammonium chloride to urea results in the production of hydrogen ion and thus leads to an acidosis.

DR. SENDROY: How do you know that the amount of ammonium salts you gave did not act *per se* as a convulsant?

DR. GOTTLIEB: The only way we know that these concentrations were not in themselves convulsant was by observations made on control animals, identically treated, but kept outside the chamber. In addition, I should like to point out that the ammonia-treated animals had a longer preconvulsive latency than did the untreated animals. Thus, it would be unlikely that ammonia convulsions would detract from our conclusions.

UNIDENTIFIED SPEAKER: Was the temperature outside the same as the temperature within?

DR. GOTTLIEB: Yes, within 1 to 2 degrees. The temperature was approximately 23°C.

UNIDENTIFIED SPEAKER: It is known, of course, that oxygen toxicity follows the temperature very closely.

DR. GOTTLIEB: I am not sure how this relates to my work. As I understand it, oxygen toxicity follows body temperature and not the ambient temperature. I think that ambient temperature will affect the sensitivity of animals to oxygen toxicity only insofar as it affects the animals' ability to gain or lose heat. Since we were operating at essentially a constant temperature, I do not think that temperature affected our work at all.

UNIDENTIFIED SPEAKER: The temperature of the gas mixture you breathe is the most important factor.

DR. GOTTLIEB: I do not think it is. The effect of environmental temperature on oxygen toxicity has been studied by many people (Aerospace Med. 35:563, 1964, and Amer. J. Physiol. 206:49, 1964). It is my impression that when one works with an animal that loses heat primarily through the respiratory system, any change of ambient temperature that will result in prevention of heat loss will increase the animal's susceptibility to oxygen toxicity.

UNIDENTIFIED SPEAKER: Just recently, work has been done at the Broad Naval Physiological Establishment, in Portsmouth, England (unpublished data), that shows that the temperature of the inhaled gas may be quite critical.

DR. GOTTLIEB: This may be so. However, since my control and experimental animals were exposed simultaneously to oxygen in the same chamber, I do not see how this study, which I cannot critically evaluate, has any bearing on my results or conclusions.

DR. F. DICKENS (*London, England*): I was very interested in Dr. Gottlieb's biochemical observations, and, while it is difficult to explain the action of some of the compounds which proved effective, there is perhaps a little more direct evidence about the action of ammonium salts on the metabolism of cerebral cortex slices. My colleague, Dr. H. Weil-Macherbe (Biochem. J. 32:2257, 1938) has shown that ammonium chloride has peculiar actions on brain metabolism, in that concentrations of about 0.001 molar have effects similar to high potassium chloride concentrations. That is, the brain respiration is doubled, and at the same time there is a large outpouring of lactic acid. I think that possibly some of these effects might be related, that if they were actually due to this type of metabolic change, I would expect much lower concentrations of ammonium chloride than you used to be effective.

DR. GOTTLIEB: I can't answer that, Dr. Dickens. One thing we want to do is to measure the actual ammonia concentrations that reach the brains of these animals, which, I think, would give us a better idea. We are administering the ammonia on a milligram per kilogram basis, and it is, of course, distributed throughout the entire body, but how much of that actually gets into the brain we do not know.

DR. D. JAMIESON (*Melbourne, Australia*): Did you ever try THAM in your experiments? We found this agent to be very effective against post-OHP paralysis in rats, and Dr. Nahas has had considerable experience with this agent.

DR. G. G. NAHAS (*New York, N. Y.*): It has been shown by Dr. Gottlieb and by Dr. Bean that THAM confers a significant amount of protection against onset of convulsion in rodents, but how that observation could account for the present observation of Dr. Gottlieb, I do not know. I personally believe that the mechanisms would be entirely different.

DR. A. R. BEHNKE (*San Francisco, Calif.*): If lactic acid is infused in large amounts, one gets the same type of protective effect. We tried to separate acidosis associated with hypercarbia and acidosis induced by infusion of an organic acid that reduced the pH to 6.9. There was a protective effect associated with the infusion procedure, so we concluded that the effects which have been described by Dr. Bean were specific carbon dioxide effects and not dependent upon a lowering of pH *per se*.

DR. J. W. BEAN, *Session Chairman* (*Ann Arbor, Mich.*): I have always been interested in carbon dioxide and acidosis, and our earliest experiments showed the importance of these in oxygen toxicity. Dr. Behnke has also pointed this out, and so this question of reversal is of special interest to me. Could it be that you have depressed the centers to a point where you got an anesthetic effect from your procedure?

DR. GOTTLIEB: This is a possibility that we had thought of, but the animals that were treated with ammonium chloride were depressed. Whether this was anesthetic effect or not I don't know. Of course, the depression could be explained by the theory that if you increase the GABA concentration you should essentially have a depressant effect.

Acute Oxygen Toxicity as Influenced by Multiple Exposures to OHP and by Alterations of Lipolysis

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Since oxygen at high concentrations produces toxic manifestations in almost all species of animals and plants, a single mechanism of O_2 toxicity seems unlikely and O_2 in toxic concentration might be presumed to have multiple metabolic and functional effects.[1] To further test the hypothesis that O_2 toxicity has multiple causes, we designed two series of experiments—the first to determine whether repeated exposure to clinical ranges of OHP could produce residual effects similar to those following exposure to radiation, and the second to determine the effects of alterations in lipolysis on O_2 toxicity. These alterations were produced by starvation, which increases the release and turnover of free fatty acids (FFA),[2] and by treatment with Butoxamine (*N*-tertiary butylmethoxamine),[3] which inhibits lipolysis. In all these experiments, the index of O_2 toxicity was taken as the time required for the appearance of convulsions in mice.

Methods

Male Paris mice weighing 25–35 gm were exposed to O_2 at 3 ata in a small transparent Lucite pressure chamber described previously.[4] The floor of the chamber was covered with a CO_2 absorbent, Baralyme (barium hydroxide lime), and, before pressurization, the chamber was flushed with 100% O_2 for 5 min and then pressurized to 3 ata in 3–4 min. Gas flows were adjusted to maintain a constant pressure in the chamber while allowing some O_2 to continuously escape. The mice were constantly observed throughout the exposure to O_2, and the time of onset of any convulsions was noted.

Two exposure methods were used—intermittent and continuous. The mice subjected to intermittent exposures were pressurized at 3 ata for 15 min or at 4 ata for 5 min; they were decompressed over 3–4 min. They were then given a similar exposure after 1 hour, or at various intervals during the course of 1 week. The continuous-exposure groups were maintained in O_2 at 3 ata until all animals in the chamber had convulsed.

First Series: Residual Effects of Hyperbaric Oxygen

In the first group of experiments, 144 mice received intermittent exposures to

3 ata of O_2 for 5 days: 24 mice were exposed four times a day, 24 twice a day, 48 once a day, and 48 once every other day (three exposures). In the second group, 288 mice, divided into six subgroups, received an initial exposure to 3 ata of O_2, and each subgroup received a single additional exposure on one of the following 6 days. The delay between exposures in this second group thus varied between 1 and 6 days. In the third group of experiments, 144 mice received intermittent exposures to 4 ata of O_2 for 5 days: 24 mice were exposed four times a day, 48 twice a day, 24 once a day, and 48 once every other day (three exposures).

At the end of the procedure, the animals were sacrificed and a gross examination was made of the lungs. The testicles were removed and prepared for microscopic examination. Any mice that died during the experiments were autopsied and their lungs carefully examined.

Second Series: Alterations in Lipolysis

This series of experiments was performed on two groups of animals. One group was divided into mice fasted 24 hours, mice fasted 48 hours, and control mice fed *ad lib*. The fasted animals were weighed before starvation and again just before pressurization. Mice in the second group were given, by intraperitoneal injection, either 20 or 40 mg Butoxamine/kg body weight 1 hour before OHP exposure, or, in the case of controls, 0.5 ml of saline. No more than six animals were exposed to OHP at any one time: two animals served as controls, while the other two pairs either received the two dose levels of Butoxamine or were selected from the two different periods of starvation.

Two separate exposures to OHP at 3 ata were given to 24 animals pretreated with 40 mg of Butoxamine/kg body weight, 24 pretreated with 20 mg/kg, and 24 saline-injected mice which served as controls. Similar exposures were given to 100 mice fasted 24 hours, 100 mice fasted 48 hours, and 50 untreated control animals.

Another group of animals was given continuous exposure to OHP: 52 mice were pretreated with Butoxamine 40 mg/kg, 51 mice were given Butoxamine 20 mg/kg, and 53 saline-injected animals were used as controls. In further starvation experiments, continuous exposure to OHP was used on 49 mice starved 24 hours, 47 mice starved 48 hours, and 50 untreated mice. Another series of 50 mice were fasted 48 hours and then given Butoxamine 40 mg/kg before exposure.

Plasma FFA levels were determined by the method of Novak[5] in animals starved 24 and 48 hours, in those receiving Butoxamine 20 and 40 mg/kg intraperitoneally, and in an untreated control group. Six samples were taken from each of the above five groups of mice. In order to obtain the volume of plasma required for the analysis, two mice were decapitated and their blood pooled for each sample.

RESULTS

First Series: Residual Effects of Hyperbaric Oxygen

In the first group of experiments at 3 ata, none of the mice convulsed on first exposure. On the second exposure, 29–94% of the animals convulsed. The subgroup of mice subjected to four exposures per day did not have a higher percentage of convulsions (Table 1). Average time to onset of convulsions after full pressure was attained ranged from 4.2 to 10.8 min and could not be related to the number of exposures. The onset of convulsions following the initial pressurization also varied widely among the individual animals, ranging from 0.5 to 14.5 min.

Figure 1 summarizes results of the second group of experiments at 3 ata pressure in which six subgroups received one additional exposure, each at a different time interval. One mouse convulsed on initial pressurization and two suffered me-

TABLE 1. Incidence of Convulsions in Mice after Successive 15-Minute Exposures to OHP at 3 ata

No. exposures	No. mice	Incidence of convulsions (%) Day 1	Day 2	Day 3	Day 4	Day 5
4/day	24	0 83 88 83	96 46 42 58	100 75 79 100	100 75 79 70	100 88 67 70
2/day	24	0 29	100 83	100 100	83 88	96 92
1/day	48	0	56	72	73	66
1/alternate day	48	0	—	94	—	100

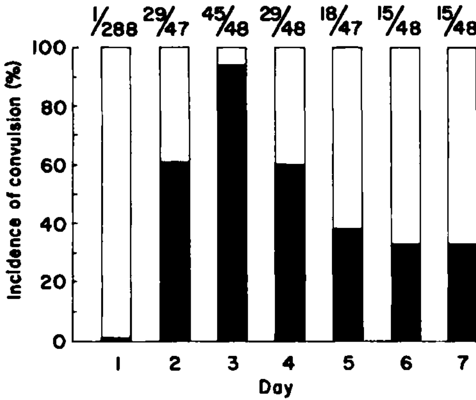

FIGURE 1. Incidence of convulsions in mice following two exposures to 3 ata of O_2 for 15 min (second exposure given 1 to 6 days after the first). (Reproduced from Proc. Soc. Exp. Biol. Med. 119:788, 1965.)

chanical trauma during the first exposure and died within 24 hours. On autopsy, these two animals had normal lungs. On second exposure, between 32% and 94% of the mice convulsed, the lowest percentages occurring during the exposure given 5 days after the first, and the highest incidence occurring when the second exposure was 2 days after the first.

Table 2 summarizes results obtained when mice were exposed to O_2 at 4 ata for 5 min. Of a total of 144 animals, two convulsed during the first pressurization (1%). None of the mice pressurized only once a day or on alternate days died. In the group receiving two exposures a day, one mouse died on the second day and another on the fourth day (4%). Two of 24 mice (8%) exposed four times a day died after the first day. On autopsy, all of these mice exhibited the classic "liver-like" lungs observed in O_2 toxicity. Convulsions occurred at any time from the beginning of full pressurization to the start of decompression. The average time of onset of convulsions ranged from 2.0 to 4.5 min.

Second Series: Alterations in Lipolysis

Table 3 summarizes the results obtained in mice receiving various pretreatments and two 15-min exposures to O_2 at 3 ata. Animals receiving intraperitoneal Butoxamine before pressurization convulsed in highly significant numbers on the first exposure. With the higher dose of Butox-

TABLE 2. Incidence of Convulsions in Mice after Successive 5-Minute Exposures to OHP at 4 ata

No. exposures	No. mice	Incidence of convulsions (%) Day 1	Day 2	Day 3	Day 4	Day 5
4/day	24	0 33 33 29	27 41 50 45	64 36 59 36	55 73 45 64	45 32 50 55
2/day	48	4 38	45 55	57 68	74 50	80 72
1/day	24	0	33	67	58	54
1/alternate day	48	0	—	63	—	69

TABLE 3. Incidence of Convulsions in Mice after Two Successive 15-Minute Exposures to OHP at 3 ata

Pretreatment	No. mice	Incidence of convulsions (%) Exposure 1	Exposure 2
None (control)	24	0	42
Butoxamine (20 mg/kg)	24	20	46
Butoxamine (40 mg/kg)	24	46	66
None (control)	50	0	42
Fasting (24 hours)	100	0	21
Fasting (48 hours)	100	0	13

amine (40 mg/kg), almost twice as many animals convulsed on the first exposure as those receiving 20 mg/kg of Butoxamine. None of the control animals convulsed during their first exposure to O_2. In contrast, none of the starved animals convulsed during their first exposure to OHP, and there was a marked decrease in the number of animals convulsing on the second exposure when compared with controls.

Figures 2, 3, and 4 summarize the results when mice were continuously exposed to O_2 at 3 ata until all animals in any one experiment convulsed. There was no significant difference between the controls for the Butoxamine experiment and

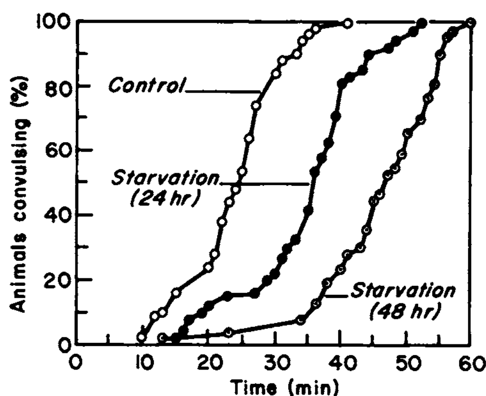

FIGURE 3. Cumulative convulsion times of fasted mice exposed to 3 ata of O_2.

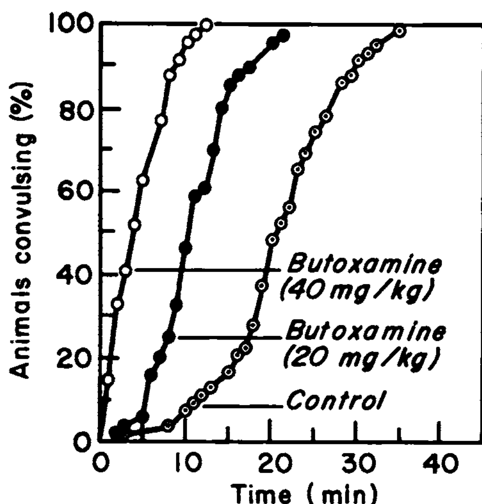

FIGURE 2. Cumulative convulsion times of mice exposed to 3 ata of O_2 following treatment with Butoxamine.

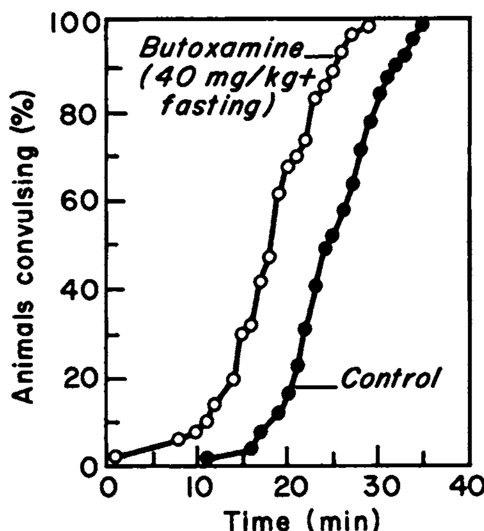

FIGURE 4. Cumulative convulsion times of mice fasted 48 hours, then given Butoxamine 40 mg/kg before exposure to 3 ata of O_2.

the controls for the fasted group. Increasing doses of Butoxamine shortened the latent period from time of pressurization until convulsions. By contrast, starvation significantly increased this latent period. Butoxamine given after a period of fasting abolished the increased latent period before the onset of convulsions regularly seen in fasted animals. Analysis of the FFA content from pretreated and control animals is presented in Table 4. The average weight loss was 13% in mice starved 24 hours and 23% in those starved 48 hours.

Discussion

First Series: Residual Effect of Hyperbaric Oxygen

In all the experiments on the second exposure to OHP, convulsions occurred in a significant number of mice although they had no apparent reaction to an earlier identical first exposure. This effect persisted 6 days after the initial pressurization. Aside from an increased mortality rate in the groups of mice exposed to 4 ata, there was no apparent pattern of cumulative toxic symptoms or of tolerance to OHP. Some of the animals did not convulse after the second exposure, indicating a wide range of individual tolerance to hyperbaric oxygen, a finding which is well documented. Administration of chlorpromazine (20 mg/kg intraperitoneally) to the mice before the first exposure did not alter the incidence of convulsions following a second exposure.

TABLE 4. Influence of Butoxamine and Fasting on Mean Serum Free Fatty Acids in Mice

Treatment	FFA (mEq/liter)
Butoxamine (40 mg/kg)	0.09
Butoxamine (20 mg/kg)	0.20
Control (untreated)	0.23
Fasting (24 hours)	0.69
Fasting (48 hours)	0.31

These observations confirm those of Almeida,[6] who reported in 1934 that rats exposed to O_2 at 6 ata until they convulsed would, in subsequent exposures, convulse within one-third to one-fourth of the initial time. Bean[7] reported that rats exposed to "subconvulsive periods" of hyperbaric oxygen two or three times daily would develop paralysis. Fenn[8] observed that daily exposures of the fruit fly to 1 ata of O_2 for 6 hours every day reduced its life span by 10–15%. This residual effect of high O_2 tension indicates that OHP profoundly alters the metabolism of the central nervous system of rodents. The mechanism of this phenomenon and the extent of its presence in man remain to be established.

Second Series: Alterations in Lipolysis

These experiments extend and confirm the results of those done by Almeida,[6] which simply indicated that fasting gave protection against the central nervous system symptoms of toxicity from OHP. His data, however, were difficult to evaluate.

Fasting has been reported to be associated with an increased turnover of free fatty acids, and this observation was confirmed in our experiments.[2] The highest FFA levels were observed in mice starved for 24 hours (Table 4). At 48 hours, the FFA declined, although still remaining above control values, and the greatest resistance against convulsions occurred in mice starved 48 hours, when fat depots were most significantly reduced.

Lipolysis associated with an increased turnover of FFA is a complex process which is dependent upon the activity of at least three enzymes: (1) adenylcyclase, which forms cyclic 3,5-adenosine monophosphate (AMP) and is stimulated by catecholamines, (2) phosphodiesterase, which inactivates cyclic 3,5-AMP, and (3) lipase, the activity of which depends upon the actual amount of cyclic 3,5-AMP present. Some of these enzymes

are also involved in carbohydrate metabolism. It is of interest to note that Butoxamine, which inhibits lipolysis and glycogenolysis[9] (probably as a result of inhibition of adenylcyclase), completely removes the protective effect of starvation on convulsions induced by OHP. This might indicate that OHP also has a depressant effect on the adenine nucleotides, AMP, ADP, and ATP,[1] which are also involved in lipid metabolism and acceleration of FFA turnover. In this case, one might expect that Butoxamine and OHP might have a synergistic effect—and the present experiments indicate that this is the case, since Butoxamine significantly accelerates the onset of convulsions.

All of these experiments suggest that the toxic effects of hyperbaric oxygen are associated with multiple and basic enzymatic and metabolic alterations, some of which may persist for as long as 6 days.

ACKNOWLEDGMENTS

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Butoxamine was kindly supplied by Dr. J. J. Burns, Burroughs Wellcome & Co., Inc., Tuckahoe, New York.

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DISCUSSION

DR. J. D. WOOD (*Toronto, Canada*): We did some experiments with rats and found no difference in susceptibility to oxygen convulsions during repeated exposures. Patel and Gowdey (Canad. J. Physiol. Pharmacol. 42:245, 1964) have done similar experiments and came to the same conclusion. However, on one occasion they did get increased susceptibility after the first exposure and, on examining the lungs, they found that there was some mild infection which they could pick up microscopically. I was wondering first whether you had done any similar studies on rats, and second, whether you had looked at the lungs of some of the mice before exposure to see if there was a mild infection.

DR. MATTEO: No, we have not done this work on rats, primarily because we needed

large numbers to get significant results, and mice were much more practical to use with our chamber. We did examine a number of untreated mice; an autopsy was routinely done on all mice pressurized and their lungs were examined. I did not mention this, but at 3 atmospheres with our intermittent 15-minute exposures, the lungs of none of these animals seemed to show any signs of oxygen toxicity. This is not to say that perhaps 5 to 10% would show the atelectasis that we all see in laboratory rodents, but it was a scattered patchy sort of atelectasis, one lobe that you see in a perfectly normal rodent. On the contrary, at 4 atmospheres with the intermittent exposures, perhaps 5 to 10% of these animals grossly showed diffuse pulmonary infiltration which was compatible with the change you see in hyperbaric oxygenation.

DR. E. NEPTUNE (*U. S. Navy*): Do infused free fatty acids or free fatty acid levels elevated by use of growth hormone have any effect in this sort of a situation?

DR. MATTEO: We have not infused free fatty acids. We have tried to approach this another way—we fed animals a pure-fat diet for several days. This appreciably raises the free fatty acid content and has absolutely no protective or detrimental effect on the animal. We feel that the level of free fatty acids *per se* is not responsible for the protection or sensitization of the animals, but that rather it reflects derangement of basic enzyme mechanisms caused by hyperbaric oxygenation.

DR. D. GILBERT (*Bethesda, Md.*): I would just like to mention that we used fasting some time ago (Amer. J. Physiol. 181:272, 1955) and noticed that with fasting prolonged over 72 hours, there was an extremely marked increase in protection in survival time of mice at 6 atmospheres of oxygen.

DR. MATTEO: I am sure that is true. We stopped at 48 hours because at 72 hours the mice began to die, and we did not feel that we could carry the experiment this far.

DR. J. W. BEAN, *Session Chairman (Ann Arbor, Mich.)*: I believe that this question of starvation was quite well documented by A. O. De Almeida (C. R. Soc. Biol. [Paris] 116:1225, 1934) and a few years later by A. J. Campbell (J. Physiol. 89:17P, 1937). To carry this somewhat further, what relationship does this have to cholesterol?

DR. MATTEO: I am sorry, but I could not even offer a guess at this.

Chemical Protection Against the Toxic Action of Hyperbaric Oxygen

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Several authors have suggested a similarity in the mode of action of ionizing radiation and hyperbaric oxygen. It is possible that both could act by causing the increased formation of oxidizing free radicals in the tissues of the body. This is consistent with the readily observed inactivation of SH-containing enzymes *in vitro* by hyperbaric oxygen. Several reports have appeared showing that substances known to exert a protective effect against ionizing radiation can, under some circumstances, reduce the symptoms of oxygen toxicity.[1]

We have studied a series of recently discovered radiation-protective agents to determine their effect on the time to death of mice exposed to hyperbaric oxygen. We have also studied the effectiveness of some oxygen-protective agents against radiation.

Methods

Fed female white mice (weighing about 20 gm) were exposed to oxygen at 45 psig in a standard Vickers baby chamber. During exposure, the mice were kept in a Lucite container consisting of individual compartments about 3 × 2 × 2 in., where they were clearly visible and identifiable at all times. The container was so arranged in the chamber that the oxygen flowed through each compartment, in through holes in the bottom, and out through a loosely fitting lid, thereby keeping the percentage of carbon dioxide in the gas breathed to a minimum. The oxygen flowed at a rate sufficient to give approximately 12–15 changes per hour.

At the start of each experiment, each animal was injected intraperitoneally with 0.5 ml of a solution of the substance under investigation. Control animals were injected with 0.5 ml of distilled water. The injections were given 10–25 min before the set pressure of 45 psig was reached. The time between reaching pressure and the cessation of visible respiration was recorded for each animal by visual observation.

Results

The average survival time for all control animals in this series (nearly 200) was 130 min. Table 1 shows the effect on average time to death of four radiation-protective agents (each tested on 24 mice). The only significant protective effect was obtained with aminoethyliso-

thiuronium bromide HBr at a dose of 4 mg/mouse. It was interesting that, at a dose of 8 mg/mouse, reported as the optimum for radiation protection, we observed death in a preliminary series of six animals at an average time of only 20 min. We failed to observe any significant protection with the other three compounds at the levels tested, although all are reportedly quite effective against radiation when used in this range of concentrations. Thus, our series offered little evidence to suggest that radiation-protective agents also protect against the toxic action of hyperbaric oxygen.

We then studied a number of other compounds which might be expected to exert some protective effect (Table 2). Thomas *et al.*[2] suggested that impaired oxidative ability of brain homogenates could be due to the destruction of thioctic or α-lipoic acid. However, injection of this compound appeared to exert no protective effect in our studies. The sodium salt of γ-hydroxybutyric acid is known to produce unconsciousness at doses in the range of 20–40 mg/mouse.[3] Doses this large were rapidly toxic in hyperbaric oxygen, even 15 mg/mouse giving an average time to death of only 70 min. γ-Aminobutyric acid, as already shown,[4] offers significant protection, but only at a high dosage, one which would be quite impracticable in man. Penicillamine was tried because of its action in complexing copper ions, and it appeared to increase the toxic action of hyperbaric oxygen slightly.

The most significant protective effect in these series was obtained with vitamin K_3 and vitamin K_1 (Table 3). Vitamin K_3 was used as the water-soluble sodium bisulfite addition compound of menaphthone and showed maximal protection at a dose of only 2 mg/mouse. Vitamin K_1, phytomenadione (an oil-soluble substance), was given by mouth in even smaller doses, and was still quite effective. Probably we have not yet used the most effective analogue of vitamin K or the most effective method of administration, and work is continuing on these aspects.

In order to determine the protective effect in another way, animals were injected with vitamin K_3 (distilled water in controls) and subjected to a near-lethal exposure to oxygen, after which the numbers of survivors were recorded (Table 4). Two groups, of 36 mice each, were exposed to oxygen at 45 psig for 120 min; mice in one group received 2.0 mg of vitamin K_3 in 0.5 ml of water by intraperitoneal injection, and the control mice received 0.5 ml of distilled water only. Mortality was much higher, both during decompression and over the next 15 days, in the control group than in the treated group.

The ultimate survival rate was nearly five times greater for the treated animals than for the controls, although the time to death was only doubled—reflecting one problem in this kind of work. In addition to oxygen toxicity, or perhaps as a contributing feature, marked diuresis occurs in animals exposed to hyperbaric conditions. Thus, in a separate project we noted that over a 150-min period, albino rats lost twice as much body weight in oxygen at 45 psig as animals in air at ambient pressure at the same gas flow rate. This weight loss represented the sum of urine excreted and the water and carbon dioxide expired, with almost all of the loss due to urine. For male rats, it amounted to nearly 15 gm lost per 250 gm body weight in oxygen but only 7 gm in air, and, for female rats, it amounted to 11.5 gm lost in oxygen compared to 5.25 gm in air. (All animals were fasted overnight.) Clearly, then, dehydration is going to affect the state of an animal kept for more than 4 hours in oxygen, and no protective substance will fully maintain its effect.

As a corollary to the determination of protection against oxygen, we also tested the effectiveness of vitamin K_3 and γ-aminobutyric acid as radiation-protective agents. Figure 1 shows the survival rate of white mice treated with 800 rads of gamma radiation approximately 20 min

TABLE 1. Effectiveness of Radiation-Protective Compounds Against Toxicity from Hyperbaric Oxygen [a]

Aminoethylisothiuronium bromide HBr		Guanylthiourea *p*-toluenesulfonate		3,5-Diaminothiadiazole		Mercaptoethylamine	
Dose (mg)	Time (min)	Dose (mg)	Time (min)	Dose (mg)	Time (min)	Dose (mg)	Time (min)
3	150	5	108	2	147	2	108
4	175	10	121	4	132	3	137
5	157	20	130	8	127	4	148
		30	77	10	126	5	127

[a] Each survival time shown above represents the average time for 24 mice exposed to oxygen at 45 psig.

TABLE 2. Protective Action of Four Compounds Against Toxicity from Hyperbaric Oxygen [a]

α-Lipoic acid		Na γ-hydroxybutyrate		γ-Aminobutyric acid		Penicillamine	
Dose (mg)	Time (min)	Dose (mg)	Time (min)	Dose (mg)	Time (min)	Dose (mg)	Time (min)
0.5	136	1	126	40	142	1	123
1	138	2	109	80	210	2	128
2	133	3	125	120	205	3	123
3	102	4	110	160	144	4	103

[a] Each survival time shown above represents the average for 24 mice exposed to oxygen at 45 psig.

TABLE 3. Protective Action of Vitamin K Analogues Against Toxicity from Hyperbaric Oxygen [a]

Menaphthone $NaHSO_3$ Vitamin K_3		Phytomenadione Vitamin K_1 (orally)	
Dose (mg)	Time (min)	Dose (mg)	Time (min)
0.5	194	0.2	178
1	220	0.4	181
2	260	0.6	139
4	184		

[a] Each survival time shown above represents the average for 24 mice exposed to oxygen at 45 psig.

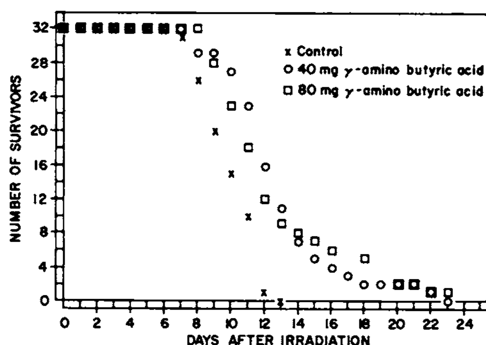

FIGURE 1. Mouse survival after 800 rads of gamma radiation. Control group received no pretreatment, while treated groups received 40 or 80 mg γ-aminobutyric acid approximately 20 min before irradiation.

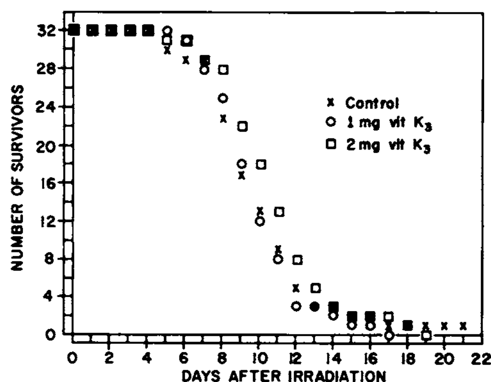

FIGURE 2. Mouse survival after 800 rads of gamma radiation. Control group received no pretreatment, while treated groups received 1 or 2 mg vitamin K_3 approximately 20 min before irradiation.

after injection with 40 or 80 mg of γ-aminobutyric acid. There was a slight protective effect, although the deaths among the control animals in this series occurred sooner than usually observed, tending to emphasize the protective effect perhaps more than is justified. When animals were treated with 1 or 2 mg of vitamin K_3 20 min before irradiation, no significant difference occurred between control and treated animals (Figure 2).

Vitamin K is believed to participate in the transfer of electrons from the substrates of Krebs cycle intermediates between the pyridine nucleotide coenzymes and the cytochromes and coenzymes Q.[5,6] Chance and Jamieson[7] have shown that the pyridine nucleotide system is inhibited by hyperbaric oxygen, and our work on the protective effect of vitamin K would seem to emphasize still further the possibility that this series of reactions may be the locus for one of the major biochemical effects of hyperbaric oxygen.

TABLE 4. Effect of Vitamin K_3 on Mouse Survival at 45 psig Oxygen

Group	No. mice	Survivors after: Decompression	5 Days	10 Days	15 Days
Control	36	16	10	7	5
2.0 mg K_3	36	29	25	24	24

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DISCUSSION

DR. D. JAMIESON (*Melbourne, Australia*): I have a comment about the site of action of coenzyme Q. Looking at Dr. Chance's rather complicated table again, it appears that he has coenzyme Q sitting in the reverse electron track, if perhaps a little tentatively. We did find in the last few days that Q changed and changed quite rapidly under hyperbaric conditions, at least in mitochondria. We have done only a few of these experiments, but Q is changing along with our DPNH being oxidized to DPN. There is much doubt about just where Q is and what it is doing, but, for the purpose of this discussion, it is quite possible that it is indeed in the reversed electron path.

DR. HORNE: Thank you, Dr. Jamieson. I was going by all I could find in the literature. I have no personal experience with Q other than this bit of work here.

UNIDENTIFIED SPEAKER: I would just like to confirm the result which was obtained here with gamma-hydroxybutyric acid. We have tried it, and it is more of a poison in high oxygen pressure experiments on rats. We have also repeated Dr. Wood's experiments as you have done with gamma-hydroxybutyric acid and we have found there is a protective action.

DR. P. V. VAN TASSEL (*U. S. Navy*): If I recall correctly, Dr. Gerschman (Proc. Soc. Exp. Biol. Med. 85:75, 1954) some years ago reported a protective effect of beta-mercaptoethylamine at 6 atmospheres of oxygen, using mice as you did. She showed something like a 70% increase in survival time. Do you know about this and whether it is due to a dosage difference or simply represents the effects of the different pressures?

DR. HORNE: Yes, as I recall, the effect of this substance was dependent both on the pressure and the drug concentration used, and it appeared to be quite variable. Perhaps Dr. Gilbert knows better about that.

DR. D. L. GILBERT (*Bethesda, Md.*): Yes, that is why I asked whether you had tried various pressures, because we did find that the effect of these substances was dependent upon the pressure (Amer. J. Physiol. 192: 563, 1958). Using survival times of mice as an index of oxygen toxicity, we found that as the oxygen pressure was decreased below 6 atmospheres, the protective action of beta-mercaptoethylamine (cysteamine) and glutathione decreased.

I would like to make one further com-

ment. This morning Dr. Dickens mentioned that cobalt was a very good protective agent. We found that cobalt did protect mice at the low oxygen pressure of 1 atmosphere. For paramecia, cobalt had a protective effect against oxygen toxicity at the high oxygen pressure of 9 atmospheres (Amer. J. Physiol. 192:572, 1958). Have you tried the influence of other pressures on the effect of these substances which you reported on today?

DR. HORNE: Our work has been limited to 45 psig.

Effects of Hyperbaric Oxygenation on Tissues in Organ Culture

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Techniques for organ culture were first developed in 1926 by Strangeways and Fell,[1] who cultured fragments of chick embryo organs in plasma clot. While this method provided new vistas for the embryologist, adult tissue stubbornly resisted life apart from the body. Parker [2] and others soon drew attention to the higher oxygen requirement of adult tissue, which limited the cultured specimens to a maximum diameter of 2 mm. Larger tissue fragments routinely showed anoxic central necrosis. To circumvent this problem, various attempts were made to culture adult organs at a high oxygen concentration. In 1948, Medawar [3] cultivated adult mammalian skin in a "rocker flask" with 70% oxygen. In 1952, Trowell [4] devised a method for culturing adult organ fragments on cotton wool in oxygen, and later he developed his ingenious Perspex chamber which uses circulating 95% O_2 + 5% CO_2.[5] More esoteric approaches included the utilization of oxygen produced by electrolysis from water (Petrovic and Heusner,[6] 1963).

In contrast to some of the successful attempts to sustain mature tissue *in vitro* by the use of increased oxygen, Rueckert and Mueller [7] found that 95% oxygen at ambient pressures caused inhibition and irreversible damage in HeLa cell cultures. Brosemer and Rutter [8] reported similar responses in AH (human sarcoma) cell cultures exposed to oxygen concentrations above 35% at ambient pressure. Fischer and Anderson [9] found damage to Rous sarcoma cells in hyperbarically oxygenated cultures, while normal fibroblasts tolerated this environment better. Heppleston and Simnet [10] reported that hyperbaric oxygenation had some cytotoxic effects on cultures of prostate, epididymis, and lung.

In the face of the above conflicting evidence, we undertook the current studies to ascertain whether increasing the pressure of a high oxygen concentration would prolong the *in vitro* growth and maintenance of fragments of adult tissue larger than 2 mm, by providing the culture with more oxygen in physical solution.

Methods

Canine and human organs were obtained aseptically from living donors and washed

* Markle Scholar in Academic Medicine.

in a balanced salt solution containing penicillin and streptomycin. The tissues were sliced 3–5 mm thick for culture.

Culture chambers consisted of Conway dishes with stainless-steel screens placed over the inner rings. Cultures were maintained on these screens on the surface of the media, and cell yield was harvested in the well below. The entire unit was placed in a 12-cm petri dish, and the excess space was filled with water-saturated cotton wool in order to minimize evaporation of the culture media. Tissue slices were turned (inverted) every 48 hours when the media were changed. Disposable 5-cm petri dishes were used for immersion cultures.

Eagle's solution containing glutamine, 10% calf serum, penicillin, and streptomycin was used as the culture medium. When Eagle's culture medium was pressurized with the standard gas mixture (95% O_2 + 5% CO_2) used in hyperoxic tissue culture, immediate severe acidosis occurred (Table 1). This finding can be accounted for by the fact that Eagle's solution is designed for use at a pCO_2 of 40 mm Hg. Reducing the percentage of carbon dioxide in the gas mixture to 2% (pCO_2 of 60 mm Hg at 45 psig) adequately corrected the acidosis (Table 2). The addition of 0.05 M tris buffer to the media helped to maintain an acceptable pH over the 48-hour culture period. Terminal pH ranged between 7.14 and 7.38 in 71 cultures.

An 8-ft^3 (30-in. diameter) cylindrical cast-iron tank, safety-coded for 75 psig, was used for our early OHP studies. The

TABLE 1. Effect of OHP (95% O_2+ 5% CO_2) on Acidity of Eagle's Solution

Atm	pH
1	7.30
2	6.95
3	6.76
4	6.65
5	6.54

TABLE 2. Effect of OHP (98% O_2+ 2% CO_2) on Acidity of Eagle's Solution

Atm	pH
1	7.55
2	7.43
3	7.32
4	7.20
5	6.95

chamber is fitted with three plexiglass ports for observation and four instrument-access plugs. A sealed heating element within the chamber is covered with a deflector; a fan, magnetically driven from outside the vessel, further insures a homogeneous temperature. The heater is regulated by a proportional-controlled thermostat to ± 1°C regardless of room temperature. For our experiments with this chamber, the culture dishes were first placed in polyethylene bags and the bags were filled with the gas mixture utilized for that experiment. The gas-filled bags were then placed in the chamber, which was pressurized with compressed air.

Later, to simplify operations, the large chamber was replaced by four smaller ones measuring 24×24×6 in. These are made of aluminum, with aluminum or 1-in. Lucite tops. They are coded for 75 psig and have four access plugs and a safety valve set at 60 psig. These chambers require no internal heat source, since they fit into standard bacteriologic incubators. They are gassed directly. A Clark oxygen electrode read through an Astrup meter and a special Beckman pressure-compensated pH electrode were tapped into the chambers for pO_2 and pH measurements.

All cultures were maintained at 37°C. The experimental cultures were pressurized in the chamber to 45 psig in 98% O_2 + 2% CO_2. Controls were run at ambient pressures in 95% O_2 + 5% CO_2 and also in room air. Cultures contaminated by bacteria or fungi were discarded.

The criteria employed to determine

viability depended on the individual tissues studied. The standard tissue culture techniques of eosin vital staining,[11] measurement of cell yield, determination of glucose consumption, histologic examination, and counting of mitoses were supplemented with autoradiography to measure the incorporation of tritiated thymidine (^{3}H-thymidine) and thus cell multiplication rates. The isotope was added to the media (8 μc/10 ml, activity 6.4 mc/mmole) for 3-hour pulses at various stages of culture. Both impressions and sections were studied with autoradiography by the methods of Craddock *et al.*[12]

Results

Tissue Survey

Ninety cultures (both human and canine) of liver, spleen, lymph node, pancreas, ureter, and skin were successfully cultured for periods of 10–14 days (Figure 1A). Mitotic counts and ^{3}H-thymidine incorporation were highest at 3–5 days (Figure 1B), dropping to less than 3% by 12 days. Pyknotic nuclei began to appear on the surface of these slices at 6 days (5–10%), and gradually extended into the core by 11–12 days. Control cultures at ambient pressure in 95% O_2 + 5% CO_2 or in room air consistently showed autolysis and central necrosis within 24–36 hours. The maximum and average mitotic rates observed at 3–4 days in three hyperbarically oxygenated cultures of each tissue are listed in Table 3.

In hyperbarically oxygenated cultures (both human and canine) of brain, lung, and thyroid tissue, no mitoses, ^{3}H-thymidine uptake, or glucose utilization could be demonstrated. Uniform cell death of neural and glial, alveolar, and acinar cells was seen. Vascular stroma and bronchial epithelium were preserved.

Cell Yield

In hyperbarically oxygenated cultures, canine lymph nodes consistently yielded $3.2–8.0 \times 10^6$ cells/gm/day for 7 days with 75–92% viability. Yields were higher and better maintained when slices were cultured on a screen at the air–fluid interface than when fully immersed in

TABLE 3. Mitotic Rates Observed at 3–4 Days in OHP Tissue Cultures [a]

Tissue	Mitotic rate	
	Maximum	Average
Liver	7%	3%
Spleen	14%	9%
Pancreas	3%	1%
Ureter	13%	9%
Skin	4%	2%

[a] Average of three cultures for each tissue.

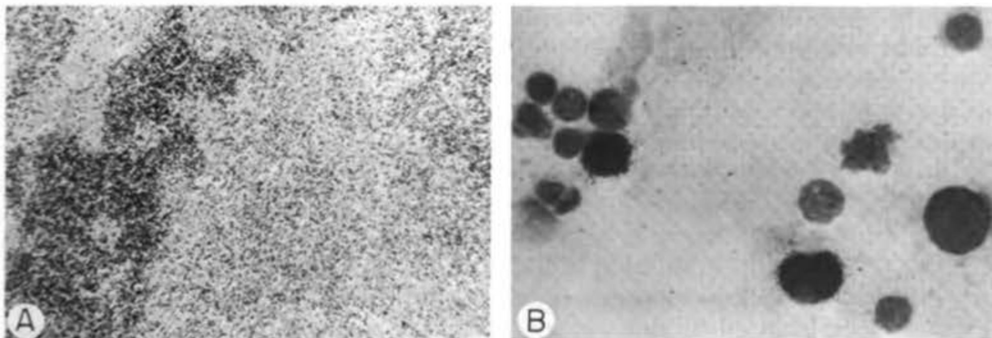

FIGURE 1. A, canine lymph node from a 5-day culture at 45 psig in 98% O_2 + 2% CO_2. B, autoradiograph of hyperbarically oxygenated canine spleen culture pulsed with ^{3}H-thymidine at 72 hours.

media (Table 4). Dog spleen slices cultured on screens tripled the original number of spleen cells in 10 days, with 83% viability. Addition of phytohemagglutinin M to the media doubled this yield further.

Compression and Decompression

Five-minute periods in the large chamber and 1-min periods in the small chambers were allowed for compression and decompression. When the gas was stored at room temperature (in G-tanks) ±5°C excursions in temperature occurred. Gassing was repeated four times to 30 psig prior to the final pressurization to 45 psig. Oxygen concentration was raised consistently to above 96% by this method. In the large chamber, the oxygen and carbon dioxide concentrations in the polyethylene bags remained unaltered at the end of the 48-hour culture periods.

Eight triplicate studies of cell yield were performed in the small chambers using both canine spleen and lymph nodes, with 1-, 3-, and 5-min compression and decompression periods. Yields were comparable at 2, 4, 6, and 8 days, suggesting that the more rapid pressure changes produced no harmful effects.

Bacterial Contamination

The contamination rate was consistently under 1% despite much handling, unavoidable turbulence, and entry of gases into culture dishes during compression.

Tumor Cultures

Slices of human tumors obtained fresh from the operating room were cultured in 98% O_2 + 2% CO_2 at 45 psig, using standard tissue culture methods.

Adenocarcinomas. Three breast, three prostate, two stomach, and two pancreas adenocarcinomas retained their histologic pattern in 6- to 15-day cultures, even in marginal outgrowths, and showed no fibroblastic transformation. Desmoplasia was diminished, and, indeed, fibrous tissue seemed to survive poorly, often developing early metachromasia and pyknosis. Metabolism was assayed by glucose consumption, which diminished after 11 days, dropping to 30% by 15 days. Mitotic counts were maximal at 4–7 days (10–14%), falling off by 12 days to less than 2%. ^{3}H-thymidine incorporation corresponded well to mitotic counts.

Epidermoid Carcinomas. Three lung, two esophagus, and two larynx carcinomas plus two melanomas (Figure 2) followed a pattern similar to adenocarcinomas. Signs of differentiation in terms of keratinization and intercellular bridge formation appeared in two 1-week-old cultures in which these traits had not originally been present.

TABLE 4. Cell Yield from OHP-Cultured Lymph Node Tissue

	Cell yield			
	On screen at air–fluid interface		Fully immersed in dish	
Day of culture	No. cells/gm of lymph node	% viable	No. cells/gm of lymph node	% viable
1	8.0×10^6	92	6.0×10^6	85
2	7.4×10^6	84	5.1×10^6	82
3	7.0×10^6	83	3.5×10^6	58
4	5.7×10^6	78	1.7×10^6	40
5	3.4×10^6	76	0.87×10^6	38
7	3.2×10^6	75	0.8×10^6	30

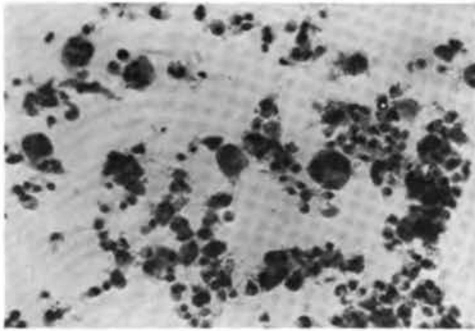

FIGURE 2. Imprint of human malignant melanoma after 7 days of culture at 45 psig in 98% O_2 + 2% CO_2.

Undifferentiated Carcinomas. Three undifferentiated carcinomas grew rather poorly, at least partly because of difficulties in obtaining satisfactory tissue slices. When plasma-clot techniques were used, fair outgrowths were obtained.

DISCUSSION

Significant inhibition of enzymatic activity, cell metabolism, and cell multiplication have been well-recognized *in vitro* effects of high oxygen tensions since the days of Paul Bert. Interference with SH-containing enzymes appears to be rather specific.[13] Normal glucose utilization is blocked in many instances,[7] presumably because of conversion to anaerobic pathways. For this reason, measurements of glucose utilization cannot be relied upon as indicators of tissue metabolism. Recent studies have shown less interference with glucose metabolism as such, suggesting instead a block in the α-ketoglutarate and pyruvate [14] utilization and high-energy phosphate bond formation.[12] Peroxide formation is apparently not involved in any of these processes, since catalase does not prevent their occurrence.[8] Metabolic studies *in vitro* have shown consistent depression of oxygen utilization at high pressure.[15] Horne's study [16] of rats taken to the point of toxicity, convulsions, and death, however, does not bear out these findings in a well-controlled *in vivo* system. The toxic effects described in some other recent studies [10] may have been partly due to different carbon dioxide concentrations and the altered pH of the media.

The effects of the above factors cannot be clearly analyzed in the studies reported. Within tissue slices, which presumably have oxygen gradients extending to their core, a variety of phenomena must occur. True hyperoxia is present on the surfaces, and early cell death in some of these zones has indeed been demonstrated. Some distance from the surface (in a region considered by some as the limit of oxygen diffusion at the pressures employed) there is a marked facilitory effect allowing good survival and rapid growth. It is unclear why this survival and growth are limited to 10–14 days and whether ultimate failure is the result of oxygen toxicity.

Certain differences between results obtained in *in vitro* brei studies and those of the slice cultures reported here must be noted. An immediate toxic effect on nervous tissue consistently occurred with both techniques. However, tissue respiration of the liver in brei was only slightly less affected by hyperoxygenation than that of the brain, whereas, in tissue slice culture, liver performed as well as other tissues considered far more resistant to OHP. In contrast, the metabolic function of lung appeared fairly resistant in brei, while in tissue slice culture it did no better than brain. The latter is easier to correlate with clinical observations (the Lorrain-Smith effect) than with the respiratory metabolic studies. A study of oxygen toxicity in this system would appear promising.

The consistently good proliferative response of lymphoid tissue is noteworthy, and of potential practical value as a source of antigen for exhaustive sensitization. Our low rate of bacterial contamination could have resulted from a bacteriostatic effect of OHP, as suggested by the studies of McAllister and others.[17]

ACKNOWLEDGMENTS

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DISCUSSION

DR. G. G. NAHAS (*New York, N. Y.*): *In vivo* high oxygen pressure has deleterious effects on two fast-growing tissues, bone marrow and gonads. Have you any data on these two tissues?

DR. HALASZ: No, I did not work with either of these myself. Dr. Jankay at the Long Beach VA Hospital has been growing bone marrow in culture and has had good luck with it. He is finding that both the myelocytic and the erythrocytic series proliferate, the latter being somewhat more vigorous (Sangre [Barc.] 9:199, 1964). As far as gonads are concerned, I do not know of anyone who has done this.

Effects of Hyperbaric Oxygen During Fetal Life

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An oxygen-pressurized immersion chamber (Figure 1) has been used as a fetal incubator in our laboratory for 3 years.[6] Within the chamber, fetal exchange of oxygen and carbon dioxide occurs through the skin as well as the lungs, and nutrients are absorbed by swallowing. Our ultimate goal is to sustain extrauterine fetuses for prolonged periods of time, providing techniques for study of fetal physiology and perhaps for clinical utilization.

Following is a report of the effects of brief exposure of mouse and rabbit fetuses to hyperbaric oxygen.

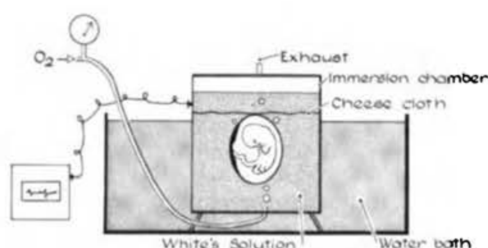

FIGURE 1. A schematic diagram of the fetal incubator. In the present study, the fetal membranes were ruptured.

MATERIALS AND METHODS

The fetal incubator is a 25-liter stainless-steel chamber, partially filled with White's balanced salt solution and 1% tris buffer, pH 8. The chamber is equipped with view-ports, oxygen inlets and exhaust, and facilities for recording electrocardiogram, tissue pO_2, immersion fluid pO_2, and pH, plus a pumping system which regularly exposes the immersion fluid to room air and ultraviolet light (Figure 2).

Estimations of tissue pO_2 were made with an Instrumentation Laboratory needle electrode in the fetal peritoneal cavity, and immersion fluid pO_2 was determined with a Beckman 777 oxygen analyzer.

Mouse fetuses (Balb/C strain) were delivered by hysterotomy at term (19 days) under ether anesthesia and rabbit fetuses (New Zealand) at term (30 days) under pentobarbital (10 mg/kg) anesthesia. After delivery, alternate fetuses were selected as controls and the remainder (experimental fetuses) were immersed within the chamber before onset of breathing. Oxygen pressure within the chamber was then raised to 150 m H_2O over 30 min; when the fetal skin became pink, the oxygen pressure was reduced to 100 m H_2O. Immersion fluid pO_2 deter-

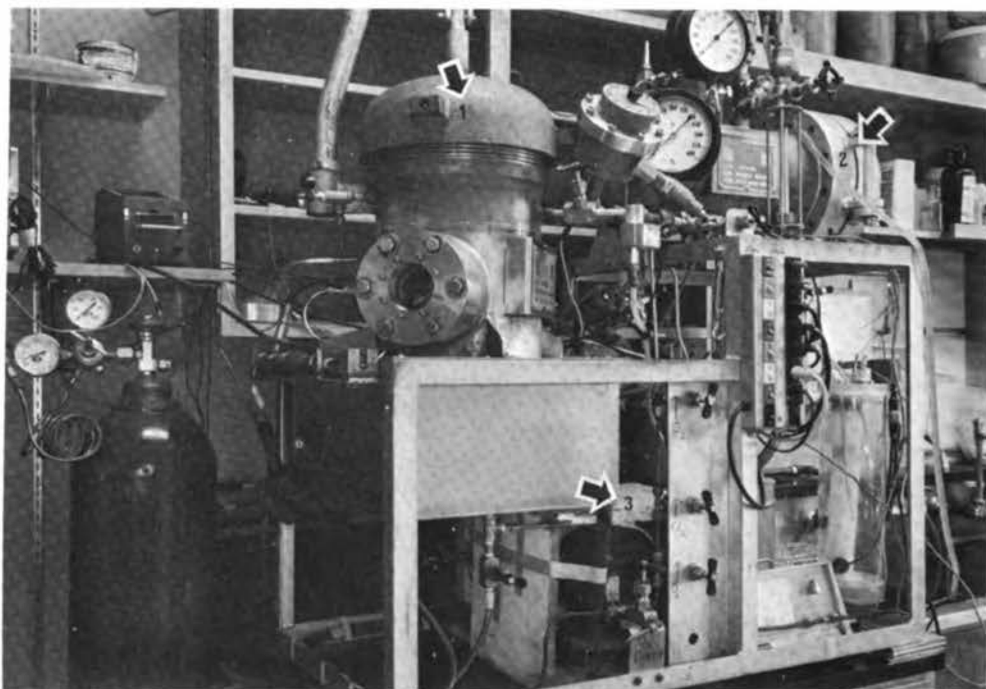

FIGURE 2. The fetal incubator: top left arrow indicates the immersion chamber, top right arrow the reserve tank, and bottom arrow the external circulating system.

minations indicated that constant vigorous stirring of the fluid was necessary to raise the oxygen tension within the fluid to the expected level. The temperature of the immersion fluid was raised from 18°C initially to 37°C during the 4 hours of fetal immersion. Control fetuses were set aside in sawdust nests at 30°C.

After decompression over 30 min, and with no effort at tracheal aspiration, newborn animals were placed in 50% oxygen until they established normal breathing pattern and activity. The next morning, both experimental and control newborn animals were placed with foster mothers.

Mixed venous blood was obtained from neck veins of rabbit fetuses before their placement in the chamber and at the time of decompression. Immediately after death, brains, eyes, liver, kidneys, and lungs were fixed in 10% formalin and histologic sections were made.

A T-maze was used to evaluate mouse intelligence, the score depending upon the number of runs required for the mouse to learn which arm contained food.

Pressure–volume characteristics were determined for fetal rabbit lungs of animals sacrificed before and after immersion, according to the techniques of Avery, Frank, and Gribetz.[1] In order to avoid injury associated with dissection after thoracotomy, the pressure–volume measurements were taken *in situ*.

RESULTS

During immersion, the breathing rates of rabbit fetuses varied from one respiration every 15 min to 50/min. Histologic studies of fetuses after immersion in fluid containing carbon particles showed carbon within many of the alveoli, indicating alveolar lavage by immersion fluid (Figure 3). Intraperitoneal pO_2 estimation of rabbit fetuses immersed in the chamber

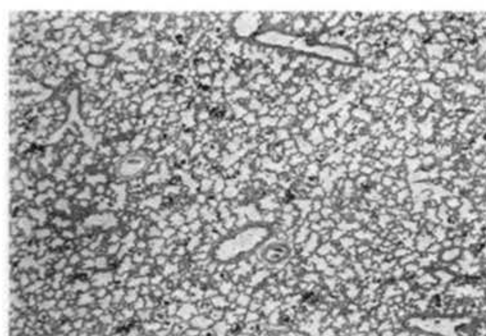

FIGURE 3. Photomicrograph of lung tissue from a 32-gm fetus, previously immersed for 6 hours in fluid containing carbon particles, demonstrating carbon in many of the alveoli. (Reproduced from Amer. J. Obstet. Gynec. 91:953, 1965.)

gave individual maximal values ranging between 45 and 230 mm Hg.

Table 1 shows the number of newborn animals alive after exposure to 4 hours of immersion. After decompression, an animal was not considered alive unless it began respiring. The high rabbit mortality reflects our difficulty in finding effective foster mothers and the high incidence of pneumonia in our colony. With immersion times of 4 hours, some fetuses of every litter always survived for at least 8 hours.

Table 2 lists the range of various laboratory values of mixed venous blood before and after immersion. The high postimmersion blood sugar reflects, in part, fetal swallowing of immersion fluid with a sugar concentration of 1500 mg%.

TABLE 2. Range of Laboratory Values of Mixed Venous Blood from Rabbit Fetuses Sacrificed Before and After 4 Hours of Immersion

	Before immersion	After immersion
No. fetuses	11	10
pH	7.0–7.5	6.6–7.2
pCO_2 (mm Hg)	32–41	84–129
Sodium (mEq/liter)	137–143	124–149
Chlorides (mEq/liter)	96–104	82–101
Potassium (mEq/liter)	4.6–5.2	5.1–12.9
Glucose (mg/100 ml)	61–85	110–568
Osmolarity (milliosmoles)	284–293	294–331

Figure 4 shows photomicrographs of lungs from newborn rabbits dying in the postimmersion period. The lungs were generally immature, demonstrating varying degrees of edema, congestion, and interstitial as well as intra-alveolar hemorrhage. Hemorrhages were absent in postimmersion apneic fetuses and appeared to be of maximum intensity in those animals dying 6–8 hours after immersion. Late deaths were due to pneumonia and expansion atelectasis, suggestive of hyaline membrane disease. Sections of other organs showed similar vascular congestion but no other obvious pathologic features.

Comparison of preimmersion and postimmersion air pressure–volume curves of the lung demonstrated reduction in volume at similar pressures in dying postimmersion fetuses, suggestive of reduced surface-

TABLE 1. Survival of Mouse and Rabbit Fetuses after 4 Hours of Oxygen-Pressurized Immersion

	No. before immersion	Survivors after decompression			
		0 hr.	6 hr.	24 hr.	12 wk.
Mice					
Experimental	58	34	26	23	17
Control	22	22	22	20	15
Rabbits					
Experimental	63	35	23	19	5
Control	51	51	48	45	9*

* Twenty-five apparently normal controls were removed from the study because of space problems.

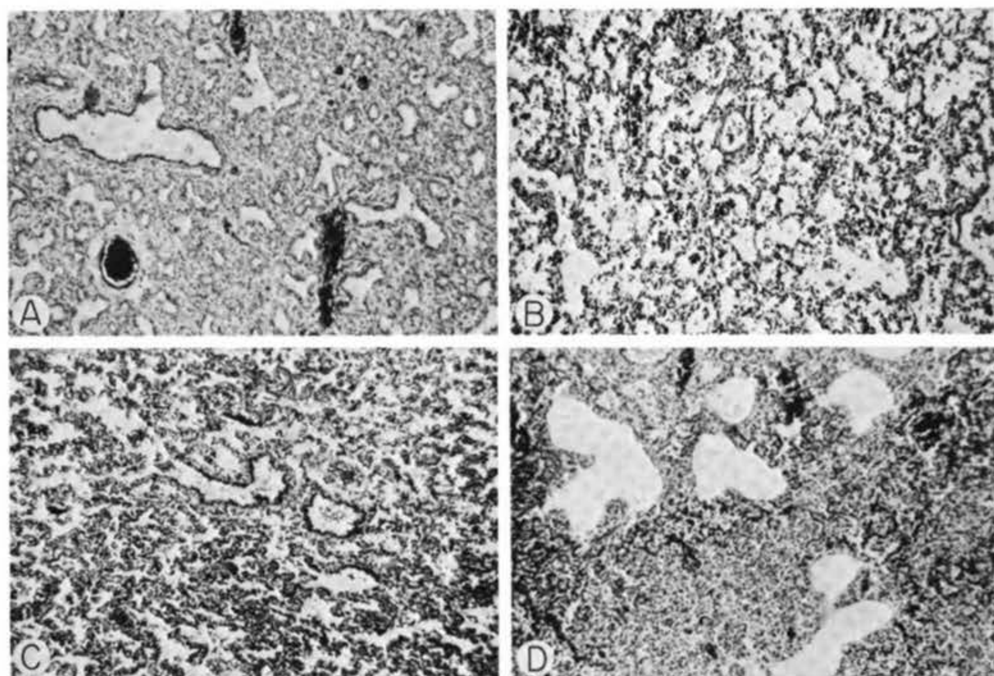

FIGURE 4. A, photomicrograph of lung tissue from 26-gm fetus, immediately after decompression, demonstrating vascular congestion. B, photomicrograph of lung tissue from 41-gm fetus which died after decompression, demonstrating interstitial and intra-alveolar hemorrhages. C, photomicrograph of lung tissue from 43-gm fetus which died 6 hours after decompression, demonstrating marked pulmonary hemorrhage. D, photomicrograph of lung tissue from a 110-gm 8-day-old rabbit, demonstrating expansion atelectasis and pneumonia.

acting agent. Preimmersion lungs, when analyzed by a staff member from the Department of Pediatrics for surfactant,[7] were judged as "active," while the lungs of fetuses dying in the postimmersion period were judged "inactive." Pressure–volume curves were also determined on a random basis in fetuses immediately after decompression and found to be normal. The abnormal lungs were found, then, only in the dying fetuses.

Mouse intelligence estimations at 3 months of age showed an average of 2.8 runs in the maze for the 17 postimmersion animals, compared with a 3.3 average for the 15 controls (no oxygen), a nonsignificant difference. Detailed studies of rabbit weight, sensory development (pain perception, sight, and hearing), and activity demonstrated no differences between control and experimental rabbits at various ages up to 6 months.

DISCUSSION

Conclusions regarding the specific effects of drugs administered to the gravid female upon intrauterine fetuses should be made with caution unless conditions of the fetal milieu are known. For example, Fujikura,[5] in studies on rabbit fetuses, found that previous exposure of the mother to 100% oxygen for 15 hours on the 26th or 27th day of gestation resulted in an increased incidence of retrolental fibrosis, retinal detachment, microphthalmia, stillbirth, and neonatal death in the offspring. As inferred by Fujikura, the toxic effects on fetuses of maternal oxygen exposure may have represented the

effects of hypercapnea, acidosis, and electrolyte imbalance rather than fetal oxygen tension levels (these determinations not having been made).[5] The fetal incubator provides an experimental tool to study the extrauterine fetus free of maternal influence and to assay its internal and external milieu.

Rabbit and mouse fetuses have established effective postdecompression breathing patterns even after 36 hours of immersion, but in our experience none have successfully made the transition to air-breathing unless immersion time was less than 8 hours. Thus, an immersion time of 4 hours was selected in order to obtain sufficient animals for study.

Fetuses appeared to have normal lung expansion curves at the time of decompression, and those dying in the postimmersion period had abnormal (or reduced) expansion curves, but the mechanism is unclear. Animals dying in the immediate postimmersion period also had pulmonary hemorrhages, and many showed laboratory evidence of severe respiratory acidosis. While the cause of death after decompression appeared to be respiratory failure, the significance of reduced surfactant was unclear.

The accepted view of oxygen toxicity is that oxygen damages alveolar lining, which, in turn, is followed by capillary congestion progressing to alveolar exudates and hemorrhage.[3] In the present study, it was frequently impossible to distinguish histologically between lungs of litter mates autopsied immediately after delivery and those autopsied immediately after decompression. In some experiments, however, lungs after decompression demonstrated vascular congestion and edema.

The inconsistent survival rates of litter mates suggested variability of pulmonary reaction to hyperbaric oxygen and fluid-breathing, but this possibility was not confirmed in our study. The fetuses were inbred strains, all delivered of anesthetized females, and equal degrees of hypothermia were utilized throughout the study; these factors are known to influence pulmonary reaction to oxygen.[2]

Durfey [4] reported that when mice were exposed to 100% oxygen for 48 hours, pulmonary hemorrhage was absent in those autopsied immediately, but it did occur in mice killed in oxygen and kept there until autopsy. The suggestion that pulmonary hemorrhage is a rapid post-mortem change due to oxygen was tested by killing five postimmersion rabbit fetuses when they appeared near death. Three of the fetuses showed marked pulmonary hemorrhages, leaving the question unanswered, as the other two could conceivably have survived.

Among the survivors of the rabbits and mice exposed to hyperbaric oxygen, there appeared to be no residue of toxic effects. The apparent benign effects of brief hyperbaric oxygen exposure on fetal development noted in the present study cannot be compared to other *in utero* data on the effects of maternal oxygenation. The cause of respiratory failure in some postdecompression fetuses is unclear and requires further study.

Summary

Sixty-three rabbit fetuses and 58 mouse fetuses were delivered at term abdominally and immersed in an oxygen-pressured salt solution for 4 hours. After decompression, there was a high immediate mortality associated with respiratory failure, but no apparent ill effects of the hyperbaric oxygen were noted in the long-term survivors.

ACKNOWLEDGMENTS

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We wish to thank Dr. M. Klaus, Department of Pediatrics, for analyzing fetus lungs used in this study.

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DISCUSSION

DR. G. MARGOLIS (*Hanover, N. H.*): I thought it would be of interest to show two examples of what might happen to animals in the fetal stage exposed to OHP at a little earlier phase than shown here, just to have these findings recorded in the conference. These figures are from my colleague, Dr. Ferm, who exposed *in utero* the fetuses of pregnant hamsters during early stages of pregnancy for either 4, 3, or 2 hours at either 3 or 4 ata (Proc. Soc. Exp. Biol. Med. 116:975, 1964). In his studies, a small but significant number of congenital malformations were encountered, including examples such as this exencephaly (Figure 1). In another experiment, he has used the combination of colchicine and OHP. Figure 2 shows the results of exposure to this pair of toxic agents. This is a far more severe malformation—neither OHP nor colchicine alone would produce such a severe deformity of the nervous system and related skeletal system.

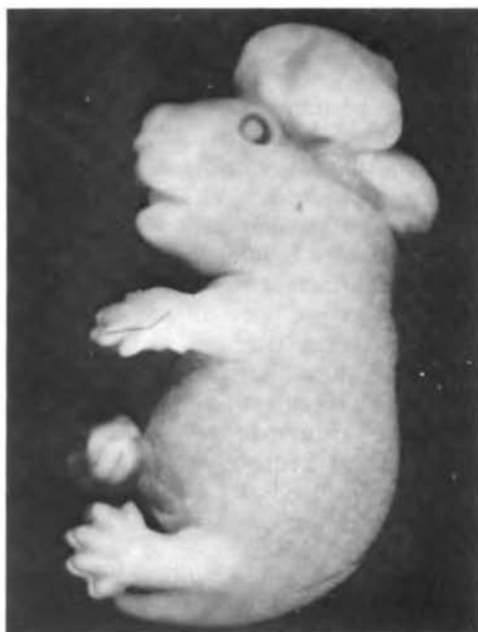

FIGURE 1. Thirteen-day-old hamster fetus from mother exposed to 40 psi of 100% oxygen for 3 hours on 6th day of gestation. Note marked exencephaly. (Reproduced with permission from V. H. Ferm: Proc. Soc. Exp. Biol. Med. 116: 975, 1964.)

DR. G. G. NAHAS (*New York, N. Y.*): This very elegant work reminds one of Dr. Kylstra's experiments in which adult mice were immersed in saline at 8 atmospheres (Trans. Amer. Soc. Artif. Int. Organs 8:378, 1962). Now, I want to ask Dr. Goodlin, what was the temperature of his medium and what was the exact pressure of oxygen in his hyperbaric chamber. Also, why was there such a high pCO_2 and low pH after 4 hours, and how much carbon dioxide absorber was there in his medium?

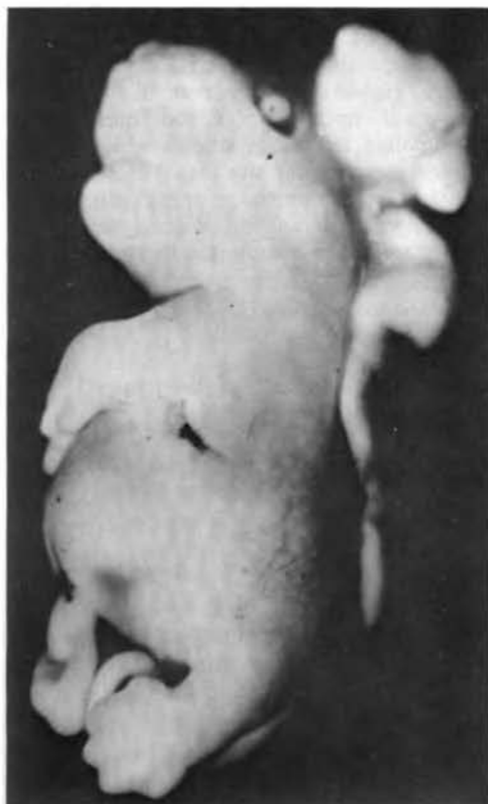

FIGURE 2. Thirteen-day-old hamster fetus from mother exposed to 40 psi of 100% oxygen for 3 hours on 8th day of gestation. Note complete craniorachischisis. (Reproduced with permission from V. H. Ferm.)

DR. GOODLIN: The temperature was initially started at 18°C because we found that the animals would not survive unless the temperature was low. As soon as the oxygen pressure on our gauge reached 225 psi and as soon as the animal began to respond and move about, we lowered the oxygen down to 150 psi and raised the temperature slowly to 37°C. You asked why the high pCO_2. Well, we added a lot of THAM to this solution, and we thought this was the buffering effect of the THAM. As a matter of fact, these animals were fairly acidotic and I think that is really why they died, since they were unable to get rid of their carbon dioxide.

DR. A. R. BEHNKE (*San Francisco, Calif.*): It seems to me that this type of investigation is of tremendous importance. The gravid animal evidently is an extremely sensitive organism for evaluation of both oxygen and drug toxicity. My question is whether the offspring of gravid animals subjected to oxygen inhalation at high pressures have been observed throughout their life span or studied systematically over shorter periods?

DR. MARGOLIS: I am encouraged by your words, Dr. Behnke. I will urge Dr. Ferm to continue this work. Within 24 hours he can tell whether any drug now on the market is teratogenic. He uses gravid hamsters at about, if I remember correctly, the eighth day of the gestation cycle, which is 16 days (Lab. Invest. 14:1500, 1965). During this time, so rapid a change is going on in the fetus that any drug or any change in the environment such as we have just discussed may induce crucial defects in development. I think this is probably the most sensitive example that I have seen of such an experiment, being essentially an almost immediate test for teratogenic activity.

DR. GOODLIN: I would just like to defend this system that I have used here. There have been other studies in which pregnant rabbits were exposed to high oxygen concentrations and fetal anomalies were subsequently found, but, of course, nobody knows what alterations of fetal environment occurred in these situations. There is a great deal of discussion as to whether the oxygen tension in the fetus is necessarily elevated just because the mother happens to be exposed to an elevated oxygen tension (Amer. J. Obstet. Gynec. 93:583, 1965).

DR. B. SMITH (*Miami, Fla.*): Pertaining to the question about hyperbaric oxygen causing teratogenic defects, we have been carrying out this kind of work for quite some time. We were a little unimpressed with the hamster as a test animal because of the lack of really uniform genetic stock and the fact that it is a hibernating, high-altitude animal. We did attempt to reproduce this work of Dr. Ferm, and found that his conditions presented an extremely challenging stress for the hamster. A good portion of these hamsters will die, and I think in going to the mammal for this type of study it is easy to disregard some of the extreme effects on physiology of the mother from

this stress. There is no argument, however, that hyperbaric oxygen can be teratogenic. We went further and used several hundred chicken embryos. We chose progressive stages of gestation for the stress and found a highly reproducible and marked increase of the sensitivity to hyperbaric toxicity with increasing age of the embryo. It is our current unsubstantiated hypothesis that this progression in sensitivity may be related to the developing enzyme systems and the dependence of the chick upon more sophisticated metabolic pathways as they grow older. However, in general, I think it is important not to infer too much from mammalian teratology studies which do not control or consider the toxic effects of the stress substance on the maternal physiology, which, when deranged, presents an uncontrolled teratogenic stress for the fetus.

A New Ocular Manifestation of Oxygen Toxicity

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Pulmonary and central nervous system sequelae have long been recognized as the major overt manifestations of oxygen toxicity, but the pathogenesis and therapeutic management of these toxic effects remain unresolved. Our studies have indicated that the eye is a consistent and early indicator of oxygen toxicity. Following is a description of this pathologic manifestation and a discussion of the possibility that multiple mechanisms of hyperoxic injury may operate simultaneously.

METHODS

Adult healthy dogs were subjected, under standard U.S. Navy compression and decompression techniques,[1] to either a single exposure or a series of daily exposures to increased oxygen pressures, according to the schedule summarized in Tables 1–3. For tests with 100% oxygen, a 35-ft^3 experimental hyperbaric chamber was used. Oxygen inflow was regulated to maintain the desired pressure while the chamber was vented at 0.5 ft^3/min, with soda lime used to extract carbon dioxide. The roles of carbon dioxide, anesthesia, and events attending convulsions (apneic hypoxia, excess utilization of oxygen and lactic acid formation associated with muscular activity, and exhaustion of high-energy compounds by discharging neurones) in potentiating the toxic action of oxygen were explored by studies in a clin-

TABLE 1. Consistent Manifestation of Cytoid Body Change in Retinas of Dogs Exposed to 100% O_2 at 30 psig for Over 4 Hours [a]

Exposure (min)	Cytoid body change
210	—
225	—
242	+
259	+++
261	++
265	+
269	+++
270	+
275	+
295	+
300	+
312	+++
326	+++
362	+++

+, few.
++, moderate numbers.
+++, numerous.
[a] At this exposure, no neurologic deficit was detectable, and pathologic change in central nervous system tissue was absent.

TABLE 2. Incidence of Cytoid Body Change in Retinas of Dogs Given Multiple Daily OHP Exposures to Onset of Convulsion [a]

Dog no.	Exposure (min)		Cytoid body change
	Average	Range	
1	15	15	—
1	18	18	—
4	63	30–105	—
15	31	3–112	(died)
19	70	22–120	+
21	20	4–59	+
22	53	9–94	—
22	36	10–75	—
22	38	9–148	—

[a] OHP consisted of 100% O_2 administered at 58 psig. At this exposure, no neurologic deficit was detectable, and pathologic change in central nervous system tissue was absent.

ical hyperbaric chamber vented at 12 liters/min. These experimental conditions enabled us to maintain respiration with a Harvard (or Bird) respirator, to administer selective anesthesia, to block convulsive activity with succinylcholine (Anectine), to sample arterial and venous pH, pO_2, and pCO_2 with IL-113 meter, and to monitor the electroencephalogram, electrocardiogram, and arterial pressure through a Gilson recorder.

In the single-exposure series, tests were run for a predetermined period, unless the condition of the animal warranted termination of exposure. In the multiple-exposure series, each test was terminated at onset of convulsive activity. Surviving animals were observed for 5–21 days after exposures, particularly for the presence of neurologic deficits, and pathologic studies were focused particularly upon the central nervous system. Artifact-free preparations were obtained by a perfusion-fixation method of sacrifice, by the technique of Cammermeyer[2] or of Malm.[3] Routine and special neurohistologic methods[4] were used. Splanchnic organs were fixed in Zenker-formol fixative (formalin concentration 10%) and prepared for study by standard techniques.

RESULTS

In all animals exposed to 100% oxygen at 30 psig for more than 4 hours, a characteristic ocular lesion was observed (Table 1) which occurred in the absence of evidence of neurologic impairment and in advance of recognizable structural damage to the central nervous system. Manifested by the presence of clusters of globular bodies in the nerve fiber layer of the retina, it presented features typical of the microscopic aspects of the "cotton-wool spot," the entity characteristic of the vascular retinopathy associated with certain syndromes in man.[5,6] The occurrence of this ocular lesion in animals given multiple exposures at higher pressures (Table 2) provides confirmatory data, albeit difficult to evaluate on a quantitative basis. In the absence of other central nervous system lesions, the focal degeneration observed in optic nerves in this series was interpreted as secondary to the eye injury, not as a manifestation of the direct toxic action of oxygen.

Table 3 clearly shows the predictable increase in oxygen toxicity produced by adding CO_2 to the respiratory gas. Manifestly, a major shift has taken place in the locus of the toxic action from the eye to the central nervous system. Convulsive activity, whether directly observed or recorded by electroencephalogram in animals maintained on Anectine, was accelerated in onset, occurred more frequently, and was more severe. A striking increase in mortality was observed, and a high incidence of functional and/or structural brain injury was recognized in survivors, even though exposure periods were necessarily shorter. The use of a short-acting barbiturate and the inhibition of convulsive activity failed to significantly influence the toxic action of oxygen. This increase in neurotoxicity took place in the face of a virtual disappearance of ocular injury. While chlorpromazine appeared to protect the eye without increasing vulnerability of the brain to hyperoxia, these studies were too limited to allow definitive conclusions.

TABLE 3. Influence of Carbon Dioxide upon Toxic Effect of Oxygen at 30 psig [a]

Respiratory gas	Anesthesia		pCO_2 [b] (mm Hg)	Exposure (hr)	Total no. dogs	Deaths	Neurologic deficit	Pathology	
	Induction	Maintenance						CNS [c]	Eye [d]
100% O_2	—	—	—	3.5–6.0	16	2	0/14	0/14	12/14
100% O_2	Chlorpromazine		—	3.5–5.0	4	0	0/4	0/4	1/4
98% O_2+2% CO_2	Nembutal	Nembutal	64–100	1.5–3.5	14	3	8/11	1/11	1/11
98% O_2+2% CO_2	Nembutal	Anectine	76–79	1.5–3.5	3	1	2/2	2/2	2/2
98% O_2+2% CO_2	Brevital	Anectine	79–84	1.5–3.5	13	8	1/5	3/5	0/5

[a] Note the increase in toxicity and shift in major locus of action from the eye to the brain. The chlorpromazine series, while still too small to be critically evaluated, indicates the possibility of a therapeutic approach which protects from retinal injury without intensifying CNS injury.
[b] Measured after equilibration.
[c] Selective neuronal necrosis.
[d] Cytoid body change.

The work reported herein represents one of the first experimental productions of the cytoid body, a lesion whose nature and pathogenesis have been the subject of speculation for the past century. There have been two earlier reports of the experimental production of the cytoid body change. In 1962, Okun and Collins[7] produced this change by photocoagulation of the canine retina and choroid, but this technique would render the analysis of the pathogenesis of the process extremely difficult. In 1964, however, Gay, Goldor, and Smith[8] created the cytoid body by occluding the chorioretinal vasculature of the canine eye by the injection of latex microspheres. Manifestly, this study, providing clear evidence that cytoid body lesions are secondary to nerve fiber ischemia, can be used as supporting evidence for our postulated basis of the pathogenesis of the retinal lesion of oxygen toxicity.

Following is a condensed description of the lesion to assure its identification, and a discussion of its pathogenesis and therapeutic implications.

The Ocular Lesion

The ocular lesion is found in the posterior polar region of the retina, neighboring and sometimes involving the margins of the optic nerve head. In the latter location, it resembles the pathologic picture of papilledema,[9] except that commonly only one margin of the papilla may be involved. The lesion is focal, sometimes perivascular or paravascular, and characterized by clusters of discrete globular and pyriform bodies in the nerve fiber layer of the retina. Strongly eosinophilic, these bodies display no specific tinctorial or histochemical features. A significant feature in some globules is the presence of a prominent, but vaguely defined, moderately hematoxyphilic central or eccentric body (pseudonucleus). The bodies are unequivocally related to swollen disrupted axones (Cajal swellings), which can be visualized in both hematoxylin–eosin and axone stains. In the latter, the termination of axones in a bulbous enlargement surrounded by a weakly argyrophilic zone of pseudocytoplasm is clearly demonstrable. An unusual and characteristic feature in these lesions is the orientation of the Cajal swellings. The bulbous severed end appears always to face the periphery of the eye and the tail of related axone to stretch toward the optic nerve head. Wolter has described this orientation as occurring in a minority of these structures, thus furnishing evidence of centrifugal fibers in the retina.[6,10,11] Necrosis of a few ganglion cells may be found in association with the cytoid body change; some cytoid bodies lie deep in the nerve fiber layer or originate from dead neurones in the ganglion cell layer, as described by Wolter.[10] The absence of changes in the visual cells of the retina is a notable feature. The photomicrographs in Figure 1 demonstrate the salient features of this lesion.

Discussion

In the past, studies regarding the effect of oxygen toxicity upon the eye have been largely limited to the work of Behnke,[12] Noell,[13] and Beehler.[14,15] In 1932, Behnke *et al.*[12] observed in man a progressive contraction of visual fields and impairment of central vision after exposure to 100% oxygen at 3 ata for periods over 4 hours. More recently, Noell[13] studied the effect of oxygen toxicity on the retina of the rabbit and found that despite the avascularity of rabbit retina, the visual cells were a sensitive target for the toxic action of oxygen, with limits of tolerance remarkably close to those reported herein. An exposure to 100% oxygen at 3 ata produced a maximal depression of the electroretinogram after 3 to 5 hours; irreversible injury was regularly recognized at 5 to 6.5 hours, and frequently with shorter exposures. Hitherto, retinal separation has been considered the most common manifestation of hyperoxic injury in the canine eye.[14,15]

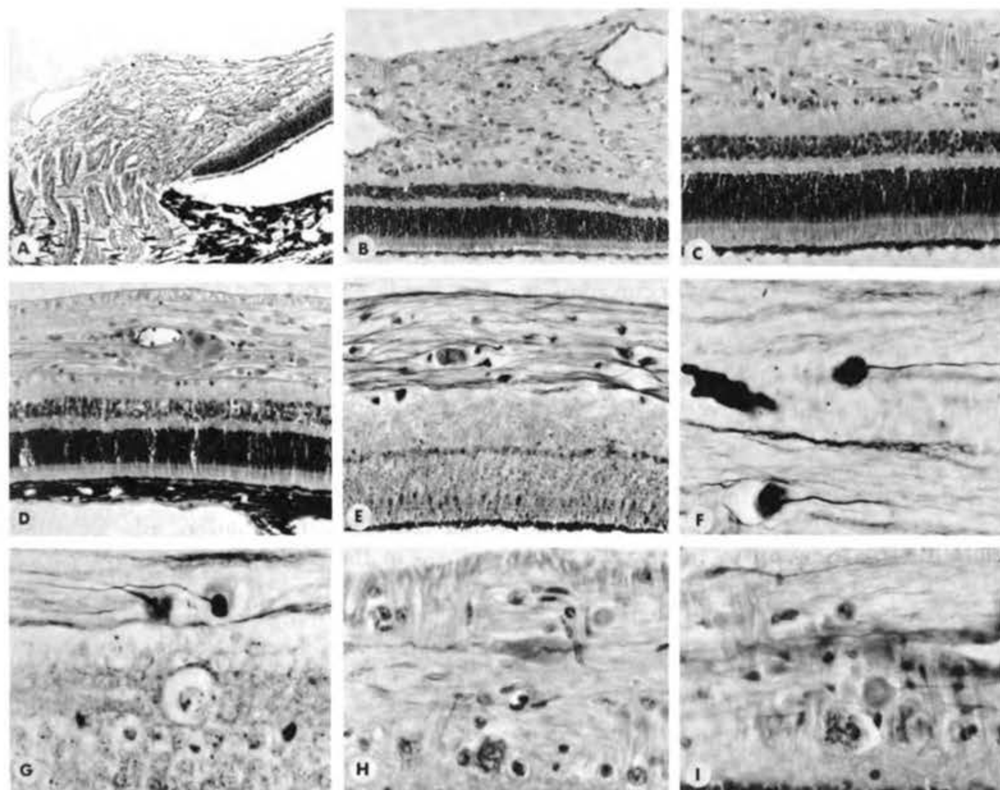

FIGURE 1. (A) Canine optic papilla and bordering retina. Scattered globular bodies are present in the nerve fiber layer. This change is not present on the opposite margin of the optic disc. Holmes axis cylinder stain (× 30). (B) Retina adjacent to papilla shown in A. Numerous globular eosinophilic bodies are distributed at all levels in the nerve fiber layer. A hematoxyphilic pseudonucleus is visible in many of these cytoid bodies. The remaining layers of the retina are not altered. Hematoxylin and eosin (× 70). (C) Canine retina showing numerous cytoid bodies in nerve fiber layer, many having a pyriform shape, with a short axonal tail extending from the apex. All of these bodies are oriented in the same direction, with the axonal extensions directed to the left, toward the optic nerve. Hematoxylin and eosin (× 70). (D) Retina showing in the nerve fiber layer a perivenous edematous zone containing numerous globular cytoid bodies. A pseudonucleus is clearly seen in the body to the right of the vessel. Grossly this lesion would probably have the appearance of a "soft exudate" or "cotton-wool spot." In the absence of acute changes affecting ganglion cells, the paucity of neurones in that layer (which varies greatly in the canine retina) is not considered abnormal. Note the absence of changes in the visual cells. Hematoxylin and eosin (× 70). (E) Canine retina with scattered interrupted axones having swollen terminal bulbs (Cajal swellings) in the nerve fiber layer. Two of these appear to be derived from the ganglion cell layer. Again, all of these structures show a similar orientation, with the bulbous endings directed to the right and away from the optic nerve head, which is to the left. This centrifugal orientation has been observed uniformly in these experimentally produced axone lesions. Holmes axis cylinder stain (× 50). (F) Canine retina showing two centrifugally oriented Cajal terminal swellings. The terminal bulb (black) shows the characteristic relationship to the pseudonucleus, and the surrounding substance (grey) to the pseudocytoplasm of the hematoxylin and eosin stain. Holmes axis cylinder stain (× 280). (G) Canine retina showing two Cajal terminal swellings which appear to be derived from necrotic ganglion cells. Again, their orientation is centrifugal, with the optic nerve at the left. The pseudocytoplasmic features are clearly shown here. Holmes axis cylinder stain (× 175). (H) Canine retina with a periaxonal eosinophilic substance enveloping a fiber which is tortuous, slightly swollen, basophilic, but not interrupted. This would appear to represent one of the earliest recognizable stages of the cytoid body formation. Hematoxylin and eosin (× 175). (I) Canine retina showing edematous nerve fiber layer with several cytoid bodies. Just beneath the internal limiting membrane is an axis cylinder exhibiting the early phase of development of a Cajal bulb and the surrounding eosinophilic substance. Just above a ganglion cell is a more advanced cytoid body, again with an axonal extension. Between these two cytoid bodies is another, in which a dumbbell-shaped pseudonucleus is seen. Hematoxylin and eosin (× 175).

The fact that this ocular manifestation was observed in the dog is particularly significant, the dog being the most common "proving ground" for therapeutic procedures in the cardiovascular field. Furthermore, the lesion has occurred after OHP at pressures close to those now applied to man.

Aside from its relationship to hyperbaric medicine, the discovery of this lesion is an important event for investigative ophthalmology, for it presents a means of experimentally producing the cytoid body, an entity of disputed pathogenesis. Once the structural identity of the experimentally induced and the naturally occurring lesions was recognized, our next step was to consider what our present knowledge of the pathogenesis of the retinal lesion of hypertension could tell us about the genesis of the hyperoxic retinal lesion, and, ultimately, about the basis of the toxic action of oxygen. The work of Ashton on the nature and pathogenesis of the cytoid body[5,16] was helpful in this regard. Using flat preparations and trypsin-digested specimens of retinas perfused post mortem with india ink, Ashton was able to identify and study cotton-wool spots which had been observed during life. The failure of the injection mass to reach the zone occupied by the cotton-wool spot, the presence of microaneurysms in the adjacent vascular bed, the occurrence of mural fatty changes, and the absence of endothelial cells and pericytes in the vessels within the lesion indicated that the patency and integrity of function of the involved vessels were doubtful. These studies provided cogent arguments favoring a vasospastic and/or ischemic background for the retinal lesion of hypertension.

The question was next posed whether the same pathogenesis applies to hyperoxic retinal lesions. Despite the strong vasoconstrictive reaction of the retina to oxygen,[17-21] increased oxygen transport in OHP may more than compensate for reduced blood flow, as suggested by observations of the arterialized appearance of retinal veins,[18-21] and by the prolongation of retinal function afforded by OHP in retinal ischemia.[22,23] Furthermore, the retina is notably resistant to anoxia. Our own studies of the protection afforded by hyperoxia against anoxic sequelae of circulatory arrest, reported at the same time,[24] have yielded not a single instance of anoxic retinal injury, even in the presence of severe brain damage. Still, the possibility had to be considered that a profound, prolonged, and excessive vasospastic response to oxygen could produce the paradoxical situation of localized anoxia in the presence of an oxygen-rich environment.

If the lesion has a vasospastic background, it would be reasonable to predict that it should be readily prevented by an elevation of pCO_2 with its vasodilating action. Conversely, if the lesion results from the direct toxic action of oxygen, its onset should be accelerated and its severity increased by the addition of CO_2[25] which would eliminate the possible protective constrictor vascular response to hyperoxia, a mechanism postulated as long ago as 1921.[26] Studies probing this question are summarized in Table 3. These results strongly favor the existence of a vasospastic mechanism for the production of the retinal lesion.

The lack of parallelism between the ocular and central nervous system manifestations of oxygen toxicity can be reasonably explained by the following recognized facts concerning the action of oxygen and carbon dioxide at high partial pressure: [27,28] (1) the retina, resistant to anoxia, is vulnerable to hyperoxic injury because of its strong vasospastic response to oxygen; (2) protection against this anoxic effect may be achieved by adding CO_2 to the respiratory gas mixture; (3) the brain, susceptible to anoxia, is vulnerable to hyperoxic injury through the direct action of oxygen, and actually may be protected by the physiologic autoregulatory response of its vascular bed to this gas; (4) potentiation of the histotoxic ac-

tion of oxygen upon the brain is produced by the vasodilating action of CO_2, resulting in an elevated pO_2 in the central nervous system; and (5) the absence or delayed onset of vasospastic injury to the brain in OHP is postulated to be based on the low degree of reactivity of the cerebral vascular bed.

To support the hypothesis in (5), a different order of reactivity must be demonstrated in the retinal and cerebral vasculature. There is abundant evidence of the superiority of the peripheral vessels over those of the central nervous system in responses to vasoactive agents.[29, 30] Studies by one of us (G.M.), using directional shunting of roentgen contrast agents and electromagnetic measurements of blood flow, have demonstrated that when one vascular bed is pitted against the other the vessels of the central nervous system play an essentially passive role, with their primary responses being readily overridden and reversed by the more powerful reactions of the peripheral vasculature.[31-33] In view of this lower order of reactivity of the cerebral vascular bed, its constrictor response to hyperoxia would be unlikely to be strong enough and sustained enough to result in focal ischemic anoxia.

The significance and even the existence of retinal vasospasm has long been debated.[34] The previously quoted studies of the physiologic responses of retinal vasculature to variations in inspired oxygen and carbon dioxide,[17-21] plus Byrom's studies on the pathogenesis of hypertensive encephalopathy,[35] have erased all doubt regarding the high reactivity of these vessels. We know of only a few studies which permit a comparison between the reactivity of the retinal vascular beds and that of the cerebral vascular beds. Hickam *et al.*[36, 37] have shown that with advancing arteriosclerosis a parallel impairment of reactivity occurs in both of these vascular beds. Byrom's studies[35, 38] demonstrated a strong spastic response in both trees in the presence of severe elevations of blood pressure. Frayser and Hickam[21] concluded that the reactivity of the retinal vessels to both oxygen and carbon dioxide was greater than that of cerebral vessels. Porsaa[39] has published an extensive review of the older literature concerning retinal vasomotor activity and has reported his own intensive study of the effects of pharmacologic agents, variations in systemic blood pressure, and changes in intracranial pressure upon the caliber of the retinal vessels of the cat. As a result, he concluded that "retinal arteries . . . and those of the brain . . . in all respects behave physiologically alike" but did not himself make parallel observations between these two vascular beds. The recent studies of Lende and Ellis[40, 41] were concerned particularly with the spasmolytic effects of pharmacologic agents. Studies of dynamics of retinal blood flow, using a fluorescent indicator[42-44] and measurements of retinal venous blood oxygen saturation,[45] and the possible use of reflective densitometry to measure ocular blood flow and volume[46] under conditions of chronic hyperoxia could add critically needed information in this area of incomplete knowledge. Studies based on our own experimental model, pitting the cerebral and extracerebral vascular beds against each other,[32, 33] could be readily adapted to the pursuit of this knowledge.

An important therapeutic lead is suggested by these studies. Two distinct mechanisms of hyperoxic injury appear to be operative, and therapy directed toward the protection from one may potentiate the other. Thus, these findings complicate rather than simplify the problem of the treatment of oxygen toxicity. To obtain maximum protection from the toxic effects of oxygen it may, therefore, be necessary to use a double-barreled therapeutic approach. Conceivably, if an effective prophylactic is developed against the direct histotoxic effects of oxygen, carbon dioxide may be needed in the respiratory gas to protect the eye from the vaso-

constrictor action of oxygen. It is theoretically possible, however, that a single agent may inhibit the eye lesion without accelerating or accentuating brain damage. Our own limited trials of chlorpromazine (based on earlier studies of Bean[17]), if confirmed by extended studies, would point to this likelihood. Tranquilizers, because of their dual inhibitory effects upon vascular smooth muscle[48] and upon neurone function, might theoretically be expected to exert a dual protective action against the toxic action of oxygen.

Summary

A characteristic ocular injury is described in dogs as the earliest irreversible manifestation of oxygen toxicity. This injury is manifested as a focal lesion in the nerve fiber layer of the retina, comparable to the microinfarcts associated with retinal vascular disease in man, and featured by the cytoid body change. Protection from this injury is achieved by adding carbon dioxide to the respiratory gas mixture. This observation, and the pathologic features of the lesion, support the interpretation that the injury has a vasospastic background. The protective effect of carbon dioxide for the retina is compounded by the acceleration and intensification of central nervous system effects of oxygen toxicity. Thus, two distinct mechanisms of hyperoxic injury appear operative—one vasospastic, and the other histotoxic—and therapy directed toward protection from one may potentiate the other. The problem of the therapy of oxygen toxicity may, therefore, be more difficult than hitherto considered. It is conceivable that a double-barreled therapeutic approach may be required, in which CO_2 may be prerequisite for the protection of the eye.

(A preliminary report of this work has recently appeared elsewhere,[49] and a more detailed report of the pathologic features of the lesion is in preparation.[50])

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DISCUSSION

DR. E. J. BURGER (*Boston, Mass.*): I have just a couple of points upon which I would appreciate your comments. Dr. Noell (in Schaefer, K. E.: *Environmental Effects on Consciousness*, Macmillan, New York, 1962, pp. 3–18) chose rabbits for his experiments on the effect of oxygen on the eye because the rabbit lacks a retinal blood vessel system and the retinal nutrition seems to be entirely from the choroidal vasculature. The point of all this is that the rabbit presumably could not suffer from a disease like retrolental fibroplasia, which originates from changes in vascular caliber in the presence of high oxygen tensions, but does show apparent direct toxic changes among visual cells. My first question is whether the dog retina resembles our own or that of the rabbit in terms of vascular supply.

DR. MARGOLIS: To answer this question graphically, Figure 1 shows three beautiful examples of capillaries in the nerve fiber layer. This is an injection preparation, so that the capillaries stand open. You can see other injected capillaries in the ganglion cell layer and elsewhere, which helps differentiate between the vascular pattern of the canine and rabbit retina. According to Dr. Noell's studies (and I believe he will verify this) the visual cell is the critical site for oxygen toxicity in the rabbit.

DR. BURGER: He also observed that by increasing the partial pressure of oxygen and

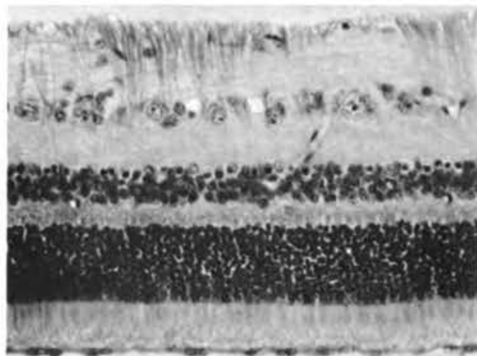

FIGURE 1. Normal retina of dog (×188, hematoxylin–eosin). The capillary network is particularly well shown in the ganglion cell layer.

by prolonging the duration of exposure, the retinal lesion appeared to proceed from the area of the macula outward toward the periphery. Was this your experience?

DR. MARGOLIS: We have seen enough to suggest that it is most commonly demonstrated early around the optic nerve head in the posterior polar region. I believe this is somewhat comparable to his studies, and when the process advances, we find that it spreads toward the retina. It sometimes extends into the optic nerve head, but this is rare. I think that he also demonstrated a depression of electroretinographic activity in the rabbit after about 4 hours (the same time at which we observed the canine le-

sion), with a permanent depression after 5½ to 6 hours. Dr. Behnke has shown that in man, after the same time under the same conditions of exposure to hyperoxia, there is constriction of the visual field and also diminution of visual acuity. How closely this time corresponds to the critical toxic period in man makes me shudder.

DR. BURGER: One final point is worth making. This is a reminder that in a recent series of experimental human exposures to 100% oxygen at low ambient pressures for varying times, photographs of the fundi showed some striking constrictive changes in vascular caliber. It would seem that this might have contributed to the picture you have described. I am puzzled by your distinction in terms of vascular reactivity to oxygen between cerebral and retinal blood vessels.

DR. MARGOLIS: I think this whole problem needs to be studied a little bit more. I would like to show these illustrations in the hope of stimulating people to study this problem more thoroughly. I have long been impressed with the tremendous reactivity of the vessels of the eye. These cerebral angiograms of the dog, made by injecting the common carotid artery with 4 ml of 70% Hypaque, illustrate how well one can demonstrate the various phases of circulation in the canine eye, and how this technique might afford an interesting model for further study.

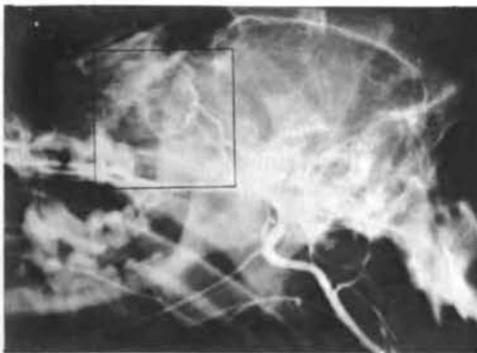

FIGURE 2. Cerebral angiogram in the dog, arterial phase. The ophthalmic arterial flow is clearly seen, and the globe is beginning to be outlined by the injection of its intrinsic arterial bed. The relatively inconspicuous component of intrinsic cerebral circulation is notable in this series.

Figure 2 shows the arterial flow to the eye. Figure 3 shows the optic globe outlined like a pale moon by the choroidal, and possibly the retinal, capillary circulation. Finally, in Figure 4 the venous return from the eye is demonstrated, with the choroidocapillary phase still evident, after the venous phase of the cerebral circulation has passed. In studies we have just begun at Dartmouth, it can be demonstrated that these circulation rates may be strikingly altered by the use of vasoactive substances. As yet, we are unable to draw any direct conclusions about differential reactivity of retinal and cerebral vascular beds to variations in blood gases. We hope that such approaches will supplement information obtained from direct observations of the retinal responses to hyperoxia.

DR. B. ANDERSON, JR. (*Durham, N. C.*): I would like to ask Dr. Margolis if these animals convulsed and if, during the convulsion, they became anoxic. We have seen cotton-wool exudates in humans after hemorrhagic shock, and I, too, would believe this may be an ischemic lesion. The reactivity of the retinal vessels is well known, and perhaps there is evidence that the smaller the vessel the more constriction you get with high oxygen tensions (Lancet 2:291, 1964). I wonder if you would comment on that.

DR. MARGOLIS: In some of these experiments convulsions were deliberately eliminated by an Anectine drip. I do not know

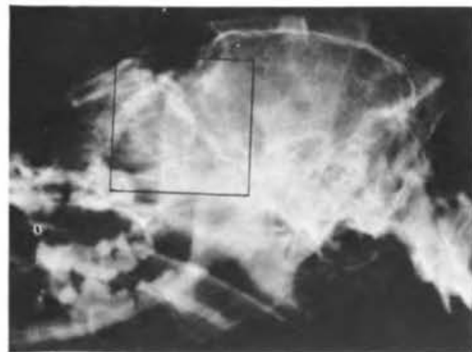

FIGURE 3. Capillary phase of cerebral angiogram in the dog. Injection of the choroidal and retinal vascular beds clearly outlines the globe against background structures.

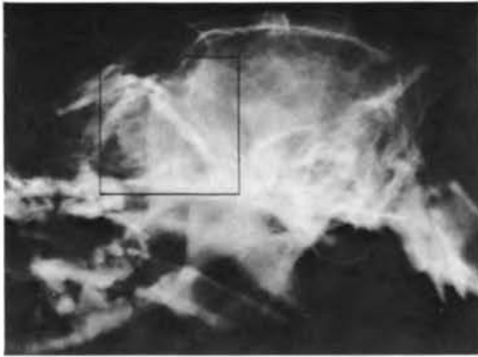

FIGURE 4. Late venous phase of cerebral angiogram in the dog. The globe is still outlined, but the large, opacified venous channel draining the eye is the outstanding feature. The venous phase of the cerebral blood flow is not evident at this time.

whether this has been completely covered, but certainly it appears significant. Perhaps Dr. Brown, who is actually running the animal chambers here, or one of his team, could comment on a more informed basis.

DR. G. MOOR (*Durham, N. C.*): The animals in 100% oxygen did have convulsions. I cannot vouch that they were not hypoxic for short periods during convulsion. The animals with the additional carbon dioxide were on a mechanical respirator with an endotracheal tube. The ones anesthetized with Nembutal did not have seizures, and I could say that they were not anoxic. The ones anesthetized lightly with Brevital and Anectine all convulsed within 30 minutes. They were also on a mechanical respirator and were not anoxic. All of these animals with the additional carbon dioxide were severely damaged.

DR. MARGOLIS: Severely neurologically impaired?

DR. MOOR: Yes, sir.

DR. MARGOLIS: But they did not exhibit retinal damage?

DR. MOOR: That is correct.

DR. W. I. HOPKINSON (*Sunninghill, Ascot, England*): I would like to ask if you have examined the lens and ciliary body in your experimental animals? Both Dr. Slack of Whipps Cross Hospital and I have observed changes in vision in patients exposed to hyperbaric oxygen. In the two cases, there was a change toward what was considered to be presbyopia. The first patient had had 60 hours of exposure at 2 ata spread over 3 weeks. Although the change was quite marked, it was comparatively short-lived, and the visual acuity returned to normal in about 3 weeks. A similar effect occurred in a second patient with 31 hours of treatment spread over 3 weeks. This man was initially myopic and after hyperbaric treatment required new spectacles. About 3 weeks later his vision returned to its normal myopic state. No fundal abnormality was found in either case. I wonder if anybody here has similar observations and perhaps has examined the lens, as this effect appears to be of lenticular rather than retinal origin.

DR. MARGOLIS: I can answer that very briefly—no.

DR. D. G. MCDOWALL (*Glasgow, Scotland*): I would like to make an anesthetist's comment which may or may not be helpful. I think Anectine is a trade name for suxamethonium, and there is some recent evidence that suxamethonium increases the eye blood flow. This is based on some evidence on intraocular pressure even after the suppression of muscle fasciculations.

DR. G. G. NAHAS (*New York, N. Y.*): This very beautiful work was interesting to us because we made similar observations in the paralyzed, mechanically ventilated monkey. This antagonistic action of carbon dioxide, beneficial to the retina and yet deleterious to the brain, is certainly perplexing. We attempted to approach it by associating administration of carbon dioxide and of THAM at the same time. By this method, one maintains a normal pH, or an elevated pH and elevated pCO_2 at the same time.

Influence of Pentobarbital Anesthesia on the Distribution of Central Nervous System Lesions in Rats Exposed to Oxygen at High Pressure

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Selective central nervous system (CNS) lesions have been produced in rats exposed daily to 1 hour of oxygen at 57 psig until they developed limb paralysis.[1] These lesions have two morphologic patterns: Type A, random necrosis of individual neurons within generally preserved nuclei, and Type B, necrosis of large parts of, or entire, nuclear groups with involvement of axons, myelin, and glia within the nuclei. The Type B lesions are always accompanied by pallor and cystic changes interpreted as edema. The two types have distinctive distributions within the CNS. Type A lesions are found principally in the spinal cord, hindbrain, and midbrain, consistently involving the medial anterior horn cells, the superior olivary complex, the ventral cochlear nucleus, and the nucleus of the spinal tract of the fifth cranial nerve. Type B lesions occur chiefly in the midbrain and forebrain, constantly affecting the substantia nigra and globus pallidus but frequently involving specific areas in the olfactory brain and thalamus. The cerebral peduncles are damaged where they are contiguous with massive necrosis of the substantiae nigrae in many animals, suggesting a correlation with the paralysis. The lesions differ in either type or distribution from those of generalized cerebral ischemia, cerebral hypoxia, and decompression illness.

The purpose of our study was to determine the influence of anesthetic dosages of pentobarbital sodium on the occurrence and distribution of the CNS lesions induced by oxygen at high pressure (OHP).

Materials and Methods

The same hyperbaric chamber used in the previous study[1] was employed. The chamber operations were identical[1] except that the ventilation rate was increased to 18–20 liters/min. Oxygen tensions from the outflow valve were measured on an Instrumentation Laboratory gas analyzer (which was used for all gas determinations) after flushing the chamber and prior to each compression, and these values were found to vary from 703 to 712 mm Hg. Carbon dioxide tensions were measured during and at the end of each exposure and were always zero.

Forty white female rats of the Osborne-Mendel strain weighing 160–175 gm were divided into two groups of 20 and each

was exposed to 57 psig of oxygen for 1 hour on consecutive days until limb paralysis developed. From 30 to 60 min prior to exposure, each was anesthetized with 6–6.5 mg (34–41 mg/kg body weight) of pentobarbital sodium administered intraperitoneally. Animals awakening during exposure (16) or dying before paralysis occurred (7) were eliminated from the study. Three paralyzed rats were omitted because of incidental CNS lesions, leaving a final basis of 14 animals reaching the experimental end-point and hence suitable for histologic study. They were sacrificed from 6 to 120 hours after their last exposure. Five control rats of the same specifications as the experimental animals were anesthetized in a similar manner on 3 consecutive days and were sacrificed 72 hours after the last day.

The method of sacrifice by perfusion with Susa's solution and the histologic techniques were identical to those previously described.[1] Sections of brain were taken at 70- to 200-μ intervals serially and sections of spinal cord at every 300 to 400 μ. All sections were stained using Luxol fast blue with hematoxylin and eosin.

Results

The onset of paralysis in the 14 experimental animals occurred after the first exposure in six rats, after the second in six rats, and after the third in two rats. The mean onset of paralysis was at 1.71 exposures. All rats had CNS lesions identical in character to those described as Type A in the previous work,[1] *i.e.*, random necrosis of neurons within generally preserved nuclei (Figure 1). Two animals had Type B lesions, *i.e.*, necrosis of large portions of (or entire) nuclear groups with involvement of axons, myelin, and glia within the nuclei (Figure 2). No white matter lesions were present.

The distribution of CNS lesions in the unanesthetized group from the previous study[1] is given in Figure 3. Figure 4 shows

FIGURE 1. Type A lesion: random necrosis of individual neurons (arrows) in the nucleus of the spinal tract of fifth cranial nerve. Luxol fast blue with hematoxylin and eosin (×445).

the distribution of CNS lesions in the anesthetized group from the present experiment. Comparison of the two figures reveals that the Type A lesions in both groups have a similar distribution. Type B lesions were found only in the pericentral grey matter of the cervical spinal cord in the animals from the anesthetized group.

The five control animals remained anesthetized 2–5 hours each day and demonstrated no CNS lesions.

Discussion and Conclusions

This investigation establishes the presence of selective CNS lesions in rats anesthetized with pentobarbital sodium and paralyzed by repeated 1-hour exposures to oxygen at 57 psig. The mean onset of paralysis was potentiated in the anesthetized rats (1.71 exposures) when compared to that of the unanesthetized rats (5.375 exposures), confirming the findings of van den Brenk and Jamieson.[2]

The rare occurrence of Type B lesions in the anesthetized animals may be related to the shorter total exposure time in this group, since it has been suggested in the previous investigation that Type B lesions occur later than do Type A.[1] The presence of Type B necrosis in the spinal cord in the present study points to a possible regional exception to this rule. Although the Type B lesions were rare in

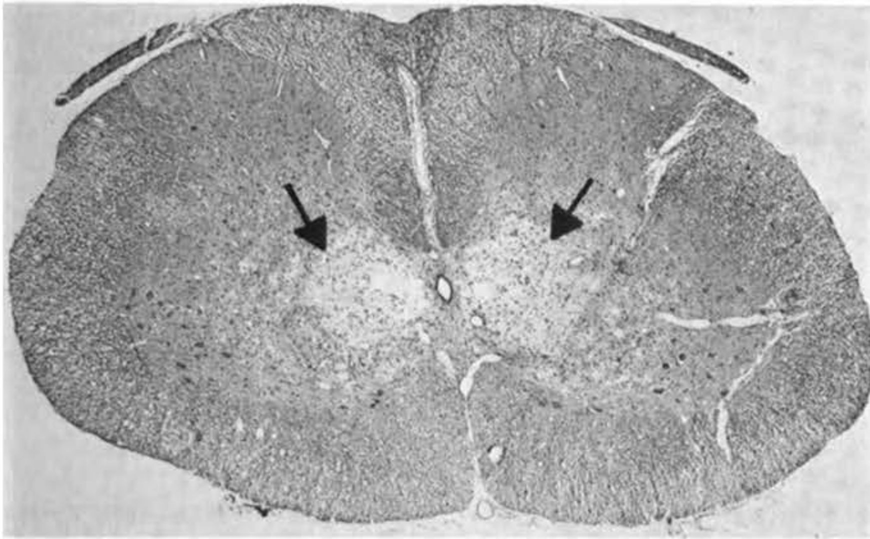

FIGURE 2. Type B lesion: necrosis of pericentral grey matter of spinal cord (arrows) at the level of the cervical enlargement. Axons, myelin, and glia in the area are destroyed as well as neurons. The lesion is accompanied by pallor and cystic changes interpreted as edema. Luxol fast blue with hematoxylin and eosin (×25.7).

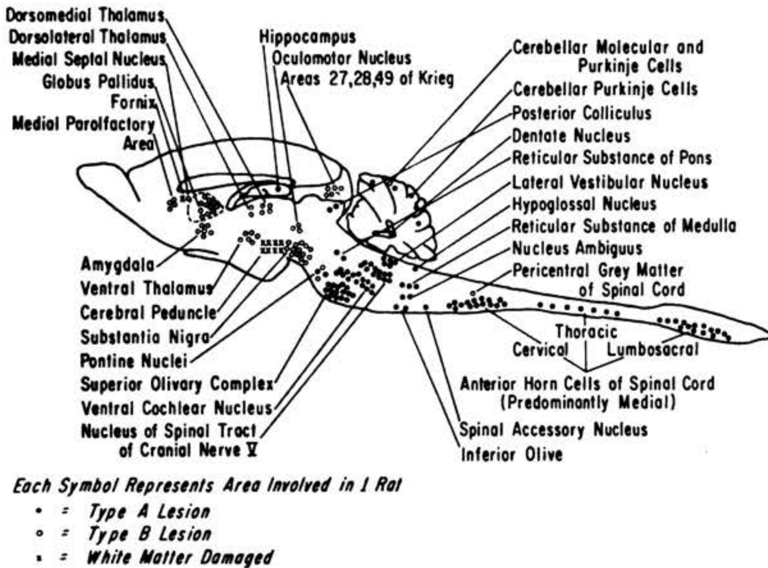

FIGURE 3. Distribution of CNS lesions in unanesthetized rats daily exposed for 1 hour to oxygen at 57 psig until the onset of paralysis. Rat brain model, sagittal plane. Type A lesions, random necrosis of neurons in generally preserved nuclei; Type B lesions, necrosis of all or large parts of nuclear groups with frequent involvement of axons, myelin, and glia within the nuclei; damage to myelin, demyelination with preservation of axons in white matter contiguous with massive neuronal necrosis.

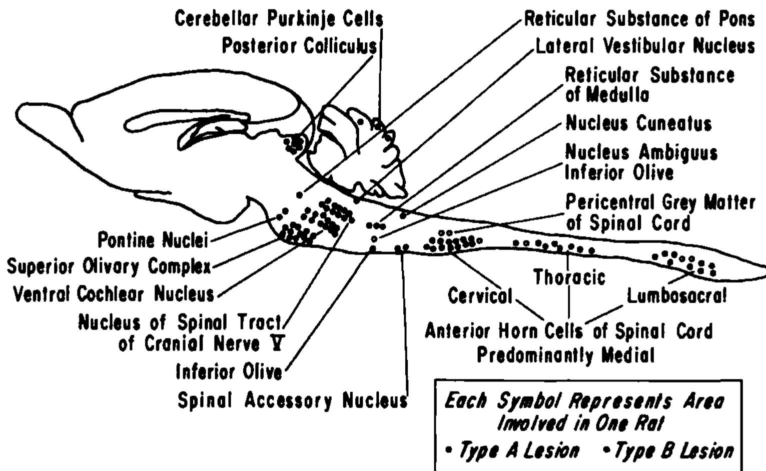

FIGURE 4. Distribution of CNS lesions in anesthetized rats daily exposed for 1 hour to oxygen at 57 psig until the onset of paralysis. Rat brain model, sagittal plane. See Figure 3 or text for description of lesions. Note rare occurrence of Type B lesions and absence of damage to white matter.

the anesthetized group, Type A lesions were enhanced, as indicated by the fact that they did not appear until after the third exposure in unanesthetized rats.[1]

The occurrence of CNS lesions in the unanesthetized rats exposed to OHP clarifies the pathogenesis of the oxygen-induced lesions only to the extent of demonstrating that they are not dependent upon prior convulsive episodes. The potentiating effects of pentobarbital anesthesia on the delayed CNS signs and the Type A lesions of oxygen toxicity remain unelucidated. Carbon dioxide retention resulting from impaired ventilation during anesthesia has been suggested,[1,2] but this may be an oversimplification of the matter in view of the complex biochemical changes in the CNS associated with barbiturate anesthesia and with OHP.[3,4] Possibly both have similar influences on some phase of cellular metabolism that may be additive in producing a toxic effect. For example, both have been shown to interfere with adenosine triphosphate production.[3,4]

A correlation between damage to the cerebral peduncles and the limb paralysis was suggested in the study of the unanesthetized rats.[1] The present investigation detracts from the likelihood of this correlation unless a preceding phase of functional paralysis is postulated. Perhaps stronger consideration should be given to the possibility that the paralysis is produced by selective lower motor neuronal necrosis, which has been consistent in both studies.

ACKNOWLEDGMENTS

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DISCUSSION

DR. J. W. BEAN, *Session Chairman* (*Ann Arbor, Mich.*): To me this is quite interesting because some years ago we did some studies on the central nervous system (Proc. Soc. Exp. Biol. Med. 55:20, 1945) and found there was a good deal of damage done, such as perivascular edema, anemic necrosis, and degeneration in the CNS among other things. Did you find some?

DR. BALENTINE: We could not readily identify or evaluate edema because the animals were perfused with fixative. In fact, the evaluation of edema is a great problem in the whole area of neuropathology. We can state, I think, that the type B lesions are associated with some change that may be reasonably interpreted as edema because of the pallor and cystic changes that are discretely localized to the area of necrosis. No discrete pallor of this sort was seen in the absence of necrosis. The perfusion artifact tended to be patchy, diffuse, and not symmetrically localized to certain areas.

DR. H. A. S. VAN DEN BRENK (*Melbourne, Australia*): I would like to ask two questions. First, did you notice any change or any difference between the symptomatology of the animals that had the barbiturate and the ones that did not? And second, did you notice whether there was any improvement in the paralysis of the animals observed for some time afterwards? In our work, we did notice some improvement of paralysis and considerable recovery in the animals that were less severely paralyzed. Had you made histologic observations on this?

DR. BALENTINE: In both groups, the animals were sacrificed very soon after the onset of the end point, so I can say nothing as to the permanency of the paralysis. We have had some animals with this model that have survived 6 months with no significant improvement of their paralysis. The only sign that we followed consistently was the paralysis. We did observe other signs between exposures, such as hyperexcitability, ataxia, spasticity, and rigidity, but we made no effort to compare these signs in the two groups or to correlate them with histologic findings.

DR. BEAN: Dr. Edstrom corresponded with us some years ago when he was interested in some work we did, and he claimed he could not find any lesion whatsoever except some very slight swelling of nuclei, an increased nucleolar size in the stellate ganglion. Dr. Balentine's paper is particularly interesting to me because it brings out one of the points which I think has been neglected—that is the possibility that permanent lesions can occur in the central nervous system under these conditions. Now, Dr. Jamieson and Dr. van den Brenk were the first investigators that I recall who found permanent lesions in these animals. What also interested me in this paper was the difference between the effects of pentobarbital and the other anesthetics. I have been under the impression that, clinically, anesthesia of-

fered a great advantage in relieving oxygen toxicity. Now, we have heard evidence that, in certain circumstances, the anesthesia probably enhances this toxicity. Is this correct?

DR. BALENTINE: The pentobarbital anesthesia does potentiate the onset of paralysis and the type A lesions, and the absence of the second type of lesion may be related to the short exposure time in the anesthetized group. It is of interest that Dr. Gutsche and myself have been engaged in drug studies concerning the effects of certain drugs on mortality and morbidity of the same type of rat, and we were relatively unsuccessful at producing signs of CNS toxicity both in rats sedated with one-half the dosage of pentobarbital and in rats on Librium. We carried four rats to 22 exposures on Librium without producing any signs; I looked at two of these in relative detail and have found no lesions. So this again presents the problem of dosage as far as drugs are concerned.

DR. BEAN: Speaking of permanency, some of the rats I used were observed for 18 months and there was no recovery evident whatsoever.

DR. G. MARGOLIS (*Hanover, N. H.*): I would like to make a comment. Dr. Balentine's studies and his own statements perhaps underestimate the role of carbon dioxide, but I think all of these things that we talked about, taken together, could indicate a greater influence of carbon dioxide. This gas would act by relieving whatever vasoconstrictive action is produced by oxygen in the nervous system. The topography of these lesions certainly suggests to me a direct histotoxic effect. We have found that multiple exposures also produce neurologic damage, even though they were terminated immediately at the onset of convulsions. In one study, we exposed a series of animals up to 22 times, and during each exposure a convulsion occurred. These animals sometimes developed the cytoid body change, at other times a definite destruction of the myelin in the optic nerve, and, occasionally, neuronal necrosis within the deep centers of the brain.

Oxygen Tolerance and Biochemical Response of Anesthetized Dogs During Oxygen Ventilation at 3 ata

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Reports in the literature vary widely as to the time during which oxygen at normal and increased atmospheric pressure is tolerated. Gillen[1] found that some healthy young males suffered convulsions within 30 min of oxygen inhalation at less than 3 ata; in contrast, Schwartz[2] reported an absence of apparent oxygen toxicity in patients exposed to 100% oxygen at 4 ata for up to 130 min. Other reports also seem to disagree with one another.[3] It may be that only small differences in the condition of man or animal or in the circumstances of the observation result in great differences in real or apparent oxygen tolerance. Some workers are even so pessimistic as to imply that the neurologic hazards of high pressure oxygen overshadow its benefits and that hence its ultimate therapeutic value is almost nil.

Our clinical and experimental experience, however, suggests OHP to be a relatively safe therapeutic tool. Exposure to oxygen at 3 ata for 2 hours (compression and decompression time included) seldom had adverse effects, although occasionally oxygen treatment had to be stopped earlier. Bean[4] has raised the question whether some data concerning the threshold and tolerance of man to oxygen are thoroughly reliable; he suggests that because of an absence of rigid controls the subjects may actually inspire oxygen in concentrations appreciably different from those supposed. His point is well taken: in most of our patients, we had no figures of the real oxygen tensions, and the same applies to many of the figures reported in the literature.

Our knowledge of what happens at 3 ata during oxygen treatment is very limited. To select a uniform exposure time of 2 hours is actually somewhat arbitrary; for some patients this is too long, but for others a much longer exposure may effect no harm to brains and lungs and have great value in the management of the disease under treatment. In a number of experiments, we exceeded the exposure time of 2 hours in order to determine the effect of long-duration oxygen treatment under special conditions—for, in selected cases, prolonged oxygen therapy could prove to be life-saving if oxygen toxicity were not too great a hazard. The time limits for OHP might be more easily defined if more experimental and control data were available.

The purpose of our investigation was to find answers to the following questions:

(1) After how much time does hyperoxic anoxia occur during oxygen ventilation at 3 ata? (2) After how much time does so much pulmonary damage occur that it results in a drop of arterial pO_2? (3) What is the biochemical response to extended oxygen ventilation at 3 ata?

Methods

Dogs weighing 13–26.5 kg were anesthetized with kemithal and injected with 0.5 mg atropine and 50 mg succinylcholine. Artificial ventilation was begun with air, using a semiclosed system without CO_2 absorber. A constant level of anesthesia was maintained by frequent injections of small doses of Nembutal. The ventilation was standardized with a high and constant gas flow and a constant inspiratory period occupying 40% of the cycle. The frequency of ventilation was 18–20 respirations/min. The femoral artery was cannulated for pressure recording and blood sampling, using a pressure transducer. The pressure recording was made inside the high pressure chamber; the left femoral vein was cannulated for blood sampling at the level of the bifurcation and for blood replacement. Electroencephalogram and electrocardiogram were monitored continuously and a recording made every 5 min. Six experiments were performed inside a high pressure chamber. Artificial ventilation was started with air. Fifteen minutes after cannulation, arterial and venous samples were drawn for determination of pH, bicarbonate, pCO_2, potassium, hematocrit, blood glucose, and lactic acid (pyruvic acid and fatty acids were also measured but the results of these experiments will be discussed separately).

After the samples had been drawn, oxygen ventilation was substituted for air ventilation; 15 min later samples were drawn again. Subsequently, the pressure in the chamber was raised to 3 ata, oxygen ventilation was continued, and arterial and venous samples were drawn after each half hour. In five cases, decompression was started after a 4-hour stay at 3 ata. Ten minutes after decompression, blood samples were drawn again; finally, air ventilation was substituted for oxygen ventilation, and, after 15 min of this, samples were drawn and the experiment was terminated. Three control experiments were performed with oxygen ventilation at 1 ata. Finally, one experiment was performed with oxygen ventilation at 3 ata for 8 hours.

All animals were slightly hyperventilated. The anesthetic level was kept constant at a rather deep level. The response in dogs with light anesthesia and high CO_2 levels will be discussed in a separate study. We tried to keep the temperature at a constant level by covering the animal with sheets, preventing spontaneous cooling. Levels of pH, pCO_2, and bicarbonate were determined using the Astrup apparatus. Potassium values were measured using a flame photometer, and lactic acid levels in the blood were determined by a modification of the enzymic method of Hohorst. Oxygen tensions were measured polarographically on the Astrup apparatus. Glucose determinations were performed using a glucose oxidase method.

All determinations were performed on decompressed samples.

Results

Four Hours at 3 ata

None of the animals subjected to hyperventilation and deep anesthesia for 4 hours at 3 ata suffered convulsions. In contrast, other experiments using light anesthesia have shown us that oxygen ventilation at 3 ata, especially when the CO_2 level in the blood is high, usually results in convulsions within 45 min. Occasionally, spike potentials that sometimes precede convulsions were observed after 90–150 min at 3 ata.

In five experiments carried out at 3 ata, one animal was sacrificed at the end of the experiment; of the four remaining

animals, one which had been used 3 days earlier for another experiment died from pulmonary complications; the three others were long-term survivors and were sacrificed after several weeks. Two of the three control animals tolerated the procedure well; the third died at the end of the experiment because of mechanical failure of the respirator.

Oxygen Tensions. The average arterial oxygen tension (Table 1, Figure 1) in Cases 1, 2, 3, 4, and 5 rose from 90 to 550 mm Hg when air ventilation was replaced by oxygen ventilation. At 3 ata, the average values stayed between 1598 and 1684 mm Hg; after decompression, the values dropped to precompression values. Also, the control arterial oxygen tensions were relatively constant during the procedure. The venous oxygen tensions rose to rather high levels in several instances shortly after compression; usually this rise was followed by a sudden drop to levels that were still significantly above the precompression values. These changes occurred without changes in ventilation and without changes in the position of the cannula. The average venous pO_2 during oxygen ventilation at 1 ata was 44 mm Hg. At 3 ata, this value ranged from 255 to 97 mm Hg. In the control experiments, the venous oxygen tensions stayed at constant levels; the average values during oxygen ventilation ranged from 69 to 46 mm Hg.

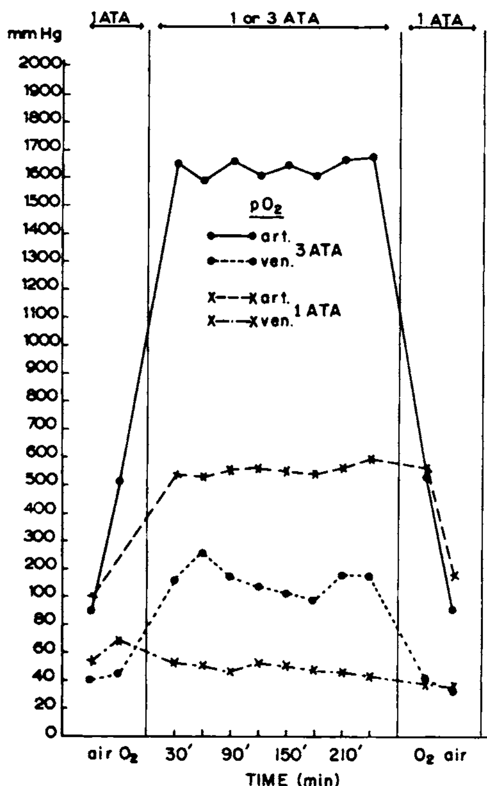

FIGURE 1. Oxygen tensions during ventilation at 3 ata for 4 hours.

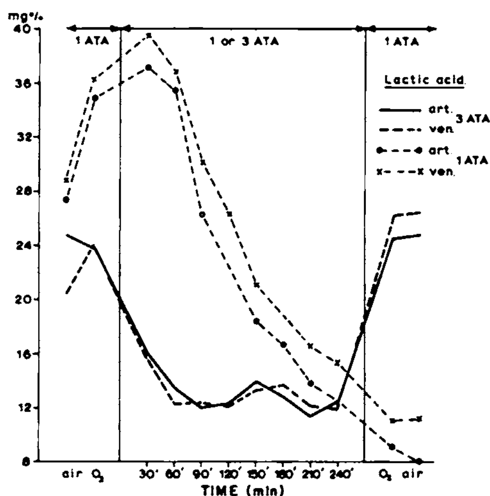

FIGURE 2. Lactic acid level during oxygen ventilation at 3 ata for 4 hours.

Lactic Acid. After the start of compression to 3 ata, the lactic acid level (Table 1, Figure 2) usually dropped rapidly and stayed at a low level during the entire 4-hour period of oxygen ventilation at 3 ata. After decompression, there was a rise to the precompression values. The arteriovenous differences were small and rather constant during the whole procedure. In the controls, the lactic acid levels dropped gradually during the procedure, and after 4 hours they were at the same level as in the high pressure group, despite the fact that they had been higher at the start of the experiment. In

TABLE 1. The Effect of 3 ata Oxygen on Canine Arterial and Venous Blood Parameters [a]

Parameter studied		Before compression		O_2 at 3 ata (compared with controls at 1 ata O_2)				After decompression	
		Air	O_2 15 min	60 min	120 min	180 min	240 min	O_2 10 min	Air 15 min
pO_2 (mm Hg)	Experimental	90	550	1598	1618	1614	1684	514	91
Aorta	Control	100	560	543	553	553	560	—	120
pO_2 (mm Hg)	Experimental	40	44	255	135	97	172	41	32
I.V.C.	Control	53	69	53	46	50	46	—	35
Lactic acid (mg%)	Experimental	23.3	23.8	13.4	12.3	12.8	12.1	24.5	24.8
Aorta	Control	27.3	34.8	37.2	26.3	18.3	13.7	—	8.0
Lactic acid (mg%)	Experimental	22.1	24.0	12.2	12.1	13.7	11.9	26.2	26.4
I.V.C.	Control	28.8	36.3	39.5	30.0	21.0	16.5	—	11.2
pH	Experimental	7.51	7.47	7.33	7.35	7.37	7.36	7.53	7.49
Aorta	Control	7.49	7.55	7.60	7.62	7.65	7.67	—	7.64
pH	Experimental	7.44	7.41	7.25	7.26	7.27	7.26	7.41	7.42
I.V.C.	Control	7.47	7.51	7.52	7.53	7.58	7.59	—	7.60
pCO_2 (mm Hg)	Experimental	28	26	46	44	43	44	24	28
Aorta	Control	33	25	22	20	20	18	—	18
pCO_2 (mm Hg)	Experimental	34	35	61	59	58	64	36	38
I.V.C.	Control	34	29	28	28	26	24	—	21
HCO_3^- (mEq/L)	Experimental	20.6	19.2	23.4	23.3	23.3	23.3	18.3	19.7
Aorta	Control	23.4	21.1	20.1	19.8	20.1	19.5	—	17.5
HCO_3^- (mEq/L)	Experimental	21.8	21.0	26.1	25.5	26.0	26.7	21.8	21.9
I.V.C.	Control	23.6	21.9	21.7	22.3	21.9	20.8	—	18.6
Glucose (mg%)	Experimental	92	88	87	80	80	94	91	93
Aorta	Control	86	86	89	107	76	78	—	99
Glucose (mg%)	Experimental	86	83	85	78	75	92	79	82
I.V.C.	Control	84	81	91	94	77	77	—	93
Potassium (mg%)	Experimental	2.7	2.5	2.9	3.1	3.3	3.5	3.3	3.0
Aorta	Control	2.6	2.6	2.4	2.3	2.4	2.4	—	2.2
Potassium (mg%)	Experimental	2.8	2.6	2.9	3.2	3.4	3.7	3.3	3.2
I.V.C.	Control	3.0	2.6	2.4	2.4	2.4	2.4	—	2.4
Hematocrit	Experimental	49	49	50	49	51	52	52	52
Aorta	Control	50	50	50	50	52	52	—	52
Blood press. (mm Hg)	Experimental	173	166	169	168	166	171	140	139
Systolic	Control	143	147	149	148	148	148	—	150
Blood press. (mm Hg)	Experimental	130	118	130	129	125	127	98	97
Diastolic	Control	113	116	111	116	116	120	—	95
Pulse rate	Experimental	168	150	135	130	117	133	178	168
(per min)	Control	179	173	172	168	179	183	—	180

I.V.C., inferior vena cava.

[a] Experimental values represent the averages of data from five dogs exposed to oxygen at 3 ata; control values are the averages of data from three animals given oxygen at normal atmospheric pressure.

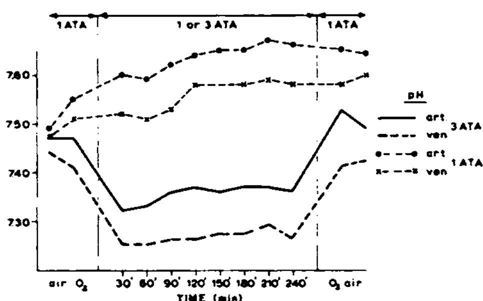

FIGURE 3. Levels of pH during oxygen ventilation at 3 ata for 4 hours.

almost all cases, the venous level was slightly higher than the arterial.

Levels of pH, pCO_2, and HCO_3^-. The levels of pH, pCO_2, and HCO_3^- are shown in Table 1 and Figures 3–5. After compression to 3 ata, the average arterial pH (Table 1, Figure 3) dropped from 7.47 to 7.32–7.33 and stayed rather constantly at this level. The same was true for the venous pH; this dropped from 7.41 to 7.25. After decompression, the pH values rose to 7.53 for arterial blood and 7.41 for venous blood. In the controls, the pH values remained significantly higher during a long procedure.

The average arterial pCO_2 (Table 1, Figure 4) rose from 26 mm Hg before compression to 45 mm Hg after 30 min at 3 ata. During 4 hours, only very small changes occurred. After decompression, the pCO_2 dropped to 24 mm Hg. The comparable figures for the venous pCO_2

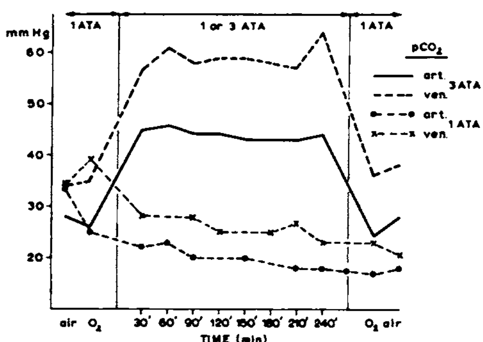

FIGURE 4. CO_2 levels during oxygen ventilation at 3 ata for 4 hours.

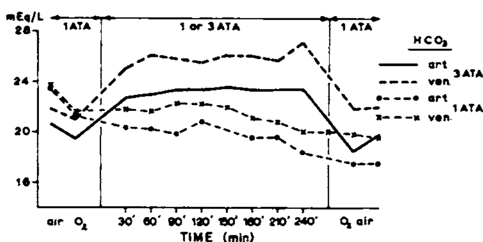

FIGURE 5. Bicarbonate levels during oxygen ventilation at 3 ata for 4 hours.

were 35, 57, and 36 mm Hg. In most of the controls, the pCO_2 values were lower than 35 mm Hg and the arteriovenous differences were smaller than at 3 ata.

The bicarbonate (Table 1, Figure 5) rose slightly after compression to 3 ata, dropping after decompression. This did not occur in the control group.

Glucose. In Cases 2, 3, and 5, the glucose levels (Table 1, Figure 6) were rather constant during the whole procedure. In Case 1, the arterial glucose level rose from 107 mg% before compression to 124 mg% after 90 min at 3 ata; thereafter there was a sudden decrease. In Case 4, a gradual and continuous rise was seen during the whole experiment. Also, in one control (Case 8) a sudden drop occurred after 2 hours of oxygen ventilation; the other figures were at a constant level.

The potassium levels (Table 1, Figure 7) in the chamber group showed a slight continuous rise from 2.5 mEq/liter before compression to 3.5 mEq/liter after 4 hours at 3 ata. In the controls, the potassium stayed at a constant level. The

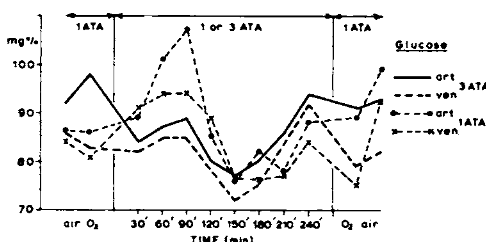

FIGURE 6. Glucose levels during oxygen ventilation at 3 ata for 4 hours.

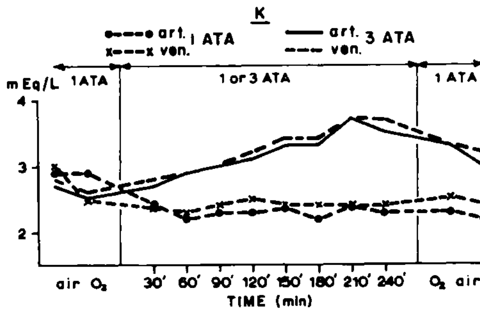

FIGURE 7. Potassium levels during oxygen ventilation at 3 ata for 4 hours.

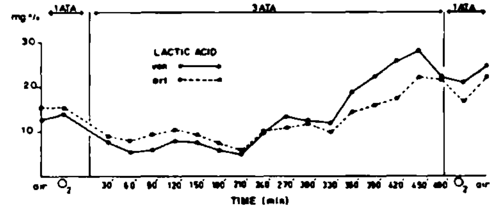

FIGURE 8. Lactic acid levels during oxygen ventilation at 3 ata for 8 hours.

hematocrit levels were constant in dogs treated in the chamber as well as in control animals. Also, the blood pressures were rather constant in chamber and control animals (Table 1). The pulse rate dropped during the period at 3 ata.

Eight Hours at 3 ata

One animal (Case 9) was subjected to 3 ata for 8 hours, during which time no convulsions occurred. The electroencephalogram showed no important changes until after 6 hours, when gradual deterioration was seen. After decompression, the blood pressure could be kept at a normal level only by administration of pressor amines. During the whole period at 3 ata, the arterial pO_2 remained at a high level: between 1800 and 2100 mm Hg. The venous pO_2 ranged between 35 and 65 mm Hg. After decompression, the arterial and venous pO_2 dropped to 82 and 27 mm Hg, respectively, with oxygen ventilation, and to 29 and 19 mm Hg, respectively, with air ventilation.

The lactic acid values during the first 4 hours were comparable to those in Cases 1–5; thereafter, a slight rise occurred, while a positive arteriovenous difference in the first period changed to a negative difference in the last 4 hours (Figure 8). Changes in the pCO_2 level occurred after 3.5 hours at 3 ata; the arterial pCO_2 rose to high levels and the arteriovenous difference became greater. After 30 min at 3 ata, the pCO_2 arteriovenous difference range was 38–44 mm Hg; after 3 hours it was 45–62 mm Hg, and after 8 hours it was 47–80 mm Hg. During the same period, the pH dropped gradually: in the first 4 hours from 7.35 to 7.25, in the last 4 hours from 7.25 to 7.15.

The glucose levels decreased slightly. Potassium levels rose slightly from 3.3 to 3.5 mEq after 4 hours and to 4.5 mEq after 8 hours. The hematocrit rose slightly, particularly in the last 4 hours.

DISCUSSION

The dangers of hyperbaric oxygenation, especially the risk of cerebral and lung damage, have been stressed in the literature; and, certainly, a thorough recognition and knowledge of the limitations and associated problems is essential to clinical application. Frequently, however, difficulties in general are emphasized in reports in which exact information about the conditions under which difficulties occurred is lacking. It seems to us that many of the hazards of OHP can be circumvented if certain conditions will be fulfilled; at least convulsions can be prevented if the anesthesia in ventilated animals is deep enough and if hyperventilation is performed. It should be noted, however, that in Case 9, the dog that was ventilated for 8 hours at 3 ata, no convulsions occurred, but at the end of the experiment a certain amount of cerebral damage was present. Thus, cerebral damage will occur without convulsions, convulsions being a symptom (probably

the earliest and most frequent). The question then arises whether the animals ventilated at 3 ata for 4 hours may have suffered cerebral damage. The autopsy reports are still under study, but three of the five animals were long-term survivors and, after the experiment, behaved quite normally. The fact that all animals were slow to awaken after the procedure may have indicated some reversible damage, but it can also be explained as a consequence of the deep Nembutal anesthesia. Electroencephalographic evidence (Figure 9), however, tends to exclude serious damage.

As long ago as 1932, Bean [5] reported that he had evidence of diminished oxidative processes with exposure of an animal to high oxygen pressures. In his study, exposure of dogs to pressures of about 5 ata was usually accompanied by an increase in blood lactic acid, occurring in 15 of 19 experiments. Recovery from the higher lactic acid levels was practically complete in six experiments on decompression. Six others showed only partial recovery, and in three more the lactic acid continued to increase during and after decompression. This was not observed in our study during a 4-hour period at 3 ata.

The possibility of blockage of enzyme systems by oxygen under increased pressure has been demonstrated several times. It can be expected that, despite great availability of oxygen, anaerobic metabolism occurs in many areas of the body. The oxidation of pyruvate, the glycolytic end-product of glucose or glycogen degradation, requires sulfhydryl-containing enzymes, and a prolonged exposure to hyperbaric oxygen might inactivate these enzymes with the result that pyruvate is converted to lactate. The enzyme studies reported in the literature were usually performed at pressures much higher than at 3 ata.

In our study, it was shown that blocking sufficient to result in anaerobic metabolism did not occur within 4 hours of oxygen administration at 3 ata. Case 9, where ventilation was carried out with oxygen for 8 hours, clearly indicated when the difficulties started. After 4 hours, the lactic acid level in this animal rose slowly and the previously positive arteriovenous difference turned negative. This probably shows that anaerobic metabolism starts in peripheral body areas where it was not present before. This change in arteriovenous difference must be explained as tissue anoxia, perhaps due to blockage of enzymes. Because it occurs when the arterial pO_2 is still at a high level, this lactic acid rise can be considered a symptom of so-called "hyperoxic anoxia." [6] The potassium rise in the last part of this study may be explained on the basis of tissue anoxia, although response to increased CO_2 levels will also play a role.[7]

At about the same time in the experiment, impairment of pulmonary gas exchange started, the arterial CO_2 level rose, and the arteriovenous difference in-

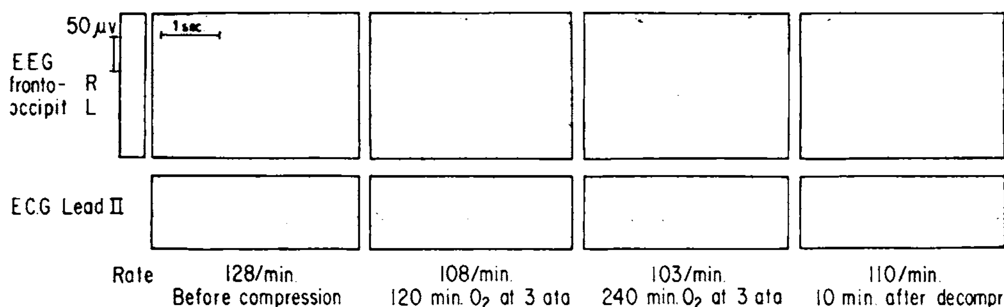

Figure 9. Electroencephalogram and electrocardiogram during oxygen ventilation at 3 ata for 4 hours.

creased. It appears that the fourth hour at 3 ata is a critical time under these experimental conditions, for in the five experiments in which decompression was started after 4 hours, the aforementioned changes did not occur. In all these cases, the lactic acid remained at a constant level, and the CO_2 did not rise after the initial rise during compression.

The blood glucose levels during oxygen ventilation under increased atmospheric pressure have been a point of debate for many years. It can be expected that glycolysis increases when oxygen blocks enzyme systems necessary to anaerobic metabolism. Although this is usually detectable in decreased blood glucose values, the blood glucose level may remain constant at the expense of glycogen stores in liver and muscles.

Historically, Bert (1878)[8] found that the uptake of glucose was diminished in animals by exposure to oxygen under increased pressure; then, in 1934, Iwanow[9] reported an increase in glucose; and in 1939, Ishikawa[10] found the blood glucose to be increased in rabbits which had been exposed to compressed air from 60 up to 130 psi. In 1957, Sullivan and Bean[11] concluded from experiments on rats exposed for 20–40 min to oxygen at 90 psi that elevation of blood glucose preceding exposure provided some protection against OHP and that the initial elevation of glucose induced by oxygen under increased pressure is a part of a defense mechanism operating through the hypothalamus and sympathetic nervous pathway. In our 4-hour exposure experiments, changes were very small; in the 8-hour exposures a slight drop occurred in the glucose level after several hours. In other experiments, where convulsions occurred, the glucose level rose after convulsions, probably as a stress effect. It is unlikely that critical changes in metabolism occur within 4 hours, when lactic acid and blood glucose levels remain relatively constant. During the 8-hour exposure, the drop in glucose level was rather small, and it may be that there was a concurrent decrease of stores in the liver.

In all animals exposed to 3 ata of oxygen, blood pH levels were lower than in control animals at 1 ata. In a previous study,[12] we found no significant differences in pH, but possibly the animals in that study were more ventilated at 3 ata. On the other hand, in this study the venous pO_2 levels were higher and the oxygenation better, because no thoracotomy was performed (higher venous pO_2 levels mean more difficulty in CO_2 removal). In these cases, we kept the gas flow constant, but we did not calculate possible changes in recording of the flowmeters by density changes of the gases.[13] The pH drop and pCO_2 rise point to the necessity of a higher pCO_2 and a greater arteriovenous CO_2 difference to keep the blood CO_2 constant. It is unlikely that impairment of the carbonic anhydrase plays an important role during the first 4 hours at 3 ata because of the rather constant levels of pH and pCO_2 after reaching 3 ata. The increase in bicarbonate can be explained as a response of the kidneys to restore the normal ratio of H_2CO_3 to $NaHCO_3$, in case of increased pCO_2. Over a 4-hour period, it appears that a constant level is reached quickly and that it persists during that period. Over an 8-hour period, changes occur toward the end of exposure time.

The fact that systolic and diastolic blood pressures remained at the same levels excludes the possibility of large areas of vasoconstriction, even after several hours; this is in agreement with previous findings for shorter periods of time. The definite decrease of pulse rate with the unaltered blood pressure is likely to result in decrease of cardiac output.

Summary

As a result of studies on nine dogs ventilated with oxygen at 1 ata and 3 ata for periods from 4 to 8 hours, the following conclusions were drawn.

If the anesthetic level is deep and hyperventilation is carried out, convulsions can be prevented. Biochemical signs of inadequate tissue oxygenation and impaired gas elimination start after 4 hours. Consequently, we believe that hyperbaric oxygenation can be safely employed in clinical situations at 3 ata for more than 2 hours if (1) the disease process makes OHP therapy advisable, (2) the abovementioned conditions are observed, and (3) electroencephalographic and biochemical monitoring are carried out.

ACKNOWLEDGMENTS

These studies were performed with the technical assistance of A. B. Bulterijs and Miss C. de Boer.

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DISCUSSION

Dr. C. Hitchcock (*Minneapolis, Minn.*): Dr. Meijne, in the animals that were under anesthesia for a long time (5 to 8 hours at 3 atmospheres) how much trouble did you have with the lungs? In our experience, we have had a significant number of animals which developed, even in that period of time, what we considered to be the Lorrain-Smith phenomenon. Also, did you do any pathologic studies on the brain, kidney, or liver of the animals that had been kept at 3 atmospheres for 3 or more hours?

DR. MEIJNE: We only studied the lungs. Those animals that were ventilated at 3 atmospheres for 4 hours and then died after a few days did have lung changes that correlated with oxygen toxicity. Those that survived were slow to recover from the anesthesia, but they appeared clinically normal, and no pathologic studies were made. Of the animals that were given carbon dioxide, many had brain damage and changes in the lungs. One important point is that they can be ventilated for a long time if they are in good condition. They do not get brain damage from one or two convulsions. On the other hand, if they are ventilated for a long time and have many convulsions, most will fail to wake up and will eventually die.

DR. I. LEDINGHAM (*Glasgow, Scotland*): Was the respirator output kept constant, while changing from air at 1 atmosphere to oxygen at 3 atmospheres? Did I understand correctly that the arterial pCO_2 rose and arterial pH fell? Were the venous samples taken from the right atrium or from the low vena cava?

DR. MEIJNE: The venous samples were drawn from the low inferior vena cava. The ventilation was a semiclosed system kept at a constant rate. The pH did fall and the pCO_2 increased. In fact, there was always some increase in pCO_2 during compression. The level of pCO_2 remained constant up to 4 hours of oxygen ventilation. After 4 hours there may be a further increase in pCO_2, which may be due to lung damage.

DR. R A. COWLEY (*Baltimore, Md.*): Dr. Meijne, did most of your animals convulse by the end of 4 hours with light anesthesia?

DR. MEIJNE: With light anesthesia, they do convulse early.

DR. COWLEY: Did the animals survive if the anesthesia was deepened after 4 hours, or had they already developed pulmonary damage?

DR. MEIJNE: If the animals had convulsed, we did not try to continue beyond 4 hours.

Biochemical Pathology of Oxygen Toxicity: Enzymic Studies at the Cellular Level

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Paul Bert, with his description of the inhibition of "ferments" by hyperbaric oxygen concentrations in 1878, was the first to recognize the toxic effect of oxygen on some enzyme systems.[1] His studies of proteolytic inhibition in meat, blood, urine, and egg albumin were largely ignored, however, and the first systematic biochemical studies of oxygen effects on enzymes involved oxidative enzymatic activity almost exclusively.[2-6] This was extremely fortunate, since the need of many oxidative enzyme systems for sulfhydryl groups led to recognition of the role of oxygen in oxidizing thiol compounds of biological importance and thereby inactivating the dependent enzyme system.[7]

It is important that one recognize that enzyme systems other than those directly in the scheme of intermediary metabolism may be affected by oxygen, especially when one seeks to explain mechanisms of oxygen toxicity in the production of specific pathology in exposed experimental animals, and even in humans. Generally, at the cell and tissue levels, interference with normal schemes of oxidative metabolism leads to alternative pathways of oxidative phosphorylation with a significantly lower energy yield, to anaerobic metabolism for varying periods, or to abrupt cessation of basic cellular metabolism and cell death. In short, the organism as a whole could not long survive under these conditions, and there would scarcely be time for any characteristic pathology to occur other than that concerned with death. On the other hand, interference with enzymic mechanisms other than those involved in basic cellular respiratory function can result in the production of specific pathology, especially in situations where the organism would still be surviving and perhaps even recover.

For this reason, we became interested in studying the proteolytic enzyme systems originally described by Paul Bert as inhibited by oxygen. We chose a series of intracellular peptidases known collectively as cathepsins because these are known to be SH-dependent and can be studied both by *in vitro* assay and by reasonably reliable cytochemical visualization methods. Furthermore, they appear to be present in nearly all kinds of cells, from single-celled protozoa to metazoan cells and tissues.

Materials and Methods

Two hundred and fifty male and female rats of the Holtzman strain (250–300 gm) were used for the experiments. For *in vitro* studies of lung, tissue was removed from the animal with only moderate aseptic precautions. All glassware used was autoclaved. Animals were exposed to 100% oxygen at normal atmospheric pressure for various times up to 60 hours. Animals were kept in cages enclosed in a polyethylene case fitted with inlet and outlet tubes for gas delivery. These were placed to achieve maximal circulation; some outlets served for temperature and gas monitoring of the contents. With this arrangement, 30 or more animals could be treated simultaneously for several days while in their own cages. Continuous monitoring of all cages for oxygen concentration was done with a Beckman polarographic oxygen analyzer. Determination of carbon dioxide with gas chromatography was undertaken every 60 min at first, but with the rapid oxygen delivery (10–12 liters/min), two or three daily monitorings for carbon dioxide sufficed to establish no accumulation.

Two kinds of biochemical determinations were performed. The first, used to indicate any changes in amount of protein in a constant mass of lung tissue, was done by a modification of the method of Hirs *et al.*,[8] involving tryptic hydrolysis. Lung tissue was homogenized in an Elvejhem glass homogenizer and denatured with 6.8 M urea; S–S bonds were split with performic acid. Protein was hydrolyzed at room temperature at pH 7.5 with 5×-crystallized trypsin (in 50% magnesium sulfate solution). Enzymatic hydrolysis took place in phosphate buffer, and sample tubes were removed at times from zero to over 72 hours of exposure to trypsin. Complete hydrolysis (100%) generally occurred by 60 hours in our experimental material. The product of hydrolysis, as determined by hydroxyl groups of liberated amino acids, was read with ninhydrin reagent at 570 mμ in a Beckman spectrophotometer. Since the time required for complete hydrolysis of lung tissue made automatic titrations desirable, this was accomplished by using salt-free solutions[9] in which hydrolysis was carried out and then measuring the alkali consumption required with time to maintain pH at a constant value. The amount of titrating solution (0.1 M triethylamine) utilized for maintenance of constant pH at 60-min intervals was taken as a direct measurement of carbon dioxide absorption from the atmosphere, as compared to alkali consumption from a blank.

The second biochemical determination was an assay for endogenous catheptic activity, using known protein substrates. For this, denatured bovine hemoglobin was digested under standard conditions by enzymic activity contained in a homogenate of normal rats' lungs as well as lungs from animals exposed to 100% oxygen at atmospheric and hyperbaric pressures. The undigested hemoglobin was precipitated with trichloroacetic acid and the remaining soluble hydrolytic product measured by ultraviolet spectrophotometry at 280 mμ.[10] This method was also used to determine catheptic activity in a variety of biological materials, including protozoa.

Results

Endogenous Enzymic (Catheptic) Activity in Normal Lungs

The normal level of catheptic activity detectable in lungs of the animals assayed is shown in Figure 1. By 8 hours, 95–98% hydrolysis of the hemoglobin substrate was achieved. Frequently, maximal hydrolysis occurred by 6 hours. We were initially concerned that some of this activity could be derived from bacterial action. Similar results, however, were obtained with lung tissue excised and kept under aseptic conditions. Indeed, the data from such excised sterile lung frag-

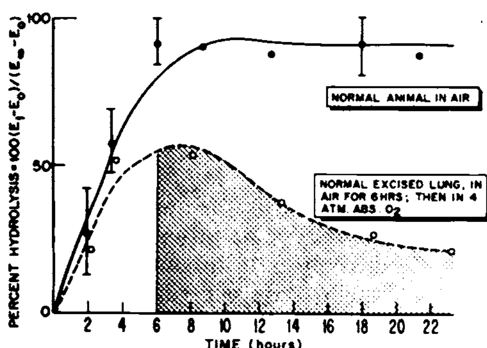

FIGURE 1. Catheptic activity in constant-volume samples of lung homogenate shown as a function of hydrolysis of substrate with time (E_t = extinction coefficient at time t; E_0 = extinction coefficient of the blank; E_∞ = extinction coefficient of complete digestion). Enzymic activity of normal lung tissue removed from a normal animal, left in air for 6 hours and then placed under 4 ata of O_2 (shaded area).

ments showed essentially the same pattern as that from lungs freshly removed and assayed with no sterile precautions. When excised lung fragments were exposed to hyperbaric conditions (4 ata of oxygen), however, there was an abrupt decline in the hydrolytic activity of the tissue toward a hemoglobin substrate (Figure 1).

Catheptic Activity in Cells and Tissue Exposed to OHP in vitro

Figure 2 summarizes some experiments designed to illustrate that pressure itself played no role in the catheptic inhibition by oxygen at elevated pressures under the conditions we employed. While the graph deals specifically with protozoa, lung homogenates showed similar results. With 7 ata of pure nitrogen superimposed on 1 ata of air, hydrolysis of hemoglobin proceeded at the level seen with cells exposed to air at normal atmospheric pressure or killed with a thermal shock (10 min at 37° C). Under 8 ata of pure oxygen, however, nearly complete inhibition of proteolytic activity occurred (Figure 2).

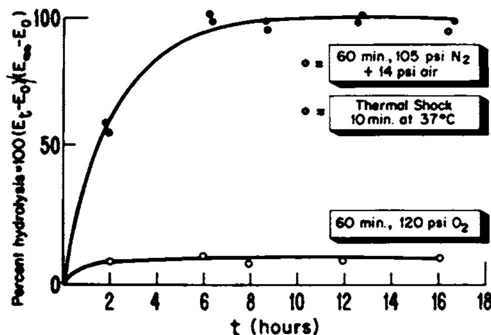

FIGURE 2. Catheptic activity with time of constant-volume samples of a homogenate of *Paramecium caudatum*. Aliquots were exposed for 60 min to 120 psi O_2, to 105 psi N_2 + 14 psi air, and to thermal shock, after which the assay was begun.

Several factors appeared to influence the degree of inhibition of catheptic activity by oxygen, including the pressures employed and the duration of oxygen exposure, both of which determined the overall concentration of gas available for inhibition of enzymic activity. A third factor, temperature, also played a significant role in the oxygen-mediated inhibition. Figure 3 illustrates differences in maximal hydrolysis obtained from homogenized protozoa after three different exposure times (*in vitro*) to 120 psi of pure oxygen. Under these pressures, there was little or no indication of catheptic activity with the 60-min exposure. With 10- and 20-min exposures to 120 psi oxygen, however, enzymic activity recovered upon return to air; with 45-min exposure to 120 psi oxygen, partial re-

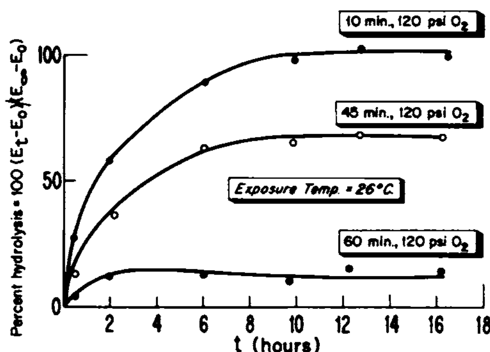

FIGURE 3. Catheptic activity with time of constant-volume samples of a homogenate of *Paramecium caudatum* as a function of exposure to 120 psi O_2 for different times.

covery appeared to take place upon return to air.

Effects of OHP on Total Tissue SH in vitro

We assayed total tissue SH in *in vitro* studies under a variety of conditions, using both normal atmospheric air and hyperbaric oxygen at several exposure times. Sulfhydryl groups were determined by an amperometric titration or by stoichiometric binding with mercaptides. These results showed that levels of tissue SH decreased with increased exposure to oxygen (Figure 4). Indeed, even the SH levels in tissue used as controls and exposed to normal air decreased. This might be expected, however, in view of the lability to oxidation on the part of these radicals. Treatment with a variety of "SH-protective" agents during oxygen exposure (glutathione, cysteine, thiosuccinate, aminoethylisothiuronium, and mercaptoethylamine) appeared to alter the rate of oxidation of tissue SH but did not provide a continuous protective effect.

Since it was now possible to relate the presence or absence of SH to enzymic activity, a second series of experiments dealt with the possible protective effect of SH reagents on pulmonary tissue catheptic activity. Using *in vitro* systems (Figure 5) similar to that described above, it could be demonstrated that glutathione or cysteine provided increased enzymic activity only following exposure of homogenate to oxygen (in other words, when the homogenate had been returned to normal air). Potentially protective compounds used while tissue homogenates were exposed to hyperbaric oxygen had no influence on inhibited catheptic activity (Figure 5).

Hydrolysis of Peptide Bonds in Pulmonary Tissue of Untreated and Oxygen-Exposed Rats

Another series of experiments, performed to extend our *in vitro* experiments outlined above, used tissue from intact animals exposed to oxygen. When normal rats are exposed to 1 atm of 100% oxygen, by 36–40 hours their lungs become filled with a proteinaceous effusion lead-

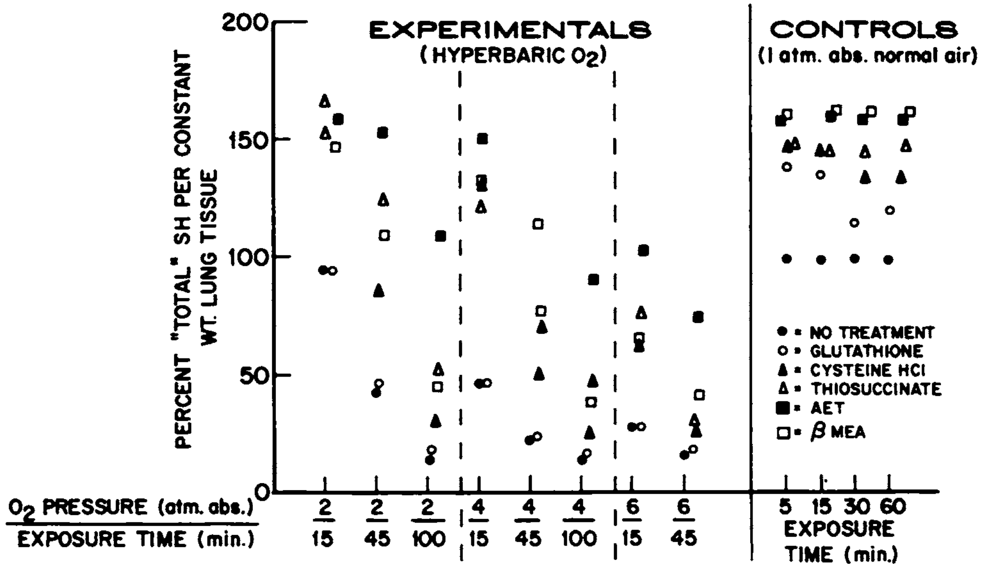

FIGURE 4. Percent of total tissue SH in lung homogenates from normal rats exposed *in vitro* to various pressures of O_2 for different times. Various protective compounds were added to the homogenates while under pressure.

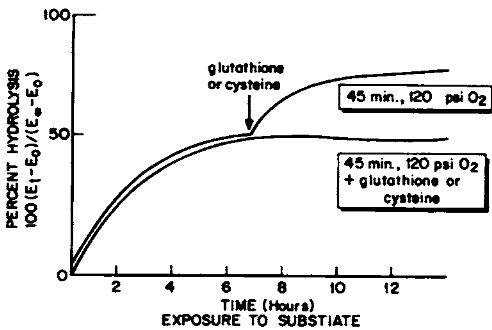

FIGURE 5. Recovery effect of SH-protective compounds on catheptic activity added after exposure of lung homogenate from a normal rat to 120 psi O_2 for 45 min. Samples of protozoa homogenate exposed to identical O_2 concentrations with SH-protective reagents showed no increase in enzymic activity (compare with Figure 3).

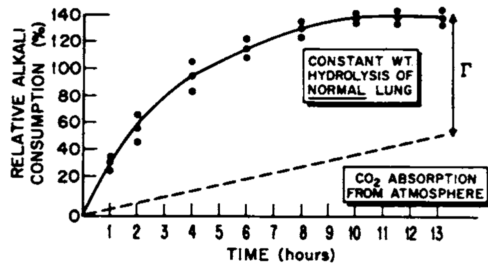

FIGURE 6. Alkali consumption (triethylamine) with time required to maintain constant pH of the tryptic hydrolysate of normal rat lung homogenate. CO_2 absorption from the atmosphere was controlled by a blank which was employed in the final determination of the Γ equivalent to relative protein concentration as a function of peptide bonds available. These values are essentially those employed on the ordinate in Figure 7.

ing to pulmonary consolidation (widely termed "oxygen pneumonia"), first described by J. Lorrain Smith.[11] The effusion contains variable amounts of protein showing considerable quantitative and perhaps qualitative variation.[12,13] It is doubtful that the criteria separating transudates from exudates in humans [14] can be applied to studies with rats, and we are as yet uncertain how to more accurately describe this protein.

When aliquots of homogenized normal rat lung were assayed by trypsin hydrolysis for the amount of peptide bonds present (as a measure of protein content), maximum alkali consumption based on the automatic titration of the products of hydrolysis occurred by 10 hours. On a constant-weight basis, these values can serve as a base line for protein content of normal rat lung (Figure 6). For these experiments, we preferred this method to the more standard nitrogen determinations, especially since our protection experiments used high levels of compounds themselves containing nitrogen.

Rats maintained in pure oxygen at normal atmospheric pressure were killed at various times after initial exposure, and aliquots of their homogenized lungs were examined by the trypsin hydrolysis method for quantitative assay of peptide bonds. As indicated in Figure 7, for up to 16 hours the level of demonstrable peptide bonds fell at or below the range of 1.0 established as normal by our experiments with lungs from normal control animals. With 24-hour exposure to 100% oxygen, there was a significant rise in the amount of peptide bond material (protein) which reached a plateau by 28 hours. There was a second, less abrupt rise in protein after 40-hour exposure to oxygen. These assays for the presence of peptide bonds signified to us the increase in protein trapped in the alveolar spaces as a result of pulmonary effusion. At present, we are unable to distinguish

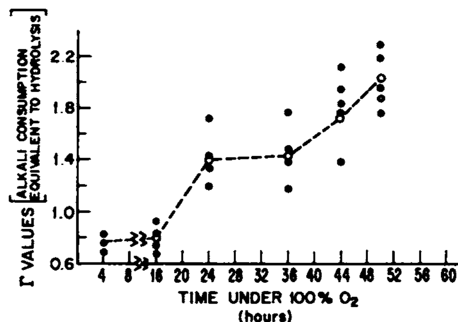

FIGURE 7. Increase in alkali consumption as indicative of the amount of peptide bonds available in lung homogenate from rats exposed to 100% O_2 at normal atmospheric pressure for 4, 16, 24, 36, 44, and 50 hours.

any possible qualitative differences between the "early" and "late" effusion. We are currently exploring this problem with electrophoretic and cytochemical methods.

In association with this data, the endogenous proteolytic capacity of lungs from such oxygen-exposed animals decreased steadily as the animals were subjected to oxygen for increasing times (Figure 8).

Under pure oxygen, therefore, the level of proteolytic activity in the lungs of living animals appeared to fall, as described for *in vitro* lung homogenates (Figure 1) and for protozoa under hyperbaric concentrations of oxygen (Figure 3).[15]

Discussion

The observations presented here show the capacity of hyperbaric oxygen to suppress or totally inhibit some kinds of cellular proteolytic activity. We have previously reported on the prevention of cytolysis in protozoa[15] and in amphibian eggs.[16] Aside from Bert's early observations, no studies have dealt with hyperbaric oxygen in producing such inhibitions, although several investigators have reported inhibition of necrosis under conditions favoring local oxidation.[17,18]

The inhibitory action of oxygen on proteolytic enzymic activity has implications with respect to etiology of the pulmonary pathology in oxygen toxicity. This becomes most evident in experimental situations where animals exposed to oxygen are removed from such atmospheres and recover. These animals invariably show beginnings of alveolar protein accumulation, but it does not appear to be of the same nature as that seen in the acute stage characteristic of the Lorrain-Smith "oxygen pneumonia." This early pathology is reversible and shows a characteristic morphology, including many macrophages with high levels of esterase and acid phosphatase activity. Also, similar enzymic activity is present in the alveolar epithelium of such animals.[19] Most of this esterase activity is fluorophosphate-resistant and thus appears related to *c*-type cathepsins, the same class of enzymes that we are here concerned with biochemically. This same class of enzymes is also known to be SH-dependent. Possibly oxygen acts, in this early reversible stage of oxygen poisoning, to inhibit that kind of enzymic activity which ordinarily breaks down protein, and the oxygen thus provides a more suitable substrate for subsequent phagocytic activity by macrophages. It is doubtful whether inhibition of cathepsins or any SH-dependent protease activity would have any direct effect on those effusions taking place during the terminal stages of oxygen toxicity in the rat, presumably by increases in capillary permeability.[20] The action of oxygen on SH-labile proteases is perhaps the basis for the recent methods of organ preservation using hyperbaric oxygen in conjunction with decreased temperatures.[21]

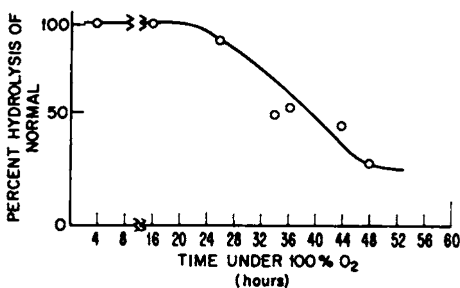

FIGURE 8. Catheptic activity in lung homogenates from rats exposed to 100% O_2 at normal atmospheric pressure for various times. Activity is expressed as percent of normal.

The question remains whether our *in vitro* data with rat lung homogenate can be applied to the situation in the intact animal exposed to oxygen. While the rapid oxidation of tissue SH in homogenates exposed to oxygen might be expected, in this material even atmospheric concentrations of oxygen readily cause loss of unstable SH radicals. Whether this occurs so rapidly in intact animals is questionable. For instance, continuous turnover of redox states occurs in intact tissue and, as yet, we have been unable to test

the capacity of this tissue to maintain reduced SH under hyperbaric oxygen concentrations.

A final question concerns the site of oxygen damage at the cellular level, especially with respect to the catheptic enzymes that we are here concerned with. One possibility is that the SH-dependent enzymes themselves are directly affected by oxygen, a situation which would be similar to that described for oxidative enzymes.[4,5,7] On the other hand, many intracellular proteolytic enzymes are restricted to subcellular particulate structures, the lysosomes,[22] which themselves may be affected by oxygen. We have elsewhere suggested[15,16] that oxygen in sufficient concentration may prevent the disruption of the lysosomal lipid membrane, which could prevent leakage of enzyme into cytoplasmic compartments. Alternatively, stabilization of the lysosome membrane by oxygen in sufficient concentration could prevent substrate from coming into contact with retained enzyme. We are currently exploring this question, using tissue-culture methods and techniques employing protozoa similar to those discussed in this report.

ACKNOWLEDGMENTS

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DISCUSSION

UNIDENTIFIED SPEAKER: I would like to comment on lysosomes and lipid peroxidation, as they explain some of the phenomena of oxygen toxicity. We have observed very little, if any, effect on the lysosomal enzymes under 100% oxygen. However, I would disagree partly with the opinion expressed that peroxidation attacks lysosomal membranes. We have done some experiments that show that this is not the case.

DR. ROSENBAUM: It is certainly equivocal whether bound hydrolases are released from all mammalian tissues by oxygen and whether they can then be inactivated by further exposure to it. The easiest *in vitro* system to employ for a study of effects of oxygen on lysosomal membrane is, of course, liver. The enzyme assay of choice would, in this case, be acid phosphatase. However, acid phosphatase activity within lung is largely confined to macrophages where there is a rich lysosomal population. This localization makes it quite difficult to separate activity in pulmonary epithelium from that within macrophages. There is a very high activation in pulmonary epithelium, and therefore most of our studies employ cathepsin activity. Sometimes, the oxygen inactivation of the lysosome–enzyme complex takes so long (up to 18 hours) that we feel there may be stages in the inactivation. Perhaps this reflects stages in the oxidation of the lipid membrane. We are still quite uncertain whether the lysosomal membrane is affected by oxygen, or whether the effect is primarily on released or contained bound hydrolase. Perhaps it is both. Finally, I suspect that, especially with *in vitro* experiments with oxygen inactivation, one must always be aware of the problem of adequate amounts of gas being at the proper site for a sufficient length of time. For this reason, hyperatmospheric pressures should be used, even with final oxygen concentrations of 100%.

Quantitative Electron Microscopic Studies of Murine Lung Damage after Exposure to 98.5% Oxygen at Ambient Pressure: A Preliminary Report

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The therapeutic application of oxygen at high partial pressures has brought with it the hazard of lung damage resulting from prolonged exposure to a hyperoxic environment. This danger has been well documented.[1,2] The most important alterations known to occur in the respiratory tract of men and animals are: changes in airway structure,[3-8] vascular bed,[3,9,10] and lung tissue[3,7,11] and the appearance of exudate and hemorrhage in the chest cavity.[7]

Changes in airway structure include: (1) atelectasis,[3] (2) alveolar exudate with accumulation of leukocytes, erythrocytes, and macrophages,[3-8] and (3) formation of "membranes" at the alveolar wall.[3]

Changes in the vascular bed include: (1) hyperemia,[9,10] (2) agglomeration of blood cells in capillaries,[3,10] and (3) capillary proliferation.[10]

Changes in lung tissue include: (1) peribronchial and perivascular edema,[3,7] (2) accumulation of fluid and cells in the interstitium,[3,7] and thickening of the barrier,[3,11] and (3) swelling of mitochondria in the alveolar epithelium.

These pathologic changes are observed, for the most part, in terminal phases of oxygen toxicity. The observations reported in this paper are concerned with events prior to terminal toxicity and trace the time-course of alterations from their inception until the final stages. Morphometric methods were used to quantitatively evaluate the results.

Materials and Methods

Seventy-six purebred male Sprague-Dawley rats born on the same day were divided into four groups and simultaneously exposed in individual cages to 98.5% oxygen at 765 mm Hg for 6, 24, 48, and 72 hours. Fourteen rats, kept in room air under comparable conditions, were used as controls. (Vital statistics of the animals studied appear in Table 1.) Exposure of the test groups to oxygen was done in one of the environmental chambers at the 6570th Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio. Technical design and facilities of this chamber have been described elsewhere.[12] A special study

TABLE 1. Exposure of Sprague-Dawley Rats to 98.5% Oxygen

Group	O_2 exposure (hours)	Age at sacrifice (days)	Body wt. (gm)	Vol. of fixed lungs (ml)
1	6	47	116 ± 1.6	5.2 ± 7.4
2	24	48	119 ± 7.8	6.4 ± 0.41
3	48	49	123 ± 9.6	6.6 ± 0.37
4	72	50	102 ± 13.3	4.4 ± 0.33
Control	0	44	123 ± 5.7	5.6 ± 0.60

±, standard deviation.

failed to demonstrate the presence of toxic contaminants.[13] The conditions of the chamber atmosphere during the course of the experiments are shown in Table 2.

At the end of the exposure period, the animals were deeply anesthetized by intraperitoneal injection of pentobarbital (Nembutal, 5 mg/100 gm body weight), brought out of the chamber, and weighed. A tracheostomy was performed immediately and the chest was punctured to collapse the lungs. A fixative solution of 2.5% glutaraldehyde buffered to pH 7.4 with 0.03 M potassium phosphate was instilled into the lungs through a tracheal cannula. The instillation pressure was standardized at 20 cm H_2O. The trachea was then ligated and the heart and lungs were removed *en bloc* and submerged in the fixative for 2 hours. After dissection of heart and mediastinal tissue from the lung, the lung volume was measured by fluid displacement. Subsequently the lungs were sliced into alternately thick (3–5 mm) and thin (1 mm) slices. The thick slices of each lung were embedded in celloidin–paraffin for light microscopy. The thin slices were cut into about 250 small cubes,

TABLE 2. Conditions of Exposure in Chamber

O_2 concentration	98.5 ± 1%
CO_2 concentration	< 0.1%
Total ambient pressure	765 mm Hg [a]
Relative humidity	46 ± 1%
Temperature	23.3 ± 1°C

±, standard deviation.
[a] 740+25 mm Hg.

approximately 2–3 mm^3, washed in three changes of 0.11 M potassium phosphate buffer for 2 hours, placed in 1% OsO_4 buffered to pH 7.4 for 90 min, and embedded in Epon 812 according to the method of Luft.[14] Sections of 600–900 Å in thickness were obtained on an LKB Ultrotome using a DuPont diamond knife; they were picked up on 150-mesh copper grids fitted with a thin Formvar film reinforced by carbon. Section contrast was enhanced by lead citrate.[15]

All measurements were made using stereologic principles.[16,17] The volumetric composition of the tissue was analyzed by point-counting.[18] Surface areas were estimated on the basis of probability of intersection with randomly placed linear probes,[19] and surface:volume ratios were calculated by the method of Chalkley.[20] Arithmetic mean and harmonic mean of the air–blood barrier thickness were obtained by methods previously described.[21]

A systematic procedure of random sampling of the various sections was utilized so that only a minute sample of the total material need be studied.[16] This technique was applied in: (1) sampling of sections for light microscopy, (2) sampling of sections for electron microscopy, and (3) sampling of test points for measurement.

RESULTS

Gross Observations at Autopsy

The first two experimental groups breathed oxygen for 6 and 24 hours. At

autopsy, the pleural cavity was free of exudate and the gross appearance of the lungs was normal. In animals removed after 48 hours of exposure, the pleural surface of the lung was mottled; dark red and light patches a few millimeters in diameter were present. In all cases after 48 hours of exposure, the pleural cavity contained a yellowish exudate which was occasionally hemorrhagic. After 72 hours of exposure, the pleural cavity of all animals contained large amounts of partly hemorrhagic exudate, and the mottling of the pulmonary surface was pronounced.

Changes in Fine Structure of the Alveolocapillary Region

Alveoli. In the first three groups and in the controls (Figure 1A), alveoli appeared normal in the light microscope with the exception of a few scattered foci of exudate formation in the lungs of animals that had breathed oxygen for 48 hours. In contrast, the light microscope revealed striking changes in a large fraction of alveoli of those lungs which had been exposed to oxygen for 72 hours (Figure 1B). These alveoli were filled with exudate which often contained a network of fibrin strands and numerous macrophages, erythrocytes, and leukocytes. Volumetric analysis showed that 65% of the terminal airways were obliterated by this exudate, while 35% appeared air-filled (Table 3). Profuse alveolar exudate developed, then, during the third day of oxygen-breathing.

The total alveolar epithelial surface area was measured as 0.38 m^2 in electron microscope preparations. Figure 2 shows that no significant changes in this alveolar surface area were observed. This may be interpreted to indicate that the architectural structure of the lung did not change in the course of the experiments, although electron microscope studies showed no clear differentiation between

TABLE 3. Volume Fractions of Normal and Damaged Alveoli

	Alveolar volume	
Type of alveoli	Untreated controls	Rats exposed to 72 hours of O_2 at 1 atm
Normal	100%	35 ± 0.5%
Obliterated by edema fluid	—	39 ± 0.5%
Fibrin-containing	—	26 ± 0.5%

±, standard deviation.

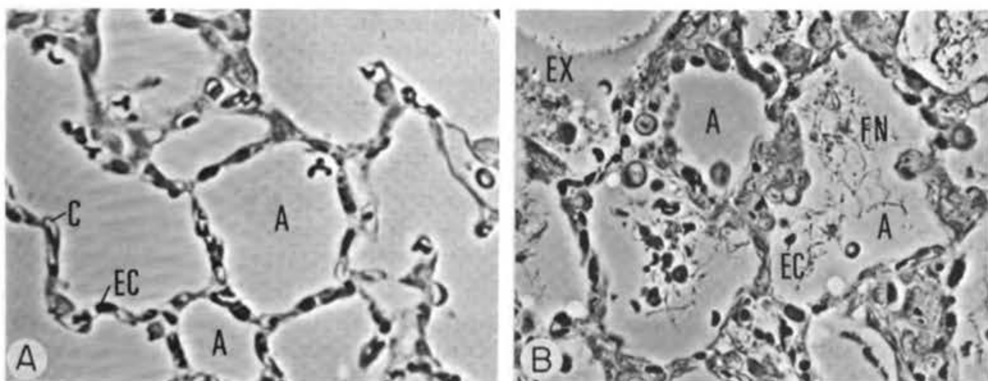

FIGURE 1. A, phase-contrast micrograph of a control lung (× 340). Normal alveoli (A) and capillaries (C). Note red blood cells (EC) in the capillary bed.

B, phase-contrast micrograph of lung after 72 hours of oxygen exposure (× 340). Alveoli (A) filled with exudate (EX) containing erythrocytes (EC), cell debris, and fibrin threads (FN). Note marked thickening of interalveolar septa with increased number of cells and obliteration of capillaries.

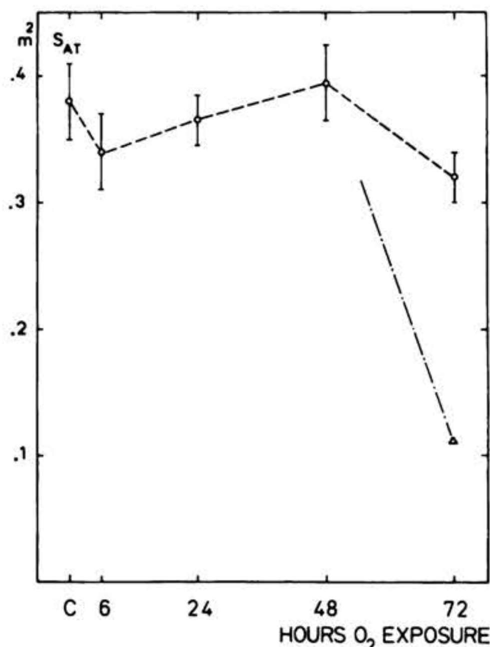

FIGURE 2. Total alveolar surface area of rats exposed to 98.5% oxygen. Circled points (o) represent measurements of *total* alveolar surface area (S_{AT}). Triangular point (Δ) is an estimate of the alveolar surface area available for gas exchange based on measurements of that fraction of total alveolar volume which was filled with exudate.

fluid-filled and air-filled alveoli. Volumetric determination of free lung space made it apparent, however, that after 72 hours of oxygen exposure no more than one-third of the total alveolar surface remained in contact with the gas phase.

The changes seen were distributed throughout the lungs with no particular lobular localization. Under light microscopy, edema was seen primarily in those alveoli which had alterations in the interalveolar septa. Even in the heavily damaged lungs (after 72 hours of exposure to oxygen), however, there were areas in which no pathologic changes were apparent by either light or electron microscopy.

Capillary Bed. The light microscope revealed no striking changes in the pulmonary capillary bed after any of the periods of oxygen exposure. Electron microscope examination, however, showed the first signs of destruction of capillary endothelial cells after 48 hours of exposure. In some scattered areas, the endothelial lining lost its continuity and sharp definition. After 72 hours, the capillaries of large areas of the lung were almost unrecognizable as such; the capillary endothelial cells were necrotic and the lumen contained distorted and fragmented red blood cells. In some areas, the endothelium had completely disappeared and erythrocytes were in direct contact with the boundary membrane (Figure 3). Morphometric measurements

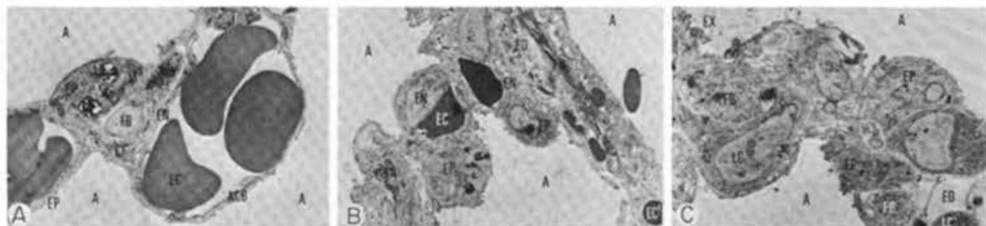

FIGURE 3. A, electron micrograph of control lung (× 2400). Alveolocapillary tissue barrier (ACB). Interstitial space, containing a fibroblast (FB) and collagen fibrils (CF). Alveolar epithelial cell (EP) with lamellated bodies (LB). Capillaries containing erythrocytes (EC) and thrombocytes (T). Alveoli (A), capillary endothelial cell (EN), mitochondria (M).

B, electron micrograph of lung after 72 hours of oxygen exposure (× 1600). Interstitial edema (ED) containing fibrin threads (FN) and erythrocytes (EC′). Note marked thickening of the interalveolar septum and destroyed capillaries.

C, electron micrograph of lung after 72 hours of oxygen exposure (× 1600). Interstitial edema (ED) with leukocytes (LC′). Alveoli contain exudate (EX). Note absence of red blood cells and increased number of other cellular elements.

confirmed the microscopic observations; during the second and third days of oxygen exposure, the capillary volume dropped from 0.38 ml to 0.17 ml (Figure 4) while the surface area of capillary endothelium fell from 0.34 to 0.17 m² (Figure 5).

Air–Blood Tissue Barrier. The tissue barrier which separates air and blood comprises the alveolar epithelium, an interstitium, and the capillary endothelium. In the normal animal, the interstitium disappears in some regions, so that the boundary membranes of alveolar epithelium and capillary endothelium are contiguous. In other regions, the interstitial space is wider and contains fibroblasts, collagenous fibrils, and elastic fibers. It should be emphasized, however, that the ground substance between these formed elements normally occupies only a very narrow space (Figure 3A). In the lungs of the control animals, this air–blood barrier had an average thickness of 1.5 μ, the alveolar epithelium measuring 0.67 μ, the interstitium 0.55 μ, and the capillary endothelium 0.28 μ (Figure 6).

During the first 24 hours of oxygen exposure, there were no significant changes from these values. Between the first and second day, however, a striking enlargement of the interstitium could be observed (Figure 7A, 7B) in scattered areas of the lung, which accounts for the doubling of the average thickness of the

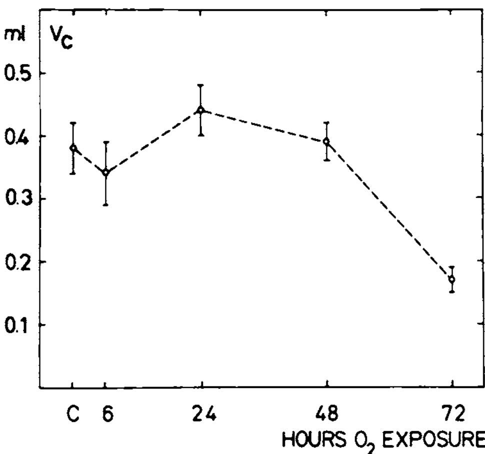

FIGURE 4. Capillary volume (V_C) changes during oxygen exposure.

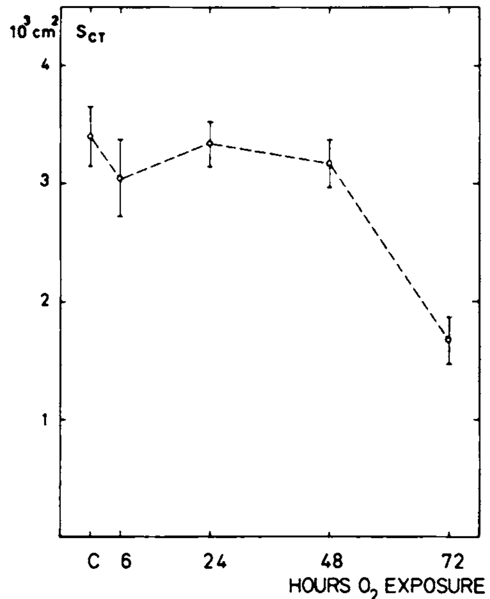

FIGURE 5. Capillary endothelial surface area (S_{CT}) changes during oxygen exposure.

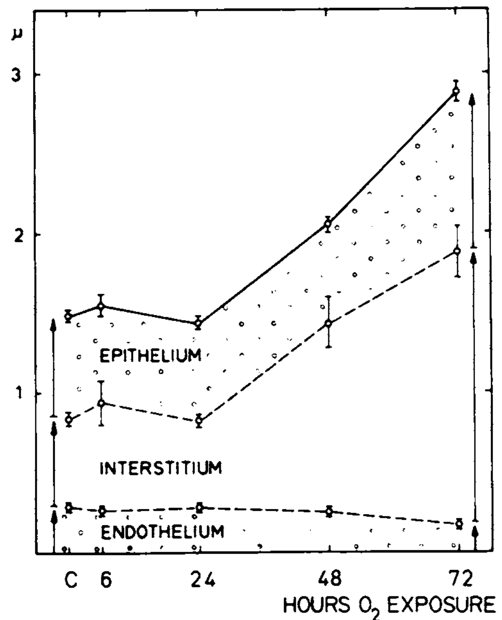

FIGURE 6. Changes in distribution of components of alveolocapillary barrier during oxygen exposure.

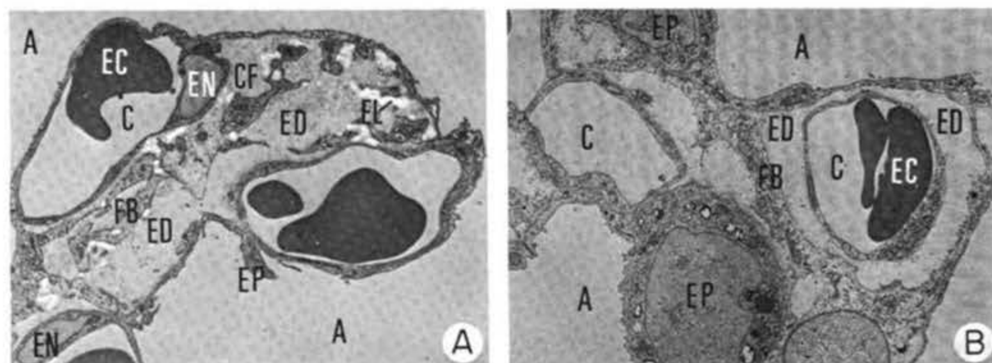

FIGURE 7. A, electron micrograph of lung after 48 hours of oxygen exposure (× 2600). Note enlargement of the interstitial space due to imbibition with edema (ED), separation of capillaries (C) as well as of collagen fibrils (CF) and elastic fibers (EL). Capillary endothelium (EN) and alveolar epithelium (EP) are apparently intact.

B, electron micrograph of lung after 48 hours of oxygen exposure (× 2600). Note capillary (C) completely surrounded by edema (ED).

interstitial space shown in Figure 6. This enlargement of the interstitium was due to accumulation of fluid in the ground substance, which caused a wide separation of formed interstitial elements as well as of alveoli and capillaries. As a result, the thickness of the air–blood barrier increased to about 2.0 μ. In more severely affected regions, infiltration of the tissue by leukocytes and interstitial fibrin formation occurred. At this stage (48 hours), epithelium and endothelium still remained normal in appearance.

At the end of the third day, the interstitial edema fluid was largely replaced by numerous interstitial cells (some of which could be identified as polymorphonuclear leukocytes and lymphocytes), by fragments of destroyed cells, by thrombocytes, and by fibrin strands (Figure 3B, 3C). The interstitial space had tripled its average thickness to 1.7 μ (Figure 6). This enlargement, combined with the thickening of the epithelium by 50%, led to a total thickness of the air–blood tissue barrier of 2.9 μ—despite a decrease of the average thickness of the capillary endothelium by 60% due to destruction of capillaries. The changes in the harmonic mean thickness (τ_h) of the air–blood barrier paralleled the changes in average thickness ($\bar{\tau}$) (Figure 8).

Interestingly, the alveolar epithelial cells did not undergo dramatic morphologic changes, although there was an apparent increase in their number during the period between 48 and 72 hours.

The sequence of events may be summarized as follows. Toward the end of the first day of oxygen exposure, fluid

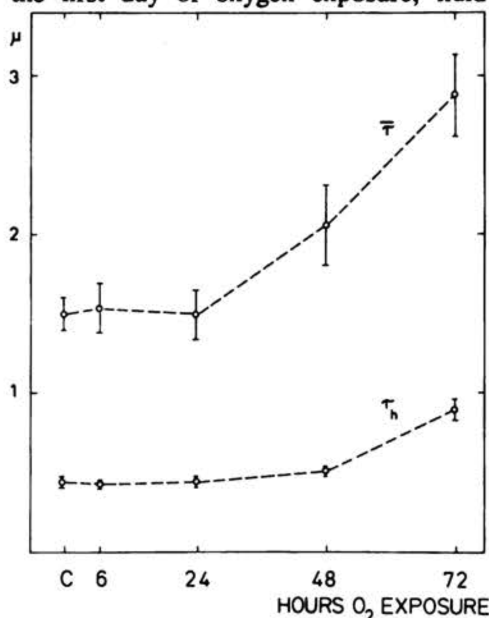

FIGURE 8. Changes in mean ($\bar{\tau}$) and harmonic (τ_h) alveolocapillary tissue barrier thickness during oxygen exposure.

starts to move from the capillary bed into the ground substance of the interstitial space. At this stage, no morphologic changes are observed in the endothelium. During the second day of exposure, the imbibition of fluid by the tissue is associated with the first signs of visible damage to the capillary endothelial cells and the interstitial space becomes widened by edema accumulation, causing a separation of the alveoli from the capillaries. During the third day, this process accelerates. Capillary endothelial cells become necrotic, and numerous erythrocytes undergo fragmentation. In some areas, the altered endothelial lining disappears completely. Cellular elements and fibrin infiltrate the edematous interstitium. Copious exudate, composed of protein-containing fluid, leukocytes, macrophages, cell debris, and fibrin strands, fills 65% of the peripheral airways. The air–blood barrier becomes markedly thickened and the capillary bed of the lung is concomitantly reduced in size.

Discussion

Our findings on the morphologic appearance of the terminal stages of oxygen toxicity are in general agreement with previously cited observations of others. Our results differ, however, in several respects.

First, with regard to the ultrastructure of these changes, we found no generalized edematous swelling of alveolar epithelial cells. We would tend to characterize the thickening of alveolar epithelium which we observed in terminal stages as cell proliferation rather than cytoplasmic swelling. The cytoplasmic changes accompanying the alteration of the capillary endothelial lining likewise differed in many respects from simple cytoplasmic edema; in heavily damaged areas, a clear disintegration of endothelial cells could be observed.

In the terminal stages, we did note occasional swelling of mitochondria in some alveolar epithelial cells, but our results did not suggest this change to be a characteristic lesion of oxygen toxicity; rather, it appeared to be related to other degenerative signs in these cells.

Finally, in contrast to Pratt's [10] reported finding of capillary proliferation in human lungs after oxygen therapy, we observed no increase in capillary volume, surface of the capillary bed, nor in the volume of capillary endothelium. On the contrary, the capillary volume dropped during the second and third day to half its original value. The limited resolving power of the light microscope does not allow clear recognition of destroyed capillaries, and this may account for these discrepancies. The importance of engorged blood vessels, on the other hand, may actually be overestimated unless objective quantitative methods are employed.

Studies of physiologic changes in men breathing oxygen under the same conditions as those of the present study showed a drop in diffusing capacity to 81% of the control value after 48 hours and to 73% after 74 hours of exposure. The total lung capacity fell to 72% of the control value after 74 hours.[13] These results might readily be explained on the basis of thickening of the air–blood tissue barrier, a decrease in pulmonary capillary surface area, and alveolar edema formation, as observed in the present study.

Since, in the experiments reported here, principal structural elements related to the air–blood barrier diffusing capacity could be measured, it was possible to estimate its capacity for gas exchange from the proportionality relationship:

$$D_m \propto \frac{S_{CT}}{\tau_h}$$

where D_m is the gas-exchange capacity of the air–blood tissue barrier, S_{CT} is the capillary surface area, and τ_h is the harmonic mean thickness of the air–blood tissue barrier.

The results, expressed as percent of control value, indicated a fall of diffusing capacity of the air–blood barrier to 25%

after 72 hours of oxygen exposure (Table 4, Figure 9). Taking into consideration the obliteration by edema of 65% of the functional air units at 72 hours, the estimated diffusing capacity fell to 9% of the control values (Figure 9).

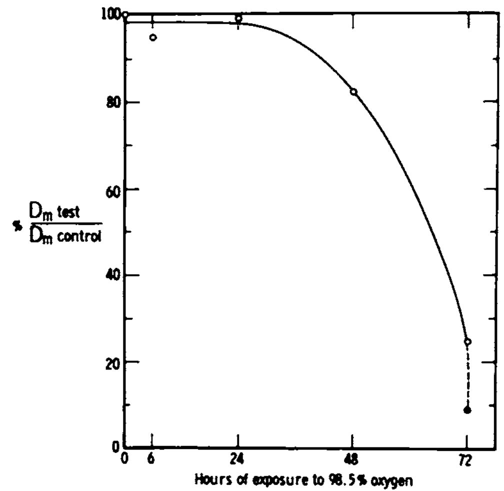

FIGURE 9. Decrease in estimated diffusing capacity (D_m) of the air–blood tissue barrier during exposure to 98.5% oxygen. The closed circle represents the additional effect of functional obliteration of 65% of the air units by edema.

SUMMARY

Rats were exposed to 98.5% oxygen at 765 mm Hg in a controlled-environment chamber, while a control group breathed room air. Groups exposed to oxygen were sacrificed at 6, 24, 48, and 72 hours, and the lungs were prepared for electron and light microscopic examination. Rats which breathed oxygen for 6 and 24 hours showed no observable changes in lung structure. After 48 hours of exposure, the interstitial space of the air–blood tissue barrier had become enlarged by accumulation of fluid, and early destructive changes of the capillary endothelial lining were found.

After 72 hours, the widened interstitial space contained numerous leukocytes, thrombocytes, and other cells, and fibrin strands were numerous. There was marked destruction of the pulmonary capillaries. Sixty-five percent of all alveoli were filled with an exudate containing leukocytes, erythrocytes, macrophages, and fibrin strands. Capillary blood volume and endothelial surface area had decreased in the 72-hour group. The thickness of the air–blood barrier was increased after 48 hours and doubled after 72 hours, the barrier thickening being mainly due to an increase in the interstitial space. Terminally, the epithelium was also thickened, although the endothelium became thinner, on the average, due to destruction.

As a result of these alterations, there was a marked fall in estimated gas-exchange capacity of the air–blood tissue barrier.

TABLE 4. Estimated Decrease in Diffusing Capacity (D_m) of Air–Blood Barrier During Oxygen Exposure

	Exposure to 98.5% Oxygen (hours)				
	0	6	24	48	72
Total alveolar surface area (m^2)	0.38	0.34	0.36	0.39	0.32
Capillary surface area (m^2)	0.34	0.30	0.33	0.32	0.17[a]
Harmonic mean thickness, τ_h (μ)	0.45	0.42	0.44	0.51	0.90[a]
D_m test/D_m control (%)	100	95	99	83	25

D_m, gas exchange capacity of the air–blood tissue barrier.
[a] $P < 0.01$.

ACKNOWLEDGMENTS

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DISCUSSION

Dr. R. Rosenbaum (*New York, N. Y.*): This was a beautiful presentation. Did you see any indication of hemolysis of the trapped red cells?

Dr. Kistler: After 72 hours of oxygen exposure, a number of erythrocytes in heavily damaged lung capillaries had indeed a decreased electron density. This may well indicate a loss of cell content, that is, of hemoglobin. Such brighter red blood cells could be found in scattered areas of many of our sections.

Dr. Rosenbaum: Did you see any separation of cell membranes from adjacent alveolar lining cells that would indicate intercellular pathways permitting leaking between cells?

Dr. Kistler: No, we did not. At the end of the second day, the epithelium appeared actually normal. After 72 hours of oxygen exposure, we noted an apparent increase in the number of alveolar epithelial cells, some of which had signs of intracellular edema. There were no indications for intercellular leakage.

Dr. F. Spencer (*Lexington, Ky.*): I wondered if you have observed any animals taken out of oxygen after 48 hours, then examined 1 or 2 weeks later to see if these changes were reversible?

Dr. Kistler: No, we sacrificed all our animals immediately after bringing them out of the chamber. The only thing I can say is that after 72 hours of exposure almost all animals would have died. When brought back to room air, the rats started gasping and became rapidly cyanotic. Some died within a few minutes.

Dr. P. C. Pratt, *Session Chairman* (*Columbus, Ohio*): Such studies have been made, and I think there is little doubt the changes seen after only 48 hours are highly reversible.

Dr. C. J. Lambertsen (*Philadelphia, Pa.*): The question I raised about reversibility is a rather important one, and relates to whether the actual toxic process is reversible or whether healing of tissue takes place, as if one had a chemical wound of the lungs with a replacement of the cells by the normal process of healing and growth. This differs from the reversal of a toxic process.

Dr. Kistler: Well, I think there are two possibilities. First, the interstitial edema can be reabsorbed, leading to a complete healing. On the other hand, it is known that edema can be followed by cell infiltration. We have seen this in the lungs after 72 hours of oxygen exposure, where the initial interstitial edema was more or less replaced by cells. These cells had to come from somewhere, and, since we could not observe dividing cells, we concluded that they came from the blood vessels. With their appearance, the alveolocapillary tissue barrier increased once more in thickness.

Dr. E. J. Burger (*Boston, Mass.*): Is there any suggestion that there is a primary change in vascular caliber which precedes any of the things you spoke of?

Dr. Kistler: No, we did not observe anything like that. There is obstruction in the capillary bed beginning after 48 hours and proceeding rapidly between 48 and 72 hours. We did not note earlier changes in the diameter of the capillary lumen.

Dr. Pratt: Before one could make observations along that line, it would be necessary to know more about your fixation procedures and solutions, *etc.*

Dr. Kistler: We used standardized procedures throughout our experiments. After puncture of the chest on both sides to collapse the lungs, we instilled buffered isotonic glutaraldehyde through a fine polyethylene catheter inserted into the trachea. The initial hydrostatic pressure was always 20 cm and flow continued until equilibrium was reached. The trachea was then ligated, and lung, mediastinum, and heart were removed together and immersed in glutaraldehyde for 2 hours. None of the changes described in the animals exposed to oxygen could be seen in our controls, which were processed in the same way.

Pathophysiology of Pulmonary Oxygen Toxicity

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That animals exposed continuously to a single atmosphere of pure oxygen die in several days with respiratory distress has long been known,[1] but the mechanism of this response remains unclear. The oft-described pulmonary manifestations of oxygen-breathing include severe atelectasis, vascular congestion, hyaline membranes, and pulmonary edema.[1–4] Thickening of alveolar septa and pulmonary capillary endothelial proliferation have also been reported.[5–7]

Apparently, many factors influence pulmonary susceptibility to oxygen toxicity: body temperature, partial pressure of oxygen, metabolic state of the organism, and age, among others. Although no mammal is known to be fully resistant to the toxic effects of oxygen, the threshold of susceptibility varies among species, and intraspecies variations are often marked.

Some workers believe that the pulmonary response to hyperoxia might be due to histamine release,[8,9] while others have suggested that the toxic effects of high oxygen concentrations are related to sulfhydryl inactivation. More recently, experiments employing oxygen toxicity as a "model" for studying the respiratory distress syndrome of the newborn have indicated abnormal alveolar surface tension properties,[10,11] suggesting that loss of pulmonary surfactant causes alveolar instability, leading to atelectasis. Neuroendocrine stimulation [12] resulting from the severe stress imposed by exposure to high oxygen tensions, as well as altered acid–base balance, has also been considered as the fundamental disorder of oxygen toxicity.

These diverse opinions of the pathophysiology of oxygen toxicity prompted us to undertake the present studies, with the hope of clearly delineating some of the tissue changes and the mechanisms which bring these about.

Materials and Methods

Male albino rats of the Holtzman strain (160–180 gm) and albino guinea pigs (300–325 gm) were divided into six experimental groups (Table 1) and depleted of histamine and 5-hydroxytryptamine as described below.

During oxygen exposure, the animals were placed in a small hyperbaric chamber in their usual cages with food and water. Pure oxygen ($> 99\%$) at 1 ata was al-

TABLE 1. Injection Schedule for Depletion of Histamine and 5-Hydroxytryptamine [a]

No. animals	Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
60	Polymyxin B [b]	—	2.5 mg/kg	5 mg/kg 2×	7.5 mg/kg	7.5 mg/kg	—	—
60 [c]	Polymyxin B [b]	—	2.5 mg/kg	5 mg/kg 2×	7.5 mg/kg	7.5 mg/kg	—	—
	and promethazine	—	—	—	—	—	50 mg/kg	50 mg/kg
60	Polymyxin B [b]	2.5 mg/kg 2×	5 mg/kg	7.5 mg/kg	7.5 mg/kg	—	—	—
	and reserpine	—	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg	—	—
55	Reserpine	—	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg	—	—
53 [c]	Promethazine	—	—	—	—	—	50 mg/kg	50 mg/kg
40	None (controls)	—	—	—	—	—	—	—

[a] Exposure to 100% oxygen began on the seventh day.
[b] Given in dosage of 7266 units/mg.
[c] In addition to the treatment indicated in the table, these animals received promethazine 50 mg/kg daily during oxygen exposure.

lowed to flow through the chamber, and its concentration was continuously monitored and recorded with a Beckman polarographic oxygen analyzer. Oxygen flow was maintained at 10–12 liters/min. The flow rate was controlled with Fisher–Porter flowmeters corrected for oxygen. Oxygen concentrations after the initial 20-min flushing never fell below 99%. Temperature was continuously recorded and remained between 25°C and 26°C throughout the exposure period. Initially, carbon dioxide concentrations were determined with a Scholander apparatus but later were done by gas chromatography. Carbon dioxide levels always remained below 0.5%.

Histamine and 5-Hydroxytryptamine Depletion

Because histamine and 5-hydroxytryptamine (5-HT) have been suggested as important factors in the pathogenesis of oxygen toxicity,[8,9] we wished to consider the possible roles of these two compounds under hyperoxic conditions. Parrot and West[13,14] demonstrated that rat lungs contain large amounts of 5-HT as well as histamine, and high concentrations of histamine in the guinea-pig lung have been known for many years.

Rats and guinea pigs were depleted of their body stores of histamine by intraperitoneal injections of polymyxin B on a weight basis (Table 1). More than 90% of the total body histamine, including more than 85% of the lung histamine, was released. Recovery occurred slowly over a 56-day period. Tissue histamine levels were determined on a Farrand microspectrophotofluorometer by the method of Shore.[15] A second group of animals received reserpine to deplete the tissue 5-HT stores. By this method, over 92% of the total body 5-HT was released, as determined with the microspectrophotofluorometer by the method of Udenfriend.[16,17] A third group received polymyxin B and reserpine. A fourth group received the antihistamine promethazine (Phenergan) on a body-weight basis. A fifth group served as untreated oxygen controls. A series of normal air controls was maintained for each treated group.

Degassing Experiments

Although total atelectasis has been reported as a regular feature of pulmonary oxygen poisoning, it seemed possible that atelectasis may have resulted from terminal resorption of oxygen. To test this hypothesis, 180-gm male albino rats of the Holtzman strain were placed in a small hyperbaric chamber in their usual cages with food and water. They were exposed continuously to either 50% O_2 + 50% N_2 at 1 ata, 35% O_2 + 65% N_2 at 3 ata, or 20% O_2 + 80% N_2 at 5 ata. Temperature was maintained at 25–26°C. Gas concentrations were monitored continuously as described above.

Morphologic Observations

Lung tissue from each experiment was fixed in either ice-cold calcium formol or Rossman's fixative, or was fresh-frozen at −70°C. Sections were stained with Harris' hematoxylin and eosin, periodic acid–Schiff (PAS) and elastica–van Gieson. Frozen sections of formalin-fixed lungs were cut at 10 μ, air-dried, and mounted in glycerin jelly after brief hydration in distilled water. They were examined with a Zeiss ultraviolet microscope. Excitation was with the entire emission of a Zeiss-Osram HBO–200 mercury burner; Zeiss −65 and 44 barrier filters were used for observation.

Results

Histamine and/or 5-HT depletion in either guinea pigs or rats, with or without promethazine, had no influence on survival time (55–60 hours), compared with survival times of untreated oxygen-exposed controls.

Postmortem examination usually revealed bilateral pleural effusions of clear thin fluid, with a volume up to 7 ml in rats and about 10 ml in guinea pigs. Mild ascites was frequently found in both species. Total atelectasis was absent in only five of 328 animals exposed to 100% oxygen at 1 ata.

Histologic examination of lungs from each of the experimental and oxygen-exposed controls revealed no appreciable differences. It was usual to find severe edema and congestion of the alveolar septa (Figure 1A,1C). A proteinaceous exudate was noted in the alveolar space (Figure 1C). Smaller pulmonary artery and arteriolar constriction was evident throughout the parenchyma, resulting in marked narrowing and frequently almost complete obliteration of the vessel lumen (Figure 1). Many foci of arterial and arteriolar vasculitis were noted in both species; pulmonary phlebitis was more frequently observed in rats (Figure 1B,1D).

Acute inflammation was unusual, although occasional scattered areas did reveal polymorphonuclear leukocytes and fibrin strands within alveolar spaces. Interlobular septa and peribronchial, periarterial, and periarteriolar edema were characteristically found (Figure 1B,1C, 1D).

Animals exposed to 5 ata of 20% O_2 + 80% N_2 died with minimal atelectasis in the usual time (Figure 2A). The lungs were pink but heavy with edema fluid, and pleural effusion was present. The lungs of animals exposed to 50% O_2 + 50% N_2 at 2 ata showed partial atelectasis with only patches of "hepatization"; the lungs of animals that died in 35% O_2 + 65% N_2 at 3 ata (Figure 2B) revealed atelectasis approximately between that of the 20% O_2 + 80% N_2 group and the 50% O_2+50% N_2 group.

Ultraviolet microscopy of oxygen-poisoned lungs (Figure 2D) revealed a diminution in or absence of the characteristic green autofluorescent alveolar lining (Figure 2C). (These studies will be reported in more detail elsewhere.)

Discussion

Previous studies of pulmonary oxygen toxicity in experimental animals[8,9] have indicated that antihistamines prevent or delay the onset of pulmonary edema, atelectasis, and vascular congestion. Our findings, however, failed to confirm this. Moreover, examination of pulmonary tissues by a variety of techniques suggests that continuous exposure to high oxygen concentrations for short periods of time provokes severe arterial and arteriolar vasoconstriction, vasculitis, and possibly arterial medial hyperplasia. We believe that these vasoconstrictive changes lead to pulmonary hypertension and possibly to reduced blood flow. An occasional observation of the beating heart of an oxygen-poisoned animal in the terminal state usually revealed a prominent right ventricle and a dilated distended conus arteriosus. Furthermore, direct toxic effects of oxygen on alveolar cells and anoxemia may lead to "leaking" cell membranes as well as to altered metabolic and synthetic capacities. Such metabolic changes are indicated by the absence of the alveolar phospholipid lining layer and *in vitro* hydrolytic enzyme inactivation of pulmonary parenchyma, as well as *in vivo* sulfhydryl inactivation.

Pulmonary vascular resistance measured in the presence of elevated intra-alveolar oxygen tensions has been reported to either increase[18] or decrease.[19,20] Pratt[5,7] has described alveolar capillary proliferation in man as a result of hyperoxia, and he relates this change to capillary dilatation. Increased pulmonary arterial pO_2, however, has frequently been associated with pulmonary arteriolar vasoconstriction,[21,22] and it has been suggested that pulmonary venous or venular constriction is the basis of the resulting hypertension.[18] Our histologic evidence indicates that arterial, arteriolar, and venular constriction occur frequently. We have been unable to demonstrate capillary proliferation, but perhaps such changes are peculiar to human pulmonary endothe-

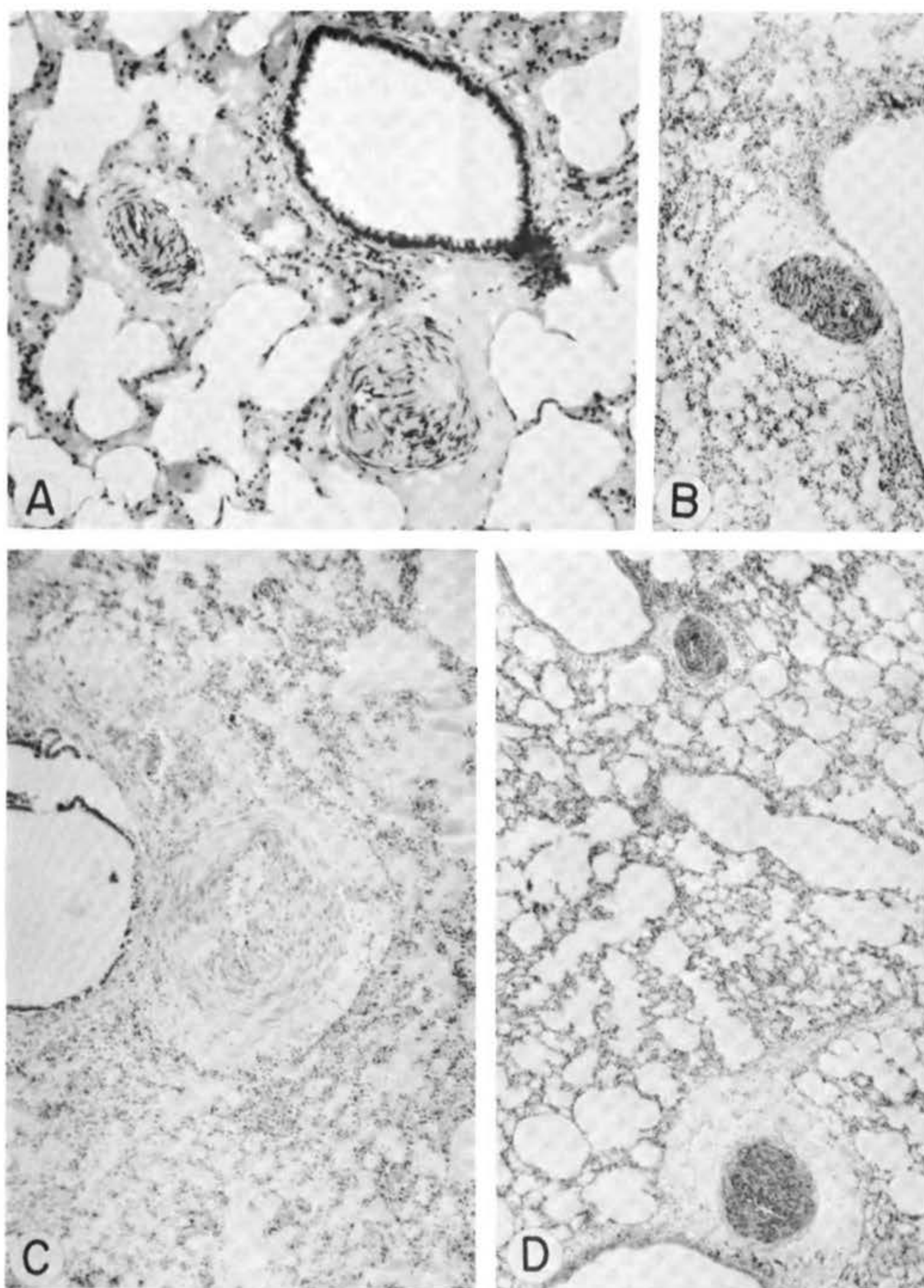

FIGURE 1. A, section of lung of rat that died after 58-hour exposure to 100% O_2 at 1 ata; note severe vasoconstriction and edema. B, section of lung of rat that died after 54-hour exposure to 100% O_2 at 1 ata; note marked vasoconstriction and perivascular edema. C, section of lung of guinea pig that died after 61-hour exposure to 50% O_2 + 50% N_2 mixture at 2 ata. Vasoconstriction and pulmonary edema are prominent, and periarterial edema and vasculitis with a moderate infiltrate of inflammatory cells are evident. D, section of lung of rat that died after 58-hour exposure to 20% O_2 + 80% N_2 mixture at 5 ata; arterial vasconstriction and perivascular edema are prominent.

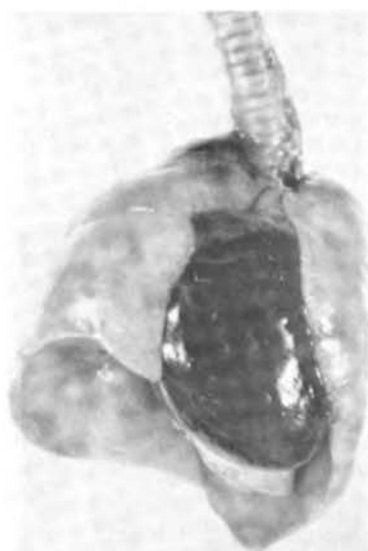
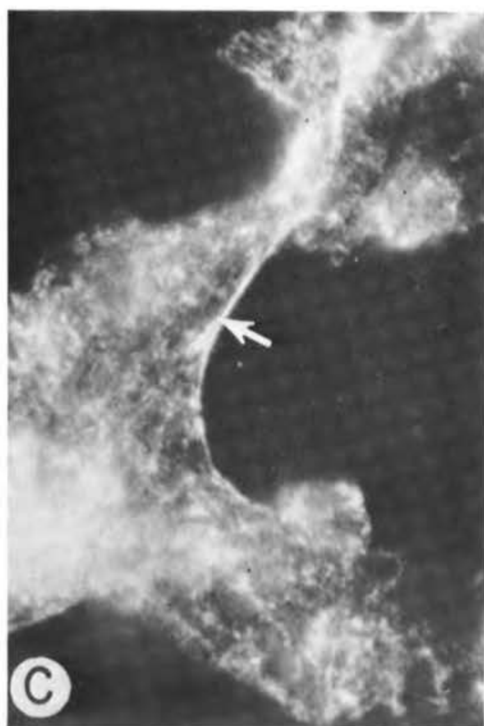
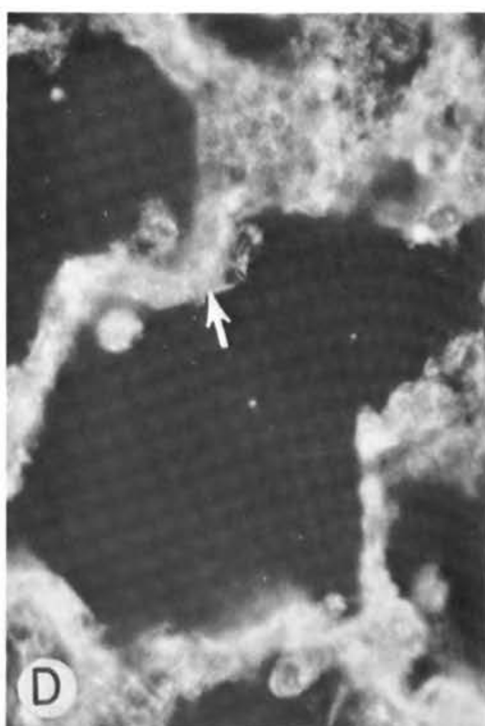

FIGURE 2. A, lung described in Figure 1D; note general pale color (pink). Atelectasis is minimal. B, lung from rat that died in 35% O_2 + 65% N_2 mixture after 56-hour exposure. Focal areas of complete collapse appear as darker areas. Hepatization would appear grossly similar to that of the heart, the dark structure in the center of the photograph. C, autofluorescent (bottle-green) alveolar lining layer (arrow) in normal rat lung. D, absence of fluorescent lining in lung of O_2-poisoned rat.

lium. Our observations resemble those previously described in rats chronically exposed to air at the equivalent of 83.6% oxygen.[23–25]

Atelectasis and hepatization, regarded as common features of oxygen poisoning, may actually be unimportant or may not even represent genuine lesions of oxygen poisoning. Our experimental animals, continuously exposed and eventually killed in 20% oxygen at 5 ata, consistently revealed only minor degrees of atelectasis (Figure 2A); however, alveolar stability was markedly diminished, as judged by their inability to retain air when artificially inflated. This instability may have reflected diminished or absent alveolar lining layer (*i.e.*, surfactant), as evidenced by diminished autofluorescence under ultraviolet microscopy. Possibly oxygen inactivates surfactant, as *in vitro* studies have indicated,[26,27] or redistributes surfactant within alveoli as a result of pulmonary edema fluid "washing off" surfactant from alveolar surfaces. Complete atelectasis ("hepatization") seems to be a terminal event, inasmuch as the experiments at 5, 3, 2, and 1 ata have clearly demonstrated that the degree of atelectasis varies directly with the oxygen percentage in the gas mixture. In support of this, it was demonstrated that atelectasis could be delayed by artificial periodic pulmonary insufflation.[28]

Finally, the question arises as to the importance of pulmonary surfactant in relation to atelectasis. Animals (rats, guinea pigs, hamsters, or mice) in 100% oxygen, sacrificed during the first 24–48 hours of exposure, never showed discernible lesions typical of oxygen toxicity. Only in the last few hours before death (as judged by comparison with litter mates) were many of the typical changes found. Vasoconstriction, alveolar instability, and atelectasis were absent. Correlated with these findings, fluorescence and surfactant measurements were within normal limits during these first 48 hours, becoming abnormal only just before death, although respiratory distress was evident after 24–30 hours. If there are early morphologic counterparts of these symptoms, they must be readily and rapidly reversible.

Figure 3 summarizes our current concept of the mechanism of pulmonary oxygen toxicity. Although not all aspects of this proposal are proven, we believe it comes close to explaining many of the findings of this most perplexing disease. Our *in vitro* and *in vivo* studies[29–32] have clearly demonstrated enzymic inhibition associated with exposure to high oxygen concentrations—which could certainly account for the proposed alveolar cell defects leading to the complex gross and microscopic pulmonary changes.

Summary

1. Hyperbaric oxygenation causes marked pulmonary vasoconstriction affecting small muscular arteries, arterioles, and venules.

2. Vasculitis commonly affects these vessels.

3. Histamine and serotonin are not responsible for these vasconstrictive changes.

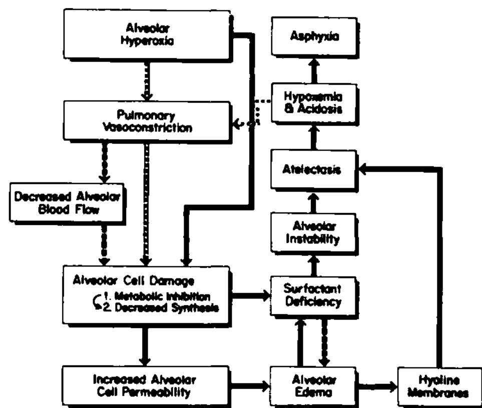

FIGURE 3. Sequence of interrelated pathophysiologic events leading to pulmonary oxygen toxicity in mammals.

4. Total atelectasis with "hepatization" of the lung parenchyma is a result of terminal resorptive atelectasis.

5. The alveolar lining layer, as visualized by autofluorescence, is absent in oxygen-poisoned animals. This may be responsible for alveolar instability typically found in these animals.

6. A pathophysiologic correlation is suggested.

ACKNOWLEDGMENTS

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DISCUSSION

DR. K. H. SMITH (*Fort Collins, Colo.*): Have you found that this pulmonary vasoconstriction has a central origin or is directly caused by oxygen tension? We have noticed that in hypoxic cattle with reduced oxygen tension we get a vasoconstriction from an increase of the media in vascular tissue.

DR. WITTNER: We do not know whether it is central, but we think that the vasoconstriction is probably secondary to increased pO_2 and pCO_2 changes, and these may act both directly and indirectly on pulmonary vessels.

DR. P. C. PRATT, *Session Chairman* (*Columbus, Ohio*): The present situation, though, is that hyperoxia and hypoxia both produce vasoconstriction. This seems questionable to me.

UNIDENTIFIED SPEAKER: I believe you showed two lungs, one of which was redder than the other, and I thought you said it was exposed to 50% oxygen. Why was there a greater hepatization at 50% oxygen, less at 35% oxygen, and even less at 20% oxygen?

DR. WITTNER: We think that what happens is that hepatization results from terminal resorption atelectasis of the oxygen from alveoli. The experiment we presented demonstrated that the degree of atelectasis varied directly with the amount of oxygen in the gas mixture, keeping in mind, of course, that in each experiment the pO_2 was always close to 760 mm Hg (*i.e.*, the equivalent of 100% oxygen at 1 ata).

DR. PRATT: We have confirmed this interpretation in a different way by exposing mice to 100% oxygen, killing them, and autopsying them, some immediately and some 3 hours after death. We find greater collapse and congestion of the lung in those with delayed autopsy.

CAPT. G. BOND (*Washington, D. C.*): There were two other factors not mentioned: (1) the increased density of the breathing medium, which would naturally add to the work of breathing, and (2) the nitrogen narcotic effect. Would you care to comment on how much that might play a part in your picture?

DR. WITTNER: The animals that were at 5 ata with the 80% nitrogen and 20% oxygen died within the same time range as our controls that were in 100% oxygen at 1 ata. We really did not notice anything particularly different about them.

DR. J. A. MENDELSON (*Edgewood Arsenal, Md.*): I just wanted to make a practical point that may not apply to your slides particularly, but we have noticed that in some rabbits perivascular thickening and constriction do, at least, appear somewhat similar to those shown in your slides. It was pointed out by our veterinary pathologist that this could excite people a great deal except that it occurs very often under other conditions in rabbits and perhaps in other small animals.

DR. WITTNER: We know this occurs very rapidly in guinea pigs and rabbits following endogenous histamine release as a result of hypersensitivity or anaphylaxis. Moreover, injection of histamine and/or 5-hydroxytryptamine usually produces lesions similar to ours. The animals in our experiment, however, were depleted of their tissue amines so that we know this was not a factor in causing vasoconstriction. We think these effects are real since our control series never showed these changes.

DR. J. D. HACKNEY (*Downey, Calif.*): I have one comment. In judging lung stability and instability and resorption when animals are breathing 100% oxygen, it would be worthwhile to expand the specimen fully just before the observations are made, then let them come down to low or zero distending pressure, and then judge grossly whether they are unstable. It is even better to do pressure–volume measurements, though this is sometimes very difficult because of holes in the specimen. However, if you are going to use gross appearance to judge lung stability, I think it would be worthwhile to expand the specimen just before the observations.

DR. WITTNER: We have done numerous pressure–volume curves using inflation techniques similar to those described by Gruenwald at Johns Hopkins (Anat. Rec. 139:471, 1961) and Craig (Amer. J. Dis. Child 106: 174, 1963). We consistently found that greater work was necessary to move a known volume of air in an oxygen-poisoned lung than in a normal lung. Poisoned lungs also showed a greater tendency to collapse; that is, they were markedly unstable. This was true for animals killed at 5 ata in 20% oxygen as well as at 1 ata in 100% oxygen. Furthermore, one cannot expand the oxygen-poisoned lungs with liquid, since the fluid runs through them just like water through a saturated sponge. At this time, we have no really concrete ideas as to why this occurs. We do think, of course, that the cell membrane, as well as the alveolar lining layer (surfactant), are disrupted or impaired. I think the electron micrograph studies just presented at this session support our suggestions as to the reason for these leaking membranes, and may be part of the reason why we cannot inflate lungs with any kind of fluid.

Fatal Pulmonary Injury from Prolonged Inhalation of Oxygen in High Concentrations

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The experiments described in this report were designed to measure specifically the effects of prolonged breathing of oxygen at different partial pressures (pO_2 140–700 mm Hg) upon dog lungs. This work evolved from our experiences in 1963 with fatal pulmonary injury in a patient after mechanical ventilation for several days with high concentrations of oxygen. Serious preexisting pulmonary disease prevented evaluation of the specific role of oxygen in the production of the fatal pulmonary lesion.

The increasing use of mechanical ventilators in man, capable of delivering oxygen at partial pressures over 600 mm Hg, has made recognition of the hazards involved in administering oxygen in different concentrations extremely important. Following is a report of our experimental data on dog studies and our clinical findings in patients in whom respiration was being assisted by mechanical ventilator.

Materials and Methods

Experimental Studies

The studies were performed in an air-conditioned building (temperature 21–24°C, humidity 38–50%). Healthy mongrel dogs (weighing 6–20 kg) were placed in a barrel modified to permit continuous exposure to oxygen of different concentrations (Figure 1). The capacity of the barrel was about 180 liters (length 75 cm, width 55 cm). Food, water, and a small container of soda lime to absorb carbon dioxide were placed in the barrel with the dog. The open end was then sealed with a lid of transparent rigid plastic material through which the animal could be observed. Small openings in the plastic lid were made for the entrance and exit of oxygen. The rate of oxygen flow into the barrel varied from

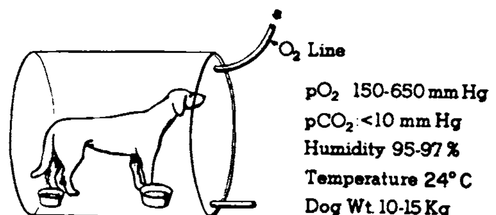

FIGURE 1. Sketch of barrel used for experiments. The open end was closed with rigid transparent plastic. Openings provided entry and exit for oxygen. Food, water, and a small container of soda lime to absorb carbon dioxide were placed in the barrel.

TABLE 1. Group 1 Experiments: Exposure of Dogs to Compressed Air [a]

Dog no.	Wt. (kg)	O_2 tension daily range (mm Hg)	Duration of observation (days)	Pulmonary findings at autopsy
1	9	140–150	5	N
2	12	—	14	N
3	14	—	14	N
4	13	—	14	N
5	9	135–140	14	N
6	8	135–143	14	N
7	7	137–149	12	N

N, lungs normal on both gross and microscopic autopsy examination at end of observation period.
[a] Chamber humidity was 75–95%; carbon dioxide tension was less than 10 mm Hg.

5 to 20 liters/min, depending upon the oxygen concentration desired.

The animal moved about freely inside the barrel, eating and drinking. No restraints, anesthesia, or cannulas were used. Two or three times daily the atmosphere inside the barrel was sampled to determine concentrations of oxygen, carbon dioxide, and water vapor. Except for one time each day when food and water were changed, the atmospheric conditions inside the barrel remained uniform throughout the period of study.

Thirty-three dogs were studied, and these were divided into three groups: (1) In control experiments, seven animals were exposed to a continuous flow of compressed air (5 liters/min) for 5–14 days (Table 1). (2) Eight animals breathed oxygen at a partial pressure of 295–345 mm Hg for 10–14 days (Table 2). (Originally, this group comprised nine animals, but the dog in experiment 16 died of unknown causes after 8 days.) At the end of the experimental period, the animals were killed by the intravenous injection of sodium pentobarbital and autopsy was performed. (3) Seventeen animals breathed oxygen at a tension between 300 and 720 mm Hg (Table 3) until death occurred from respiratory insufficiency.

TABLE 2. Group 2 Experiments: Exposure of Dogs to Oxygen at Tensions of 295–345 mm Hg [a]

Dog no.	Wt. (kg)	O_2 tension daily range (mm Hg)	Duration of observation (days)	Gross pulmonary findings at autopsy [b]
8	—	300–320	10	N
9	15	305–345	14	N
10	14	305–325	14	N
11	15	305–315	11	N
12	15	305–315	14	N
13	15	310–325	14	N
14	11	295–305	14	N
15	10	300–310	14	N
16	8	205–325	8 [c]	N

N, lungs normal on gross examination.
[a] Chamber humidity was 75–95%; carbon dioxide tension was less than 10 mm Hg.
[b] Microscopic examination of lungs at autopsy (at end of observation period) showed frequent alveolar thickening.
[c] This animal died of an unknown cause before termination of the experiment.

TABLE 3. Group 3 Experiments: Exposure of Dogs to Oxygen at Tensions of 300–720 mm Hg [a]

Dog no.	Wt. (kg)	O_2 tension daily range (mm Hg)	Survival time (days)	Pulmonary findings at autopsy
17	8	300–515	5½	Atelectasis, edema
18	10	360–460	2½	Hemorrhage, edema
19	9	380–415	2½	Edema
20	11	310–410	2½	Edema
21	13	420–550	3½	Hemorrhage, consolidation
22	13	380–490	4	Hemorrhage, edema
23	20	350–640	2½	Edema, consolidation
24	15	620–720	4½	Edema, consolidation
25	13	620–642	2½	Edema, consolidation
26	13	420–640	2½	Hemorrhage, edema, consolidation
27	12	670–680	2	Hemorrhage, edema, consolidation
28	12	490–535	2½	Massive hemorrhage, consolidation
29	15	460–560	2½	Edema, consolidation
30	15	465–660	4½	Edema, consolidation
31	10	600–615	2½	Patchy edema, consolidation
32	6	620–700	3	Hemorrhage, edema
33	6	300–580	9	Sacrificed, lungs normal

[a] Chamber humidity was 75–95%; carbon dioxide tension was less than 10 mm Hg.

Autopsy was performed upon each animal, with examination of the heart, lungs, and abdominal viscera. Care was taken to occlude the trachea with a clamp before the thorax was opened in order to prevent postmortem collapse of the lungs. Microscopic examination of the lungs was routinely done, with histologic sections stained with hematoxylin and eosin. Histologic examination of the heart, liver, and kidneys was performed in only a few experiments, as no significant abnormalities were found.

Clinical Studies

Thirty-two patients in whom respiration was supported with a pressure-cycled mechanical ventilator because of postoperative respiratory insufficiency (usually following cardiopulmonary bypass for valvular heart disease) were studied twice daily. The ventilator was connected to the patient through a cuff tracheostomy tube. Oxygen tensions in the inspired gas immediately proximal to the tracheostomy were measured simultaneously with oxygen tensions in arterial blood. The discrepancy between the oxygen concentration indicated by the oxygen dilution valve on the ventilator (usually 40–60%) and that measured in the inspired gas was noted.

RESULTS

Experimental Studies

Group 1 Experiments. Animals breathing compressed air showed no changes during the 5–14 days of observation. They ate and drank normally and showed no adverse effects from confinement in the barrel. At autopsy, the lungs were normal on both gross and microscopic examination (Table 1).

Group 2 Experiments. All nine animals breathing oxygen at a tension of 295–345

mm Hg for 10–14 days survived but one (Dog 16, Table 2). This death was probably due to an unrecognized intercurrent illness, for no pulmonary injury was found. During the experiments, the only abnormality noted was a tendency for the animals to become drowsy and not eat well. Dyspnea was not apparent. At autopsy, the lungs appeared normal on gross examination, but often microscopic examination revealed thickening of the alveolar walls (Figure 2). Hemosiderin deposits were also frequently seen, suggesting previous hemorrhage into the alveoli. There was no cellular infiltration or accumulation of fluid in the alveoli.

Group 3 Experiments. All but one of the 17 animals breathing oxygen at a tension above 300 mm Hg died in 2–5½ days from respiratory insufficiency (Table 3). The sole survival (Dog 33) can probably be explained by inadvertent interruption in the continuous flow of high concentrations of oxygen, for the measured oxygen concentrations varied widely, and the lungs appeared normal at autopsy. In all other experiments, the animals first became drowsy and stopped eating. Soon afterward, dyspnea appeared, gradually becoming more severe, until death occurred in 12–24 hours. There was no correlation between oxygen concentration and survival time, for the clinical course of animals breathing oxygen at a tension of 400–500 mm Hg was similar to that of animals breathing oxygen at a tension of 600–700 mm Hg (Figure 3). Neither was there a correlation between body weight and survival (Tables 1–3).

At autopsy, the lungs were usually a fiery red color from intense congestion and hemorrhage (Figure 4A, 4B). Severe pulmonary consolidation was often found, with large amounts of pulmonary edema fluid in the trachea and bronchi. When the lungs were incised, large amounts of fluid poured from the cut surfaces (Figure 4C). Often the areas of consolidation were irregularly distributed throughout the lungs, the lower lobes being most frequently involved.

In two animals, the pulmonary artery to one lung was ligated 6–8 weeks before the animal was placed in the oxygen barrel to determine whether the absence of mixed venous blood in a lung would influence pulmonary sensitivity to oxygen. Autopsy examination of both dogs showed the degree of injury in the lung containing the intact pulmonary artery to be similar to that found in the lung with the ligated pulmonary artery.

Histologic sections of the lungs showed varying degrees of congestion, edema, hemorrhage, and deposition of fibrin within the alveoli (Figure 5A, 5B). Except for the occasional deposition of

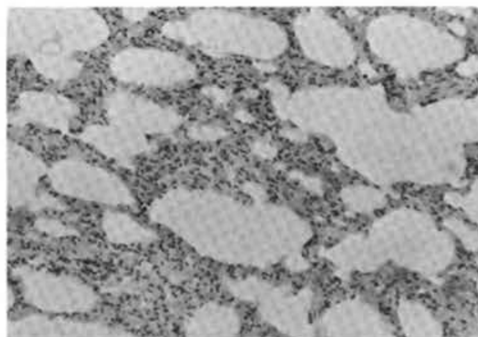

FIGURE 2. Histologic section of lung from Group 2 experiment, showing thickening of the walls of the alveoli. No fluid is seen in the alveolar lumen, nor any inflammatory cells.

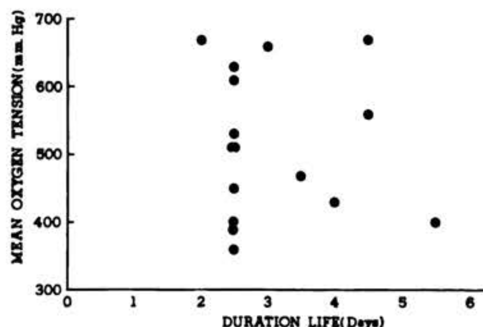

FIGURE 3. Comparison of the mortalities in dogs exposed to different concentrations of oxygen showed no correlation between oxygen concentration and mortality once the oxygen tension exceeded 400 mm Hg.

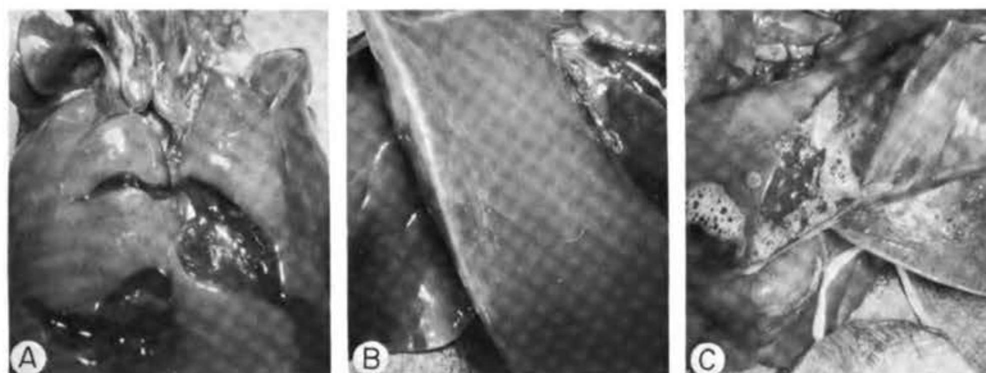

FIGURE 4. A, photograph of lungs at autopsy in Group 3 experiment. There was massive consolidation and edema and a fiery red color consistently found from congestion and hemorrhage. B, view of the margin of one of the lobes of the lungs shown in A, illustrating the liverlike appearance due to massive consolidation. C, incision of the same lobe, showing the large amount of pulmonary edema fluid that poured from the cut surface.

strands of fibrin within the alveoli, most of the pathologic changes appeared to be of recent origin. Infiltration of cells was seldom found. No intrinsic changes were seen in the cells lining the alveoli except for those due to the distortion produced by the extensive congestion and edema.

Clinical Findings

In the 32 patients ventilated with a pressure-cycled mechanical ventilator, great variation was found in the oxygen concentrations in the inspired gas, depending upon the degree to which the nebulizer attached to the ventilator was used. Because the nebulizer was activated by oxygen flowing through it, gas leaving the nebulizer had a high oxygen concentration which accordingly raised the oxygen concentration in the gas from the ventilator when mixed with it. In 23 patients ventilated with one type of respirator, the oxygen dilution valve was adjusted to

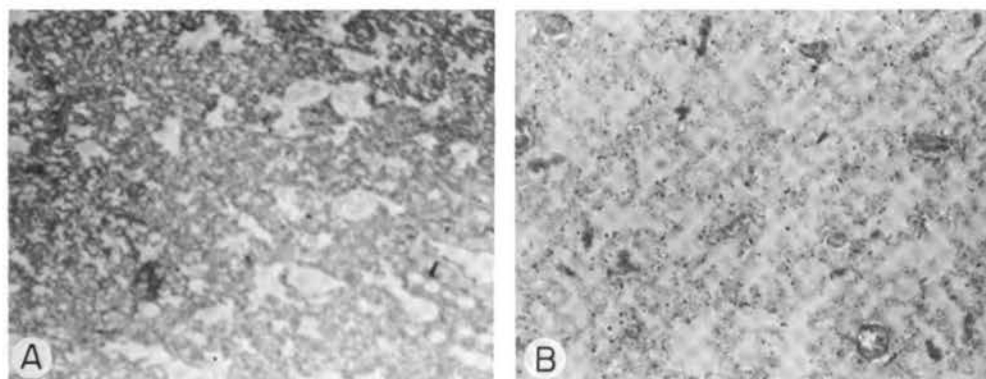

FIGURE 5. A, histologic section of lung in Group 3 experiment, in an area of moderate injury. There was patchy consolidation and congestion throughout the lung field. B, high-power view of lung tissue in Group 3 experiment, showing the typical massive edema and congestion regularly found in these experiments.

deliver an oxygen concentration of 40%. When the nebulizer was not used, the oxygen concentration in the inspired gas varied from 48 to 63%. When the nebulizer was activated with oxygen, the concentration in the inspired gas rose to 78–96% (Table 4).

In another type of pressure-cycled ventilator, studied in nine patients, where the oxygen dilution valve was set to deliver an oxygen concentration of 40%, the oxygen concentration when the nebulizer was not used varied from 43 to 46%. When the nebulizer was used, however, the oxygen concentrations ranged from 49 to 68%, depending upon the peak pressure developed by the ventilator during inspiration (Table 4).

Observations were made on 11 patients ventilated with a volume-cycled piston respirator, in which varying amounts of oxygen were introduced into the gas mixture entering the piston chamber. In all of these, the partial pressure of oxygen in the gas from the ventilator was below 250 mm Hg. This oxygen tension was sufficient to maintain an arterial oxygen tension between 88 and 108 mm Hg.

TABLE 4. Oxygen Concentration Variation in Inspired Gas with Pressure-Cycled Respirators: Oxygen Dilution Valve Set to Deliver 40% Oxygen

		Inspired O_2 concentration	
Respirator used	No. pts.	Without nebulizer	With nebulizer
A	23	48–63%	78–96%
B	9	43–46%	49–68%

Most of the patients were ventilated for only 1–2 days. In this period of time, no pulmonary injury was recognized as having resulted from the oxygen tensions employed. The fact that serious pulmonary disease was present, however (which was the reason the ventilator was used), made it impossible to detect any subtle harmful effects from oxygen.

Two patients who were treated before these studies were undertaken developed fatal pulmonary injury after ventilation with high tensions of oxygen (450–500 mm Hg) for 4–5 days. In both patients, serious preexisting pulmonary disease made it impossible to separate the effects of oxygen from other factors. At autopsy,

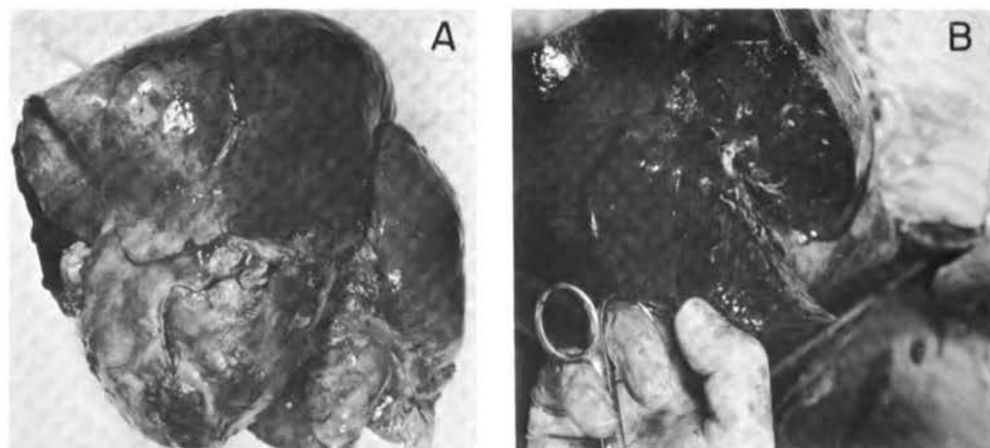

FIGURE 6. A, photograph of heart and lungs removed at autopsy from a patient who died after ventilation for 5 days with oxygen tensions greater than 400 mm Hg. The diffuse hemorrhagic consolidation of both lungs closely resembled that seen in the experimental studies (Figure 4A, 4B). B, incision of one of the consolidated lobes of the lungs shown in A, showing a diffuse hemorrhagic consolidation throughout the pulmonary tissue.

extensive pulmonary congestion and consolidation was found in one patient (Figure 6A,6B), which closely resembled the changes found in the lungs of the dogs in the Group 3 experiments (Figure 4A, 4B).

Discussion

Pulmonary Injury from Oxygen in Dogs

The experimental results clearly demonstrate the uniformly lethal action of oxygen in dogs exposed to environmental oxygen tensions above 350–400 mm Hg. The absence of any pulmonary injury in the control studies in the Group 1 experiments with compressed air would seemingly exclude any etiologic factor other than oxygen. It was of interest that the minimum lethal partial pressure of oxygen was near 350–400 mm Hg, since animals consistently survived oxygen pressures of 300–350 mm Hg (Group 2). Above 40 mm Hg, however, there was little correlation between the rate at which the fatal pulmonary injury developed and the actual oxygen tension. Dogs breathing oxygen at a tension of 400 mm Hg had a course very similar to that of dogs breathing oxygen at a tension of 600 mm Hg. From the data available, one cannot make any inferences with certainty. Only 17 experiments were performed. Possibly a larger series might reveal a correlation between the actual oxygen tension and the rate of development of pulmonary changes. Another possibility is that individual animals vary in susceptibility to injury from oxygen. Such a spontaneous variation has been found in experiments with smaller animals and could easily obscure a correlation between oxygen tensions and pulmonary injury.

The results in these experiments are very similar to those reported in 1941 by Paine, in studies of dogs inspiring oxygen at concentrations of 95–100%.[1] Oxygen concentrations of 75–80% were well tolerated for 7 days, but only two experiments were performed. Many investigations of the effects of oxygen on smaller animals (mice, rats, guinea pigs, and rabbits) have been performed, one of the best early studies being that of Karsner in 1916, who found that high concentrations of oxygen caused fatal pulmonary injury in rabbits.[2] His descriptions of the pathologic alveolar changes of congestion and edema closely resemble those in this report. An excellent summary of the different experimental studies appeared in Bean's detailed report on oxygen poisoning in 1945.[3] Fatal pulmonary injury was almost invariably found in experiments on small animals, but often additional factors such as carbon dioxide concentration or humidity were not controlled. Hence, the applicability of the findings in small animals to large animals and man has long been debated.

The pulmonary manifestations of oxygen toxicity have been consistently found to be a combination of congestion, hemorrhage, and edema, producing a massive hemorrhagic consolidation in the lung. This picture suggests a direct irritant effect of oxygen on the pulmonary capillaries and alveoli. Signs of chronic inflammation, such as infiltration by inflammatory cells, have not been found, although the occasional finding of strands of fibrin within the alveoli indicates a subacute injury. Whether a chronic sublethal pulmonary injury can be produced by oxygen is unknown. Such a possibility is suggested by the irregular thickening of the alveolar walls in the Group 2 experiments after exposure to oxygen for 14 days, but only limited observations were made. Possibly such changes may subside when oxygen inhalation is stopped.

Hazards of Pulmonary Injury from Oxygen in Man

With the experimental findings described in the preceding paragraphs, the risks of pulmonary injury from oxygen in man

have naturally long been considered. As early as 1783, Lavoisier expressed concern over the risk of pulmonary injury.[3] In 1899, J. Lorrain Smith considered in detail the experimental findings indicating pulmonary injury from oxygen.[3] In later years, however, more than one experienced investigator expressed the opinion that there was very little risk in man of pulmonary injury from oxygen. Undoubtedly these opinions arose partly from the fact that methods of administering oxygen to man before 1955 seldom produced oxygen concentrations greater than 50%. In 1945, Bean stated that a fatal pulmonary injury in man had not been recognized.[3]

One of the best early studies of oxygen toxicity in man was performed in 1945 by Comroe *et al.*,[4] who administered 97–99% oxygen to 90 healthy adult volunteers for 24 hours. The most frequent symptom produced was substernal distress, occurring in 82% of the subjects. Signs of nose and throat irritation were also common. The vital capacity was significantly reduced, although the chest roentgenogram was normal. In parallel studies with inhalation of 50% oxygen, no symptoms were produced.

Similar findings were later reported by several other investigators. In 1947, Ohlsson studied six normal adults in an oxygen chamber for 50–60 hours.[5] The longest studies were performed in 1962 by Dolezal, who studied 12 adults remaining in an oxygen chamber for 42–110 hours.[6] In 1963, Lee *et al.* demonstrated a decrease in pulmonary diffusion capacity associated with other symptoms of oxygen toxicity in volunteers breathing 98% oxygen at 1 atm.[7] The data now available have fairly well defined the clinical picture resulting from continuous breathing of 100% oxygen. Symptoms appear between 6 and 30 hours, the usual limit of tolerance being 50–75 hours. Substernal pain is the earliest and most frequent symptom. Other symptoms include irritation of the nose and throat, anorexia, and paresthesia. The chest roentgenogram remains normal, and symptoms subside soon after oxygen inhalation is stopped.

The oxygen tension at which symptoms develop is apparently near 400–425 mm Hg. Michel *et al.* maintained six men at an oxygen tension of 418 mm Hg for 7 days and found that substernal distress developed but did not prevent completion of the experiment.[8] This level of oxygen tension is close to the minimal lethal oxygen tension of 350–400 mm Hg found in our experiments, described herein. What changes evolve from longer periods of exposure to oxygen in lesser concentrations is uncertain. Welch *et al.* have recently reviewed the existing data in this regard.[9]

The risks of oxygen injury to the lungs have been largely overlooked in the clinical use of mechanical ventilators employed with a tracheostomy.[10,11] Part of this oversight is due to the faulty design of the pressure-cycled ventilators, with nebulizers that negate dilution of oxygen in the ventilator.[10] A false sense of security easily results from relying upon the oxygen dilution valves in the ventilator. By contrast, with volume-cycled piston respirators, utilizing oxygen only to enrich the inhaled concentration to levels required to maintain normal arterial tension, inadvertent administration of oxygen in high concentrations has not occurred.

Another reason for the difficulty in detecting pulmonary injury from oxygen is that ventilators are frequently used only for serious pulmonary disease. Hence, if a death occurs after use of a ventilator, one can easily attribute it to progression of the original disease, not recognizing additional injury produced by high oxygen tensions. While proof cannot be obtained, it is highly probable that such a lethal pulmonary injury was produced by oxygen in the two patients described in this report.

At present, it would seem best to monitor the inspired gas for each patient in whom respiration is supported with a mechanical ventilator by *measuring the*

oxygen tension in the inspired gas at least twice daily. Only in unusual circumstances should oxygen tensions above 300 mm Hg be used and then only for short periods of time.

SUMMARY

1. Thirty-three unrestrained dogs were exposed to different concentrations of oxygen in a closed chamber for 5–14 days. In control studies using compressed air, the animals showed no ill effects. With oxygen tensions at 300–350 mm Hg for 14 days, the animals became drowsy but not dyspneic. Thickening of the walls of the pulmonary alveoli was the only abnormality found at autopsy. Oxygen tensions above 350–400 mm Hg regularly produced fatal pulmonary injury in 2–6 days. The prominent pathologic changes were congestion, edema, hemorrhage, and consolidation.

2. Pressure-cycled ventilators used in man for mechanical ventilation through a tracheostomy have a serious mechanical defect, as a result of which oxygen may be given in much higher concentrations than the oxygen dilution valves on the ventilator indicate. The error results from activation of the nebulizer in the ventilator with undiluted oxygen. Two fatalities from pulmonary insufficiency have been seen in man after ventilation with oxygen at a tension above 400 mm Hg for longer than 4 days. Although exact conclusions cannot be reached, the data strongly suggest that the pulmonary injury was related to the high concentrations of oxygen used.

3. When a patient is ventilated with a mechanical ventilator through a tracheostomy, the oxygen tension in the inspired gas proximal to the tracheostomy should be measured twice daily. For most patients, the oxygen tension should be kept well below 300 mm Hg.

ACKNOWLEDGMENTS

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DISCUSSION

DR. R. L. YANDA (*Los Angeles, Calif.*): Dr. Spencer, you stated that the dogs were breathing on their own and the patients were being ventilated. Do you think your results with the dogs would have been different if you could have ventilated them?

DR. SPENCER: This is difficult to answer, and we spent 6 months trying to design an experiment with dogs using a ventilator so that we could compare the two. A ventilator requires prolonged anesthesia, however, which itself causes pulmonary complications. I suspect that oxygen injury may have been frequently produced in man during mechanical ventilation and overlooked at autopsy because ventilators are only used on people with pulmonary disease. Hence, if someone dies on a ventilator, it may be readily assumed that this is due to his primary pulmonary disease, and separating this effect from a possible effect of oxygen is virtually impossible at that time.

DR. J. S. MCLAUGHLIN (*Baltimore, Md.*): When we set up our shock unit and began studying pulmonary functions on patients on the Bird respirator, we noted a high oxygen concentration during certain phases of the respirator, but not throughout the entire cycle. The oxygen concentration went from 25% to nearly 90% during inspiration, averaging about 40%. I wonder if you have made measurements throughout the entire cycle.

I might add that to preclude this problem in studying pulmonary function we have been using the Bird respirator set on 100% oxygen (with the 100% oxygen button pushed in) and then using bottled oxygen at 40% concentration, to give a standard 40% oxygen mixture throughout the respiratory cycle.

DR. SPENCER: The concentrations were measured at different times and varied widely, from as much as 65 to 95%. We found that there are two factors; one is the nebulizer, and the other the amount of inspiratory pressure used in the ventilator. This apparently alters the Venturi effect in the nebulizer, so if one ventilates with 25 cm of pressure, it is different than 10 cm, and thus a wide variation may occur. Because of the unreliability of the device, we have given up using oxygen with ventilators, and we ventilate all patients with compressed air or with the oxygen mixture you described.

UNIDENTIFIED SPEAKER: Does the oxygen concentration you describe give you an arterial tension between 90 and 100%?

DR. SPENCER: Precisely. That is the monitor we have utilized. One does not encounter this problem if one uses the volume-cycled respirators such as the Merck, Airshields, Engstrom, and others, in which oxygen is admitted to the system. By using an oxygen tension in the ventilator between 200 and 250 mm Hg, one can hold arterial oxygen tension at about 90 to 110 in the majority of patients.

DR. J. JACOBSON (*New York, N. Y.*): I would like to tell you about a case relevant to the general discussion, though not entirely pertinent to Dr. Spencer's paper presented. This was a 16-year-old boy admitted to our hospital about 2 months ago with a diffuse pneumonia thought to be viral in origin. On admission, he appeared in fairly good condition, but he went downhill rapidly. We were asked to see him when he was almost terminal. By this time he had had a tracheostomy and had been placed on 100% oxygen on a Bird respirator. In the absence of adequate oxygenation by conventional methods he was brought to the hyperbaric chamber for treatment, as his only chance for survival. On entry to the chamber, arterial pO_2 was 35 mm Hg. He had a bradycardia that looked terminal. Pressure was raised to 2 ata, which brought his arterial pO_2 up to 90. This treatment was continued for the next 24 hours, testing every hour or two to lower the pressure. We were able to decompress about half of the time to 1½ ata, always giving the patient 100% oxygen. At the end of the twenty-fourth hour, we were able to take him out of the chamber.

Some 8 hours later, he again became deeply cyanotic. The anesthetist who was

squeezing the bag was forced to do it energetically with both hands. The patient was brought back to the chamber. We were concerned about oxygen toxicity, but he appeared to be dying. We put him in the chamber once again and carried him for 16 hours at 1½ to 2 atmospheres of pressure. At the end of 16 hours he was able to tolerate normal atmospheric pressure, and from there on had an uneventful recovery.

He is back in school now and does not seem to be any the worse for the wear. The thing that disturbs us is that he wants to become a physician, and we were wondering whether there was some brain damage from our therapy.

DR. P. C. PRATT, *Session Chairman (Columbus, Ohio)*: You did say he was at 2 atmospheres of 100% oxygen for 16 hours?

DR. JACOBSON: He was at 2 atmospheres for about 14 hours and at 1½ atmospheres for 10 hours for the first 24 hours—then an 8-hour interval at normal pressure, followed by 16 hours at 2 ata and 1½ ata divided about equally as to time. He is probably the most gratifying single case we have had.

I would like to comment that in treating some of these hyaline membrane diseases, the attitude should not be to raise pressure as much as we can, but rather to strive toward achieving a normal oxygen saturation of the blood. This was what we were trying to do in this youngster, and it may be that just 1¼ or 1½ atmospheres would be enough to tide somebody over a bad period. Why we did not get into severe oxygen toxicity in this youngster, I do not know, except that the lungs may have been protected internally by a normal pO_2 of the blood, and this is something we do not take into consideration. We are just talking about the pO_2 in contact with the alveolar membrane. It may be quite different when the pO_2 of the blood is normal.

DR. D. G. MCDOWALL *(Glasgow, Scotland)*: In dealing with a case such as Dr. Jacobson has just described, is there any value from the point of oxygen toxicity in using a higher pressure together with an inert gas rather than trying to keep the total ambient pressure down?

DR. JACOBSON: I have no comment; I hope that somebody in the next couple of days will answer that question.

Cyclical Intermittent Hyperbaric Oxygenation: A Method for Prolonging Survival in Hyperbaric Oxygen

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Hyperbaric oxygen appears to be proving its value in the treatment of various acute and chronic pathologic conditions. When OHP is used during cardiac surgery, during radiation therapy for cancer, as a treatment for decompression sickness, or for carbon monoxide poisoning, toxicity is minimized by the relatively brief periods of exposure. Considerably longer periods of treatment, however, are required for numerous chronic conditions such as circulatory insufficiency (due to cerebral vascular accidents, myocardial infarction, peripheral vascular disease, decubitus ulcers, frostbite, burns, pedicle skin grafts, and fractures and wounds with marginal blood supply), gas gangrene and other anaerobic infections, and chronic respiratory insufficiency (pulmonary emphysema, severe pneumonia, and postoperative pulmonary conditions). Logically, many of these diseases should be best treated by continuously administered hyperbaric oxygen for days or even weeks, but due to the danger of oxygen toxicity, exposures are generally limited to 1–3 hours, several times a day, leaving periods of 12–20 hours when the patient receives little or no benefit from the hyperbaric oxygen exposure. Several reports have appeared in the literature of patients with these chronic diseases who have received obvious benefit while in the compression chamber, only to revert to their previous pathologic state shortly after decompression.[1,2]

It seems reasonable, therefore, that any technique for administering hyperbaric oxygen that could permit almost continual tissue hyperoxygenation and yet decrease toxicity significantly would find considerable clinical and experimental use. During the course of our studies on tissue oxygen tensions in experimental animals under a variety of conditions, such a technique suggested itself and has been tested successfully in experimental animals.

TISSUE OXYGEN TENSION STUDIES

Tissue oxygen tensions were measured in healthy New Zealand albino rabbits, lightly anesthetized with pentobarbital

(Nembutal), by means of a Beckman 160 gas analyzer and polarographic microelectrode. The electrode was inserted into the hind-limb musculature through an 18-gauge Riley needle. Readings of the tissue oxygen tension as a reflection of the current flow through the electrode were recorded continuously during control and experimental periods. Oxygen tension levels were expressed as "arbitrary units," as recorded on the gas analyzer.

The tip of the electrode used for these studies is covered by a polyethylene membrane, beneath which a platinum cathode and silver anode are bathed in an electrolyte solution. A constant polarizing voltage of −0.68 volt from the amplifier is applied to the platinum cathode. When the electrode is placed in a sample, oxygen molecules migrate through the membrane and are reduced at the cathode. The reduction of oxygen molecules creates a flow of current which is proportional to the pO_2 of the sample.

Although many other investigators have performed studies with similar equipment,[3-12] it is generally acknowledged that these polarographic electrodes have certain limitations, most important of which is the inability to obtain precise oxygen tension calibration in tissue. This means that an exact numerical value of oxygen tension in millimeters of mercury cannot be given. Even so, such valuable information has been obtained as the direction of acute changes in tissue oxygen tensions, the magnitude of change compared to control levels, the rate of the change (response time of the electrode being only a few seconds), the rate at which oxygen tensions return to normal levels after termination of experimental conditions, and the comparative effects of several different techniques in raising oxygen tensions.

The effects of breathing 100% oxygen at atmospheric pressure in a plastic tent were determined in 19 studies (Figure 1). In most studies, the oxygen tensions began to rise within 1 min, reaching peak levels

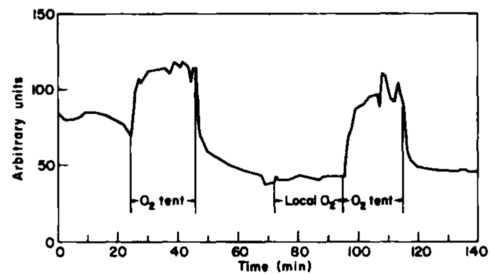

FIGURE 1. Response of tissue oxygen tension in hind-limb musculature of rabbits during exposure to 100% oxygen-breathing in a tent and during local exposure of just the limb to 100% oxygen. Note rapid rise of pO_2 during exposure to oxygen in the tent and slower decline of pO_2 when oxygen tent was removed.

averaging 2.33 times control levels. When the oxygen tent was removed, the values began to fall within 30 sec and returned to control levels within a mean of 13 min. When the hind limb was exposed to an oxygen-rich environment produced by placing a plastic bag around the leg, no significant change in muscle oxygen tensions was measured. Tissue oxygen tensions were measured during exposure in a small animal hyperbaric chamber to 100% oxygen at 15 psig. The tissue oxygen tension levels rose rapidly within 1 min after compression was started. The levels usually rose in steps as the pressure was increased. The peak levels averaged 4.44 times control levels in 20 studies. The elevated oxygen tension levels in the muscle began to fall within 1 min after the start of decompression. An average of 27 min was required for the tensions to return to control levels. These results are summarized in Table 1.

The effects on tissue oxygen tension of 100% oxygen at 15 psig, compressed air at 15 psig, and 100% oxygen at 0 psig were compared (Figure 2). In seven studies, compressed air at 15 psig significantly increased muscle oxygen tension, averaging 2.07 times control levels. When oxygen was substituted at the same pressure, a further increase in oxygen tension occurred, with peak levels averaging 4.57 times control levels. Sim-

TABLE 1. Response of Tissue Oxygen Tensions to Oxygen Environment

	Exposure conditions	
	100% O_2 at 0 psig [a]	100% O_2 at 15 psig [b]
Time of pO_2 rise from start of O_2 exposure	1 min	40 sec mean 15 sec median
Time of pO_2 fall from termination of O_2 exposure	30 sec	1 min mean 15 sec median
Time of return to control pO_2 levels	13 min	27 min
Ratio of peak:control pO_2	2.33	4.44

[a] Nineteen studies performed under these exposure conditions.
[b] Twenty studies performed under these exposure conditions.

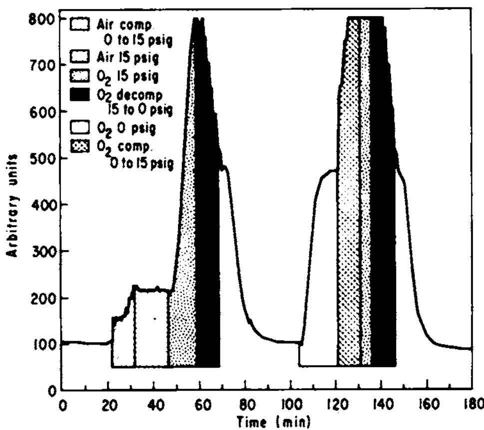

FIGURE 2. Comparison of tissue oxygen tensions resulting from exposure to air and oxygen at normal and hyperbaric pressures. The hindlimb pO_2 rose rapidly when the animal was exposed to high pressure oxygen and fell more slowly when oxygen concentration was decreased. Peak:control pO_2 ratios were highest during exposure to 100% oxygen at 15 psig.

ilarly, in eight studies, 100% oxygen at 0 psig increased oxygen tension with peak:control ratios averaging 2.65. When the pressure was raised to 15 psig, the muscle oxygen tension rose further, reaching a mean peak:control ratio of 5.01 (Table 2). Under all conditions, the tissue oxygen tensions rose rapidly as the concentration of oxygen in the chamber was increased, and they fell more slowly as the oxygen was decreased.

In order to try to rule out the possibilities of artifacts in the pressure-chamber studies, the effects of pressure *per se* on the electrode were tested. With the electrode lying free within the chamber, the pressure was increased to 15 psig with 100% nitrogen (Figure 3). The oxygen tension readings rapidly dropped to zero. When oxygen was substituted for nitrogen at the same pressure, the values rose to over 800 mm Hg, or higher than this system can record. To rule out the possibility of seepage of oxygen down into the muscles along the electrode needle tract, several additional experiments were carried out. In several studies, the electrode was tunneled subcutaneously

TABLE 2. Ratio of Peak to Control Tissue Oxygen Tensions

Exposure conditions	No. studies	Peak:control ratio (mean and SE)
100% O_2 at 0 psig	8	2.65 ± 0.66
100% O_2 at 15 psig	8 [a]	5.01 ± 1.12
Air at 15 psig	7	2.07 ± 0.42
100% O_2 at 15 psig	7 [a]	4.57 ± 0.48

SE, standard error of the mean.
[a] Same animals used as those directly above; this second group of studies performed after a change in exposure conditions, as indicated.

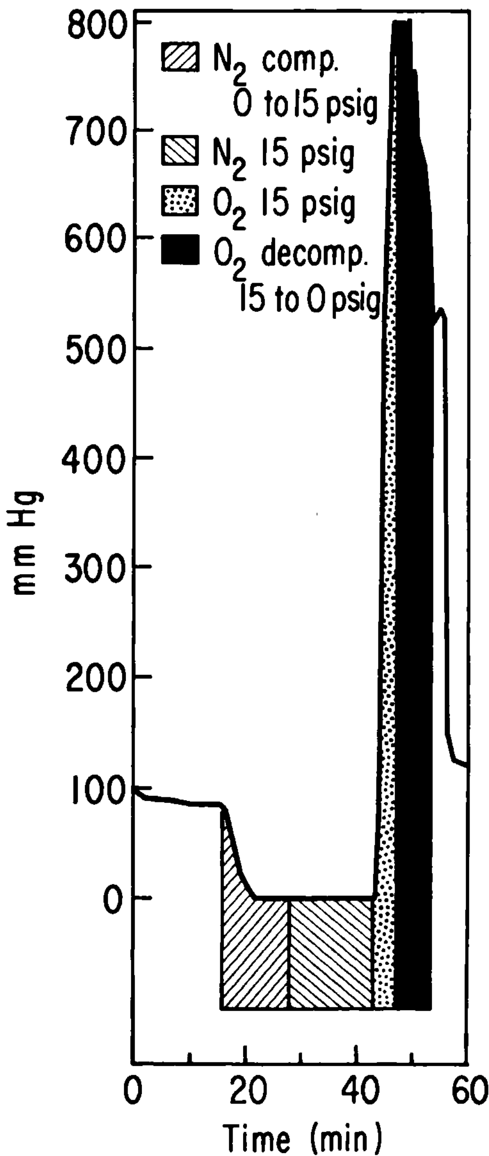

FIGURE 3. Effect of hyperbaric nitrogen and oxygen on polarographic microelectrode. With 100% nitrogen at 15 psig, the electrode within the chamber recorded pO_2 at 0 mm Hg. When oxygen was substituted at 15 psig, pO_2 levels rapidly rose above 800 mm Hg.

from midabdomen to hind leg, and in other animals the entire electrode assembly was sewn into the peritoneal cavity with only the tip of the needle penetrating from the peritoneal cavity into the leg muscle directly. In all experiments, significant increases in muscle oxygen tensions were recorded when the animals were exposed to 100% oxygen at 15 psig.

Cyclical Intermittent Hyperbaric Oxygenation

Since the tissue oxygen tension studies described above indicated an almost instantaneous rise in tissue tensions when the animals were exposed to hyperbaric oxygen and a slower fall when decompression was begun, we felt that with repeated intermittent periods of hyperbaric oxygenation one could obtain levels almost continuously elevated. This would avoid the necessity of an uninterrupted period of high pressure, and, as a result, toxicity might be decreased. This hypothesis has been tested by observing survival times of experimental animals during exposure to hyperbaric oxygen.

Sixteen healthy unanesthetized rabbits were exposed continuously to 100% oxygen at 15 psig with a steady flow of oxygen maintained through the chamber. The rabbits died from oxygen poisoning at 12.5–24.5 hours, with a mean time of 17 hours. Other animals were maintained on cycles of intermittent hyperbaric oxygenation. The first group of six rabbits was placed on a repeated cycle of 15 min of compression from 0 to 15 psig with 100% oxygen, 2 hours of 100% oxygen-breathing at 15 psig, 15 min of decompression to 0 psig with 100% oxygen, and 30 min of air-breathing at 0 psig with the chamber door open. The 30 min of exposure to room air was adopted so that the period of low tissue oxygen tensions would be relatively short. The survival time of these animals varied from 24 to 62 hours, with a mean of 46 hours. In an attempt to improve survival time further, the exposure to 100% oxygen at 15 psig was reduced to 1 hour. On this 15:60:15:30 (min) cycle, the mean survival time of six rabbits was 87 hours, with a range of 78–104 hours.

The best results obtained thus far have been on a cycle of 15 min of compression, 30 min of 100% oxygen at 15 psig, 15 min of decompression, and 30 min of room air at 0 psig. Of 23 animals kept on this cycle, 22 survived the entire run of over 100 hours, although three animals died during the next 2 days. Thus, 83% survived almost continuous hyperbaric oxygenation for over 100 hours under the 15:30:15:30 (min) cycle. These results are summarized in Table 3.

Tissue oxygen tension determinations were made during several cycles of intermittent oxygenation (Figure 4). During the period of exposure to room air at 0 psig, the oxygen tension levels slowly drifted down to the control range. The oxygen tensions remained at the control level for only a brief period. As compression with oxygen was resumed, the oxygen tensions rose rapidly to a significantly elevated range.

The experience with cyclical intermittent hyperbaric oxygen has been extended to studies with other laboratory animals and to exposures at higher pressures. Healthy young Sprague-Dawley rats were exposed continuously to 100% oxygen at 15 psig at a flow of 3–5 liters/min. The mean survival time was 15 hours (range 13.5–19.5 hours). On a 15:30:15:30 (min) cycle, the mean survival time was extended to over 45 hours (range 34–57.5 hours). When the rats were exposed continuously to 100% oxygen at 30 psig, mean survival time was 9 hours (5–11.5 hours). Survival time was increased to 24 hours (range 18–33.5 hours) when the rats were maintained on a 15:30:15:30 (min) cycle. These data are summarized in Table 4.

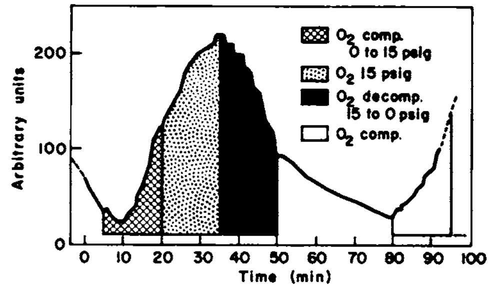

FIGURE 4. Response of tissue oxygen tension to cyclical intermittent hyperbaric oxygenation. Hind-limb pO_2 gradually drifted to control values during exposure to air at 0 psig and rose rapidly at beginning of next cycle. The pO_2 was elevated above control levels during most of the cycle.

DISCUSSION

Cyclical intermittent hyperbaric oxygenation appears to have increased survival time of the experimental animals in these studies significantly during exposure to almost continuous hyperoxygenation. Whether this treatment procedure can be of practical benefit to patients with chronic diseases still remains to be proved, especially in comparison to the regime of hyperbaric treatment commonly employed. The effects of cyclical intermittent hyperbaric oxygenation are currently being evaluated in studies with ischemic tissues in our laboratories.

The 15:30:15:30 (min) cycle used successfully in our studies is not necessarily ideal. Where oxygen is administered under pressures greater than 15

TABLE 3. Effect of Cyclical Intermittent Hyperbaric Oxygenation on Survival Time in Rabbits

Exposure to 100% O_2 at 15 psig	No. rabbits	Survival time (hours)
Continuous	16	17 (2.5–24.5)
15:120:15:30 (min) cycle	6	46 (24–62)
15:60:15:30 (min) cycle	6	87 (78–104)
15:30:15:30 (min) cycle	23	> 100 [a]

[a] Eighty-three percent survival.

TABLE 4. Effect of Cyclical Intermittent Hyperbaric Oxygenation on Survival Time in Rats

Exposure conditions	No. rats	Survival time (hours)
100% O_2 at 15 psig		
Continuous	6	15 (13.5–19.5)
Cyclical intermittent [a]	6	45 (34–57.5)
100% O_2 at 30 psig		
Continuous	12	9 (5–11.5)
Cyclical intermittent [a]	12	24 (18–33.5)

[a] Cycle consisted of 15 min 100% oxygen-breathing during compression, 30 min 100% oxygen-breathing at full pressure, 15 min 100% oxygen-breathing during decompression, and 30 min air-breathing with the chamber door open.

psig, the return of tissue oxygen tension to control levels during the decompression period probably takes considerably more than 27 min. If the chamber pressure is raised to 45 psig, for example, it may be possible to expose the animal to room air at atmospheric pressure for an hour or more without losing the effects of hyperoxygenation. This might increase the survival even longer during exposure to higher pressures.

Although most clinical and experimental studies have limited the use of hyperbaric oxygenation to 1–3 hours several times a day, there have been some reports of continuous use of high pressure oxygen. The use of "intermittent hyperoxygenation" has been reported in the therapy of some patients with peripheral vascular disease, and it has appeared to give favorable results without untoward effects from the oxygen.[13] The periods of decompression were considerably less frequent than those described in the present study.

We believe that equally good results could be achieved, with less chance of toxicity, if repeated cycles similar to those described in this paper were employed. About 10 years ago, similar intermittent cycles in guinea pigs [14,15] were reported in the literature, and survival time was also prolonged in these studies. Mention was made of successful field experiments with these techniques among diving groups in World War II. With the addition of the tissue oxygen tension determinations made in the present study, one can design intermittent cycles to increase the safe period of exposure to hyperbaric oxygen and still allow almost continuous tissue hyperoxygenation.

SUMMARY

Tissue oxygen tension studies in experimental animals have indicated that an almost immediate rise in tension occurs when the animal is exposed to hyperbaric oxygen, and there is a slower fall in tension when the pressure is discontinued. The use of cyclical intermittent hyperbaric oxygenation with repeated cycles of 15 min of compression with 100% oxygen, 30 min of 100% oxygen at elevated pressures, 15 min of decompression with 100% oxygen, and 30 min in room air at 0 psig with the chamber door open has significantly prolonged survival times in experimental animals. The belief that animals would receive almost continual hyperoxygenation by this method is supported by tissue oxygen tension studies performed during such cycles. These techniques of administering hyperbaric oxygen may have useful clinical and experimental applications.

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DISCUSSION

DR. R. ROSENBAUM (*New York, N. Y.*): The thought has occurred to us at various times over the past years that when you treat pulmonary patients in oxygen tents or with respirators of various kinds, might not their own lesions protect against oxygen toxicity? Pneumonias or some types of effusion might well protect the pulmonary epithelium from direct contact with oxygen, at least for a time. Naturally, this could only be possible if the patient's tissues were not permitted to be saturated with oxygen. Certainly the case that Dr. Jacobson described would normally have killed a rat, or certainly it would have caused some pulmonary pathology in a rat or a dog.

DR. P. C. PRATT, *Session Chairman* (*Columbus, Ohio*): And in most people too, wouldn't it?

DR. ROSENBAUM: Yes indeed—so the question is, could that which we consider a lesion of oxygen toxicity actually serve to protect, and hence have survival value?

DR. C. J. LAMBERTSEN (*Philadelphia, Pa.*): Dr. Ackerman may, during his student days at Pennslyvania, have missed some experiments in pharmacology concerning prolongation of oxygen tolerance by alternating high and normal oxygen tensions in animals. The animals (guinea pigs) were different from those used here, but the experiments involved the same kind of time sequence. Intermittent exposure to the 3 atmospheres of oxygen

resulted in an approximately fivefold increase in oxygen tolerance. This system does work, and I think the adding of tissue oxygen tension is a fine idea in the present study if one should try to pick the tissues of greatest interest. I would welcome information concerning rise of oxygen tension and the rate of fall of oxygen tension in the myocardium, the brain, and the kidney during intermittent exposures. It would be very wise to consider putting oxygen-sensing needles in a number of definite tissue sites.

Secondly, the question must be raised whether the technique of interrupted oxygen administration increases oxygen tolerance in man. We can answer this because it was tried, though so long ago that it has almost been forgotten. I experienced this myself about 20 years ago in World War II during oxygen-diving. When twitching (the central nervous symptom of oxygen poisoning) developed and one surfaced, the symptoms would go away even without discontinuing the oxygen-breathing. If one went down again, a long waiting period once more existed before symptoms occurred again. Therefore, both in humans and in animals, it is possible to re-establish oxygen tolerance to some degree, at least in the central nervous system, by brief interruption of exposure to high oxygen pressures. This procedure is now in use in the oxygen treatment of bends, as well as in the oxygen treatment of gas gangrene. Whether it is also possible to restore the pulmonary tolerance to oxygen is an entirely different question. Did your animals die from pulmonary damage or from central nervous system damage?

DR. ACKERMAN: We became interested in techniques of this sort primarily because we were studying the ischemic legs of rabbits. Thus, we were actually measuring the responses and tissues in which we were most interested. In such preparations, we have had some very promising, although as yet preliminary, findings in the protection of animals with surgically produced ischemic legs, after 100 hours of therapy. The animals died from pulmonary damage. As far as human studies are concerned, there are numerous clinical reports of patients who have been treated for long periods with hyperbaric oxygen and have had intermittent interruption of their therapy. I believe the group here at Duke has used this technique on a number of patients, and Dr. Ledingham has also reported several cases of this sort. We agree that this probably will work very well.

DR. N. G. MEIJNE (*Amsterdam, Netherlands*): Could the patients not be kept under pressure all the time, but the gas mixtures changed to air or to oxygen and helium?

DR. ACKERMAN: I think that procedure could easily be substituted, and we would expect to obtain similar results.

DR. H. S. WEISS (*Columbus, Ohio*): I have a comment on the interaction of pathology and oxygen toxicity. We completed a few short studies with mice and rats and found that their survival time in 100% oxygen at 1 atmosphere was less than that of conventional animals. We came to the general conclusion that the chronic infection was probably protecting the conventional animals to some small extent.

DR. R A. COWLEY (*Baltimore, Md.*): I was interested in hearing Dr. Ackerman speak about the polarographic electrode, and also to hear Dr. Lambertsen's comment. We have reported on this with animals in shock. During bleed-out, the reading dropped, but when they were put in the chamber it rose to a very high level. This was measured in the muscle and also in the liver. When the animal was taken out of the chamber the reading fell back to the normal level. Dr. Attar has done this study repeatedly and the findings are always reduplicated. Nevertheless, our physiological colleagues, Dr. Ackerman, continue to tell us that this is not so, and I would like your comments. Is it a relative measurement, or do you think one should call it "oxygen potential"? Perhaps we should measure it in voltage.

DR. ACKERMAN: This is a very difficult problem. Perhaps it is about time that some evaluation should be made as to whether polarographic studies are valid or not. This is a difficult technique, and one can only extract certain material from it. There have been all sorts of exorbitant claims in studies with polarographic electrodes, particularly in relation to tissue calibration. I think it takes considerable experience and exposure to the equipment to realize where the limitations are, and what the possible applications are for its use.

Effects of Prolonged Exposures of Rats to Increased Oxygen Pressures

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Before oxygen at high pressures can be used in spacecraft cabin atmospheres for prolonged periods, and before its medical applications can be expanded, many problems must be elucidated and overcome. The major limitation for hyperbaric use of oxygen is the inability of the central nervous system,[1] lung,[2] and eye[3] to tolerate a sustained increase in pO_2. Pulmonary edema and convulsions associated with inhalation of oxygen at high pressures (760 mm Hg and above) are well-documented syndromes in man[2,4] and animals.[5] Although these overt responses are recognized, however, the mechanism by which oxygen acts to initiate the response remains obscure. Also to be determined are tolerance levels for high partial pressures of oxygen over sustained periods of time. These problems are further complicated by a lack of information on the physiologic needs for inert gases.

The subject of this investigation is the effect of prolonged uninterrupted exposure of rats to 100% oxygen at varying pressures: those known to induce pulmonary damage (550–760 mm Hg)[6] and levels presently assumed near the threshold for oxygen toxicity (425 mm Hg).[7,8] It was anticipated that uninterrupted exposure of animals to a high pO_2 would reveal information on biological parameters most susceptible to increased oxygen tensions and on tolerance levels for oxygen partial pressures, and that it would provide a better understanding of the action of molecular oxygen on the living animal.

METHODS

Male Sprague-Dawley rats, housed in our animal environmental chambers,[9] were subjected to various oxygen pressures for selected time periods (Table 1). Rats of different ages were used so that the effect of age on oxygen susceptibility could be evaluated.

The individual chambers (Figure 1) provided uninterrupted exposure to 100% ($99.8 \pm 0.2\%$) oxygen at the desired total pressure. Animals were transferred into clean chambers daily (Figure 2) with no deviation in chamber conditions. Control animals, housed in chambers identical to those of the oxygen groups, breathed air at sea level for the duration of the experiments. Food and water consumption was recorded daily for all animals, and animals

TABLE 1. Experimental Protocol and Results of Exposures of Rats to 100% Oxygen for Selected Periods

Group	Chamber pO_2 (mm Hg)	Exposure (days)	Animal wt. (gm)	No. animals		% Surviving		Significant diff. between exposed and controls		
				O_2	Air	O_2	Air	Blood	Nutr.	Histol.
I	450	64	133.0 ± 5.0	12	6	100	100	no	no	no
II	600	28	186.0 ± 5.0	14	7	100	100	yes	yes	yes
III	600	28	542.0 ± 13.0	14	7	100	100	yes	yes	yes
IV	760	34	132.0 ± 4.0	14	7	57	100	yes	yes	yes
V	760	4	552.0 ± 30.5	14	7	0	100	—	yes	yes

were weighed routinely throughout the experiments.

Immediately upon termination of a particular experiment, the animals were anesthetized with sodium pentobarbital, sacrificed, and examined for gross pathologic lesions. A cardiac puncture was performed and 5–6 ml of blood was removed for the following analyses: complete blood cell counts, hematocrit, hemoglobin, osmotic fragility, Heinz bodies, glutathione, and red blood cell glucose-6-phosphate dehydrogenase activity. Weights were obtained for the heart, testes, thymus, and adrenal glands. A histologic survey was performed on the lungs, liver, kidneys, heart, brain, gastrointestinal tract, thymus, and adrenal glands for pathologic conditions. The hematopoietic activity of the animals was assessed by complete bone marrow differential counts and study of sections of decalcified femurs.

Statistical comparisons between the oxygen and control groups were performed with an analysis of variance (split-plot design[10]) for growth rates, for food and water consumption, and for the other variables measured.

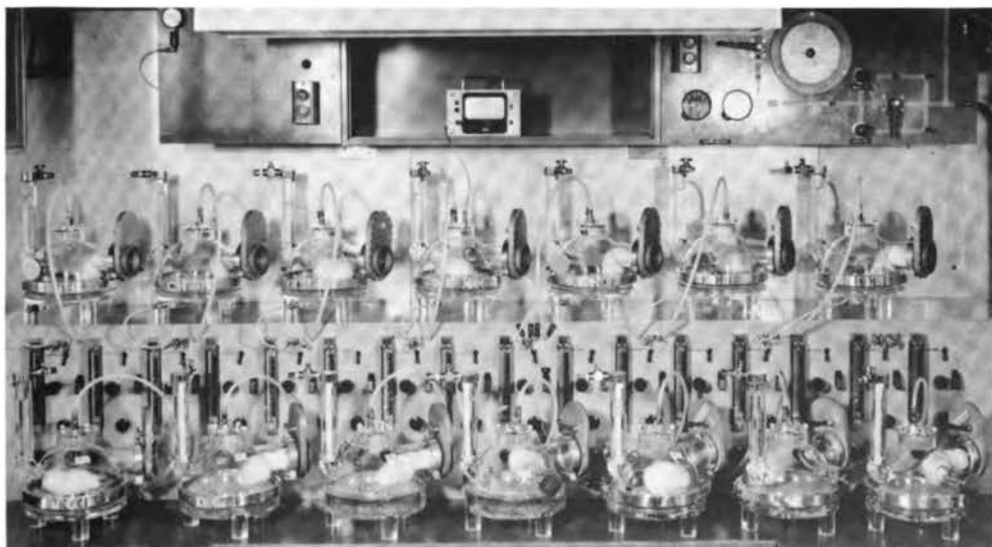

FIGURE 1. Individual chambers used for exposing rats to 100% oxygen or air under controlled conditions. (Reproduced from Aerospace Med. 37:243, 1966.)

FIGURE 2. Method of transferring rat into a clean chamber. Chamber on the right is disconnected for cleaning. (Reproduced from Aerospace Med. 37:243, 1966.)

RESULTS

As shown in Table 1, oxygen was lethal only at a sustained pressure of 760 mm Hg. After 64–78 hours of exposure to this pressure, the animals died, presumably from the extensive pulmonary edema found upon autopsy.

Nutritional data (Figure 3) indicated that only rats exposed to oxygen pressures above 450 mm Hg showed evidence of decreased nutritional well-being. Growth rates were depressed, food consumption was low, and in some cases reduced food efficiency values (weight gain ÷ weight of food eaten) revealed significant nutritional and/or metabolic disturbances in all the rats at high oxygen tensions.

Significant changes in the circulating blood elements were hard to discern. Except for what appeared to be a microcytic shift in erythrocytes in rats exposed to 600 and 760 mm Hg, most of the data (some of which appear in Table 2) suggested relative resistance of the erythrocyte to elevated oxygen pressures. Heinz-body counts, osmotic fragility, and red blood cell glucose-6-phosphate dehydrogenase activity were not affected by exposure to oxygen in any of the animals. White cell counts were elevated only in animals inhaling oxygen at 760 mm Hg for 34 days. In most cases, mean corpuscular hemoglobin was low in the oxygen-exposed animals, probably due to the apparent decreased mean corpuscular volume; this was verified by the fact that mean corpuscular hemoglobin concentrations remained normal for all the animals. The depression of reduced glutathione levels in the blood of the oxygen-exposed animals (Table 2) did indicate that significant hemoglobin oxidation was present with increased oxygen pressures *in vivo*. It is interesting that

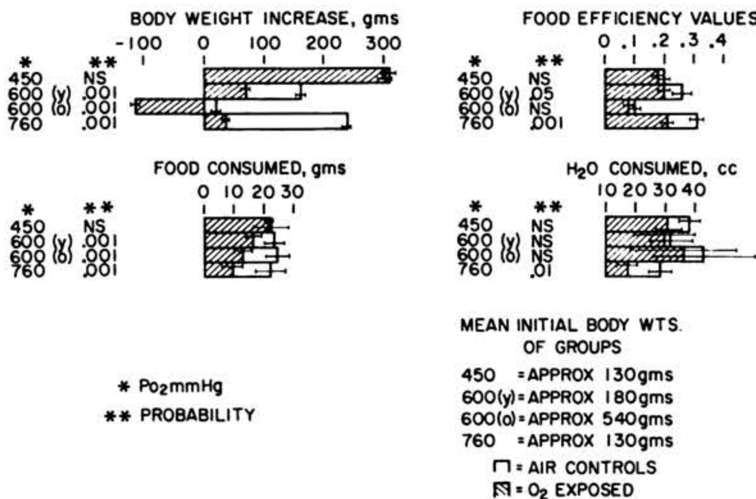

FIGURE 3. Summary of results for nutritional parameters measured. Those values found to be significantly different from the controls are indicated. Values expressed are means and standard deviations for the various groups.

TABLE 2. Means and Standard Errors of Selected Blood Variables for Various Oxygen Concentrations and Animal Body Weights

Variable	760 mm Hg — 34 days 130 gm		600 mm Hg — 28 days 180 gm		600 mm Hg — 28 days 540 gm		450 mm Hg — 64 days 130 gm	
	Control	Oxygen	Control	Oxygen	Control	Oxygen	Control	Oxygen
Hematocrit (vol %)	44.6 ± 1.4	43.7 ± 1.7	46.3 ± 1.4	40.7 ± 0.5[c]	45.5 ± 0.5	36.3 ± 1.4[c]	45.3 ± 0.2	43.3 ± 0.7
RBC ($10^6/mm^3$)	5.644 ± 0.231	6.713 ± 0.263[b]	5.800 ± 0.213	6.324 ± 0.699	7.520 ± 0.130	6.620 ± 0.100[c]	6.665 ± 0.196	5.794 ± 0.164[b]
WBC (per mm^3)	9386 ± 104	19,862 ± 289[b]	9783 ± 717	11,214 ± 636	9429 ± 923	8565 ± 384	5191 ± 354	6050 ± 247
Reticulocytes (%)	4.4 ± 0.2	4.5 ± 0.2	3.2 ± 0.1	3.3 ± 0.1	2.0 ± 0.2	4.1 ± 0.7[a]	3.4 ± 0.2	3.0 ± 0.1
Hemoglobin (mg/100 ml)	12.4 ± 0.3	12.4 ± 0.4	12.8 ± 0.1	11.9 ± 0.2[b]	12.3 ± 0.2	9.6 ± 0.3	12.9 ± 0.1	12.0 ± 0.2[c]
MCV ($micron^3$)	79.3 ± 2.4	65.4 ± 2.4[c]	79.8 ± 1.3	65.0 ± 1.7[c]	60.4 ± 0.7	56.5 ± 0.8[b]	68.3 ± 1.7	75.4 ± 2.1
MCH ($\mu\mu g$)	22.2 ± 1.6	18.5 ± 0.6[c]	22.1 ± 0.4	18.9 ± 0.4[c]	16.4 ± 0.2	14.7 ± 0.2[c]	19.5 ± 0.5	20.8 ± 0.5
MCHC (%)	27.9 ± 0.4	28.5 ± 2.7	27.7 ± 0.4	29.2 ± 0.4	27.2 ± 0.4	26.1 ± 0.4	28.5 ± 0.2	27.8 ± 0.6
Glutathione (mg/100 ml)	35.6 ± 1.0	30.9 ± 1.6[a]	38.8 ± 1.3	34.5 ± 0.9[a]	33.8 ± 0.9	24.6 ± 0.9[c]	37.9 ± 0.8	36.1 ± 1.1

MCV, mean corpuscular volume.
MCH, mean corpuscular hemoglobin.
MCHC, mean corpuscular hemoglobin concentration.
[a,b,c] Significantly different from controls at 0.05, 0.01, and 0.001 levels, respectively.

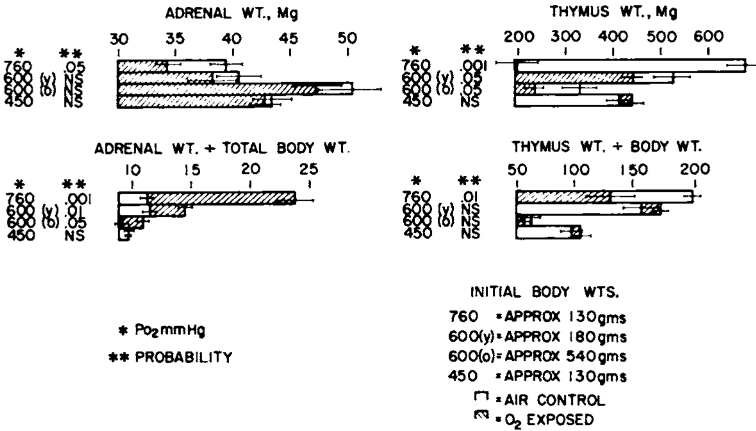

FIGURE 4. Adrenal and thymus gland weight changes following exposure to 100% oxygen at various pressures and selected durations. Those values found to be significantly different from the controls are indicated. Values listed are means and standard errors for the control and exposed animals.

this reduction in glutathione was 27.2% of the control value in the older group at 600 mm Hg compared to an 11.1% reduction in the younger animals at the same pressure.

No significant changes were observed in the bone marrow studies. This, along with no indication of reticulocytosis, discouraged thought of increased erythropoiesis or red blood cell destruction in oxygen-exposed animals.

Morphologically, increased adrenal and decreased thymus gland relative weights (organ weight ÷ body weight) indicated a significant stress response in animals exposed to pure oxygen at pressures above 450 mm Hg (Figure 4). (Glandular weights of this type are usually associated with general stress conditions in the rat.) These results were also supported (Figure 5) by the fact that in all cases, except during the 450 mm Hg exposure, the rela-

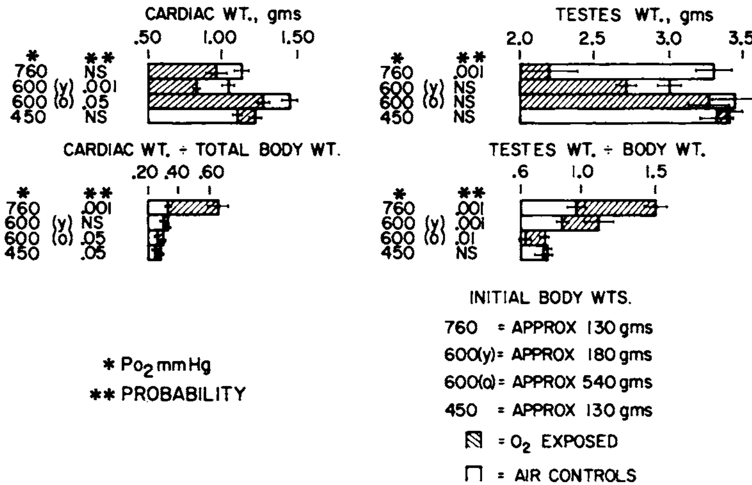

FIGURE 5. Cardiac and testicular weight changes following exposure to 100% oxygen at various pressures and selected durations. Those found to be significantly different from the controls are indicated. Values listed are means and standard errors of the controls and oxygen-exposed groups.

tive testicular weights were significantly higher for the oxygen-exposed animals than for the controls. The major animal response appeared to be a reduced growth rate, as the absolute testicular weights were only slightly lower than those of the controls—indicating the testes to be relatively resistant to stress. It should be pointed out, too, that in all exposures the relative cardiac weights of the oxygen-exposed animals were higher than those of the controls.

Histologically, tissue changes were observed only in the lungs of the oxygen-exposed animals; all other tissues appeared normal. Lesions were confined to rats maintained at 600 and 760 mm Hg, and the pulmonary changes were distinct at these two pressures. For example, the response observed at 600 mm Hg after 28 days was typified by thickening of the alveolar wall and edema within the lung parenchyma (Figures 6, 7). In 1000 alveoli counted, the alveolar spaces in the oxygen-treated rats averaged 29.5 microns in diameter compared with 62.0 microns for the controls. The alveolar wall thickness of 3.2 microns in the controls, compared with 6.0 in treated rats, gave another indication of the extent of pulmonary tissue changes.

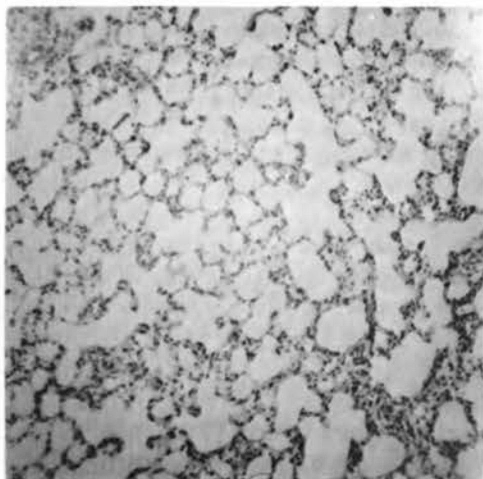

FIGURE 6. Lung of control animal exposed to air at sea level for the duration of the treatment (28 days). (Hematoxylin and eosin, × 18).

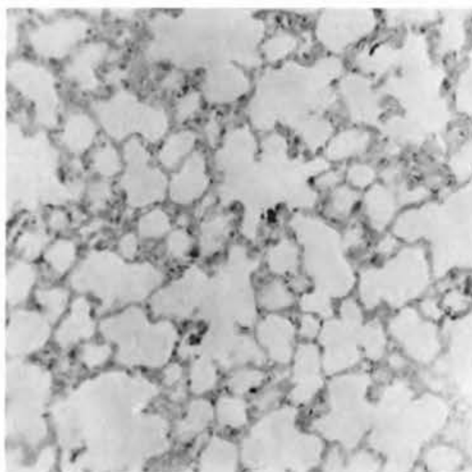

FIGURE 7. Lung from animal exposed to 100% oxygen at 600 mm Hg for 28 days. Prominent features are the lack of well-defined nuclei and generalized thickened appearance of the septa. (Hematoxylin and eosin, × 32.)

It was interesting to note that the lungs from rats exposed to 760 mm Hg appeared grossly and histologically typical of obstructive pulmonary emphysema (Figures 8, 9).

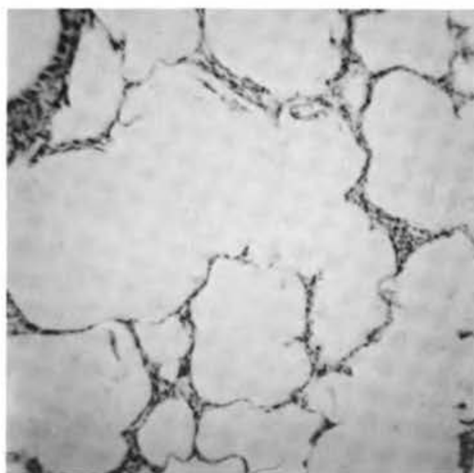

FIGURE 8. Lung of rat exposed to 100% oxygen at 760 mm Hg for 28 days. Notice enlarged alveoli, ruptured alveolar septa, and generalized emphysematous appearance. (Hematoxylin and eosin, × 37.)

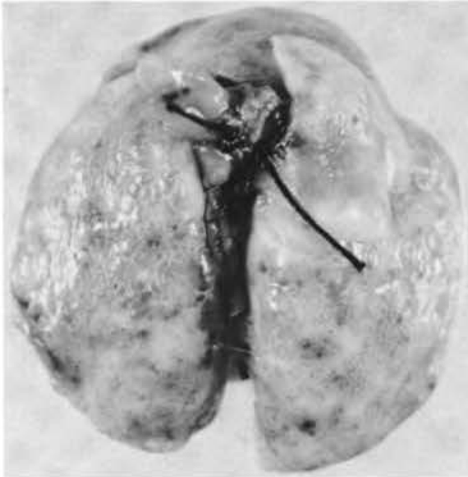

FIGURE 9. Lung of rat (anterior aspect) exposed to 100% oxygen at 760 mm Hg for 28 days. The lung appears grossly emphysematous and is characterized by extensive bullae.

DISCUSSION AND CONCLUSIONS

Caution must, of course, be used when the effects of increased oxygen pressure are compared in animals and in man. A difference in response to oxygen has even been demonstrated between strains of rats,[11] and sensitivity to oxygen is known to be related to temperature and humidity.[12] While sensitivity to oxygen may vary, however, the mechanism or mechanisms by which high oxygen tensions are deleterious to living systems may well be common to all species. In this light, the present study does provide information on the sensitive parameters for a prolonged exposure to an elevated pO_2.

The most salient feature of this study is the lack of evidence for toxic influences of oxygen during uninterrupted exposure to oxygen at a pO_2 of 450 mm Hg. This lack of evidence, in contrast to the dramatic toxic manifestations seen at 600 and 760 mm Hg, may indicate (at least in the rat) that a rather definitive threshold exists for sensitivity to oxygen. This is supported by findings of Dickerson,[6] and it suggests that the rat can tolerate sustained exposure to an elevated pO_2, provided this pressure does not exceed a certain range (presumably between 450 and 550 mm Hg). Studies on humans also suggest a tolerance level for man, probably around 450 mm Hg.[7,8,13]

The two most sensitive indicators for sustained exposure to increased pO_2 levels in the rat seem to be pulmonary histology and nutritional well-being, although declining nutritional status may simply reflect serious impairment of pulmonary function or generalized malaise. Histologic changes were observed only in the lung, even though other tissues were theoretically subjected to similar increased oxygen tensions. This may suggest something unique in the susceptibility of the pulmonary parenchyma. Also, the mechanism of pulmonary response, widely different at oxygen pressures only 150 mm Hg apart, implies that the mechanism of action of oxygen on the tissue may differ markedly at only slightly different pressures.

The apparent resistance of the erythrocytes and other blood elements is interesting in light of other work suggesting oxidative hemolysis[14] or lipid peroxidation[15] of red blood cells after exposure to 100% oxygen at elevated pressures. While admittedly our pressures were below that where lipid peroxidation has been reported to occur in humans (30–60 psia), our studies would indicate a high amount of resistance to *in vivo* changes in the red cells with a prolonged exposure to elevated oxygen tensions. This is supported by a lack of major changes even in rats exposed to oxygen at 760 mm Hg for 34 days.

In contrast to the results of other authors,[16] this study indicates that oxygen sensitivity is age-dependent. Two facts support this: 100% mortality was observed in old animals at a pO_2 of 760 mm Hg, while younger animals had a 43% mortality; and the older rats at 600 mm Hg suffered a more marked nutritional depression than the younger group. This is illustrated by the average 115-gm weight loss in the older (larger) animals and the 70-gm gain in younger animals. Also,

there was more marked depression in reduced glutathione levels for the older rats at 600 mm Hg. The significance of this remains to be determined.

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DISCUSSION

DR. P. C. PRATT, *Session Chairman (Columbus, Ohio)*: Could you repeat the conditions that produced the emphysema?

DR. BROOKSBY: We found that if we put a group of young animals (*i.e.*, no larger than 150 grams) on 100% oxygen, a 50% mortality occurred within 3 to 4 days. The 50% which survived this treatment were kept alive for about 5 weeks. We have repeated this experiment several times and found that without exception animals kept on oxygen for longer than 3 weeks developed clinical symptoms of pulmonary obstructive emphysema.

DR. PRATT: Clinical, not morphological?

DR. BROOKSBY: Well, it was actually a morphological study.

DR. K. P. PASSAMANECK (*Richmond, Va.*): Did the animals which died exhibit thickened alveolar membrances and decreased alveolar volume or number of alveoli, while the ones which survived got a thinning of the membrane or an increase in size of the alveoli?

DR. BROOKSBY: All the animals that died at 1 atmosphere of pressure showed the general response of the Lorrain-Smith syndrome, *i.e.*, pulmonary congestion, hemorrhage, and edema. In the surviving emphysematous animals, however, this condition began to improve, and we found that after about 3 weeks of treatment, the vasculature was essentially fully recovered, even though the animals had been maintained on 100% oxygen.

DR. C. J. LAMBERTSEN (*Philadelphia, Pa.*): This session deals largely with pulmonary pathology, and a fine question was asked earlier about the separate effects of oxygen upon bronchi and bronchioles as compared with the effects upon the alveolar membranes. Most of the emphasis has been on oxygen effects upon alveolar membranes. However, we know that if one exposes man to oxygen at high pressure for certain periods of time, the sensations are those of delineation of the bronchial tree. At the end of that time, when irritation and coughing are almost intolerable, there seems to be no gross impairment of oxygenation from alveoli to arterial blood. Certainly the alveolar membrane will eventually be destroyed, but evidently the histologic condition of the bronchial tree also bears investigation.

DR. BROOKSBY: We looked at the bronchial tree, particularly when we were studying this emphysema problem, more or less because we thought we had a good model for the pathogenic development of emphysema. We observed no structural changes in the bronchi, bronchioles, the terminal alveolar ducts, or any of the airway tree structures until after about 3 weeks of exposure to oxygen. At this time, we began to get much exfoliation of the bronchial epithelium and obstruction of the ducts, and we think that this obstruction eventually led to the condition of emphysema in these animals. The bronchi themselves were relatively resistant until after 3 weeks of exposure, when significant histologic lesions appeared in these structures.

UNIDENTIFIED SPEAKER: We did some culture studies on lung tissue slices of 3 to 5 mm and found that in this situation, using 45 psig, the alveolar tissue specifically was injured and damaged. The bronchial and vascular supporting tissues were not injured, at least within this particular system. There is a specific alveolar toxicity which is comparable to the toxic effect on glia and neural cells, as well as to a multitude of other diseases.

Oxygen Convulsions in Man

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Before widespread interest had been generated in the therapeutic use of hyperbaric oxygen, the specific needs of the Naval services had already stimulated studies on oxygen toxicity in diving.[1-3] Then, around 1960, Boerema began arousing interest in the clinical application of OHP.[4] This brought in its wake a need for increased understanding of the toxicology of oxygen under high pressure. While excellent reviews on the subject of oxygen toxicity have been written,[5-8] actual observations of the clinical manifestations in man have been limited. The findings from diving medicine can, therefore, offer instructive material to the clinician in this regard.

Following is a report of the clinical signs and symptoms of acute cerebral oxygen toxicity occurring in 63 men during U.S. Navy diving selection tests, training exercises, recompression treatment, standard decompression, and research activities. This material was gathered from the files of the U.S. Navy Experimental Diving Unit, Washington, D.C. Twenty-four of the 63 men evidenced signs of oxygen toxicity during the oxygen tolerance test (OTT), administered as a screening procedure for diver candidates, and the remaining 39 men had passed the OTT but later had oxygen poisoning under one or more of the other circumstances mentioned above.

Acute Cerebral Oxygen Toxicity During the Oxygen Tolerance Test

Selection for deep-sea diver training in the U.S. Navy requires a physical examination of high standards, a pressure-exposure test to 50 psig (4.4 ata), and an oxygen tolerance test (OTT),[9] the last given to identify persons highly susceptible to cerebral oxygen toxicity. High susceptibility must be determined because: (1) oxygen is used during some standard decompressions, and (2) oxygen is used during most recompression, or treatment, exposures. To avoid unnecessary exposure of highly susceptible persons to OHP, the OTT is required and regularly administered to all candidates for diving training.

The test is scheduled to last 30 min. The candidate is accompanied in the chamber by a qualified attendant. Oxygen is delivered through a dilutor-demand regulator and a tight-fitting oronasal mask. Chamber pressure is controlled by attendants outside the chamber. The candidate sits quietly on a bench in the dry gaseous environment under direct

observation of attendants inside and outside the chamber and, if available, a qualified diving medical officer outside. Occasionally, the pressure-exposure test and the OTT are combined. The candidate is first exposed to approximately 4.4 ata of pressure, breathing air for 1–10 min, and he is then decompressed to the selected pressure for the OTT.

Among all the men given the OTT, 24 cases of acute cerebral oxygen toxicity have occurred. (Seven of the 24 men did not have pressure tests as part of the OTT.) In three instances, the tests were conducted with the chamber pressure at 2.52 ata; in the remainder, the pressure was 2.82 ata. In the three men with acute cerebral oxygen toxicity occurring at 2.52 ata, first symptoms occurred after 15, 20, and 28 min of exposure. In the remaining 21 cases at 2.82 ata, all symptoms occurred at varying times after 18 min of exposure. (Onset of symptoms never occurred after return to normal atmospheric pressure.) The differences could not be statistically evaluated.

Sixteen of the 24 men had convulsions as the first apparent clinical evidence of oxygen poisoning. Of the other eight, two had facial spasms or twitching, two had nausea (one also had hiccups), two had hiccups alone, one had dizziness, and one had twitching of the right shoulder followed by vertigo (Table 1). All but two of these last eight progressed to convulsions, Total incidence of convulsions in the 24 was 92%.

Typical of those who evidenced oxygen toxicity was a 19-year-old candidate for diver training who had completed the pressure exposure test and was given the OTT at 2.82 ata. After 27 min he became nauseated; the oxygen was immediately stopped, but he had a generalized convulsion lasting 3 min. Postictal confusion persisted with amnesia for about 3 hours. Subsequent examinations after 24 and 48 hours revealed no abnormalities.

Preconvulsive variability is illustrated by the case of a 22-year-old candidate who, after 28 min on oxygen at 2.82 ata (during the OTT, following completion of the pressure-exposure test), developed a tingling sensation in the nasal mucosa. This was immediately followed by hiccuping, and before oxygen could be stopped generalized seizures started. Within 3 min, the seizures stopped spontaneously and the patient stated that he felt quite normal. Examination revealed no abnormalities.

TABLE 1. Initial and Later Signs and Symptoms of Acute Cerebral Oxygen Toxicity in 24 Diver Candidates

	No. occurrences
Onset sign or symptom	
Convulsion	16
Nausea	2
Hiccups	2
Facial twitching	2
Focal shoulder twitching	1
Dizziness	1
Later signs and symptoms	
Convulsion	6
Retrograde amnesia	5
Confusion	4
Hiccups	3
Respiratory arrest	3
Dilated fixed pupils	3
Aphasia, focal muscle twitching, absent pupil reflexes—2 each	6
Nausea, jaw dislocation, flushed feeling, profuse sweating, vomiting, vertigo, maniacal behavior, tinnitus—1 each	8

The variety of symptoms that occurred after the initial symptoms can be seen in Table 1. Retrograde amnesia frequently occurred as a temporary symptom, usually clearing before the last examination. In five instances, it persisted beyond the last examination, which was considered quite unusual. The rest of the symptoms all disappeared by the last examination. The variety of signs and symptoms recorded in this group was neither exhaustive nor exclusive. Complete and thorough neurologic observations were rarely made at the time of the oxygen convulsion, es-

pecially as the inside attendant usually had limited formal medical training or experience. The high incidence of convulsions differs from other reports of human hyperoxic toxicity in navy divers,[2,3] perhaps because of the inexperience of the candidates in the OTT group (the other groups represented military divers in most instances).

Acute Cerebral Oxygen Toxicity During Other Diving Exposures

In contrast to the group with high susceptibility to oxygen toxicity, discovered during the OTT, is the group of 39 men who had previously passed the OTT and, yet, under a number of conditions, experienced oxygen convulsions or other manifestations of acute cerebral oxygen poisoning. The major differences between the two groups were: (1) a lower incidence of convulsions in the group of 39 experienced divers, and (2) a greater variety of other signs and symptoms in the experienced divers. These differences can perhaps be explained by: (1) greater clinical sophistication in the experienced divers, enabling them to recognize their symptoms and terminate the oxygen exposure soon enough to prevent progression to convulsions, and (2) the increased likelihood of these men reporting minor suggestive symptoms that permitted termination of the oxygen exposure, without actual oxygen poisoning being imminent. Although an attempt was made to exclude from this analysis reports of symptoms unrelated to oxygen toxicity, without personal interviews some errors in our judgment may have occurred.

Oxygen poisoning occurred 46 times among the 39 persons who had previously passed the OTT. All causative exposures were at less than 3 ata, and there was no relationship between oxygen tension of the inspired gas and time of clinical onset. The initial and later signs and symptoms are listed in Table 2. Focal twitching and convulsions accounted for about one-third of the onset phenomena, and were observed as later symptoms 18 times.

TABLE 2. Initial and Later Signs and Symptoms in 46 Episodes of Acute Cerebral Oxygen Toxicity Among 39 Divers

	No. occurrences
Onset sign or symptom	
Focal twitching[a]	9
Nausea	8
Generalized convulsion	6
Dizziness	4
Dyspnea	3
Visual symptoms	2
Paresthesia	2
Fatigue	2
Throbbing Pain	2
Throat pain	2
Fainting, progressive weakness, headache, disorientation, adversive fit, focal fit—1 each	6
Later signs and symptoms	
Convulsion	11
Focal twitching	7
Unconsciousness	6
Daze	6
Fatigue	5
Amnesia	5
Gastric sensations	4
Sleepiness	4
Visual symptoms	4
Dizziness, headache, nausea, vomiting—3 each	12
Faintness, weakness, dyspnea, tinnitus, aphasia—2 each	10
Diplopia, paresthesia, slurred speech, anisocoria—1 each	4

[a] Focal twitching was listed as an initial symptom only when it definitely appeared alone. If it appeared in association with a generalized convulsion, then convulsion was listed as the initial symptom. Four of these nine men with focal twitching did eventually advance to convulsions.

Discussion

Several features of the signs and symptoms collected here serve to distinguish this clinical process from air embolism, which may often be the principal differential diagnosis. Limb weakness is quite

uncommon among individuals with acute oxygen poisoning, overall morbidity is much less, and death is rare. Compared to manifestations of cerebral air embolism, oxygen convulsions are relatively benign, essentially self-limited, and quite unpredictable with respect to threshold.[10]

Several factors may have contributed to the occurrence of oxygen poisoning in the group of 39 Navy divers who had previously passed their OTT. Eleven of the men had their oxygen symptoms during treatment for decompression sickness or air embolism, and while the oxygen exposure was in a dry chamber under supervision, previous injury (the reason for recompression treatment) may have lowered the threshold to oxygen convulsions. Eleven individuals had their signs and symptoms during decompression from helium–oxygen dives. Six of these dives were in the open sea, the others in wet chambers at training facilities. All but one instance of oxygen poisoning during decompression from helium–oxygen dives occurred when decompression schedules were being used which required 10 min of oxygen exposure at a 60-ft depth in sea water. (Since experience has shown excessive danger to the diver with this schedule, it is no longer used; the first oxygen stop is now 50 ft.)

The remainder of the episodes occurred during research programs conducted by the U. S. Navy, except for one instance in a civilian research facility. In the Naval research groups, all but three were exposures in wet chambers, with programmed exercise during the exposure. Two of this small group occurred during recompression treatment after experimental decompression from helium–oxygen dives, and one was an open-sea field trial during a swim-duration study. Three cases occurred during swimming trials designed to determine depth-duration limits during oxygen-breathing. Five were similar dives, but gas mixtures of nitrogen, helium, and oxygen were breathed in place of pure oxygen alone. Seven persons having 13 episodes of oxygen poisoning were involved in a depth-duration experimental program on unique closed-circuit underwater breathing equipment. All of these exposure conditions (wetness, swimming, forced activity, and limited caloric intake) are known to reduce the tolerance to oxygen under pressure.

The cause of the oxygen convulsion is unknown. An adequate theory to explain the mechanisms of oxygen poisoning has not yet been formulated, although several have been proposed. Direct testing of any of the hypotheses has been the limitation in all instances. The large variety of mechanisms that modify the seizure threshold in the otherwise normal person makes controlled analysis of clinical experience difficult. In none of the cases described here could a specific factor be found to account for the threshold modification. One person participating in a nonmilitary research program had not undergone proper pre-research examinations. After a typical convulsion occurred during an experimental procedure, subsequent electroencephalograms and medical history revealed that the individual had previously had minor seizures, that he came from a family with a history of seizures, and that during the research program he had experienced preconvulsive phenomena on exposure to hyperbaric oxygen.

In the diving group using closed-circuit diving apparatus, the possibility of carbon dioxide retention cannot be excluded,[11] although direct tests were not made. Many experienced underwater swimmers learn to control their breathing to conserve air supply. This inevitably leads to hypoventilation, with a relative hypoxia and a definite retention of carbon dioxide. The major hazards are the decreases in threshold for oxygen convulsions and the loss, through training and accommodation, of normal reflex responses to elevations in arterial pCO_2; *i.e.*, increases in ventilation do not occur, and hypoventilation continues until consciousness is lost or convulsions occur.

Treatment of oxygen convulsions is simple and direct. The oxygen partial

pressure must be reduced drastically to nontoxic levels. This is easily accomplished in a dry chamber by removing the oronasal mask, as the chamber contains compressed air with the pO_2 at less than toxic pressures. When the individual is wearing a diving helmet, the reduction is slower, as valves need adjustment and low pO_2 mixtures must come to the diver through a diving hose. When a self-contained closed-circuit diving apparatus is being used, the delay may be even longer, as some systems do not have adequate purging systems to dilute the oxygen. A second technique is to reduce the total pressure of the exposure, automatically reducing the pO_2. When decompression is unnecessary, or when rapid ascent can be effected without danger of pulmonic overdistention and air embolism, this can be done.

Several of the convulsions observed during the OTT occurred in the short period between completion of the 30-min exposure and pressure reduction to sea level. The candidates usually continued breathing oxygen in this interval. Among our animal experiments,[12] convulsions often occurred during pressure reduction, and occasionally within 1 min of reaching normal atmospheric pressure. These two observations cast some doubt on the efficacy and safety of pressure reduction *per se* as the technique for reducing pO_2. Drug therapy, in the absence of nervous system disease, is not indicated. The convulsions are self-limited. The greatest hazard in diving is drowning under the influence of altered consciousness.

Within the military services, high standards for selection and training are maintained in an attempt to promote safety and avoid hazards such as oxygen convulsions. Similar selection cannot be performed among patients in need of hyperbaric oxygen therapy. The physical standards required of military personnel could never be met by patients, and the restriction imposed by the 4.4-ata pressure test or the oxygen tolerance test would virtually preclude any therapeutic application of high partial pressures of oxygen. In fact, just the persons normally excluded by pre-exposure tests are very apt to be selected for the oxygen therapy. It can be expected, therefore, that as enthusiasm for hyperbaric oxygenation increases, the hazard of oxygen convulsions will increase. It is possible that some protection may result from the use of drugs prophylactically, if the individual patient's condition permits.

Summary

Seventy examples of acute cerebral oxygen toxicity among 63 navy divers were described. Twenty-five had convulsions as the first clinical manifestation of the toxicity, 10 had focal twitching, and 13 more progressed to convulsions despite attempted immediate therapy. The convulsions were self-limited when the pO_2 was reduced to nontoxic levels. The morbidity was minimal, retrograde amnesia being the only deficit persisting beyond 24 hours. No deaths occurred in this series.

Re-examination of the clinical data revealed only one instance where more careful pre-exposure selection may have prevented the convulsion. All exposures were at less than 3 ata, and all but four were for 30 min or less.

ACKNOWLEDGMENTS

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I am grateful to the Officer-in-Charge and the Senior Medical Officer of the U. S. Navy Experimental Diving Unit for their help in gathering the data for this report.

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DISCUSSION

Dr. J. D. Wood (*Toronto, Canada*): Since Dr. Gillen has been discussing sensitivity to oxygen poisoning, I thought it pertinent to mention some work we have been carrying out with rats. We have been studying the effect of heredity and have been able to develop a strain of rats which is more than normally susceptible to oxygen. We took rats at random from a colony and exposed them three times at weekly intervals. Those rats which convulsed severely on all three occasions were rated as susceptible, while the rats that had failed to convulse on all three occasions were labeled resistant. We then bred the two strains. The offspring from the susceptible parents proved to be considerably more susceptible than the randomly selected rats from the colony. The offspring of the so-called resistant parents, however, showed exactly the same susceptibility as the randomly selected first generation. We did not succeed in increasing resistance. We have so far added one more generation, and the observed increase in susceptibility is as great as, if not more than, that observed in the first experiment.

Dr. Gillen: I think we can expect to be able to accomplish this by selective breeding, because we have evidence from humans that some families have a considerably lower seizure threshold than others. You find families that have almost 100% incidence of febrile seizures among children before the ages of 2 or 3 years, and other families who never have it, even though they have the same type of febrile experiences.

Mr. A. E. Miller (*Lancaster, N. Y.*): I was wondering, Dr. Gillen, if you have ruled out the possibility that some of this represents susceptibility not so much to oxygen as to the suggestion?

Dr. Gillen: We have tried to rule out this possibility. When an individual reported a minor symptom or a single symptom, I often eliminated him because we could never be

sure. Is a man who says he feels dizzy when breathing oxygen really suffering from oxygen poisoning or not? Those questionable cases I threw out. The ones that I kept are, I feel, actual examples of oxygen toxicity.

MR. MILLER: My reason for raising the question was the fact that at the various conventions where we exhibit oxygen equipment, every morning a string of people come to us for a hangover cure. I have cured a lot of hangovers with compressed air, as we never fill our equipment with oxygen at these meetings because of the fire hazard.

DR. A. HEYMAN (*Durham, N. C.*): In our experience here at Duke, only two patients have had seizures during hyperbaric oxygenation. We were fortunate to have EEG monitoring during these episodes. We had hoped that the EEG would provide an indication of an impending seizure; unfortunately it did not. Electrical abnormalities developed almost immediately prior to the seizure. The only aura that the patient had was apprehension and a sense of impending doom. I would like to ask whether anyone has observed any EEG abnormalities prior to seizure which serve as a predictive index.

DR. S. ATTAR (*Baltimore, Md.*): We have been able to predict the development of oxygen toxicity and convulsions in dogs exposed to 3 atmospheres of 100% oxygen for 4 hours. All dogs that developed tachycardia, tachypnea, and hypertension eventually had convulsions and died of oxygen toxicity. Could you comment on this?

DR. GILLEN: A number of things will change seizure thresholds; once you interfere with gas exchange and pulmonary and circulatory efficiency, you change the way the brain is working. I suspect that this may be part of your problem. This, to me, is a different set of circumstances than oxygen toxicity because you can take any animal and run it through this cycle of pulmonary embarrassment, but the first thing that happens in humans is a convulsion. There are no prior pulmonary changes or circulatory changes.

DR. C. A. FOSTER (*London, England*): Coming back to the EEG, we have had one patient who was anesthetized and breathing oxygen for 45 minutes at 50 psig. Four minutes before a convulsion she had the typical spiked and slow wave activity on the EEG.

Proceedings of the Third International Conference on Hyperbaric Medicine
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SESSION III

Physiologic Responses to High Oxygen Tension

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Relationship of Tissue Oxygen Tension to Autoregulation

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"Autoregulation" is the ability of each tissue to regulate its own intrinsic blood flow. If, for example, the arterial pressure goes up or down, autoregulation prevents marked change in the blood flow despite the pressure change. Because of this, many circulatory physiologists have defined autoregulation as "maintenance of a constant blood flow in the face of pressure changes," but this definition is too narrow. Each individual tissue controls its blood flow in response to changes in its metabolism just as much as in response to changes in arterial pressure.

A more inclusive definition of autoregulation is "the ability of each individual tissue to regulate its own blood flow in response to the momentary demands of that particular tissue." Indeed, most tissues seem to regulate their blood flow by their need for nutrients rather than by other demands, with two notable exceptions—the kidneys and the brain. In the kidneys, local regulation of blood flow is determined by the electrolytes of the body fluids; in the brain, it is determined by carbon dioxide concentration.

The metabolic autoregulation of tissue blood flow manifests itself most markedly during exercise. Figure 1 illustrates this phenomenon in relation to the entire body, illustrating that the cardiac output increases almost linearly with the rate of metabolism. To express this another way, the overall blood flow through the tissues of the body (as determined by cardiac output) is a direct function of the rate of use of metabolic substrates. This figure also demonstrates the direct proportionality of the increase in cardiac output to rate of oxygen usage by tissues, a correlation which makes one suspect some direct relationship between oxygen usage in the tissues and the regulation of blood flow in each tissue. Various kinds of currently available evidence appear to confirm this.

Effect of Perfusing Tissues with Hypoxic Blood

One of our earlier studies [1] on the effect of oxygen on local blood flow is summarized in Figure 2, which shows the average results from 10 experiments in which the hind limbs of dogs were perfused with blood of varying levels of oxygen saturation. Initially, the oxygen

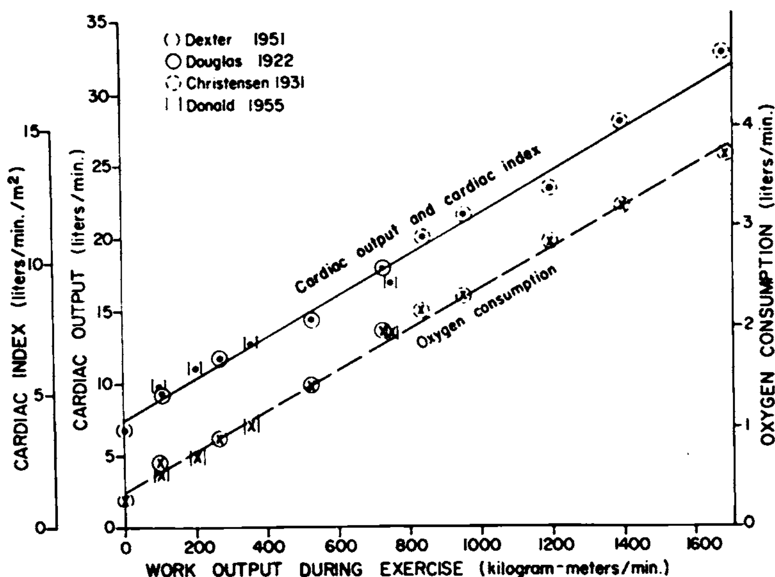

FIGURE 1. Effect of increased metabolic load during exercise on cardiac output, cardiac index, and rate of oxygen usage. (Reproduced from *Circulatory Physiology: Cardiac Output and Its Regulation,* W. B. Saunders, 1963, p. 5.).

saturation was 100% in each animal, decreasing in steps down to 30%. This was achieved by mixing arterial blood with markedly desaturated venous blood in varying proportions. The blood flow increased about 250% as the oxygen saturation fell from 100% to 30%. This increase in blood flow could almost compensate for the deficiency in oxygen saturation of the blood, implying that almost as much oxygen was still being delivered to the tissues during 30% oxygen saturation as during 100% saturation.

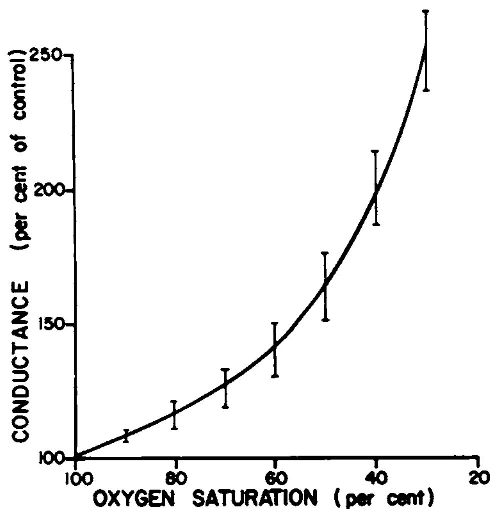

FIGURE 2. Effect of decreasing arterial oxygen saturation on blood flow (conductance) through isolated hind limbs of 10 dogs. Brackets indicate standard errors of means. (Reproduced with permission from D. G. Crawford: Amer. J. Physiol. 197:613, 1959.)

Many objections were raised to this experiment, however, because venous blood was used as the source of unsaturated hemoglobin. It was suggested that venous, but not arterial, blood contains a vasodilator agent independent of the low oxygen saturation. As a result, this experiment was repeated with some modifications. The effluent blood from one lung of a dog was surgically diverted from its normal circulation, and the lung was used to oxygenate or deoxygenate blood perfusing an isolated hind-limb preparation.[2] This time, all the blood passed through a lung and therefore was arterial in all characteristics except for oxygen content. Also, the carbon dioxide concentration of the blood could be controlled to any desired pCO_2 by respiring the lung with different gas mixtures.

In none of the experiments was a significant effect on blood flow caused by changes in pCO_2 within normal physiologic ranges. On the other hand, changing the oxygen saturation of the perfusing blood caused almost exactly the same quantitative effects on blood flow through the hind limb as observed previously when venous blood was used as the source of the desaturated hemoglobin. We concluded, therefore, that no vasodilator substance is present in venous blood; rather, the vasodilator properties of venous blood result from its low oxygen content.

Role of Oxygen Deficiency in Reactive Hyperemia (A Type of Autoregulation)

When blood flow to a tissue is completely stopped, the blood vessels in the tissue usually begin to dilate, reaching a state of full dilatation in 1–5 min. When blood flow is reinstituted several minutes later, the flow rate rises to three to seven times the original control flow. Although this phenomenon is usually called "reactive hyperemia," it is actually an attempt by the tissue to increase its blood supply, presumably because of the extreme nutritional deficiency imposed upon the tissue.

Deficiency of which nutrient causes the reactive hyperemia? To help answer this question, Fairchild *et al.*[3] attempted to test the possible role of oxygen deficiency as the cause of the reactive hyperemia. Figure 3 illustrates a typical reactive hyperemic response which they found after occlusion of blood flow to the hind limb for 10 min. A modified experiment was then performed (Figure 4) in which, during occlusion of blood flow to the limb, the blood in the perfusing system was changed from 100% saturated blood to completely desaturated (0%) blood. Upon removal of the occlusion, the hind limb was perfused with blood containing no oxygen. As long as the limb was perfused with this anoxic blood (10 min) there was absolutely no recovery from hyperemia.

The same results were obtained in eight separate experiments; they remained unaltered whether the occlusion period lasted 3, 5, or 10 min. We conclude, therefore, that recovery from reactive hyperemia requires the presence of oxygen; we can

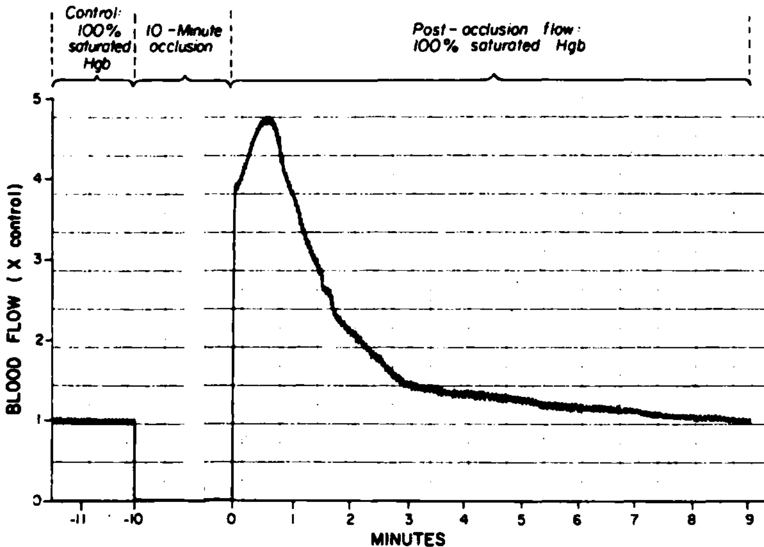

FIGURE 3. Typical reactive hyperemia response in the hind limb of a dog. (Reproduced with permission from H. M. Fairchild: Amer. J. Physiol. 210:490, 1966.)

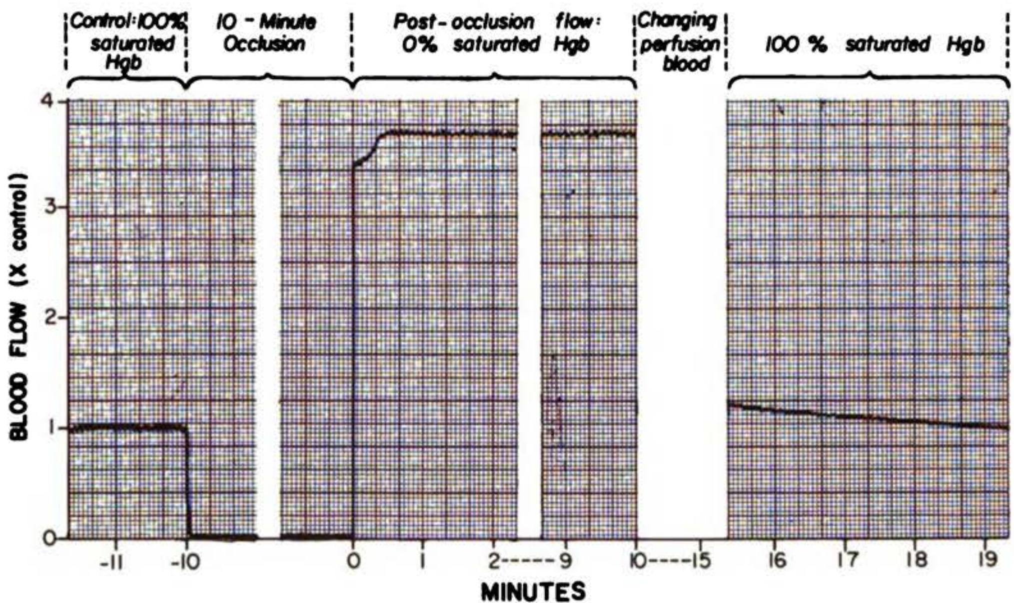

FIGURE 4. Reactive hyperemia response in same type of preparation shown in Figure 3, except that the limb was perfused with completely desaturated blood for the first 10 min after occlusion. (Reproduced with permission from H. M. Fairchild: Amer. J. Physiol. 210:490, 1966.)

also surmise that lack of oxygen during the occlusion period causes the vasodilatation characteristic of reactive hyperemia.

EFFECT OF HYPERBARIC OXYGENATION ON LOCAL BLOOD FLOW

One of us (J.R.W.),[4] in recent studies on the effect of hyperbaric oxygen on blood flow through isolated hind limbs of dogs, perfused the limbs with Tyrode's dextran solution that had been equilibrated with oxygen at 1–18 atm. Figure 5 illustrates the results from one animal, showing the decrease in perfusate flow (as would be expected) with the increase in oxygen availability.

Hyperbaric oxygen is known to decrease cardiac output.[5] These experiments point out that any attempt to explain the relationship of tissue oxygen tension to autoregulation must be able to account for the *decrease* in blood flow at high oxygen tensions, as well as the increase at low oxygen tensions.

OTHER EVIDENCE THAT OXYGEN PLAYS A MAJOR ROLE IN LOCAL TISSUE AUTOREGULATION

While the above experiments represent some of our own work concerning the relationship of oxygen to local tissue autoregulation, studies from other laboratories have yielded similar results. Jones and Berne,[6] for example, demonstrated that oxygen deficiency in blood perfusing iso-

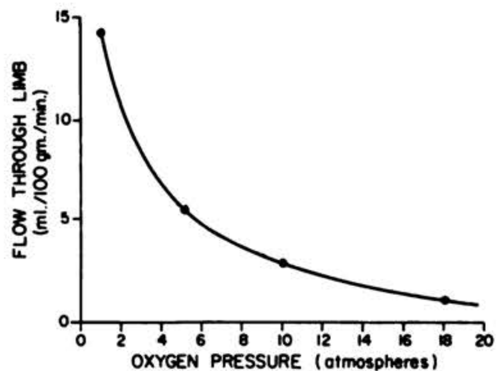

FIGURE 5. Effect of hyperbaric oxygen on flow of Tyrode's dextran solution through hind limb of a dog.

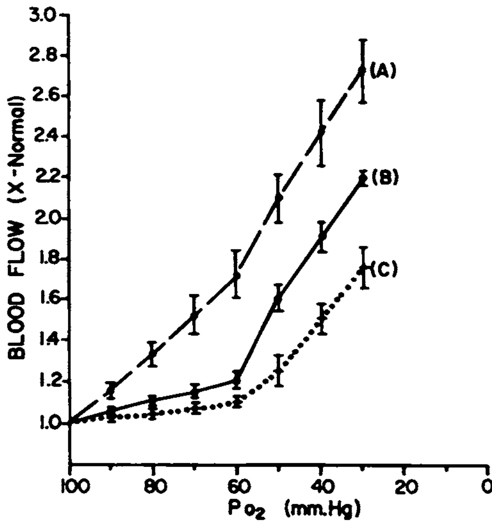

FIGURE 6. Curves relating blood flow in minute isolated arteries to oxygen tension. Ten smallest vessels (A) had initial flows of 1.0–3.5 ml/min. Ten medium vessels (B) had initial flows of 4.0–6.5 ml/min. Largest vessels (C) had initial flows of more than 10 ml/min. Brackets indicate standard errors of means. (Reproduced from Amer. J. Physiol. 206:951, 1964.)

lated skeletal muscle caused a typical local vasodilator response; Berne,[7] Coffman and Gregg,[8] and Driscol *et al.*,[9] among others, showed that oxygen deficiency or ischemia of the heart caused an almost proportional compensatory coronary vasodilation.

Another important study, performed by Stainsby,[10] demonstrated that the level to which blood flow is autoregulated is directly influenced by the degree of metabolism in the tissue. When the metabolic rate is high (and consequently the rate of oxygen utilization), the blood flow is autoregulated to a high level, and changes in arterial pressure upward or downward fail to alter this high level of flow greatly. On the other hand, when the metabolic rate is low, the level to which the blood flow is autoregulated is also low, whether the arterial pressure is high or low.

Thus, autoregulation can occur in response to *changes in tissue demand* for the substrates of metabolism as well as in response to changes in supply of the substrate. These studies also suggest that no basic difference exists between the mechanisms for regulating blood flow in conditions that increase the metabolic rate (*e.g.*, exercise) and those for regulating flow in the face of changes in arterial blood pressure or changes in availability of oxygen in the arterial blood. In other words, this is a unifying concept for the regulation of local blood flow in response to the local tissue needs for metabolic substrates and, more particularly, for oxygen *per se*.

The Mechanism by Which Tissue Oxygen Tension Regulates Local Blood Flow

Two theories have been postulated for the mechanism by which changes in tissue oxygen tension regulate local tissue blood flow: (1) oxygen deficiency causes the tissue to elaborate a metabolite with vasodilator properties,[7] which diffuses to the arterioles and causes an increase in blood flow, and (2) diminished oxygen availability to the tissues also decreases oxygen supply to the smooth-muscle cells of the vascular walls, which, in turn, weakens the vascular wall and allows the pressure head within the arterioles to effect dilatation and consequently increase local blood flow.[11]

Either of these two mechanisms could explain the vasodilator effect of oxygen deficiency. To explain the vasoconstrictor effect caused by oxygen excess, one would have to modify the vasodilator metabolite theory to assume that some finite amount of vasodilator metabolite is constantly formed in the tissues under normal conditions and that oxygen excess causes the rate of formation of this vasodilator metabolite to diminish. It is somewhat easier to explain the vascoconstriction on the basis of the direct effect of oxygen on the blood vessels by simply assuming that excess availability of oxygen increases

the strength of contraction of the local vessels, thus causing them to constrict.

In an attempt to evaluate the validity of the theory of oxygen deficiency *per se* as opposed to the vasodilator metabolite theory, we have performed two types of experiments in our laboratory. First, we [12] demonstrated that a pO_2 decrease from 100 to 30 mm Hg increased blood flow 200-300% through isolated arteries of only 0.5–1.0 mm in internal diameter (Figure 5). Furthermore, the increase in blood flow became progressively greater the smaller the vessel size. If these data were to be extrapolated to the minute arterioles, we might conclude that decreasing the oxygen tension in these minute vessels could increase blood flow five, 10, or perhaps many more times. Especially important is the fact that in these experiments there were no tissue cells other than those of the blood vessels. Therefore, the response of the small arteries to oxygen deficiency must have occurred in the walls of the blood vessels themselves, with no dependency upon a vasodilator metabolite from surrounding tissues.

The data from these experiments have been corroborated by D. Smith [13] of the University of Vermont School of Medicine; they are also supported by the findings of Detar and Bohr,[14] who showed that smooth-muscle strips isolated from small arteries lost much of their contractile strength with reduction of the pO_2 from 100 to 30 mm Hg.

The question remains unanswered as to how the vascular wall can dilate in response to changes in rate of tissue metabolism, since it has now been shown that the response is not dependent upon a vasodilator metabolite from the surrounding tissue. A possible answer might be that the parenchymal cells and the smooth-muscle cells of the vascular walls compete for the oxygen available in the blood.[11] Therefore, if the parenchymal cells utilize excessive amounts of oxygen, the amount of oxygen available to the vascular wall should become diminished. The argument against this concept is that the smooth-muscle response of the arterioles is probably governed by the intra-arterial oxygen tension rather than the tissue-fluid tension. This is not necessarily true of the precapillary sphincters, however, which lie close to the surrounding tissues and could indeed be competing with the tissue cells for oxygen.

A second type of experiment that we have performed to compare the validity of the theory of oxygen deficiency *per se* and the vasodilator metabolite theory has been to observe whether anoxic blood can wash a vasodilator metabolite out of the tissues after the onset of reactive hyperemia.[11] It can be shown mathematically that, if an accumulated vasodilator metabolite should be washed out of the tissues by perfusion with anoxic blood after a period of occlusion, this would theoretically cause an initial "overshoot" in blood flow, followed by a secondary diminution in flow. Since we were never able to demonstrate such an overshoot in eight separate experiments in which the period of occlusion varied from 3 to 10 min, we failed to confirm the existence of such a vasodilator.

An objection to this experiment is that a vasodilator metabolite removed from the tissue extremely rapidly by intratissue destruction rather than by washout would not give an overshoot. Berne,[7] on the basis of his experiments on the coronary circulation, believes that adenosine is a vasodilator metabolite with these characteristics. However, he has been unable to demonstrate the release of adenosine in skeletal muscle either in the normal resting state or in the hypoxic state. Thus, one cannot, at present, explain the control of blood flow in skeletal muscle on the basis of adenosine being a vasodilator metabolite. Neither do the present data support the concept that an adenosine mechanism could cause vasoconstriction when excess oxygen is available, as under hyperbaric conditions.

Because a vasodilator metabolite has never been found to explain all the experi-

mental results, and because our own experiments indicate that a vasodilator metabolite feedback from the parenchymal cells to the blood vessels is unnecessary, we currently believe that the theory of oxygen deficiency *per se* is the more likely explanation for vasodilatation when the availability of oxygen is diminished.

The Role of Oxygen in Long-Term Autoregulation

Thus far, we have discussed only acute tissue responses to oxygen lack or excess. Yet, we know that many days of continued oxygen deficiency increase tissue vascularity, and ischemia of tissues caused by occlusion of arteries causes a collateral circulation to develop. Both of these phenomena are types of autoregulation that take place over a long period of time and result from actual structural changes in blood vessels. There are many reasons to believe that these changes come about as a direct effect of diminished oxygen tension in the tissues and, conversely, that excess availability of oxygen in the tissues will cause regression of tissue vascularity.

This concept has been supported by studies of retinal capillary changes in premature animals treated with 100% oxygen therapy.[15] Normal growth of the capillaries into the retina ceased, and many of the capillaries that had already appeared actually degenerated. Upon removal of the excess pO_2, there was an explosive growth of capillaries in the retina—so rapid that some capillaries even grew from the retina into the vitreous humor. It is these vascular bridges to the vitreous humor that cause retrolental fibroplasia. These phenomena are also extremely important from a basic theoretical point of view, however, because they indicate that the degree of tissue vascularity is an inverse function of the availability of oxygen.

Thus, we might postulate that all the tissues of our bodies undergo a continual turnover of small vessels, some degenerating and some forming. There is good reason to believe that the rate of formation of new vessels is inversely related to the adequacy of tissue oxygenation. Therefore, when an animal is exposed to low ambient oxygen levels or when increased tissue metabolism demands increased oxygen supply, the degree of vascularity of the tissue increases. Under opposite conditions, the rate of degeneration of the small vessels outpaces the rate of formation of new vessels, resulting in diminished vascularity.

Expressed another way, one might postulate, on the basis of available evidence, that the ratio of vessel density to tissue mass is determined by a long-term autoregulatory process dependent mainly on tissue oxygen tension. To go still further, since cardiac output represents the total blood flow to all the individual parts of the body, this long-term autoregulation in the tissues would be the long-term regulator of cardiac output as well.

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DISCUSSION

Dr. C. J. Lambertsen, *Session Chairman* (*Philadelphia, Pa.*): This presentation has gotten us off to a good start by describing a possible mechanism whereby oxygen effects can play a role in their own delivery to the tissues.

Dr. R. Penneys (*Philadelphia, Pa.*): As one who works in the field of ischemia and oxygenation of the human limb, I think this gives us a monumental understanding of many mechanisms which we have talked about for 20 years, but have not understood. I want to ask about one specific situation, and wonder if it is an exception to the autoregulatory power, or control, of oxygen. If one places the hand of a human subject in cold water, he produces vasoconstriction immediately in the other hand. The blood flow goes down to zero, as does the tissue oxygen (J. Clin. Invest. 31:1042, 1952). It seems, in this case, that the tissue oxygen does not promote a secondary increase in blood flow.

Dr. Guyton: Yes, as a matter of fact you can see this same effect under many other conditions. For instance, when an animal hemorrhages, you see vasoconstriction occurring throughout the entire body for a certain period of time. This is undoubtedly a problem of parallel control systems; that is, the nervous vascular control system is parallel to the local oxygen control system. Now, which of these control systems will be "prepotent"? When you do the experiment that you mentioned, under certain conditions you get terrific vasoconstriction at first, and later you get sudden vasodilation. Therefore, I would guess that the nervous system is prepotent first, and later the other becomes prepotent as the tissues reach a state of severe nutritional deficiency. In shock, the same effects occur. If you bleed an animal down to 30 mm Hg arterial pressure, you find terrific vasoconstriction for the first half hour, or perhaps as long as an hour. After another hour, however, the blood vessels begin to open up throughout the body, and I would say that the local control system is then becoming prepotent over the nervous system. So I think this is probably just a problem of parallel control systems. They are both important.

Dr. R. F. Bond (*Winston-Salem, N. C.*): I have a question regarding your preparation using the isolated limb. Did you make any effort to isolate the cutaneous vasculature from the muscle vasculature?

DR. GUYTON: No, we did not. Other people have, however, and obtained the same results. Drs. Jones and Berne (Amer. J. Physiol. 204:461, 1963), for example, isolated the muscle by itself and got essentially the same effects.

DR. BOND: Bearing in mind that cutaneous and skeletal muscle tissues have different metabolic requirements, would you expect the cutaneous vasculature, which does not exhibit either reactive hyperemia following an occlusion of 1 to 3 minutes' duration, or autoregulation to respond to an oxygen deficit in the same manner as the skeletal muscle vasculature?

DR. GUYTON: In the first place, I would not say that you do not see autoregulation in the skin. People have said this many times, but have you ever sat absolutely still for an hour and gotten up? Are you white where you have been sitting or red? If you are red that is reactive hyperemia, isn't it? That is autoregulation.

DR. E. BURGER (*Boston, Mass.*): What are your thoughts on the effects of increased partial pressures of oxygen on the vascular caliber in the lung?

DR. GUYTON: This is a real problem, because we have been taught for many years, until yesterday, that whenever oxygen is increased in the lungs, vasodilation occurs. I have no thoughts on this, but I do understand that isolated vessels from the lungs, at least under certain circumstances, vasoconstrict with oxygen.

DR. LAMBERTSEN: Dr. Guyton, do you intend the term "regulation" to mean a physiological mechanism or, without changing the concepts that you have described, could it not merely reflect the failures of control that occur in the maintenance of tone when oxygen tension becomes so low that a pathological situation results? I have considered "anoxic regulation" simply a failure of chemistry in the smooth-muscle cells. Moreover, an effect of very high oxygen pressures may be a toxic effect of oxygen. Must we think of the regulation you describe as being physiological?

DR. GUYTON: We believe, just as you stated, that the dilation caused by low oxygen results from the fact that oxygen is required for the vascular muscle to remain contracted. Therefore, when oxygen is not available, dilation occurs. In fact, Drs. Detar and Bohr at the University of Michigan (Physiologist 8:152, 1965) have in the past few months loaded thin spiral strips cut from minute arteries and then changed the available oxygen, and they find that the degree of contraction changes terrifically between pO_2's of 30 and 100 mm Hg, which is just the range with which we are dealing. Since they find such great changes in degree of contraction of smooth muscle in that range, all because of changes in oxygen *per se*, the simplest explanation would be that lack of oxygen causes blood vessels to dilate because of decreased ATP and other oxygen-dependent substances that are required to maintain contraction.

DR. LAMBERTSEN: Would it not be then that the vessel has failed in its regulation and that the internal hydrostatic pressure of the blood has simply distended it?

DR. GUYTON: This, I think, is a matter of semantics. Actually, the factor being regulated is the tissue pO_2, and the vessels act as a control element in tissue pO_2 regulation.

Effect of Hyperbaric Oxygenation on Blood Flow in the Internal Carotid Artery of the Baboon

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Previous investigators have shown that a reduction in cerebral blood flow is associated with hyperbaric oxygenation,[1-3] but whether the decrease in flow is due to the increased oxygen tension, to changes in tissue or blood levels of carbon dioxide, or to some other mechanism is unresolved. The study described below was undertaken to evaluate the effect of hyperbaric oxygenation on continuously measured flow in the internal carotid artery of the baboon and, further, to determine the effect on flow of adding CO_2 to the inspired gas mixture under hyperbaric conditions.

METHODS

Ten male baboons (African papiopapiogera) weighing 7.1–10.0 kg (mean weight 8.7 kg) were anesthetized with 0.5% alphachloralose intravenously. After femoral cutdown, the femoral vein and artery were each cannulated with polyethylene catheters. The femoral vein catheter was used for the administration of alphachloralose. A cuff endotracheal tube was inserted after spraying the larynx with 1 ml of 5% hexylcaine HCl (Cyclaine), and the cuff was inflated with water. An anterior midline cervical incision was made extending from just below the mandible to a point just above the manubrium sterni. The right common, external, and internal carotid arteries were dissected free and the right external carotid artery ligated. Thus, measurement of flow in the right common carotid represented only that flow into the right internal carotid artery. The left common carotid artery and both vertebral arteries were ligated. The vertebral arteries were ligated just below the transverse process of the sixth cervical vertebra. Thus, with both vertebral arteries and the left common carotid arteries ligated, it was felt that flow in the right common carotid artery would perfuse the brain adequately, and measurement of this flow would provide a good estimate of total blood flow to the brain.[4,5] Finally, bilateral myringotomy was performed.

Mean internal carotid artery flow was measured quantitatively with a square-wave electromagnetic flowmeter (EMF). Calibration of the EMF was carried out at both 0 and 30 psig by passing known

quantities of physiologic saline through the probe in a given period of time. No difference was noted in the calibration factor at the two levels of atmospheric pressure. The EMF probe (usually an 8-mm probe) was placed on the intact right common carotid artery and the vessel constricted only enough to obtain an adequate signal. Zero flow reference was obtained by temporarily occluding the artery distal to the EMF probe.

Arterial pressure was measured through the femoral artery catheter with a Statham P-23 Db transducer, with mean pressure obtained by electrical integration and the level of the right atrium used for zero reference. Arterial pCO_2, pO_2, and pH determinations (using the IL-113 instrument) were made in the compression chamber and always at the same atmospheric pressure as that to which the animal was exposed when blood was drawn. (Measurements were made immediately after withdrawing the arterial blood sample.) All recordings of pressure and flow were made on a Gilson macropolygraph.

A nonreturn direction valve was fitted into the end of the endotracheal tube. The animals were paralyzed with succinylcholine chloride (Anectine); initially 1 mg/0.5 kg body weight was given intravenously and thereafter 0.5 mg/0.5 kg was given intravenously at 10- to 15-min intervals throughout the experimental procedure. The animals were respirated during the experimental procedure with a Harvard respirator at a rate of 16/min and a stroke volume varying between 90 and 100 ml, depending on the weight of the animal.

At normal atmospheric pressure (0 psig, or 1 ata), blood flow and arterial pressure measurements were made as the animal breathed, successively, the following gas mixtures: (1) room air for 5 min, (2) 90% O_2 + 10% N_2 for 5 min, (3) room air for 5 min, and (4) 90% O_2 + 4% N_2 + 6% CO_2 for 5 min. The hyperbaric chamber was then sealed and pressurized to 30 psig (3.04 ata) over a period of approximately 10 min. At this level of pressure, the animal was allowed to breathe, successively, the following gas mixtures: (1) room air for 5 min, (2) 90% O_2 + 10% N_2 for 10 min, (3) room air for 5 min, and (4) 90% O_2 + 8% N_2 + 2% CO_2 for 5 min. A 5-ml arterial sample was withdrawn at the end of each 5-min period for determination of pCO_2, pO_2, and pH. Following the last observation, the chamber was decompressed according to the staged decompression tables developed by the U.S. Navy for divers.[6,7] Although continuous flow and pressure measurements were made, the values chosen for analyses were obtained at the end of each 5-min experimental period.

RESULTS

Effect of 90% Oxygen + 10% Nitrogen at 0 and 30 psig

The data obtained at 0 psig are shown in columns 1 and 2 of Table 1. There was a slight but significant decrease ($P < 0.01$) from the average control value (66 to 60 ml/min) in internal carotid flow associated with breathing the 90% O_2 + 10% N_2 gas mixture. No change was observed in arterial pressure. The pCO_2 fell from a control value of 37 mm Hg to 33 mm Hg, a statistically significant change ($P < 0.01$). There was an obvious increase in arterial pO_2 and a slight but significant increase ($P < 0.01$) in arterial pH.

The data obtained at 30 psig are shown in columns 3–5, Table 1. There was a significant decrease ($P < 0.01$) in flow at both the 5- and 10-min intervals associated with breathing the 90% O_2 + 10% N_2 mixture. Average control value of 62 ml/min fell to 54 and 51 ml/min at the 5- and 10-min intervals, respectively. Control pCO_2 of 38 mm Hg fell to 35 mm Hg at both the 5- and 10-min intervals, a statistically significant change ($P < 0.01$). There was a marked increase in pO_2, a slight but significant increase in pH

TABLE 1. The Effect of Breathing 90% O_2+10% N_2 Gas Mixture at 0 and 30 psig [a]

	0 psig		30 psig		
	Room air (control) 5 min	90% O_2+10% N_2 5 min	Room air (control) 5 min	90% O_2+10% N_2 5 min	90% O_2+10% N_2 10 min
Int. carotid flow (ml/min)	66 ± 14	60 ± 13	62 ± 13	54 ± 12	51 ± 11
Mean arterial pressure (mm Hg)	123 ± 13	120 ± 11	122 ± 12	119 ± 14	116 ± 11
pCO_2 (mm Hg)	37 ± 4	33 ± 3	38 ± 5	35 ± 7	35 ± 7
pO_2 (mm Hg)	102 ± 12	427 ± 79	402 ± 68	1584 ± 250	1562 ± 236
pH	7.46 ± 0.07	7.48 ± 0.10	7.48 ± 0.07	7.50 ± 0.07	7.49 ± 0.07

[a] The data shown represent average values from 10 animals. In each case, the mean value and standard deviation (SD) are listed. These data were obtained at the end of each experimental period.

($P < 0.01$ at both 5- and 10-min intervals), and no significant change in mean arterial pressure associated with breathing 90% O_2+ 10% N_2 at 30 psig.

Effect of Breathing CO_2 at 0 and 30 psig

The data obtained during exposure to 90% O_2+4% N_2+6% CO_2 at 0 psig are shown in columns 1 and 2 of Table 2. There was a significant increase ($P<0.01$) in internal carotid flow from control of 55 ml/min to a value of 88 ml/min. There was a slight but significant rise in mean arterial pressure ($P < 0.01$), a significant increase in pCO_2 ($P < 0.001$) and pO_2 ($P < 0.01$), and a significant decrease in arterial pH ($P < 0.01$).

The data obtained during exposure to 90% O_2+8% N_2+2% CO_2 at 30 psig are shown in columns 3 and 4 of Table 2. There was an increase in flow ($P < 0.01$) from control of 52 ml/min to a value of 67 ml/min. There was a marked rise in arterial pO_2. The changes in pCO_2, pH, and mean arterial pressure were all significant (P value for each < 0.01), and similar to those obtained with the animals breathing 90% O_2+4% N_2+6% CO_2 gas mixture at 0 psig.

TABLE 2. The Effect of Breathing CO_2 at 0 and 30 psig [a]

	0 psig		30 psig	
	Room air (control) 5 min	90% O_2+4% N_2 +6% CO_2 5 min	Air (control) 5 min	90% O_2+8% N_2 +2% CO_2 5 min
Int. carotid flow (ml/min)	55 ± 15	88 ± 20	52 ± 12	67 ± 15
Mean arterial pressure (mm Hg)	119 ± 19	125 ± 14	119 ± 13	124 ± 15
pCO_2 (mm Hg)	34 ± 5	54 ± 3	37 ± 7	55 ± 6
pO_2 (mm Hg)	104 ± 15	427 ± 105	409 ± 75	1603 ± 80
pH	7.48 ± 0.07	7.31 ± 0.05	7.48 ± 0.07	7.35 ± 0.05

[a] The data shown represent average values from 10 animals. In each case, the mean value and standard deviation (SD) are listed. These data were obtained at the end of each experimental period.

DISCUSSION

Kety and Schmidt, using the nitrous oxide method for determination of cerebral blood flow, showed that inhalation of a high O_2 mixture (85–100%) in man at 1 ata resulted in a 13% decrease in cerebral blood flow.[8] A proportionately greater reduction in flow has been observed under conditions of hyperbaric oxygenation. Lambertsen *et al.*[3] found a 25% drop in cerebral blood flow in man breathing 100% O_2 at 3.5 ata, and Jacobson, Harper, and McDowall [1,2] observed a 21% fall in flow in dogs at 2 ata. Our results are consistent with these investigations. There was a significant decrease ($P<0.01$) in flow (13% and 18% reduction at the 5- and 10-min intervals, respectively) in the internal carotid artery of the baboon associated with breathing 90% O_2+10% N_2 at 30 psig (3.04 ata).

A significant decrease ($P<0.01$) in arterial pCO_2 associated with the inhalation of 90% O_2+10% N_2 at both 0 and 30 psig was observed in the present study. This finding, also noted by Lambertsen *et al.*,[3] suggests that the reduction in cerebral blood flow during hyperbaric oxygenation may be due at least partly to a decrease in arterial pCO_2 rather than solely to the increased pO_2. Lambertsen *et al.*[3,9] attributed the decrease in arterial pCO_2 during hyperbaric oxygenation to hyperventilation. However, this mechanism would not explain the decrease in pCO_2 associated with the inhalation of O_2 in the present experiments, as these animals were on controlled respiration and unable to hyperventilate. One conceivable mechanism may be this: the increased pO_2 associated with O_2 inhalation at 0 and 30 psig results in a smaller proportion of reduced hemoglobin,[3] which, in turn, results in less hemoglobin available for CO_2 transport. This theoretically could account for the lower arterial pCO_2 observed in the present study. However, Jacobson *et al.*[1,2] and Behnke *et al.*[10] in their studies on dogs did not observe significant changes in arterial pCO_2 associated with hyperbaric oxygenation.

A significant increase in blood flow (52 to 67 ml/min) was observed in our study when 2% CO_2 was added during hyperbaric oxygenation. This finding further implies that the level of CO_2 may be a major determining factor in the regulation of cerebral blood flow under conditions of hyperbaric oxygenation. It is noteworthy that despite the maintenance of a high pO_2, a significant increase in internal carotid flow can be obtained by raising the level of CO_2.

Several experimental observations indicate that vasoconstriction is a characteristic response to an increase in blood O_2 tension.[11–17] The reduction in blood flow associated with the inhalation of O_2 is due to an increase in cerebral vascular resistance caused by vasoconstriction. The same physiologic change, but more pronounced, also occurs during hyperbaric oxygenation. Lambertsen *et al.*[3] measured a 55% increase in cerebral vascular resistance in subjects breathing O_2 at 3.5 ata, which was approximately twice the value obtained when the subjects breathed O_2 at 1 ata. Saltzman *et al.*[14] demonstrated significant vasoconstriction of retinal arterioles in man breathing O_2 at 3.5 ata. Although the mechanisms responsible for the vasoconstriction are unknown, the role of this physiologic response is probably protective in that it tends to prevent excessively high O_2 levels in brain tissue by reducing the cerebral blood flow. However, Bean showed that this mechanism is not totally effective.[18] A shift from breathing air to breathing O_2 at 1 and 5 ata, respectively, raised the availability of O_2 to the brain. Bean concluded that cerebral vasoconstriction which may have occurred in hyperbaric oxygenation was apparently insufficient to prevent pronounced elevation of cerebral O_2 levels or to protect against O_2 toxicity.

SUMMARY

1. Blood flow in the internal carotid artery and arterial pressure were continuously measured in 10 baboons during inhalation of 90% O_2+10% N_2 gas mixture, both at 0 psig (1 ata) and 30 psig (3.04 ata). The effect of adding CO_2 to the inspired gas mixture at 0 and 30 psig was also studied.

2. There was a significant decrease ($P < 0.01$) in flow (13 and 18% reduction at 5- and 10-min intervals, respectively) associated with the inhalation of 90% O_2+10% N_2 at 30 psig.

3. A significant decrease ($P < 0.01$) in arterial pCO_2, from an average control value of 38 mm Hg to 35 mm Hg (at both 5- and 10-min intervals) occurred in association with hyperbaric oxygenation. This finding suggests that reduced cerebral blood flow during hyperbaric oxygenation may be at least partly due to a decrease in arterial pCO_2 rather than solely to the increased O_2.

4. There was a significant increase ($P < 0.01$) in blood flow (52 to 67 ml/min) when 2% CO_2 was added to the gas mixture during hyperbaric oxygenation. This finding further implies that the level of CO_2 may be a major determining factor in the regulation of cerebral flow under conditions of hyperbaric oxygenation.

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DISCUSSION

DR. C. J. LAMBERTSEN, *Session Chairman* (*Philadelphia, Pa.*): I certainly hope, Dr. Tindall, that you are going to explain the fall in arterial pCO_2 for this group. Would you start with that question?

DR. TINDALL: Speaking as a neurosurgeon and not as a physiologist, I am not sure that I can. The fall in arterial pCO_2 was observed in all these baboons and was statistically significant. On controlled respiration, the pCO_2 dropped whether they were breathing oxygen at 1 atmosphere or 3 atmospheres. The decrease may be related to a reduction of hemoglobin available for carbon dioxide transport, but beyond that I cannot comment.

DR. LAMBERTSEN: My reason for asking the question is clear. Neurosurgeons and physiologists both can do arithmetic and, seeing your results, it appeared that a mathematical problem existed. If arterial pCO_2 fell, either metabolism had to have been depressed or else the alveolar ventilation had to have been increased. Is there any possibility that the performance of the ventilating apparatus was not maintained the same at high pressure as at low, such that there may have been an unwarranted change in alveolar ventilation?

DR. TINDALL: We checked this out repeatedly with Dr. Herbert Saltzman, and it was the same at 1 atmosphere and 3 atmospheres.

DR. J. JACOBSON (*New York, N. Y.*): We have done a small number of carotid arterial reconstructions in patients in the hyperbaric chamber. In two instances, patients had complete or nearly complete occlusions on both sides, a surgeon's nightmare. In these situations, at the end of 5 minutes of carotid arterial occlusion, blood samples were taken from the jugular vein up very high, and the venous oxygen tension was in the normal arterial range of 100. Do you have measurements of venous oxygen tensions, which seem to us very important from the standpoint of indicating whether anoxia exists in the brain?

DR. TINDALL: No, we did not measure venous oxygen in these studies.

DR. G. G. NAHAS (*New York, N. Y.*): I noticed that when your animals were breathing pure oxygen at atmospheric pressure there was a fall of about 10% flow, and, at the same time, a decrease of 5 mm pCO_2. I wonder what would have happened to flow in your carotid vessel if the pCO_2 was increased by 5 mm? Can a 5-mm fall of pCO_2 in the arterial blood account for a 10% fall in cerebral blood flow?

DR. TINDALL: I think that in the range in which we are working, a 5-mm change in pCO_2 can certainly change cerebral blood flow. I wish that we could have adjusted

the pCO_2 to have made it rise 5 mm in these studies, but it was difficult to get that concentration of gas to do this, and we settled for the 2%. I think that a small change in pCO_2 in this range of, say, 35 to 40 mm Hg can certainly change cerebral blood flow.

DR. C. LUNDGREN (*Lund, Sweden*): Do you have any idea of the oxygen consumption or carbon dioxide production of your preparation? Would it not be reasonable to take into account the need of ventilation in terms of need for carbon dioxide elimination? My point is that this probably was a situation of hyperventilation in relation to the need for elimination of carbon dioxide.

DR. LAMBERTSEN: The question was, have you some idea of whether metabolism in terms of carbon dioxide production did, in fact, decrease? I could add to this that I do not know of any studies in which high oxygen pressures, except in extreme toxic ranges, have been shown to depress metabolism.

DR. TINDALL: We had no indications that metabolism was altered.

DR. LAMBERTSEN: In our experiments involving oxygen administration at sea level, where arterial pCO_2 fall was prevented by adjusting carbon dioxide tension upward toward the normal range, oxygen did not produce a cerebral vasoconstriction in human subjects. To my knowledge, this type of experiment has not been done at high pressures. Oxygen at a reasonable dose might do one thing, while a very high dose might begin to exert powerful biochemical poisoning of arteriolar smooth-muscle cells. It may well be that we are dealing not with one dose-response curve for effects of oxygen upon vessels, but with a physiological influence and a toxicological influence. These could be overlapping effects with one giving way to another as tension increases. I do not think we want to consider that increasing the tension of oxygen does one thing only.

Cerebral Cortical Blood Flow Under Hyperbaric Conditions

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The response of the cerebral vasculature to high pressure oxygen is a subject of interest and controversy. A more detailed knowledge of the mechanism of this response might help to provide a rational basis for applying OHP in the treatment of cerebral hypoxia. In addition, the relationship between the cerebrovascular response to hyperbaric oxygen and oxygen toxicity is not well understood.

Previous workers have shown that conscious human subjects exposed to oxygen at 1 and 3.5 atm have cerebral vasoconstriction as determined by the nitrous oxide method for measuring total cerebral blood flow.[1,2] Lambertsen *et al.*[2] maintained that the cerebral vasoconstriction which occurred during oxygen-breathing at 3.5 atm was due to arterial hypocapnia resulting from associated hyperventilation. Previous work carried out in this laboratory on anesthetized dogs [3] demonstrated that oxygen at 2 atm was also associated with cerebral cortical vasoconstriction. These latter workers concluded, however, that the mechanism of vasoconstriction was based upon a direct effect of oxygen, because arterial pCO_2 was held constant throughout their experiments. Quite possibly, of course, physiologic differences may exist between conscious man and the anesthetized dog in this regard.

Further investigations on the anesthetized dog at 2 and 3 atm have made it apparent that the relationship between cerebral cortical blood flow and increased arterial pO_2 may be less simple than it first appeared.

Materials and Methods

One hundred and seven determinations of blood flow through the cerebral cortex were made in eight unselected mongrel dogs by the ^{85}Kr clearance method of Lassen and Ingvar.[4] By this method, the blood flow is calculated from the rate of clearance from the exposed brain cortex of ^{85}Kr after its injection into the carotid artery. The clearance rate is measured by an end-window Geiger counter, mounted 1 mm above the brain and connected to a ratemeter and recorder.

Anesthesia was induced with thiopentone and maintained with trichloroethylene and intermittent suxamethonium chloride. McDowall *et al.*[5] have established that trichloroethylene in the concentrations used in this experiment has no significant effect on cerebral cortical blood flow. In addition, it has been shown that when this preparation is used cerebral cortical blood flow remains stable for at least

6 hours during air-breathing at normal pressure.

Respiration was controlled with a Starling ventilator, the stroke output of which was adjusted to maintain a constant arterial pCO_2. Arterial blood samples were obtained from a cannula in the femoral artery, and in one case venous blood samples were obtained from the sagittal sinus. Blood pressure was measured on a damped mercury manometer. The pharyngeal temperature was maintained at 38°C by controlling the environmental temperature.

Arterial pH and pCO_2 values were determined with two micro-Astrup apparatuses. The pCO_2 was also measured directly with a Severinghaus electrode. Arterial and venous pO_2 values were measured with a Radiometer oxygen electrode.

In each animal, blood flow estimations were made first while the animal was breathing air at normal pressure, then during oxygen-breathing at 3 atm, and again during air-breathing at normal pressure. In three of the animals, intermediate measurements were made during oxygen-breathing at 2 atm.

Results

Table 1 shows the values for cerebral cortical blood flow during eight separate experiments. All three animals exposed to oxygen at 2 atm showed a decrease in cerebral cortical blood flow, compared with the air control values. In six of the eight animals exposed to oxygen at 3 atm, the cerebral cortical blood flow rose; the two animals which had no increase had previously been exposed to oxygen-breathing at 2 atm.

Table 1. Cerebral Cortical Blood Flows with Air at 1 atm and Oxygen at 2 and 3 atm [a]

Dog	Cerebral cortical blood flow (ml/gm/min)			
	1 atm air	2 atm O_2	3 atm O_2	1 atm air
1	1.77	—	2.16	—
2	1.34	—	1.39	1.05
3	1.60	—	1.83	1.26
4	1.04	—	1.38	1.02
5	0.83	0.82	0.95	0.76
6	1.20	1.11	1.16	0.94
8	0.98	0.74	0.87	0.79
9	0.80	—	0.82	—

[a] In all but two instances, each value represents the mean of at least three separate blood flow measurements.

Table 2 shows the mean values for cerebral cortical blood flow and arterial pCO_2 in the five animals breathing oxygen at 3 atm, with no intermediate step at 2 atm. With arterial pCO_2 virtually constant, the blood flow increased during oxygen-breathing at 3 atm and fell again with air-breathing at normal pressure. The increase was not statistically significant.

Table 3 shows the mean values for cerebral cortical blood flow and arterial pCO_2 in the three animals exposed to oxygen at 2 and 3 atm. In these animals, there was no increase in cerebral blood flow at 3 atm as compared with the air control values, although there was a reversion of the decrease resulting from the 2-atm exposure.

Table 4 shows the values for arterial blood pressure, pCO_2, and pO_2 in each of the animals in the series; only the arterial pO_2 varied significantly. Alterations in

Table 2. Cerebral Cortical Blood Flows with Air at 1 atm and Oxygen at 3 atm

	1 atm air	3 atm O_2	1 atm air
Mean cerebral cortical blood flow (ml/gm/min)	1.31	1.52	1.11
SD	±0.40	±0.51	±0.13
Mean arterial pCO_2 (mm Hg)	42	43	41
SD	±4	±3	±3

TABLE 3. Cerebral Cortical Blood Flows with Air at 1 atm and Oxygen at 2 and 3 atm

	1 atm air	2 atm O_2	3 atm O_2	1 atm air
Mean cerebral cortical blood flow (ml/gm/min)	1.00	0.89	0.99	0.83
SD	±0.19	±0.20	±0.15	±0.10
Mean arterial pCO_2 (mm Hg)	44	44	43	42
SD	±2	±1	±2	±1

arterial pH and the nonrespiratory components of acid–base balance, in response to the increased arterial pO_2, were similar to alterations found under identical experimental conditions with air-breathing at normal pressure over the same period of time.

Figure 1 shows data obtained from one of the animals in the present series of studies. The mean value for cortical blood flow during air-breathing was 0.83 ml/gm/min. Immediate and rapid increase in pressure to 2 atm over 5 min was followed initially by an increase in blood flow. The first flow at 2 atm was associated with an increase of 5 mm Hg in arterial pCO_2. The remaining pCO_2 values at 2 atm and subsequently at 3 atm did not differ from the air-flow pCO_2 values by more than 2 mm Hg. Only at the end of 1 hour of oxygen-breathing at 2 atm was there an actual reduction in blood flow, in this case amounting to 25%. At this stage, compression to 3 atm of oxygen was followed by a sharp increase in blood flow, which initially rose above the air control values.

The other animals tested at 2 atm showed the same general pattern of blood flow changes, with a decrease in flow only in the latter half of the period of exposure to 2 atm. Figure 2 shows, in the five dogs exposed to oxygen at 3 atm only, that after the initial increase in cortical blood flow during the first 15 min, there was a fall toward but not below air control levels. These blood flow values were recorded in four animals beyond 1.5 hours of exposure to 3 atm and showed no tendency to fall below air control values, in contrast to the blood flow values of animals exposed to 2 atm.

After hyperbaric exposure, the normal response of the cortical blood flow to changes in arterial pCO_2 was tested and found to be within acceptable limits.

Figure 3 demonstrates the levels of pO_2 and pCO_2 found in the sagittal sinus blood during air-breathing at normal pressure and during oxygen-breathing at 3 atm in one of the animals of this series. The venous pO_2 rose from around 44 mm Hg to 110 mm Hg immediately after compression to 3 atm. Thereafter, the venous pO_2 fluctuated between 75 and 95 mm

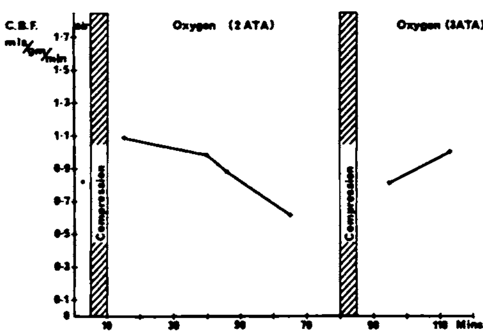

FIGURE 1. Cerebral cortical blood flow related to time and pressure.

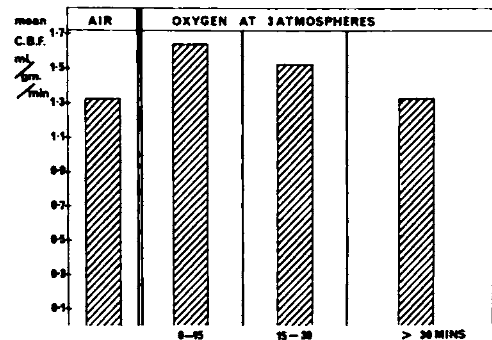

FIGURE 2. Cerebral cortical blood flow related to time at 3 atm of oxygen.

TABLE 4. Blood Pressure and Arterial Blood Gas Values (mm Hg)

Dog	1 atm air			2 atm O_2			3 atm O_2			1 atm air		
	B.P.	pCO_2	pO_2	B.P.	pCO_2	pO_2	B.P.	pCO_2	pO_2	B.P.	pCO_2	pO_2
1	130	41	97	—	—	—	145	45	1973	—	—	—
2	144	44	94	—	—	—	136	46	1730	140	40	—
3	142	37	103	—	—	—	125	39	1845	133	39	121
4	133	47	94	—	—	—	138	42	1845	120	44	84
5	136	46	70	125	44	910	130	43	1480	150	42	64
6	143	43	83	150	43	1032	136	41	1610	145	43	71
8	117	44	80	134	45	1153	135	45	1710	110	41	80
9	121	41	75	—	—	—	105	45	1729	—	—	—
Mean	133	43	87	136	44	1032	131	43	1740	133	42	84
SD	± 10	± 3	± 12	± 13	± 1	± 122	± 12	± 3	± 152	± 15	± 2	± 22

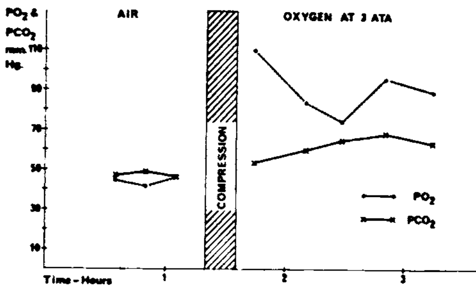

FIGURE 3. Changes in sagittal sinus blood gas values with 3 atm of oxygen.

Hg. The venous pCO_2 rose from 46 mm Hg to 54 mm Hg initially. Then, despite a constant arterial pCO_2 and blood flow by this stage, the venous pCO_2 continued to rise, stabilizing at a level of about 65 mm Hg. The data obtained during the 3-atm exposure showed that there was no alteration in cerebral cortical oxygen uptake compared with the air control values.

DISCUSSION

Although these results are admittedly of a preliminary nature, it seems that oxygen at increased pressure produces changes in cerebral cortical blood flow which vary with the absolute pressure and also with the length of exposure at a particular pressure. Our previous data, compared with the results in the initial air-breathing controls, showed that oxygen at 1 atm resulted in a 12% reduction in flow, and oxygen at 2 atm resulted in a 21% reduction in flow. The present data, however, indicate that the vasoconstriction occurring during oxygen-breathing at 2 atm may be delayed for as long as 30–45 min. In comparison, oxygen at 3 atm appears to stimulate an actual increase in cerebral cortical blood flow initially, with a later fall toward the air control values. At no stage was it possible to demonstrate a vasoconstrictive action with oxygen-breathing at 3 atm.

In the presence of a constant arterial pCO_2, the fact that oxygen at 3 atm is not associated with a reduction in cerebral cortical blood flow would seem to be in agreement with the opinion expressed by Lambertsen *et al.*[2] that the vasoconstriction at 3.5 atm in conscious humans is due to the drop in arterial pCO_2 caused by hyperventilation, rather than to a single direct vasoconstrictive action of increased arterial pO_2. On the other hand, the present studies showed a marked increase in venous pCO_2, presumably a reflection of cortical tissue pCO_2 and presumably based upon the reduced isohydric buffering capacity of venous blood. On the basis of the suggested relationship between local cerebral flow and local metabolic rate,[6] it seems possible that tissue pCO_2 has a regulating influence on flow. In these experiments, there was a marked increase in venous pCO_2 but no statistically significant change in flow. We would tentatively suggest that this points to a balance existing between the increased arterial pO_2 tending to vasoconstrict and the increased tissue pCO_2 tending to vasodilate the cortical blood vessels.

Why the blood flow should decrease only after a certain time of exposure to 2 atm and apparently not at 3 atm over the same period of time is not clear. A sound explanation based upon the available evidence would be difficult, but some connection may exist with the recognized cerebral oxygen toxicity manifestations at 3 atm.

ACKNOWLEDGMENTS

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DISCUSSION

DR. H. WOLLMAN (*Philadelphia, Pa.*): I would like to offer an alternative explanation for these changes in cerebral blood flow with time. At a constant abnormally high or low pCO_2, although the cerebral blood flow changes, it probably tends to return toward a normal value over a period of several hours. Perhaps what we are looking at here is an increase in sagittal sinus or tissue pCO_2, producing an increase in cerebral blood flow. This might be followed by the cerebral vascular compensation which I have suggested, *i.e.*, a type of autoregulatory constriction in response to increased carbon dioxide, resulting in the measured decrease in cerebral blood flow. I admit it occurs a little more quickly here than might be expected. Let me make an additional comment on the pO_2 of venous blood draining the brain, which bears on Dr. Jacobson's earlier remarks. Only those areas with some circulation can contribute blood to the venous drainage. Therefore, a high pO_2 in jugular venous blood means only that those areas of the brain being perfused have a high pO_2. Areas which may have no blood flow at all (*e.g.*, infarcts) do not contribute to jugular blood, and thus jugular pO_2 can be misleading in certain circumstances.

DR. LEDINGHAM: I think your comments pertaining to the blood-flow changes with time are perfectly reasonable. I doubt if they explain altogether the changes that we found with time, but I think that further investigation will elucidate this problem a bit more.

DR. J. W. SEVERINGHAUS (*San Francisco, Calif.*): You made an assumption that a high tissue pCO_2 might be expected to produce cerebral vasodilation, and I wondered what both you and Dr. Lambertsen think is the site at which carbon dioxide acts? I think, on the basis of some transient studies, that it seems to act at the arterial level and probably does not relate to tissue pCO_2. Dr. Lasen and I last year did an experiment in which we suddenly produced a drop of arterial pCO_2 in man from 40 to 25 mm Hg within about 6 seconds, held it there for 2 hours and followed the A-V oxygen difference. The flow, at least as measured by oxygen difference, drops within 30 seconds to its low level, while the jugular venous pCO_2, and therefore presumably the tissue pCO_2, take about 7 or 8 minutes to get down, so that correlation with flow was with the arterial and not with the tissue pCO_2. In that case, the

high tissue pCO_2 you observed probably does not relate or would not have a blocking effect in preventing a vasoconstriction from hypoxia, at least in my interpretation.

DR. LEDINGHAM: Is your comment strictly relevant to the situation which we have here, in that the change which you mentioned was from a normal pCO_2 to a very low pCO_2? I am not too sure that this excludes the possibility that high tissue pCO_2 may not affect cerebral blood flow.

DR. SEVERINGHAUS: Well, I would make the first assumption that if carbon dioxide has a site of action, it is the same one going up and down. The experiment which we did was only to determine whether the site was arterial or tissue. It seemed to be arterial, as I think everyone had assumed in the past, but if that is the case then why would the high tissue pCO_2 in your experiment, or rather the high sagittal sinus pCO_2, be preventing hyperoxia from constricting the vessels? Do you still think that this may be a tissue pCO_2 regulation as well as an arterial pCO_2 regulation of flow?

DR. LEDINGHAM: I think both arguments are possible. It seems convenient to suggest that arterial pCO_2 is the primary mechanism, but I think in the pathological situation in which we have to regard hyperbaric oxygen, it may be that a different mechanism plays a role. It is a very difficult problem. Perhaps Dr. Lambertsen would like to comment on this at the moment.

DR. C. J. LAMBERTSEN, *Session Chairman (Philadelphia, Pa.)*: I understand what Dr. Severinghaus is getting at. It relates to Dr. Guyton's talk this morning wherein it was evident that the locus of action of oxygen and of carbon dioxide in providing any regulatory mechanism must be determined. Dr. Severinghaus asks whether it is possible to use information of this sort to help us determine the normal locus of action in regulation. For example, if we can produce an accumulation of carbon dioxide in a tissue such as brain, and this does nothing to the rate of blood flow through that tissue, then I personally would feel that the rise of pCO_2, which does nothing to flow, could hardly, at that tissue location, be considered a controlling factor. This we have demonstrated already in man.

DR. SEVERINGHAUS: I wonder if you could also tell us what the sagittal sinus pH was under these conditions, because it is very probable that the effect of carbon dioxide is through pH, not in the blood, but in the extracellular space or possibly intracellular space of the muscles of the arterioles.

DR. LEDINGHAM: Yes, we did in fact measure sagittal sinus pH, but I cannot at the moment recall what the exact values were. I think I am correct in saying that they changed absolutely with the change in pCO_2, but not having the absolute values, I would not like to comment on that at the moment. It is very difficult to know just how to solve this problem between an increase in arterial pCO_2 and an increase in tissue pCO_2. I would like very much to hear of the possibility of an experimental model which could allow us to measure these two separately, because we would like to have an answer to this problem.

Effects of Hyperbaric Oxygenation on Reactive Hyperemia in Man

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The factors producing increased blood flow immediately after a period of temporary arterial occlusion are not fully understood. The important role of lowered tissue oxygen tension was suggested 30 years ago[1] and has been recently reaffirmed by Guyton and co-workers.[2] Furthermore, Bird and Telfer have reported that exposure to oxygen at 1 and 2 ata caused reductions of 11.2% and 18.9%, respectively, in the resting forearm blood flow as measured by mercury-in-rubber strain gauges.[3] These workers also reported decreases of up to 30% in the blood flow of reactive hyperemia when human subjects were exposed to 2 ata of oxygen pressure, but they commented that the records were unstable and difficult to reproduce.[4]

The following investigation was designed to study alterations in human muscle blood flow as measured by the clearance rate of injected ^{133}Xe, while subjects were exposed to 100% oxygen at 3 ata.

Methods

Muscle blood flow was measured in both arms of five adult male volunteers. Radioactive ^{133}Xe dissolved in sterile saline (75–100 μc in 0.1–0.2 ml) was injected directly into forearm muscle using a 25-gauge needle which was maintained in position for 30 sec to prevent leakage of injected material along the needle track. Scintillation detectors were positioned above the injection sites and recordings were begun 2 min after injection. Conventional ratemeters (2-sec time constant) were used and their output recorded on logarithmic potentiometers. In this way, the data could be easily calculated in terms of blood flow by utilizing the following equation: muscle blood flow $= 161 \times D$ ml/100 gm/min, where D represents the fraction of a decade which the tangent drawn to the curve at any instant decreases in 1 min.[5]

In addition, the decrease in the record over any arbitrary time period (again expressed as the fraction of a single decade) multiplied by the same factor, 161, yielded the cumulative flow value for the period of time measured. This calculated value of average flow was found useful during reactive hyperemia when instantaneous flow rates were rapidly changing.

To reduce extraneous influences on the

blood flow, only subjects experienced in hyperbaric technique were selected. Despite this fact, the studies were difficult in that the subject remained motionless during the 1.5 hours of recording. Both the base-line and OHP measurements were made within the closed hyperbaric chamber with each subject breathing either air or oxygen through a scuba mouthpiece or face mask. Bed rest (0.5 to 1 hour) preceded initial base-line measurements. Because of emotional effects on blood flow, distracting conversations and noises were avoided.

Following duplicate base-line determinations, subject and operator were pressurized to 3 ata and measurements were repeated with the subject breathing pure oxygen after a 5-min equilibration period. Thirty minutes of re-equilibration time was allowed before final base-line recordings were obtained. Each experimental sequence included measurement of blood flow for 5 min during rest, for 10 min during arterial occlusion, and for 5 more min during reactive hyperemia and recovery.

Results

In each of the five subjects, a significant reduction in resting muscle blood flow was seen between the first and second baseline observations. The arithmetic means (Table 1) were 2.1 and 1.0 ml/100 gm/min. There was a slight further reduction in resting flow during OHP exposure. Thirty minutes after returning to airbreathing at 0 psig, resting blood flow remained at the same low level observed during hyperbaric oxygenation.

The maximal rate of blood flow attained during reactive hyperemia (Table 1, column 2) was greatest during the first control period and decreased in subsequent measurements. When the response during OHP was compared to the first and second base-line values, there were reductions of 38% and 18%, respectively. Thirty minutes after decompression, however, the base line showed further reduction in maximal blood flow rate during the reactive hyperemia.

When cumulative blood flow was calculated for the entire period of reactive hyperemia, successive decreases also appeared during subsequent base-line and OHP determinations. Thirty minutes following decompression, this value also failed to demonstrate a return to initial base-line values.

Discussion

These results suggest that exposure to hyperbaric oxygenation may promote some reduction in the muscle blood flow during reactive hyperemia. The percentage reductions obtained over the initial control values agree in general with the 15–30%

TABLE 1. Effects of OHP on Blood Flow Measurements in Resting Forearms of Human Subjects [a]

Ventilating gas	Muscle blood flow (ml/100 gm/min)		Cumulative flow during first minute of reactive hyperemia
	Resting	Reactive hyperemia	
Air at 1 ata	2.1 ± 0.1	21.8 ± 14.0	29.5 ± 19.5
Air at 1 ata[b]	1.0 ± 0.1	16.5 ± 8.5	23.0 ± 11.2
O_2 at 3 ata	0.8 ± 0.4	13.5 ± 5.2	14.0 ± 4.5
Air at 1 ata	0.8 ± 0.4	9.8 ± 3.5	13.0 ± 6.0

[a] Figures represent arithmetic means for $n = 10$ and values for one standard deviation.
[b] Duplicate base-line measurements obtained before OHP exposure.

reductions that were reported by Bird and Telfer.[3,4] The instability of the response as seen in the wide range of standard deviation values also agrees with the experience of these workers.

Previous work in our laboratories using anesthetized dogs under similar conditions but with longer oxygen exposure has suggested that the level of tissue oxygenation prior to arterial occlusion is an important factor in producing the reduced blood flow observed and may be associated with greater stability and reproducibility of results. Possibly comparable long periods of exposure to oxygen in our subjects may have produced larger reductions in blood flow of reactive hyperemia, albeit at the risk of inducing clinical oxygen toxicity.

Persistence of reduced blood flow after return to ambient pressure might reflect a continuing decline in the response of the vascular system due to trauma of repeated arterial occlusions or multiple injections of the saline containing ^{133}Xe. In addition, fatigue due to the lengthy procedure may have reduced the ability of the system to respond. That the level of tissue oxygenation attained during the hyperbaric exposure may have caused a sustained constriction of the nutrient blood vessels in muscle may also account for failure of final base-line values to demonstrate recovery. Vasoconstriction from hyperoxygenation has been observed in retinal vessels,[6] suggesting a role of arterial pO_2 in regulating tissue perfusion. The same concept has been demonstrated in perfused skeletal muscle.[2] Unfortunately, tissue oxygen concentrations in the present experiments were not measured.

One other possibility is the suggestion that the final observation is a true baseline value and represents a gradual reduction of flow over the course of the study. If this were so, OHP would then have produced no significant reduction in blood flow to muscle.

SUMMARY

Blood flow in human forearm muscle was measured in both arms of five male volunteer subjects using the ^{133}Xe clearance technique. Flow was measured twice before, once during, and once 30 min after exposure to 100% oxygen at 3 ata. A marked reduction in the resting and reactive hyperemia blood flows occurred between the first and second base-line runs. When flows during OHP exposure were compared to either the first or second base lines there were reductions of 38% and 18%, respectively, in the response during reactive hyperemia. Resting blood flows were similarly reduced. Base-line flows determined 30 min after OHP failed to show recovery from experimental values.

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Renal Response to Hyperbaric Oxygen

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Oxygen has been reported to cause cerebral vasoconstriction,[1-3] and hyperbaric oxygen therapy has been shown to be contraindicated in focal cerebral ischemia.[4] Nevertheless, Smith *et al.*[5] reported that the totally hypoxic dog brain could be protected if oxygen were breathed at 2 ata, and Harper *et al.*[3] showed that cerebral vasoconstriction did not occur in the canine brain if 2 ata oxygen were breathed, provided that the brain was rendered hypoxic by reducing the arterial blood pressure to 50 mm Hg by hemorrhage.

Cowley *et al.*[6] have demonstrated the protective effect of hyperbaric oxygen in animals suffering from hemorrhagic and endotoxic shock, and it seemed desirable to measure the effect of oxygen on renal blood flow and to compare the response with that which had been obtained in the brain.

METHODS

Series 1

Ten unselected mongrel dogs weighing 12.9–25.4 kg were anesthetized with minimal doses of sodium thiopentone to allow the insertion of a cuffed Magill endotracheal tube. The animals were allowed to breathe spontaneously and anesthesia was maintained with trichlorethylene administered by a non-rebreathing circuit. Both femoral arteries were cannulated: the one to allow sampling of central aortic blood and the other to allow measurement of the central aortic blood pressure by means of a mercury manometer. A cannula placed in the right atrium allowed both sampling of mixed venous blood and the measurement of central venous pressure by means of a water manometer. A #8 esophageal tube was then passed down the right jugular vein and guided into the left renal vein, through a midline laparotomy incision, where it was ligated permanently in position. The left renal venous blood was collected into a measuring cylinder and then returned to the animal through the left femoral vein by means of a Sigmamotor pump. Thus the venous effluent from the left kidney could be measured directly, using a stopwatch. Before the abdomen was closed, the left gonadal vein was ligated and the animal given 4 mg/kg heparin sulfate.

Following a 15-min period of postoperative equilibration, the animal was given oxygen at 1 ata to breathe for 10 min. The renal blood flow was then measured three times at 5-min intervals, and blood was removed for the estimation of arterial, mixed venous, and renal venous pO_2 and oxygen saturation. These mea-

surements were made with a Radiometer oxygen electrode and Kipp's hemoreflector. Arterial and central venous pressures were also measured, as was the acid–base status of arterial blood, by means of the Astrup microtechnique.

These measurements were then repeated when the animal breathed oxygen at 2 ata, air at 1 ata, and finally oxygen at 1 ata. The mean of the three blood flow measurements was reported as the blood flow for the particular inspired pO_2 under consideration.

In all cases, the arterial pH, pCO_2, and standard bicarbonate were within the normal range when the measurements were made. The arterial blood pressure was maintained close to the value measured at the beginning of the experiment, by infusion of blood if necessary.

Series 2

Five unselected mongrel dogs weighing 10–31 kg were prepared as in Series 1, and the renal blood flow was measured while the animal breathed air at normal atmospheric pressure. The arterial blood pressure was then reduced to 60 mm Hg by allowing blood to accumulate in the reservoir. Following a 15-min period for equilibration at a blood pressure of 60 mm Hg, the renal blood flow was measured when the animal breathed air at 1 ata, oxygen at 1 and 2 ata, and finally air at 1 ata again. In total, 60 measurements of blood flow at 60 mm Hg were made.

In these experiments, the acid–base balance was adjusted 15 min prior to each series of flow measurements, so that, despite the development of the metabolic acidosis of the shocked state, each series of blood flow measurements was made in an animal whose arterial pH, pCO_2, and standard bicarbonate were within the normal range.

RESULTS

Table 1 shows the renal blood flow of the 10 normotensive dogs when breathing air and oxygen at 1 and 2 ata. Blood flow was significantly reduced at both 1 and 2 ata of oxygen. Compared with air, oxygen at 1 ata resulted in a 17% reduction in flow, while oxygen at 2 ata resulted in a 33% reduction. The mean blood flow when oxygen at 1 ata was breathed for the second time nearly returned to baseline values, indicating only slight deterioration in the preparation. No significant difference occurred between the two

TABLE 1. Measurements of Renal Blood Flow in Normotensive Dogs Breathing 1 ata Oxygen, 2 ata Oxygen, 1 ata Air, and Finally 1 ata Oxygen

	Renal blood flow (ml/100 gm/min)			
Dog no.	1 ata oxygen	2 ata oxygen	1 ata air	1 ata oxygen
1	277	199	324	—
2	297	218	372	300
3	400	361	496	406
4	176	120	231	179
5	134	112	147	147
6	218	180	273	201
7	323	252	367	255
8	401	386	462	385
9	279	253	338	273
10	250	185	306	234
Mean & SD	276 ± 80	227 ± 95	332 ± 96	264 ± 84

SD, standard deviation.

TABLE 2. Comparison of Arterial, Renal Venous, and Mixed Venous pO_2 in Normotensive Dogs Breathing Air and Oxygen at 1 and 2 ata

Ventilation gas	Arterial pO_2 (mm Hg)	Renal venous pO_2 (mm Hg)	Mixed venous pO_2 (mm Hg)
1 ata air	101 ± 29	66 ± 18	36 ± 21
1 ata O_2	517 ± 93	183 ± 78	91 ± 30
2 ata O_2	1124 ± 205	422 ± 147	162 ± 50

groups of blood flow measurements when oxygen at 1 ata was breathed.

Table 2 shows the measurements of oxygen tension made on arterial, renal venous, and mixed venous blood. The renal venous pO_2 lay between arterial and mixed venous values. The renal venous blood was fully saturated when oxygen at 1 ata was breathed, while both the renal venous and mixed venous blood were fully saturated when oxygen at 2 ata was breathed.

The renal blood flow measurements made when the arterial pressure was maintained at 60 mm Hg appear in Table 3. It can be seen that no difference in values was obtained between the first and last series of measurements made in each dog, when air at normal atmospheric pressure was breathed, indicating no deterioration in the preparation during the measurements. Also, no difference in renal blood flow occurred between oxygen breathed at 1 ata and air breathed at the same pressure. Again, however, renal blood flow decreased when oxygen at 2 ata was breathed. Compared to the flow values during air-breathing, renal blood flow fell by 28% when oxygen at 2 ata was breathed at this level of arterial pressure.

Figure 1 shows the relationship between renal blood flow and inspired oxygen ten-

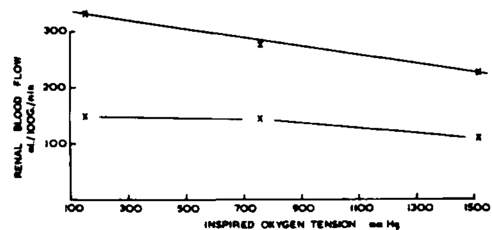

FIGURE 1. The upper curve shows the relationship between renal blood flow and inspired oxygen tension in normotensive dogs; the lower curve shows the same relationship in dogs when arterial blood pressure was maintained at 60 mm Hg.

TABLE 3. Measurements of Renal Blood Flow in Hypotensive Dogs Breathing 1 ata Air, 1 ata Oxygen, 2 ata Oxygen, and Finally 1 ata Air [a]

	Renal blood flow (ml/100 gm/min)			
Dog no.	1 ata air	1 ata oxygen	2 ata oxygen	1 ata air
1	226	219	170	207
2	132	132	103	132
3	163	182	121	190
4	100	110	65	100
5	118	83	77	112
Mean & SD	148 ± 44	145 ± 50	107 ± 36	148 ± 42

SD, standard deviation.
[a] Arterial pressure was maintained at 60 mm Hg throughout experiment.

sion observed in normotensive animals and hypotensive animals. In normotensive animals, the fall in renal blood flow varied linearly with the inspired oxygen tension, but the fall in renal blood flow, when the arterial pressure was maintained at 60 mm Hg, did not occur until 2 ata of oxygen was breathed.

The measurements of arterial, renal venous, and mixed venous pO_2 made in the hypotensive series appear in Table 4. The same relationship existed among the measurements made from the three sites in this series as in the normotensive group, and the arterial values were in the same range. Both renal and mixed venous values were lower, but the renal venous blood was still fully saturated with oxygen when oxygen at 1 or 2 ata was breathed. Only when 2 ata of oxygen was breathed, however, was the renal venous blood supersaturated with oxygen.

Discussion

The mean initial value of renal blood flow of 276 ml/100 gm/min agrees fairly closely with that of Rennie and Knox,[7] who measured renal blood flow by the whole-blood extraction method using ^{131}I Diodrast. These authors also noted a fall in renal blood flow, which declined exponentially as an inverse function of inspired oxygen tension until, at 4 ata oxygen, it was 57% of the control, which was measured during oxygen-breathing at 1 ata.

The fall of 17% in renal blood flow during oxygen-breathing at 1 ata and 33% at 2 ata compares with the fall in cerebral blood flow of 12% on changing from air to oxygen at normal atmospheric pressure and of 21% on changing from 1 ata to 2 ata of oxygen, noted by Jacobson *et al.*[8]

When the arterial blood pressure was reduced to 50 mm Hg, Harper *et al.*[3] were able to show no evidence of cerebral vasoconstriction during breathing of oxygen at 2 ata rather than air. In our studies, evidence of renal vasoconstriction was still apparent, however, when 2 ata of oxygen was breathed instead of air, at an arterial pressure of 60 mm Hg; this did not occur during oxygen-breathing at 1 ata. In these experiments, the renal venous pO_2 was still high when the hypotensive dogs breathed oxygen at 2 ata; it is possible that vasoconstriction would not occur if the arterial pressure were lowered still further when the dogs breathed 2 ata of oxygen. The renal venous blood observed in the hypotensive animals breathing oxygen at atmospheric pressure was just fully saturated and no vasoconstriction was observed.

This vasoconstrictor phenomenon has the appearances of a further mechanism, designed to protect the renal tissue from possible damage if hyperbaric oxygen is breathed. Little evidence has yet been found, however, that renal function is impaired when hyperbaric oxygen is breathed, although Dickens[9] observed a fall in the oxygen consumption of kidney exposed to OHP *in vitro.*

TABLE 4. Comparison of Arterial, Renal Venous, and Mixed Venous pO_2 in Hypotensive Dogs Breathing Air and Oxygen at 1 and 2 ata[a]

Ventilation gas	Arterial pO_2 (mm Hg)	Renal venous pO_2 (mm Hg)	Mixed venous pO_2 (mm Hg)
1 ata air	88 ± 19	47 ± 7	26 ± 6
1 ata O_2	554 ± 133	102 ± 85	45 ± 9
2 ata O_2	1256 ± 51	387 ± 190	95 ± 16

[a] Arterial pressure was maintained at 60 mm Hg throughout experiment.

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DISCUSSION

DR. N. G. MEIJNE (*Amsterdam, The Netherlands*): Do you think that your flow reduction in the renal vessels reflects a changing cardiac output, or is it possible that there are local changes in the flow distribution?

DR. NORMAN: I really do not know why this effect is taking place. The obvious extension of this work is to consider first in what way the flow distribution within the kidney is altered by this mechanism. It is more likely to be a local phenomenon in the kidney than a reflection of a change in cardiac output, although I have no real evidence upon which to base this view.

DR. C. J. LAMBERTSEN, *Session Chairman* (*Philadelphia, Pa.*): In studying effects of oxygen upon renal blood flow, we do not pay much attention to the glomeruli themselves. However, in terms of oxygen poisoning, the glomeruli represent a capillary bed which should receive almost as high a dose of oxygen as the alveolar capillaries. We should look for indication of a poisoning of the glomerular capillaries. In any of your studies, have you attempted to do this?

DR. NORMAN: No, not to date. In the second group of five normotensive dogs, we measured urinary volume changes and noted that these seemed to alter with the blood flow. The volume of urine went down with the blood flow. So far, we have done no more than that.

Effects of Hyperbaric Oxygenation on Excess Lactate Production in Exercising Dogs

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Exercise at normal atmospheric pressure results in skeletal muscle tissue hypoxia which is manifested by the production of lactic acid[1-3] and excess lactate,[4-9] the amount of lactic acid formed during exercise at normal atmospheric pressure varying in proportion to the percentage of oxygen in the inspired gas.[10-12] The present study was designed to determine the degree to which the production of lactic acid and excess lactate could be further modified in exercising dogs ventilated with oxygen at 3 ata.

METHODS

Twenty-four mongrel dogs weighing 14–24 kg were anesthetized with pentobarbital sodium (24 mg/kg body weight). Respiration was controlled by means of an endotracheal tube and a Harvard respirator operated at a fixed rate of 16 strokes/min with a stroke volume of 400 ml; this produced a state of forced hyperventilation at rest. Catheters were placed in the pulmonary artery and right common carotid artery in all animals. Hind-limb exercise was induced by electrical stimulation, electrodes being placed in the lumbar and extensor calf muscles of the dogs and then connected to a Corbin-Farnsworth cardiac pacemaker. Generally, the 6-volt stimulation at a rate of 100/min resulted in vigorous repetitive extension of the hind limbs. Each animal was stimulated in this manner for 4 min at the same rate and voltage during each study period.

The animals were divided into three groups of eight dogs each (Table 1); each animal was exercised twice. Animals in Group I were exercised for 4 min at 1 ata during air-breathing and, 45 min later, at 3 ata during 100% oxygen inhalation. The animals in Group II were exercised initially at 3 ata while breathing 100% oxygen and then, 45 min later, after decompression on air, at 1 ata while breathing air. Animals in Group III were exercised twice at 1 ata on air with a 45-min interval between the two periods.

TABLE 1. Sequence of Exercise Periods in Three Groups of Eight Dogs

Group	Exercise #1	Exercise #2
I	1 ata air	3 ata O_2
II	3 ata O_2	1 ata air
III	1 ata air	1 ata air

Arterial and venous samples were drawn during rest and the fourth minute of exercise in all groups. Blood pO_2, pCO_2, and pH were measured at pressure on the Instrumentation Laboratories pH and blood gas analyzer especially adapted for use in the hyperbaric chamber. In addition, 5-ml samples of arterial and venous blood for lactate and pyruvate determinations were drawn during rest and the fourth minute of exercise and were immediately denatured in iced perchloric acid. Blood lactate and pyruvate levels were determined by enzymatic methods[13,14] and excess lactate was calculated.[4]

Results

Mean arterial pO_2 values at 1 ata during air ventilation approximated 100 mm Hg in each group (Figure 1); the response to exercise at 1 ata was essentially the same in all animals with decreases in arterial pO_2. Resting pO_2 values at 3 ata of oxygen exceeded 1800 mm Hg in Groups I and II; exercise produced a decrease in arterial pO_2 to slightly below 1800 mm Hg.

Mean venous pO_2 values at rest were comparable at 1 ata of air (Figure 2) and rose above 100 mm Hg at 3 ata of oxygen; with exercise at 3 ata during oxygen ventilation, the venous pO_2 fell in Groups I and II, but still exceeded the resting pO_2 value of animals at 1 ata during air-breathing.

The pCO_2 values consistently rose and

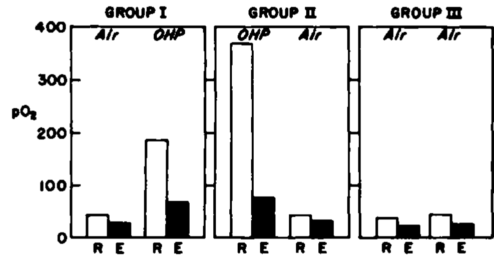

FIGURE 2. Mean venous pO_2 values of the three groups of dogs, during rest (R) and exercise (E) at 1 ata during air ventilation and at 3 ata during oxygen-breathing.

the pH values consistently fell with exercise in all three groups.

Excess lactate values from arterial blood showed interesting changes in all groups (Figure 3). In Group I, the difference in excess lactate at the two atmospheric pressures was significant ($P < 0.01$). In Group II, excess lactate production was minimal and no significant differences could be seen at the two atmospheric pressures, strongly suggesting a lasting effect from prior exposure to oxygen at high pressure. Group III animals served as controls and showed consistent significant rises in excess lactate during exercise, although a small but significant decrease in excess lactate levels was found during the second exercise period, suggesting a slight conditioning effect. Nevertheless, comparison of excess lactate levels of Group I animals during the second exercise period on oxygen under pressure with the excess lactate levels of Group III animals during the second exercise period

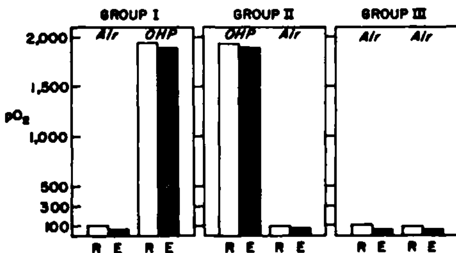

FIGURE 1. Mean arterial pO_2 values of all three groups of dogs, during rest (R) and exercise (E) at 1 ata during air ventilation and at 3 ata during oxygen-breathing.

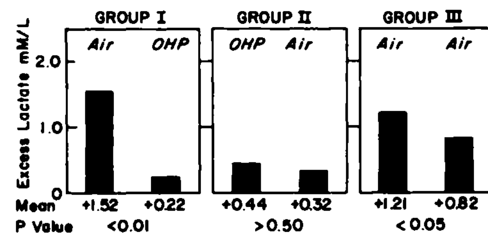

FIGURE 3. Mean arterial excess lactate levels of the three groups of dogs during exercise at 1 ata in air and at 3 ata during oxygen-breathing.

at 1 ata during air ventilation still revealed a significant difference ($P < 0.05$).

Lactate/pyruvate ratios correlated well with the excess lactate levels (Table 2).

TABLE 2. Mean Arterial Lactate/Pyruvate Ratios at 1 ata Air and 3 ata O_2

Group	L/P ratio Rest	L/P ratio Exercise	L/P ratio Rest	L/P ratio Exercise
	(1 ata air)		(3 ata O_2)	
I	16/1	26/1	19/1	19/1
	($P < 0.005$)		($P > 0.50$)	
	(3 ata O_2)		(1 ata air)	
II	14/1	17/1	17/1	21/1
	($P < 0.20$)		($P > 0.50$)	
	(1 ata air)		(1 ata air)	
III	12/1	21/1	13/1	18/1
	($P < 0.01$)		($P < 0.02$)	

DISCUSSION

The excess lactate levels observed during exercise at 3 ata during oxygen-breathing were significantly lower than the levels in the same animals exercised at 1 ata in air. Furthermore, reversal of the sequence of exposure to oxygen at 3 ata resulted in significantly lower excess lactate levels both during the exposure to high pressure oxygen and 45 min later during exposure to 1 ata of air. We postulate three possible explanations for these results: (1) that an increased removal of excess lactate was made possible as a result of stimulating oxidative enzymatic processes, (2) that the oxygen provided to the exercising muscle tissue during OHP exposure was sufficient to decrease anaerobiasis and subsequent excess lactate production, or (3) that OHP produced inhibition of glycolytic enzymes and thus resulted in suppression of lactate formation during hyperbaric oxygenation and after decompression.

Although the first possibility, the increased removal of excess lactate by stimulation of normal oxidative enzymatic processes, may explain the observed results, such enzymatic acceleration has not been demonstrated *in vitro* or *in vivo* under hyperbaric oxygenation. All evidence indicates that inhibition and inactivation of enzymatic systems are produced by high pressure oxygen. While the second possibility, reduction of anaerobiasis, provides a reasonable explanation for some of the data, it does not satisfactorily explain the persistence of lowered excess lactate levels for periods up to 45 min following OHP. The third possibility, the inhibition of glycolytic enzyme systems,[15–20] may more logically explain the decrease in excess lactate levels noted following prior exposure to high pressure oxygen.

The inhibitory effects of hyperbaric oxygenation on certain enzymes, particularly the sulfhydryl enzyme glyceraldehyde-3-phosphate dehydrogenase in glycolysis, have been well documented in simple biochemical systems.[16,18,20] Such an inhibition in our animals can readily explain the decreased lactate levels observed because of impaired anaerobic glycolysis at the level of glyceraldehyde-3-phosphate dehydrogenase activity. The persistence of this effect for 45 min after exposure to OHP could then explain the results in the Group II animals.

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DISCUSSION

DR. W. STAINSBY (*Gainesville, Fla.*): Have you any evidence that the lactate really came from the exercising muscles?

DR. WEGLICKI: We were able to obtain samples from several of the animals from the abdominal inferior vena cava which did show that the excess lactate levels in this area of drainage were significantly elevated. However, our samples were mixed venous blood in the pulmonary artery and in the carotid artery.

DR. P. WEBB (*Yellow Springs, Ohio*): A fourth possibility may be a persistence of elevated temperature in the exercising muscle and a consequent increased circulation which would persist for the 45 minutes of observation. Have you a comment on that?

DR. WEGLICKI: We do not have any evidence of flow in the muscle; however, the possibility of a conditioning effect that we commented on regarding our control animals is certainly a good one. Nevertheless, as I showed in the slide, the conditioning effect, although present to a slight degree, was felt to be a response to increased flow, but was still not sufficient to significantly decrease the excess lactate that we observed

at pressure. In other words, the pressure phenomenon could be separated from the conditioning effect.

DR. C. J. LAMBERTSEN, *Session Chairman* (*Philadelphia, Pa.*): Some of us, a number of years ago, thought that if one exercised at very high oxygen pressure, the improvement of oxygenation of the muscle might greatly increase the capacity for muscular work without exaggerated respiratory response. When you do such studies in humans, there occurs a decrease in the amount of fixed acid produced and also a decreased respiratory response. However, there seems to be no tremendous increase in work capacity. Do you personally think that, at very high oxygen tensions, if one could counteract the poisonous effects of oxygen on brain and lungs, there should be a greater work capacity?

DR. WEGLICKI: Here again, I would like to refer to Dr. Guyton's remarks that the effect observed from certain levels of oxygen may be something more than a physiological one after passing a certain threshold. Although we have no definite evidence from the results of our studies, we can postulate that efficiency of work may be decreased at a certain level. The threshold level is not known, but if there is a toxic effect and if the enzymes providing energy for muscle metabolism are inhibited, as mentioned yesterday, the efficiency of work may be decreased.

Electroretinographic Changes During Hyperoxia*

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Recent advances in the use of hyperbaric oxygen in clinical medicine have renewed interest in the early manifestations of toxic effects of oxygen. The toxic effects on the lung and the brain have been well recognized but only few of the manifestations in the visual system have been defined and investigated. Over three decades ago, subjective visual impairment was noted by Behnke,[1] and more recently Beehler[2] and Comroe[3] reported ocular hypotonia and irritation of the iris as well as of the conjunctiva. Retinal injury following 40-hour exposure to 100% oxygen has been described by Noell[4] as marked reduction in size of the outer nuclear layer. Noell also found significant attenuation of the *b* wave of the electroretinogram in animals exposed to 1 atm of oxygen for 25–36 hours. Beehler[2] observed reversible retinal detachment in dogs exposed to 1 atm of 100% oxygen for 3 days. The purpose of our study was to investigate the time–pressure dependency of changes in the electroretinogram during hyperbaric oxygenation.

* Also published in Archives of Ophthalmology 45:812, 1966.

METHODS

Thirty albino rabbits (2.5–3.0 kg) were used for this study and were divided into groups of five each. The five animals in each group were individually exposed to 100% oxygen at each of five different pressures (2.5, 3, 4, 6, and 7 ata). The remaining five rabbits were exposed to normal air and served as controls. All animals were anesthetized with urethane 1.8 gm/kg 30 min before onset of recording. Half of this dose was given intravenously and the other half intraperitoneally. Homatropine was used topically in both eyes to paralyze the iris.

Each of the animals exposed to hyperbaric oxygen was placed in an 80-liter pressure chamber and dark-adapted for 20 min while the chamber was flushed with oxygen. The animal's head was fixed in front of the chamber porthole. A Grass photostimulator (PS-2-D) was placed outside of the chamber, 25 cm from the rabbit's eye. The porthole glass transmittance was 82% as measured by a Pritchard photometer. Intensity 8 of the Grass photostimulator was used in all experiments. Carbon dioxide was absorbed and remained below 0.5%, as measured in the

chamber before the termination of each experiment. Electrocardiogram, respiratory rate, and rectal temperature were continuously recorded. External heating of the chamber was controlled to limit variations of the rectal temperature to ±1°C.

The electroretinogram was recorded from an intrastromal corneal Ag–AgCl wire electrode with a similar electrode under the skin over the upper lid. An electroretinogram was recorded every 20 sec through a differential DC amplifier. The signal was then fed into the memory of a computer of average transients (CAT). Fifteen such records were algebraically summed by the computer of average transients, and a readout of the sum was made to a pen recorder every fifth minute. Electroretinographic activity was elicited and recorded every 20 sec from 20 min before the increase of pressure until no electrical activity could be recorded from the eye.

Results

During exposure of the animals to hyperbaric oxygen, the electroretinogram showed marked changes which eventually resulted in cessation of recordable electrical events. The wave-persistence times for the *a* wave and *b* wave during hyperoxia are shown in Figure 1. The points on the graph represent the individual wave-persistence times for each group of five animals exposed to the five different pressures; the arithmetic means are joined in a curve. The wave-persistence times in hyperoxia were longer for the *a* wave than for the *b* wave at all pressures studied. The wave-persistence times were longer at lower pressures than at higher pressures. The range of data was greater at lower pressures of oxygen.

A linear relationship can be approximated for the ratio of *a*-wave persistence to *b*-wave persistence over the pressure range tested (Figure 2). The *a* wave persisted proportionately longer than the *b*

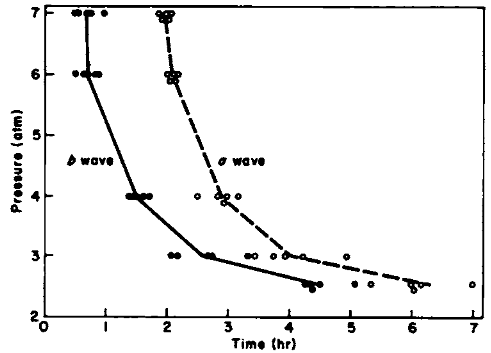

FIGURE 1. Persistence times of the *a* wave and *b* wave, measured from the onset of hyperoxia to the disappearance of the individual component. The averaged values are joined.

wave at higher pressures of oxygen. Figures 3 and 4 depict the amplitude changes of the *a* wave and the *b* wave, respectively, during the period of exposure to hyperoxia, expressed in relative units. Each point represents the normalized averaged amplitude of 75 electroretinograms (15 electroretinograms for each of five animals) as obtained from the computer of average transients. The averaged amplitude for each 5-min period was normalized (observed value/control value) to the same 5-min period of the control series in order to minimize the possible electroretinographic changes induced by further dark adaptation and anesthesia. (Data not included in the present report suggest that urethane reduced the amplitude of the

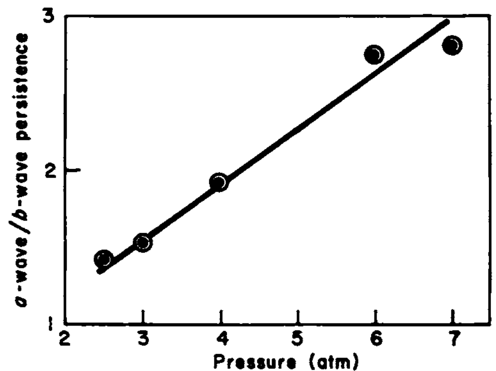

FIGURE 2. Ratio of times of *a*-wave persistence to *b*-wave persistence relative to oxygen pressure.

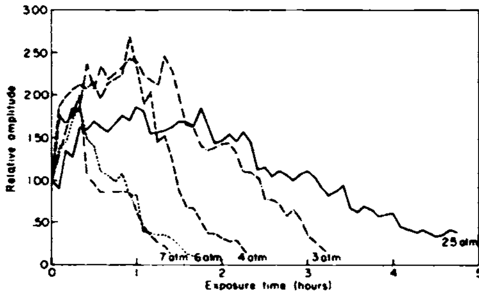

FIGURE 3. Alterations in *a*-wave amplitude during hyperoxia. Each individual amplitude is expressed relative to the last preexposure value; this value is normalized to the corresponding 5-min control period at normal atmospheric pressure.

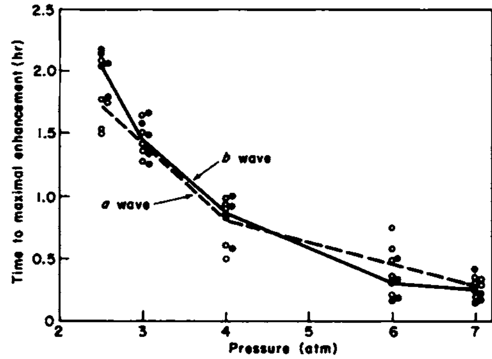

FIGURE 5. Time to maximal enhancement of *a* wave and *b* wave. The time is measured from the onset of hyperoxia to the point of maximal enhancement in each experiment. The averaged values are joined.

electroretinogram.) The *a*-wave amplitude was measured from the isoelectric line to the trough of the *a* wave, and the *b* wave was measured from the trough of the *a* wave to the peak of the *b* wave. In these data, a value greater than 1.0 represents a supranormal response, an enhancement. Such a response began at all oxygen pressures studied within 10 min after the onset of hyperoxia. The enhancement progressed to a maximum which was followed by a decline to the point of extinction. The duration of enhancement was pressure-dependent.

Figure 5 shows a plot of the time to maximal enhancement of the *a* wave and the *b* wave of every experiment at the five pressures studied. There was no difference in the time to maximal enhancement between the *a* wave and the *b* wave. The time to maximal enhancement was pressure-related.

Alterations in the electrocardiogram and respiratory pattern were observed, consisting of cardiac arrythmias, occasional ventricular fibrillation, decrease or increase in rate and depth of respiration, and respiratory arrest. Spontaneous remissions of the cardiac and respiratory manifestations were noted. These changes occurred late during hyperoxia and did not systematically affect the electroretinogram. All animals showed cardiac and respiratory activity at the termination of the experiment.

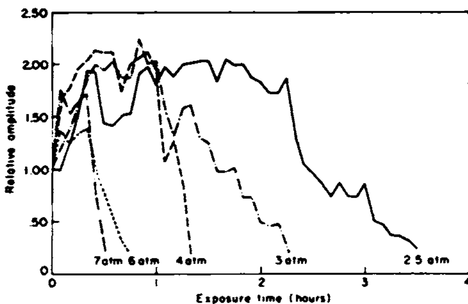

FIGURE 4. Alterations in *b*-wave amplitude during hyperoxia. Each individual amplitude is expressed relative to the last preexposure value; this value is normalized to the corresponding 5-min control period at atmospheric pressure.

DISCUSSION

Although the uncertainty of the anatomic origin of the electroretinographic activity imposes certain limitations in interpretation, the electroretinogram remains a useful tool for the evaluation of retinal functional integrity.

The wave persistence for the *a* wave and *b* wave was found to be an inverse function of the oxygen pressure, with the *a* wave persisting at all pressures longer than the *b* wave (Figure 1). Electro-

retinographic abnormalities are not expected, however, to result solely from the increase in barometric pressure as used in this study. Kuhnke and Klensch,[5] for example, reported no marked changes in the electroretinogram of the isolated frog retina up to pressures of 80 atm.

The *b*-wave persistence at 4 ata compares closely with that reported by Noell for this pressure.[6] Noell found partial reversibility of *b*-wave changes in animals removed from the hyperbaric oxygen environment at the onset of *b*-wave alterations; however, only a small portion of the loss was reversible. Reversibility was not noted in our study, because the animals were maintained in a hyperoxic environment until complete extinction of electroretinographic activity. The greater susceptibility of the *b* wave to homeostatic changes in the retina, which has been described in anoxia and with other noxious stimuli,[7] is supported by our findings.

The ratio of *a*-wave persistence to *b*-wave persistence doubled over the range tested (Figure 2). If the inhibition of the *a* wave and the *b* wave were of similar mechanism but different only in threshold of response, the ratio would remain constant. This observation suggests a difference in susceptibility of the mechanisms responsible for the two electroretinographic components.

The *a*-wave enhancement cannot be merely that known to follow *b*-wave suppression, because in the present experiments both components increased simultaneously. The enhancement occurred at all five pressures of hyperoxia studied and was progressive to the point of maximal enhancement, after which the amplitude of the wave declined to the point of complete extinction. Enhancement of the *b* wave has been reported in anoxia, in cyanide poisoning, during trichloroethylene administration, and after administration of small doses of pentobarbital.[7] Also, a selective *b*-wave enhancement with *a*-wave suppression has been reported to result from hyperventilation.[8] No reports of enhancement of electroretinographic activity during hyperoxia could be found in the literature. Henke[9] suggested increased wave activity to be a response of the retinal elements in a state of heightened irritability after a derangement of their metabolic status. Based on the present study, this hypothesis may be expanded by stating that after the enhancement the response becomes subnormal when the adverse environmental conditions persist unaltered. The electroretinographic enhancement may be the earliest detectable evidence of retinal injury during hyperoxia. It is a transient phenomenon requiring frequent recording for detection.

Three possible mechanisms should be mentioned when considering retinal oxygen toxicity: the oxidation of SH groups, the oxidations of certain lipids, and the respiratory metabolic pathway. The oxidation of SH groups includes a large number of enzymes leading to an interruption in homeostasis at numerous sites. Recent work with vitamin E inhibition of red-cell lysis at high oxygen pressures[10] has reemphasized the involvement of certain lipids, particularly unsaturated fatty acids. That the visual cell may lend itself to such a mechanism seems reasonable when one considers that approximately 35% of the outer segment of rods is composed of lipids. Toxic conditions affecting the respiratory metabolic pathway, such as anoxia and cyanide poisoning, produce transient electroretinographic enhancement, whereas glycolytic poisoning is not followed by such changes.[11] The enhancement has been suggested to represent a disturbance in cell respiration at the glutonic acid level, which in turn disrupts the potassium gradient at the nerve-cell level.[11] The enhancement may incriminate certain metabolic sites of involvement.

Our study demonstrated electroretinographic changes during hyperoxia, which were similar to changes after poisoning the respiratory metabolic pathway. The range of observations was smaller at higher pressures than at lower pressures, perhaps in-

dicating either a more complete single inhibitory mechanism or a greater number of inhibitory mechanisms at higher pressures. The retinal vascular constriction which occurs in hyperoxia may influence this latter mechanism.[12]

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DISCUSSION

DR. G. MARGOLIS (*Hanover, N. H.*): This paper is an interesting follow-up of yesterday's presentation, in which we discussed the difference between the susceptibility of the brain and the eye to oxygen toxicity. At that time, we wondered whether there was an inverse relationship between these two toxic manifestations. We speculated that the lesion in the eye was related to a vasospastic phenomenon, as you indicated in your introduction, and that the brain vasculature might be incapable of a strenuous constrictive reaction and hence would not then be injured by a vasospastic response, as was the eye. Further, when we used carbon dioxide, there appeared to be an inversion of this phenomenon. The eye was protected, but the brain was damaged; so the problem of the therapy of these toxic effects is more complex than upon first consideration.

DR. H. A. SALTZMAN (*Durham, N. C.*): Although the retina and the brain are close anatomically, substantial evidence indicates that their metabolism is quite different and that the response to hyperoxia might not be at all in parallel, although the directions would be the same.

DR. C. J. LAMBERTSEN, *Session Chairman (Philadelphia, Pa.)*: There is one other aspect to consider. Many investigators tend to think of the brain as seriously poisoned when the subject goes into convulsions. However, this increase in electrical activity does not necessarily indicate a harmful degree of toxicity for an organ like the brain, even though it results in considerable disruption of our overall performance. When we go into convulsions, the brain is just electrically uncoordinated. I wonder

how you would detect a similar slight degree of toxicity in the eye. I hope Dr. Bridges can provide a definition for the term "ophthalmological convulsion."

DR. BRIDGES: That is a new term for me. We did observe in these rabbits what we interpreted to be convulsions or evidence of CNS toxicity. During this period, however, we did not observe any demonstrable difference or change in the electroretinogram. Also, continuous recording of the heart rate and respiratory rate revealed wide variations in the former, from various types of arrhythmia to ventricular fibrillation with corresponding changes in respiratory rate and depth. Even during these episodes, no significant change was noted in the electroretinogram.

Protective Effect of THAM on the Retinal Vasculature at High pO_2

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A major unresolved clinical problem confronting ophthalmologists is retinal vascular occlusion. The therapy for this often irreversibly blinding disease has neither changed nor improved in many decades. Despite greater attention by the medical community to the rising incidence of vascular disease in our expanding and aging population, little progress has been made in preserving vision after retinal artery occlusion.

With the renaissance of hyperbaric oxygenation, a new and dynamic therapeutic tool for the treatment of vascular occlusion became available. Analysis of the pathologic events following obstruction of the central retinal artery reveals constricted retinal vessels and diminished retinal blood flow with obvious ischemia of the optic nerve and sensory elements of the retina. Ideally, effective therapeutic measures could correct this ischemia by (1) increasing delivery of oxygen to the anoxic retina, and (2) simultaneously increasing the retinal blood flow, as well as the uveal blood flow, which, although not compromised by retinal vessel obstruction, can provide an important secondary source of blood to the retina. That increased oxygen delivery would be beneficial to retinal function has been impressively demonstrated by Anderson and Saltzman[1] who showed in the experimental situation that hyperbaric oxygenation can be applied to the clinical problem of retinal artery occlusion. The results in the few well-studied cases reported to date,[2] however, have been discouraging. The explanation for these disappointing results may be inferred from the physiologic effects of high oxygen tension upon the eye, *i.e.*, significant decrease in both retinal[3] and uveal[4] blood flow. This decrease in blood flow occurs at a time when the retina, already deprived of blood, requires an increase or at least maintenance of its blood supply to sustain its unusually high metabolic demands.[5]

The vasoconstriction and decreased retinal and cerebral blood flow associated with hyperoxia can be considered a homeostatic mechanism which protects the central nervous system and retinal structures from the well-known toxic effects of oxygen.[6] However, to achieve the previously stated goals of "ideal" therapy in retinal artery occlusion, it seems obvious that homeostasis must be overcome, at least for a time sufficient to prevent retinal cell death. The present study was therefore

undertaken to explore methods of overcoming the retinal vasoconstriction which accompanies hyperbaric oxygenation.

A schema (Figure 1) modified from a report by Lambertsen[7] summarizes one suggested pathway for the development of retinal vasoconstriction during OHP therapy. In this proposed sequence, the decrease in retinal blood flow is much more dependent upon a secondary hypocapnia than upon the influence of oxygen. If this schema is correct, then by interrupting the events leading to hypocapnia by buffering accumulated excess carbon dioxide and hydrogen ion in the respiratory cells, homeostasis might be subverted sufficiently to ensure an adequate blood flow to the retina and uvea. It has been previously shown that THAM, a powerful intravascular and intracellular buffer,[8] can protect the central nervous system of rats against the toxic effects of OHP.[9] Similarly, it is suggested that THAM may protect the retinal vessels from the undesirable effects of hyperoxia during hyperbaric oxygen therapy for retinal artery occlusion.

The experimental protocols were designed to investigate (1) the effect of hypercapnia on the retinal vasculature in dogs and monkeys, (2) the *in vivo* effects of THAM on the retinal vasculature under conditions of hypercapnia, and (3) the influence of hypocapnia on the retinal vessels under conditions of hyperoxia. The experimental procedure was the same for both dogs and monkeys (schematically diagramed in Figure 2). The techniques for measuring the arterial blood gases and cerebrospinal fluid pressure and for photographing and measuring the retinal vessels have recently been described elsewhere[10] and need not be detailed here other than to stress that retinal vessel measurements were obtained under rigidly standardized conditions throughout all experiments.

A recent modification of the animal preparation has been the introduction of

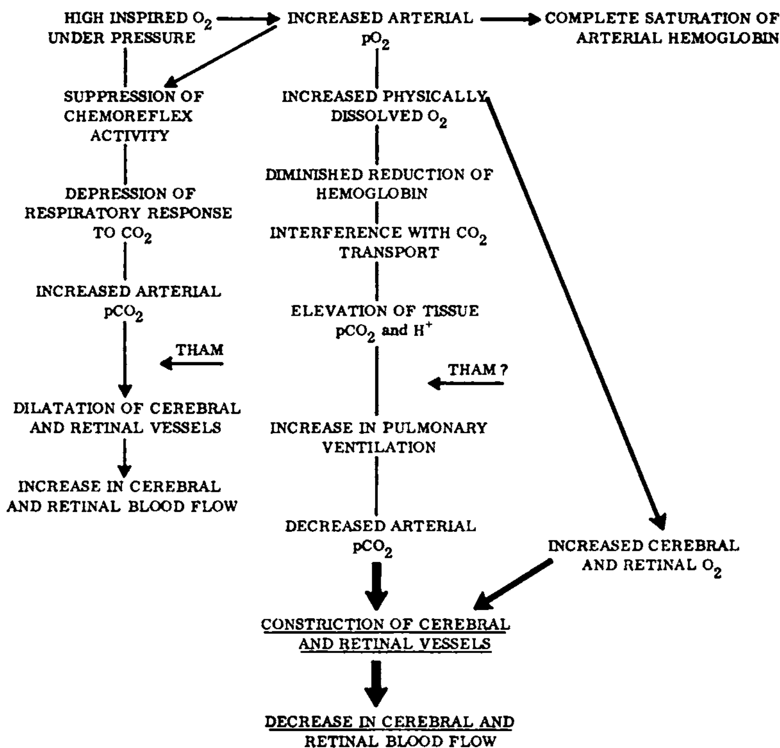

FIGURE 1. Proposed schema of physiologic effects of hyperoxia; modified from Lambertsen.[7]

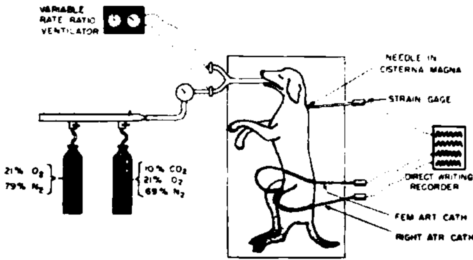

FIGURE 2. Schematic representation of animal preparation (dog and monkey).

a 25-gauge needle into the anterior chamber of the eye in order to obtain a continuous record of the intraocular pressure. Fundus photography was not interfered with when the needle was properly in place.

Figure 3 shows the results of the initial experiment designed to investigate the effect of hypercapnia on the retinal vessels. As might be expected, the profound increase in cerebral blood flow produced by hypercapnia[11] was reflected in the eye by a marked dilatation of the retinal vessels with hyperemia of the disc and choroid. These changes were further demonstrated to be independent of the hypertensive effect of increased carbon dioxide tension on cerebrospinal fluid pressure. As shown in Figure 3, when the cerebrospinal fluid pressure was artificially maintained at normal levels during hypercapnia, the retinal vessels dilated to the same degree as when the spinal fluid pressure was elevated.

The changes in intraocular pressure during hypercapnia were then studied to evaluate the second of the variables which might influence retinal vessel size, the first being the cerebrospinal fluid pressure. As shown in Figure 4, the intraocular pressure rose significantly during the breathing of CO_2. Since a rise in intraocular pressure is known to constrict retinal vessels, it must be concluded that the effect of CO_2

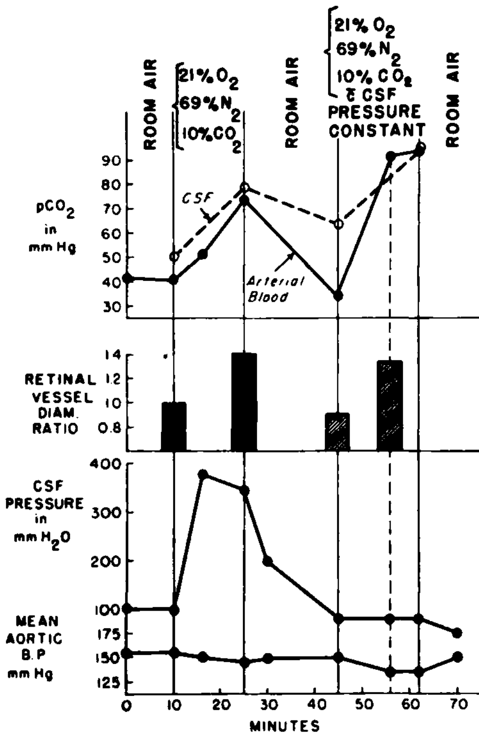

FIGURE 3. Changes of retinal vessel diameter after exposure to 10% CO_2 with and without intracranial hypertension. (Reproduced from Amer. J. Ophthal. 57:741, 1964.)

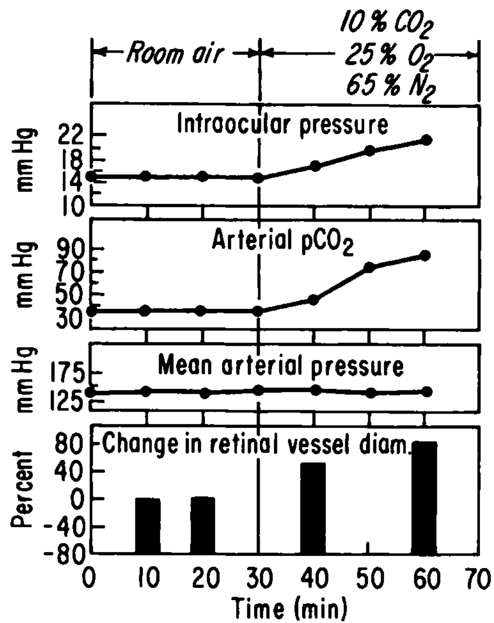

FIGURE 4. Changes of retinal vessel diameter and intraocular pressure after exposure to 10% CO_2.

on the retinal vasculature in a hypercapnic environment is not dependent upon intraocular pressure change. It appears, in fact, that the retinal vasodilatation proceeds despite the opposing action of increasing intraocular pressure.

Because the retinal vascular response to hypercapnia was not determined by the two measured variables, intraocular and cerebrospinal fluid pressure, it was concluded that increased CO_2 concentration and hydrogen ion concentration primarily control the observed retinal vasodilatation. The validity of this conclusion was tested by introducing, at this point in the hypercapnia experiments, an effective CO_2 and hydrogen ion buffer, the *in vivo* effectiveness of which could be evaluated by observing any changes in the retinal vascular response to CO_2 during administration of the buffer.

With proper titration of intravenous THAM, arterial pCO_2 and pH were restored to normal during the breathing of 10% CO_2, and this was accompanied by a prompt reversal of retinal vessel size to normal (Figure 5). A decrease in cerebrospinal fluid pressure and intraocular pressure occurred along with these retinal vessel changes.

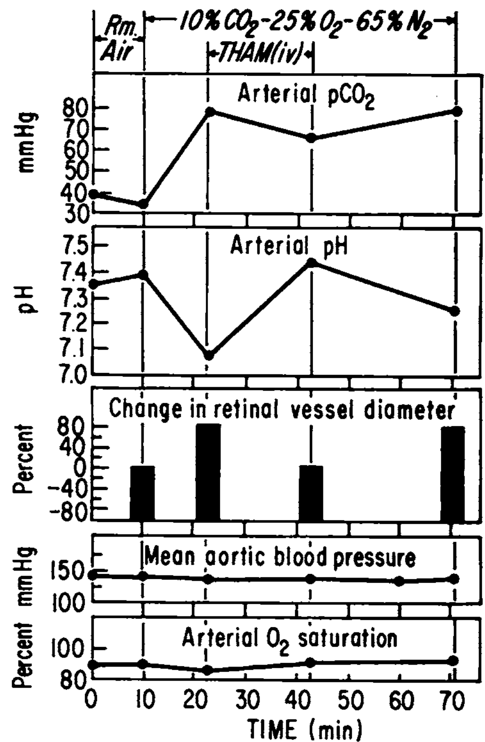

FIGURE 5. Effect of THAM on retinal vessel diameter during exposure to 10% CO_2.

The influence of decreased pCO_2 on the retinal vessels was next studied—for if, as suggested by the original schema (Figure 1), accumulation of CO_2 wastes in the respiratory center contributes to hyperventilation with hypocapnia, elimination of this physiologic mechanism by the use of THAM during hyperbaric oxygenation may prevent the observed retinal vasoconstriction which follows. The final experimental protocol, therefore, was designed to evaluate the effect of hypocapnia on retinal vessels at high pO_2.

Previous studies in humans by Saltzman and co-workers[3] failed to show further decrease in retinal vessel size following voluntary hyperventilation under conditions of hyperoxia at normal atmospheric pressure or during hyperbaric oxygenation. Our experimental findings in dogs and monkeys, however, revealed that during controlled ventilation, where the degree of hypocapnia could be established by arterial blood samplings (Figure 6), a definite and marked vasoconstriction was associated with low pCO_2. The fundus of the dogs and monkeys revealed a blanching of the disc and choroid and a decrease in retinal vessel diameter of 30–50%. Ventilation with 100% O_2 at a normal pCO_2 produced little change in the retinal vessels.

In these experiments, the combined effect of hypocapnia and hyperoxia was required to elicit significant retinal vasoconstriction. In Saltzman's work,[3] the human subjects voluntarily hyperventilated for 1 min. Since the actual arterial pCO_2 values were not recorded, it is possible that significant hypocapnic levels were not obtained; therefore, it would be difficult to conclude that hypocapnia does not influence retinal vessel size. Turner and coworkers, in fact, have shown that by ex-

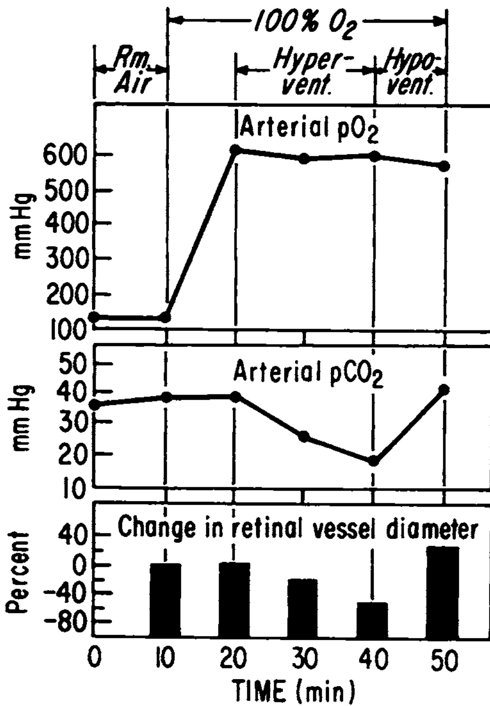

FIGURE 6. Effect of low pCO_2 on retinal vessel diameter during exposure to 100% O_2.

perimentally maintaining the alveolar pCO_2 in humans at a normal value, 80% O_2 produced no decrease in cerebral circulation compared to the control levels obtained during breathing of 21% O_2.[12] These authors concluded that the cerebral vasoconstriction normally associated with oxygen inhalation is predominantly an indirect effect, secondary to arterial hypocapnia.

CONCLUSIONS

The experimental data reported herein demonstrate the responsiveness of the retinal vessels to hypercapnia and to hypocapnia. The response of the retinal circulation to carbon dioxide has been shown to be more profound than the response to oxygen. If animals are given 90% O_2 and 10% CO_2, the vasodilator effect of carbon dioxide predominates over the vasoconstrictive effect of hyperoxia, resulting in a final increase in blood flow.[4] Similarly, in these experiments, addition of the effects of hypocapnia to those of hyperoxia results in a more profound retinal vasoconstriction than that produced by hyperoxia alone. These experiments also show that an effective intravascular and intracellular buffer, THAM, can predictably modify the effects of CO_2 on the retinal vasculature *in vivo*.

Whether THAM can interfere with the delicate homeostatic mechanism postulated, to overcome the retinal vasoconstriction induced by hypocapnia in the clinical application of OHP, is still untested. Also, we must recognize that such interference with homeostasis may permit a marked increase in tissue oxygen levels and thereby potentiate the toxic effects of hyperoxia on the central nervous system. However, with due recognition of the intrinsic hazards of interfering with normally protective mechanisms, techniques may be developed to tread the fine line between physiologic and pathologic effects in order to achieve the desirable end results of increased, not decreased, blood flow and increased oxygen delivery in the use of hyperbaric oxygenation for retinal artery occlusion.

ACKNOWLEDGMENT

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DISCUSSION

DR. H. A. SALTZMAN (*Durham, N. C.*): In this excellent presentation, an important assumption has been that hypocapnea in arterial blood plays a role in producing retinal vasoconstriction. In our studies showing retinal vasoconstriction in normal man during exposure to hyperbaric oxygen, arterial blood samples were obtained from some subjects. These samples did not, however, show a significant change in arterial pCO_2. In many studies of normal subjects in our particular chamber environment, the arterial pCO_2 has not changed significantly during exposure to hyperbaric oxygen. Therefore, unless the subjects from whom we did not obtain arterial blood differed from their colleagues, we would require an explanation for retinal vasoconstriction other than hypocapnea. One other bit of evidence which supports this, and which will be touched on in the next presentation, is the lesser effect of carbon dioxide than of oxygen in producing changes in vessel size or in retinal blood flow, when computed by a dye-dilution technique.

DR. SPALTER: In reference to the first part of your statement, I would be interested to know what the remainder of the subjects did show in terms of arterial pCO_2. We, of course, were dealing with dogs under controlled conditions. I do recall some work where you had the patients hyperventilate voluntarily, and you found no further constriction of the retinal vessels. Is that correct?

DR. SALTZMAN: We were able to measure significant small changes of about 1 or 2%.

DR. SPALTER: This to me would be more evidence against my points which, of course, we are willing to keep open to discussion.

DR. G. MARGOLIS (*Hanover, N. H.*): I was intrigued by this paper, and particularly by your opening slides which showed what you wanted for an ideal therapeutic approach and how the retinal vasoconstriction defeated you. It brings to mind the concluding statement of our own presentation, that the

therapy or the protection of the eye in hyperoxia might require the use of carbon dioxide, even though it is so poisonous. Therefore, we would have to find some other substance which would directly inhibit oxygen toxicity. At the same time, we were protecting the eye with carbon dioxide.

DR. SPALTER: The retinal vessels are easy to observe, and the constriction is obvious. However, we have a tremendous supply of blood derived from the uveal tract, and it has been shown that hyperbaric oxygenation or high oxygen pressures diminish that volume of blood, which perhaps is 70 or 80 times that carried by the retinal vessels. Equally important is the attempt to open that pathway to provide more blood during these episodes, but if we cannot get around the retinal artery occlusion because of the size of the plug or the intensity of the obstruction, at least the uveal tract may be a secondary lifeline which should be employed. Whether the methods we have outlined today will work eventually in humans, we do not know. We are treading a thin line between pathologic effects and homeostatic mechanisms.

DR. C. J. LAMBERTSEN, *Session Chairman (Philadelphia, Pa.)*: It certainly seems that work of this sort should continue. The question remains whether the retinal vessels do, in fact, behave differently from those in the brain. Certainly, if the vessels of the infant are so severely affected by high oxygen pressures, we should not lightly put aside the fact that oxygen at high pressure might also have some kind of an effect upon the adult vessels. The experiments that Dr. Saltzman described need more complete documentation by the kind of studies in which one value, such as pCO_2, is held constant while the others change. I want to ask whether THAM, which can apparently penetrate cells to some degree, might also affect the smooth-muscle cells. Could it not change the acid–base environment of the inside of the retinal vessel cells and thereby alter the reactivity to carbon dioxide?

DR. NAHAS: One of the most interesting effects of using this compound under conditions of hypercapnea is that within 10 minutes the retinal vessels return to normal size and pH under conditions of elevated pCO_2 (60 to 80 mm Hg). Most of the volume of the titrating activity which occurs in this period of time is not much larger than that of the extracellular fluid.

Retinal Vascular and Functional Response to Hyperbaric Oxygenation

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Studies of the retinal vascular and functional response to hyperbaric oxygenation have done much to elucidate both the limitations and the potentialities of OHP therapy. Inspired by the work of Carlisle and Lanphier[1] reported at the First International Congress on the Clinical Application of Hyperbaric Oxygen,[2] we have undertaken a program of investigation in this area, and our initial results were reported at the Second Congress.[3] In the last year we have expanded our observations in several areas. These findings are summarized below.

Studies on Hyperbaric Blackout

If the circulation to the eye is occluded after a period of OHP, ischemic blackout of vision is delayed, with the delay in blackout proportional to the arterial oxygen tension existing prior to occlusion. This relationship was first noted by Lambert and Bjurstedt in 1952,[4] and further observations were later reported by Lanphier.[2] Subsequent work has confirmed their findings.[1,5] The absolute values for persistence of vision after circulatory occlusion depend upon the subject, the period of oxygenation, the time since the last ischemic blackout, the type of endpoint used (greyout or blackout), and the illumination and character of the object of regard.

A study of the relationship between the duration of hyperbaric oxygenation and the delay in ischemic visual blackout has revealed that at atmospheric pressures below 40 psig, oxygenation for more than 60 sec adds little to the persistence of vision after circulatory occlusion. At 40 psig, longer periods of oxygenation do seem to have some effect, but the increase after 120 sec is small compared with that obtained initially[6] (Figure 1). If conditions are standardized, the prolongation of vision after ocular circulatory occlusion can be expressed in terms of the prior increase in arterial or tissue oxygen concentration. Conversely, if blackout of vision is the result of utilization of available oxygen, the linear change in blackout time with tissue oxygen concentration is a measure of this utilization.[1,5]

We have begun studies in which the retinal responses of patients with ocular or systemic disease are compared with those of our normal control group (Table 1). A patient with juvenile glaucoma without optic atrophy or field loss and a patient with diabetic gangrene without retino-

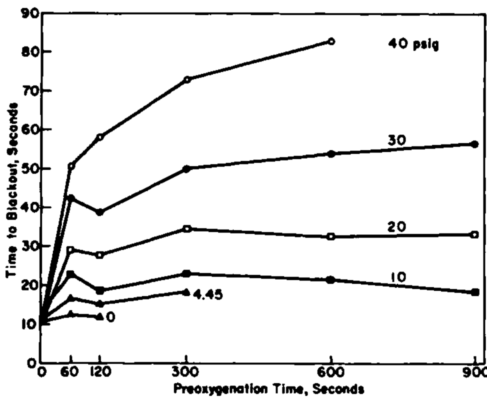

FIGURE 1. Relationship of persistence of vision after ocular circulatory occlusion to the duration of hyperbaric oxygenation prior to occlusion.

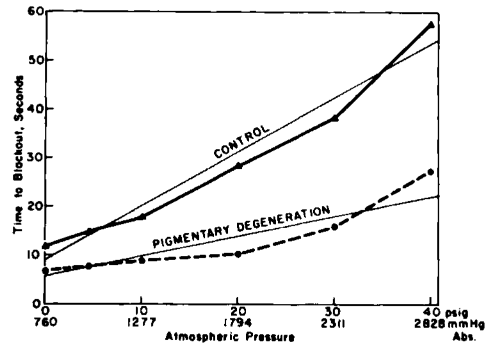

FIGURE 2. Comparison of blackout times of control group and patient with retinal pigmentary degeneration showing differences in both slope and times to blackout.

pathy were found to have essentially normal values. A patient with advanced pigmentary degeneration of the retina, however, was found to have markedly abnormal values. This patient's time to blackout was shortened at all pressure levels tested and the slope of the plotted line was reduced, compared with that of normal subjects in the control group (Figure 2). Statistical comparison of the slopes of the regression lines revealed that the difference was highly significant. Such slope differences cannot be explained merely on the basis of a uniformly smaller oxygen "reservoir," since changes of this type would result only in a shift of the lines toward shorter blackout times without slope change. The observed slope change must then either result from increased oxygen utilization in the region of the most oxygen-sensitive cells in the visual chain, or from some factor producing progressive reduction in the oxygen "reservoir" (or oxygen solubility volume)

TABLE 1. Determination of Retinal Oxygen Utilization in Normal and Diseased Eyes

Subjects	Slope and standard error of regression lines (sec/cm Hg abs)	Arterial pO_2 increase prolonging vision 1 sec (mm Hg)	O_2 utilization (ml/100 ml wet tissue/min)	Q_{O_2} (ml/gm dry wt/hr)
Controls				
1	0.150 ± 0.011	55	9.9	23.8
2	0.218 ± 0.014	38	6.8	16.3
3	0.151 ± 0.008	54	9.8	23.5
4	0.258 ± 0.015	32	7.3	17.5
5	0.277 ± 0.022	30	5.5	13.2
6	0.257 ± 0.013	32	5.8	13.9
7	0.191 ± 0.014	42	7.7	18.5
Mean control value	0.215	39	7.0	16.8
Patients				
Glaucoma pt.	0.273 ± 0.015	30	5.5	13.2
Diabetic pt.	0.222 ± 0.013	37	6.7	16.1
Pigmentary degener. pt.	0.072 ± 0.008	118	21.1	50.6

with increasing arterial oxygen tensions and atmospheric pressure. Such a factor might be the progressive increase in retinal vasoconstriction with higher arterial oxygen tensions. Since such changes in vessel caliber have been observed in the normal group,[7] a difference in magnitude must be postulated to explain the slope change noted in the patient with pigmentary degeneration. Whatever the mechanism, the retinal response to hyperbaric oxygen in this patient was significantly different from that observed in the control group.

If, instead of pure oxygen, a mixture of oxygen and carbon dioxide which produces an alveolar pCO_2 of 40 mm Hg is inhaled, the time to blackout is prolonged at all pressure levels tested. Photographic studies of the retinal vessels under these conditions have revealed that the marked vasoconstriction which occurs with the inhalation of pure oxygen is prevented or markedly reduced when carbon dioxide in low concentration is added to the inspired gas. By preventing or ameliorating hyperoxic vasoconstriction, the carbon dioxide may increase the oxygen "reservoir," which would then result in greater persistence of vision.

OHP Therapy of Retinal Diseases

A 19-year-old woman who, 10 hours previously, had developed an occlusion of the upper temporal branch of the right central retinal artery was treated with 100% oxygen at 30 psig for 20 min. The brachial arterial pO_2 under these conditions was 1770 mm Hg. No significant change in visual field, visual acuity, or cloudy swelling of the ischemic area was noted during or after OHP. This lack of significant clinical effect was consistent with our experience in three other cases with retinal vascular disease previously reported.[8] As in these earlier cases, the presence of an intact choroidal circulation within 0.15 mm of the anoxic retinal cells did not reverse the functional or observed anatomic abnormality.

A 44-year-old man with night blindness, markedly constricted visual fields, and retinal pigmentary degeneration was treated with 100% oxygen at 30 psig for 30 min. Visual fields plotted on a Goldmann projection perimeter during OHP did not differ from those obtained under normal conditions. No subjective or objective change in acuity occurred. The patient's ischemic blackout responses were markedly abnormal (Table 1). The cause of the retinal degeneration in this case was obscure. Although the clinical picture was quite typical of the hereditary form of retinitis pigmentosa, serologic tests for syphilis were positive and syphilitic retinitis could not be excluded.

Animal Studies

The persistence of vision after ocular circulatory occlusion may perhaps best be explained by the concept of an oxygen "reservoir" (or oxygen solubility volume) which requires a minute or so to fill and which is enlarged by small amounts of carbon dioxide in the breathing mixture. The vitreous body of the eye may act as such a reservoir. The amount of oxygen dissolved in the vitreous humor would be increased if vasoconstriction were prevented, for under these conditions the arteriovenous oxygen difference would be reduced. To test this hypothesis, polarographic oxygen needle electrodes were placed in the vitreous cavity of anesthetized mongrel dogs. The dogs were artificially ventilated through endotracheal tubes. Our observations revealed that the vitreous pO_2 near the retina did increase with OHP but the changes in oxygen tension when the dogs were switched between oxygen- and air-breathing were slow and required minutes rather than seconds. Our studies are incomplete, as the problem of localizing and stabilizing the electrode tip in relation to the retina in the otherwise intact eye has not yet been solved.

SUMMARY

Hyperbaric oxygenation prolongs vision after ocular circulatory occlusion despite marked constriction of the retinal blood vessels. The addition of carbon dioxide in small amounts to the inspired oxygen will prolong vision to an even greater extent after ocular circulatory occlusion; in this instance, however, retinal vasoconstriction is minimized. This enhanced visual persistence may reflect an increased accumulation of oxygen in ocular tissues as is suggested by marked rises in the vitreous oxygen tension of dogs exposed to several atmospheres of oxygen pressure. Once ischemic cloudy swelling of the retina has occurred, however, a marked increase in choroidal oxygen tension will not quickly restore function even when the retina can be shown to be viable. In one clinical case, a patient with pigmentary retinal degeneration did not demonstrate any significant increase in visual field or acuity under hyperoxic conditions. This patient did have an abnormal retinal oxygen utilization rate as determined by the persistence technique. Hyperoxic increases in arterial oxygen tension have ocular effects but such increases do not quickly reverse the effects of ischemic damage.

ACKNOWLEDGMENTS

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DISCUSSION

DR. G. MARGOLIS (*Hanover, N. H.*): I would like to add a morphological aspect to the problem that Dr. Lambertsen raised, that is, whether the effects in vessels are due to a change in the tissue or to a circulating agent that alters blood vessel tonicity. Romanul and Bannister (J. Cell. Biol. 16:78, 1962) have demonstrated, by histochemical

studies, an accumulation of alkaline phosphatase in small arteries of the most reactive order. This occurs not in the muscle, but in the endothelium. They suggest that the presence of alkaline phosphatase at that site gives vessels an active transport mechanism which enables them to sample what is flowing through the vessel. This may be a morphological or histochemical explanation of the phenomenon that we have seen.

CAPT. S. MILES (*Alverstoke, England*): I would like to make an observation which may support some of the findings which have been presented this morning from rather a different point of view. Some years ago, in a study of 126 men, we were able to lower the syncope threshold by the use of a combination of hyperventilation, postural change, and increased pulmonary pressure. We found that the syncope rate during oxygen-breathing was three times that during air-breathing. However, with addition of carbon dioxide to the oxygen, no difference was observed.

DR. C. J. LAMBERTSEN, *Session Chairman (Philadelphia, Pa.)*: Should it really surprise us if treatment with high oxygen pressure fails in the case of a patient with an occlusion of a vessel, when the primary problem is a lack of blood flow? Lack of glucose flow, and perhaps a lack of a number of other nutrients besides oxygen, have to be considered. In fact, the failure of glucose flow should be of equal importance in this situation.

DR. ANDERSON: As Dr. Guyton pointed out, oxygen is possibly the most flow-limited metabolite. We are interested not only in function, which would be lost very rapidly as a result of oxygen lack and more slowly as a result of glucose lack, but also in the functional changes in the border zone between the ischemic area and the perfused area. Several of our patients had branch retinal artery occlusions similar to the one we presented this morning, and we did visual-field studies in an attempt to see if we could expand the functioning area by a small amount. That is, we looked for an effect on the cells that were still alive but nonfunctioning. Perhaps these cells were hypoxic but had no lower glucose stores. The data indicated a slight enlargement of the visual fields, but it was so small and so clinically insignificant that glucose may well be a limiting factor in this situation.

Alveolar–Arterial Gradient for Oxygen at 2 ata

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The alveolar–arterial (A–a) gradient for oxygen has not been clearly defined for normal man breathing 100% oxygen at pressures above 1 ata. Determination of the magnitude of the A–a pO_2 under these circumstances is important, first as a measure of the efficiency of pulmonary function at raised pressure, and second as an early indicator of pulmonary oxygen toxicity. Although available evidence is inconclusive, it tends to suggest that the A–a pO_2 for normal man at increased pressure may be of the order of several hundred millimeters of mercury.[1,2]

It seemed to us necessary, therefore, to determine the A–a pO_2 during oxygen-breathing in the range of pressures used clinically. The following is a report of the results of our experiments at 2 ata. As a preliminary, it was necessary to study the performance of the oxygen electrode under conditions of raised environmental pressure and to establish the limits of accuracy of the electrode when measuring very high oxygen pressures and tensions.

Materials and Methods

The oxygen electrode used was the unstirred-system version of the Clark cell (Radiometer E-5044) with a 20-μ polypropylene membrane. The electrode and all associated equipment were situated in the chamber and the electrode was calibrated at 2 ata with 100% and 70% humidified oxygen; calibrations with tonometered water and blood were also performed. Readings at 2 ata were brought onto the normal scale by halving the amplification of the electrode current. All gas and water readings were made exactly 3 min after introduction of a sample, since it was found that the electrode signal exhibited a plateau between 90 sec and 4 min with gas and water samples.

The investigation consisted of two separate groups of experiments, designated as Studies I and II. Study I consisted of measurements in five subjects who received oxygen by face-mask; in Study II, six volunteers breathed oxygen through a mouthpiece. The systems used for tonometering blood were also different in the two studies.

All subjects had given their prior consent to this investigation. They were free from symptoms, signs, or history of respiratory disease, and their average age was 36 years (range 19–56 years). One of the investigators participated, and the remainder were patients from the general surgical wards, although none had undergone recent surgery or anesthesia.

Study I

In Study 1, a swirling-flask type of tonometer was used. Fresh blood (3 ml) was introduced into the tonometering flask and equilibrated with 100% or 70% oxygen flowing at 0.5 liter/min for 30 min (preliminary work had shown that equilibrium was achieved after 10–20 min of tonometering). The blood was then transferred from the tonometer to the electrode by manipulation of a nylon catheter into the pool of blood in the flask; the other end of the catheter was attached to the inlet of the oxygen electrode while its outlet was attached to a low-pressure suction pump. In this way, the calibrating gas occupied the catheter and the electrode right up to the moment of arrival of the blood. The reading of the electrode was noted every 30 sec after introduction of the blood for 3 min, and every minute for an additional 2–4 min. A plot of these readings made it possible to extrapolate back to zero time and obtain the oxygen tension which existed in the blood at the moment of introduction into the electrode. Several blood samples were tonometered on each day and a mean value for the day calculated.

The water used for calibration was sampled, in the same way, from a bubble-type humidifier.

In this first study, five volunteers (one studied twice) breathed oxygen from a mask known to deliver 75–95% oxygen.[3] A catheter was inserted through the mask into the nasopharynx to allow sampling of "alveolar" gas. After a variable period of oxygen-breathing (mean time 107 min, range 40–205 min), simultaneous sampling of alveolar gas and arterial blood was carried out. The alveolar sample was obtained by drawing gas through the nasopharyngeal catheter directly into the oxygen electrode while the subject slowly exhaled from the normal end expiratory position. The alveolar gas oxygen partial pressure was read 3 min after introduction of the gas into the electrode. The arterial blood which had been withdrawn by femoral puncture simultaneously with the collection of alveolar gas was then introduced and read at the same times as the tonometered blood. From the measured rate of fall of oxygen tension it was possible to determine what the blood oxygen tension had been at the moment of sampling.

Study II

The blood tonometer previously described by Torres[4] was used in this study. Fresh blood (3 ml) was introduced into a 50-ml syringe, and 15–20 ml of 100% oxygen was added. The syringe was then placed in the water bath and rotated for 30 min (equilibrium between blood and gas was reached with this system in 10–15 min with the syringe rotating at 15 rpm). At the end of this time, a nylon catheter was attached to the syringe and some of the contained gas was injected into the oxygen electrode without removing the syringe from the water bath; the oxygen partial pressure of this gas was measured after 3 min. The blood in the syringe was then injected through the catheter into the electrode, and its oxygen partial tension was read at 3 min. No attempt was made to calculate back to the oxygen tension existing at the moment of introduction of the blood into the electrode. As in the first study, several measurements of the blood:gas factor for the electrode were made on each day and a mean value calculated.

Six volunteers were studied breathing oxygen at 2 ata from a mouthpiece with the nose clipped. The mean duration of oxygen-breathing was 43 min and the range 35–69 min. An arterial blood sample was obtained from the femoral artery and was introduced immediately into the oxygen electrode. The oxygen tension of this sample was read 3 min after introduction, and no correction for oxygen consumption or leakage was made. (We assumed that the rate of oxygen tension drop was the same both in tonometered fresh blood and in the sampled arterial

blood; the possible error involved in this assumption was certainly less than that introduced in Study I when an attempt was made to correct the blood readings for oxygen consumption and leakage during the storage periods which were necessary to allow the prior readings of alveolar gas pO_2.) The arterial pCO_2 was measured with a gas-calibrated Severinghaus electrode.

In Study II, no sampling of alveolar gas was attempted; the alveolar oxygen partial pressure was assumed to equal the ambient pressure less the measured arterial pCO_2 and the water vapor pressure. As a check on the efficiency of the breathing system, a sample of mixed expired gas, collected at the time of arterial puncture, was always measured for pO_2. In all six volunteers included in this report, the mixed expired pO_2 was equal to that of 100% oxygen less the expired CO_2 and water vapor pressures within the limits of accuracy of the oxygen electrode. (In the seventh subject, who has been excluded, this criterion was not fulfilled, although careful inspection failed to reveal any fault in his breathing circuit.)

Results

An assessment of the reproducibility of the oxygen measurements at 2 ata showed that the coefficient of variation between duplicate gas readings was ±2.0% in Study I and ±1.4% in Study II. In Study I, the electrode response to tonometered water reached 93.8% (SD ±2.2%) of the equivalent gas reading. Blood gave a reading which was 92.2% (SD ±2.2%) of the gas reading. The difference between the readings on blood and gas is, of course, statistically significant, but so is the difference between water and blood ($0.01>P>0.005$). It is clear from the magnitude of the standard deviation that a wide range of blood:gas factors was obtained for the same electrode. On any one day, repeated measurements of the blood:gas factor agreed closely, but there were wide differences between different days. Consequently, the factor used to correct the arterial blood reading for each subject was the mean factor for the day of the experiment. In Study II, where a totally different tonometering technique was used, a similar mean blood:gas factor was obtained (93.1% ±2.5%).

The measured alveolar oxygen pressures for the subjects in Study I (Table 1) were within the range to be expected from the mask system used.[3] "Uncorrected" arterial oxygen tensions were the values for pO_2 read directly with the scale calibrated for gas. If these arterial readings were correct (*i.e.*, if, in fact, no difference in electrode response existed between gas and blood) then the A–a pO_2 would be very large indeed. However, application of the electrode blood:gas factor for the day of the experiment resulted in a much smaller A–a pO_2 difference (last two columns, Table 1). Comparison with the values obtained in the second study (Table 2) make it apparent that despite the

TABLE 1. A–a pO_2 (mm Hg) in Five Subjects Breathing Oxygen by Mask at 2 ata [a]

Subject no.	Alveolar pO_2	Uncorrected values: Arterial pO_2	Uncorrected values: A–a pO_2	Corrected values: Arterial pO_2	Corrected values: A–a pO_2
1	1360	1234	126	1332	28
2	1180	1002	178	1154	26
3	1264	1118	176	1288	−24
2	1150	1038	112	1102	48
4	1366	1202	164	1326	40
5	1296	1114	182	1230	66

[a] One subject was studied twice.

TABLE 2. A–a pO_2 (mm Hg) in Six Subjects Breathing Oxygen by Mouthpiece at 2 ata

		Uncorrected values		Corrected values	
Subject no.	Alveolar pO_2	Arterial pO_2	A–a pO_2	Arterial pO_2	A–a pO_2
6	1418	1238	180	1356	62
7	1426	1282	144	1428	−2
8	1428	1270	158	1414	14
9	1428	1306	122	1366	62
10	1448	1320	128	1440	8
11	1446	1308	138	1394	52

change in oxygen delivery system, in the method of calculation, and in the tonometering system, the results for A–a pO_2 were very similar in the two groups. The mean values are shown in Table 3.

DISCUSSION

Performance of the Electrode

Since our coefficient of variation of duplicate readings was similar to that reported by others at normal pressure,[5] it is clear that the oxygen electrode functions normally at increased pressure. Unfortunately, a coefficient of variation of 2% at 2 ata indicates a standard deviation of 30 mm Hg; thus, at higher pressures, the oxygen electrode may not be capable of the accuracy necessary to allow detection of small differences in oxygen pressures or tensions.

There is disagreement in the literature whether unstirred-system oxygen electrodes with small platinum cathodes respond differently to blood as compared to gas. Torres[4] has maintained that there is no difference in response. Polgar and Forster,[5] however, using an unstirred system, reported results very similar to those presented here. Their values were gas = 100% ± 2.08%, water = 96.16% ± 1.55%, and blood = 94.64% ± 2.24%. These authors also measured the difference between response to water and to blood, but in their study the difference was not statistically significant.

TABLE 3. Mean A–a pO_2 at 2 ata

Study I (face-mask group)	31 mm Hg (range −24 to 66)
Study II (mouthpiece group)	33 mm Hg (range −2 to 62)

The basis for this difference in electrode responses to blood and gas is unknown, although it has widely been ascribed to limitations in oxygen diffusion in the more viscous fluids. This would also explain the difference noted between the blood and water responses, which occurred despite correction of the blood value for cellular oxygen consumption.

Certainly the magnitude of the blood: gas factor is susceptible to many influences, well known among these being the cathode area of the electrode and the material used for the membrane. Perhaps with some systems no detectable difference in response occurs. Our results make it apparent, however, that each group of workers must determine the behavior of the oxygen electrode system employed, and for accurate work at high oxygen tensions they must measure the blood:gas factor daily. The fact that day-to-day variations occur has been noted previously,[6] and it indicates that variables other than blood viscosity, cathode area, and membrane material are involved; one of these may be variations in the width of the cathode–membrane gap.

Tonometering Techniques

Both tonometering systems proved satisfactory and gave similar results. At the slow speeds of rotation used, equilibrium was attained rather slowly, taking up to 20 min, but it was achieved without significant trauma to the formed elements of the blood (judged by packed-cell volume). Aspiration of blood directly from the tonometering flask to the electrode (as done in Study I) proved to be a most satisfactory method, but doubt was expressed about the possible effect of subjecting tonometered blood to a negative pressure. This criticism was probably unfounded since similar values for the blood:gas factor were obtained in Study II where a positive-pressure system of transferring blood was used.

Design of the Studies

The sampling of alveolar gas directly from the nasopharynx to the electrode during deep expiration gave highly consistent results for alveolar oxygen partial pressure in repeated measurements prior to arterial sampling. However, on several occasions, it was found that there was a sudden change in the value obtained for alveolar pO_2 at the moment of arterial sampling, probably due to slight changes in mask fit and in alveolar ventilation at this time. Quantification of the A–a pO_2 in this way was therefore based on a single measurement of alveolar pO_2 at a time when the value was changing. In the second study, these difficulties were avoided by using a calculated value for alveolar pO_2. This calculation involved the following assumptions: (1) that there was no residual nitrogen in the alveoli, (2) that 100% oxygen was being inspired, and (3) that the A–a pCO_2 gradient was small. Assumptions (1) and (2) were justified by measurements made of the pO_2 of mixed expired gas which reached the theoretically predicted values. Regarding the third assumption, it is difficult to conceive of an A–a pCO_2 which would be significant in comparison with the determined limits of accuracy of the oxygen electrode at this pressure.

Alveolar–Arterial Difference for Oxygen

The measured values for A–a pO_2 at 2 ata of oxygen fell within the range of values found by many other workers for normal man breathing oxygen at 1 ata.[7] The only other report of the A–a pO_2 at raised pressure is that of Lenfant,[8] who studied eight naval divers. These divers differed from the subjects in our investigation in that they had deviations from normal respiratory function, resulting from their repeated exposures to changes in environmental pressure. Among these deviations was an increase in the number of unventilated alveoli. It is impossible, therefore, to directly compare the results of the two studies.

The occurrence of an A–a pO_2 after 40 min of 100% oxygen-breathing at 2 ata of oxygen must be almost entirely ascribed to venous admixture, since nitrogen washout from even unventilated alveoli, whether open or closed, must have been almost complete. Alveoli with very low $\dot{V}_A/\dot{Q}$ ratios will still, however, have a slight influence on the A–a pO_2 (because of elevations in alveolar pCO_2) although such alveoli would be expected to be infrequent in our group of normal subjects.

Since cardiac output is thought to be lower when an individual breathes hyperbaric oxygen, as compared with oxygen at normal pressure,[9] the same percentage of venous admixture should produce a larger A–a pO_2 at pressure. Our finding of a "normal" A–a pO_2 may indicate that the percentage of venous admixture was actually smaller at pressure. Furthermore, it is likely that the influences of the various components of venous admixture were altered at 2 ata. One of these components is the drainage of bronchial venous blood into radicles of the pulmonary veins. It has been suggested that oxygen, even at normal pressure, reduces the influence

of this factor by oxygenating the blood in these thin-walled venules.[10] Hyperbaric oxygen would be more effective in accomplishing this and so would tend further to suppress the influence of this factor in venous admixture.

The component of venous admixture, however, about which clinicians employing hyperbaric oxygen therapy are principally concerned, is that which may result from the alveolar consolidation of pulmonary oxygen toxicity. The results reported here appear to indicate that oxygen at 2 ata does not produce a significant increase in the pulmonary component of venous admixture when administered to subjects with normal lungs for a period of about 1 hour. The study has therefore failed to reveal any evidence of pulmonary oxygen toxicity under these specific conditions.

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DISCUSSION

DR. P. CALDWELL (*New York, N. Y.*): You mentioned the large factor which you used to correct for metabolism in the sample of oxygen between the time that the sample was taken and the time of measurement. Can you tell us what this amount was?

DR. MCDOWALL: It is important to avoid confusion here. The large factor which was applied to the results was the factor relating to the difference in electrode signal to gas and to blood at the same oxygen partial pressure. This factor, at least in Study I, had no component due to oxygen metabolism by the blood while in the electrode. In Study I, there was, in addition, a correction made for the measured rate of oxygen consumption by the blood while in the electrode during the period required for the completion of the oxygen electrode response. This was done by measuring the blood oxygen tension at minute intervals from the third to the seventh minute and extrapolating the line of oxygen

tensions so obtained back to zero time, *i.e.*, to the time of blood sampling. The mean rate of fall in oxygen tension of blood in the electrode in Study I was 9 mm per minute, which is two to three times the value at normal pressure. We have not studied this aspect in sufficient depth to comment on this discrepancy, other than to point out that this high rate of fall was not due to faster leakage of oxygen from the electrode when at pressure, since a good stable plateau was obtained with water equilibrated with 100% oxygen at 2 atmospheres.

In Study II, the tonometered blood was read 3 minutes after introduction into the electrode, and the blood from the subject was read 3 minutes after sampling. The effect of oxygen consumption by the blood on the results was therefore assumed to have cancelled out.

DR. A. R. BEHNKE, *Session Chairman* (*San Francisco, Calif.*): Would you say then, that blood flow through the lung with oxygen-induced atelectasis had ceased? How long had the subjects been breathing oxygen?

DR. MCDOWALL: The patients had been breathing oxygen for a mean of 107 minutes in the mask group and 43 minutes in the mouthpiece group. These studies showed that there was no increase in the A–a gradient-G oxygen, but as you pointed out, if alveolar atelectasis was present and associated with redistribution of blood flow, this would not have been detected.

DR. BEHNKE: How did your results compare with those obtained by the Duke group?

DR. MCDOWALL: The Duke results showed an alveolar–arterial difference of 200 mm at 3 ata. We obtained an A–a difference of 31 mm.

Carbon Dioxide Excretion Through the Skin at 4 atm

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The recent successes of Projects Sea Lab II and Con Shelf III will undoubtedly hasten man's efforts to colonize the continental shelves. As men go deeper and stay longer, problems will inevitably arise which must be overcome before underwater exploration can proceed.[1,2] One of these problems, that of ventilatory efficiency under stress, has been of particular interest to our groups. A major aspect of this problem is the adequacy of carbon dioxide excretion. Basically, this excretory mechanism is both intermittent and dilutional. Normally it is very efficient and adequate under the circumstances in which it evolved. Failure of this "to-and-fro" system is not uncommon,[3] however, particularly in respiratory disease. Under hyperbaric conditions, the mechanism might fail in both health and disease. If this excretory system could be changed to, or supplemented by, a basically more efficient process, such as a continuous flow-through type of gas exchange, then failure under extreme conditions (such as greatly increased gas densities or severe obstructive pulmonary insufficiency) might not occur as readily. The decrease in the work of breathing under these circumstances would also be advantageous.

Behnke [4] has demonstrated that helium gas is transferred through the skin in measurable quantities. Since CO_2 has a much greater solubility coefficient than helium [5] and a very rapid rate of diffusion in biologic fluids,[6] it seemed logical to investigate its excretion rate through the skin under the conditions mentioned. A survey of the literature [7–9] disclosed that measurable gas transfer (CO_2 and O_2) occurs across the skin in normal subjects at sea level. The adequacy of the cutaneous vascular bed,[10] its response to elevated levels of blood pCO_2,[11] and the amount of blood flow that the skin receives on exercise [12] served to strengthen the supposition that this organ might, under special circumstances, act as a route for CO_2 excretion. The circumstances postulated were: (1) immersion in water, (2) cutaneous vascular bed dilated, (3) normal or increased cardiac output, and (4) the presence of an adequate CO_2 pressure gradient across the skin. To test this hypothesis, a series of animal experiments, then experiments in normal humans, and finally experiments

in patients with pulmonary disease were conducted.

MATERIALS AND METHODS

Animal Experiments

Domestic weanling pigs were selected as experimental animals, since their skin closely approximates human skin at that age. An endotracheal tube was inserted into the trachea through a tracheostomy incision and tied securely (to ensure a gas-tight seal) in the lightly anesthetized animals (Nembutal). Arterial and venous cannulas were inserted appropriately and fixed in place. Blood was taken at 5-min intervals during the experiment and analyzed for pCO_2, pO_2, pH, and lactic and pyruvic acid (the first three determined using the IL-113 system). After control samples were taken in air and in water, a one-way valve was inserted into the breathing circuit which allowed inspiration but effectively prevented expiration. During the expiratory occlusion, O_2 was added incrementally to keep the animal floating at a constant level in the water. The test sequence is outlined in Table 1. Each 15-min period of apneustic respiration was followed by a 10-min recovery period. Two animals were studied at 4 atm and two at normal atmospheric pressure. After each experiment, the animals were sacrificed and autopsy performed.

Studies on Normal Humans

Following completion of the preliminary animal experiments, the following studies were conducted on normal human volunteers. (1) The subjects were given a known O_2–CO_2 gas mixture to breathe in air and in water at 1 atm and at pressure for 15-min equilibration periods. (2) The subjects were given 100% O_2 to breathe and asked to alter their normal breathing pattern by taking short inspirations every 15 sec and a full expiration every 60 sec. In studies (1) and (2), central venous samples were taken at regular frequent intervals and were analyzed for pH, pO_2, and pCO_2. (3) During the course of another experiment, end-tidal pCO_2 was measured on the subject and two experimenters because of the circumstances surrounding that pressure exposure. Chamber air temperature was 46°C, and one experimenter was per-

TABLE 1. Outline of Animal Test Sequences at 1 and 4 atm

Code	Status	Immersion in [a]	Breathing pattern	Inhaled gas	Duration (min)
B	Control	Air	Free [b]	Air	5
C_1	Control	Water	Free	Air	5
C_2	Control	Water	Free	Air	10
C_3	Control	Water	Free	Air	15
D_1	Test	Water	Obstruct.[c]	O_2	5
D_2	Test	Water	Obstruct.	O_2	10
D_3	Test	Water	Obstruct.	O_2	15
E	Recovery [d]	Water	Free	Air	5
F_1	Test	Water	Obstruct.	O_2	5
F_2	Test	Water	Obstruct.	O_2	10
F_3	Test	Water	Obstruct.	O_2	15
G	Recovery	Water	Free	O_2	5

[a] All water temperatures were 37.5 ± 1°C.
[b] Unobstructed to-and-fro free breathing.
[c] Obstruction of inspiration only.
[d] Recovery period with free breathing for the same animal described in preceding sequence.

spiring profusely while the other was relatively dry. The subject was immersed in water and breathing quietly. End-tidal pCO_2 was monitored frequently with the IL-113 system in the chamber at 4 atm and during decompression.

Studies on Patients with Pulmonary Disease

A horizontal shower bath was arranged so that the skin could be covered with a thin film of warm water continuously (to avoid the increased breathing effort from water immersion). A Cournand needle was inserted into the brachial artery, and arterial blood gases were measured with the patient in air and in water, while breathing air and while breathing 100% O_2. The ambient 100% O_2 was used to induce hypoventilation and cause an elevation of pCO_2. Following the experiment, subjects were hyperventilated with intermittent positive-pressure breathing as necessary before being allowed to return home.

Data from these experiments were then plotted graphically to relate levels of the blood gases and pH to the passage of time and to the circumstances under which the blood samples were taken. The measuring instrument was calibrated with known buffers and gas mixtures before, during, and after each experiment.

Results

In the animal series, during the period of obstruction at 1 atm, the arterial and venous pCO_2 values rose to four times the control values. At 4 atm, they rose to not quite two times the control values (Figure 1). During occlusion, arterial pCO_2 levels exceeded the venous levels at 1 atm, but they dropped below venous levels when normal breathing was resumed. This did not occur during pressurization. The pH changes (Figure 2) were the reverse of the pCO_2 shifts. During the occlusive phase, arterial pH drop-

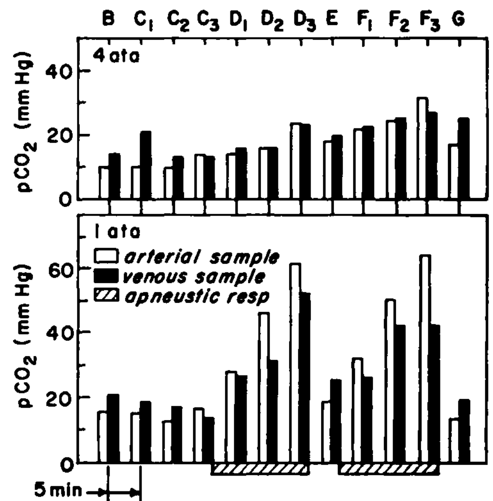

FIGURE 1. Arterial and venous blood pCO_2 levels in pigs subjected to repeated 15-min periods of apneustic respiration. Values between the diagonally striped bars were obtained on samples taken during periods of total expiratory obstruction but with inhalation of 100% O_2. All other samples were drawn during periods of unobstructed respirations.

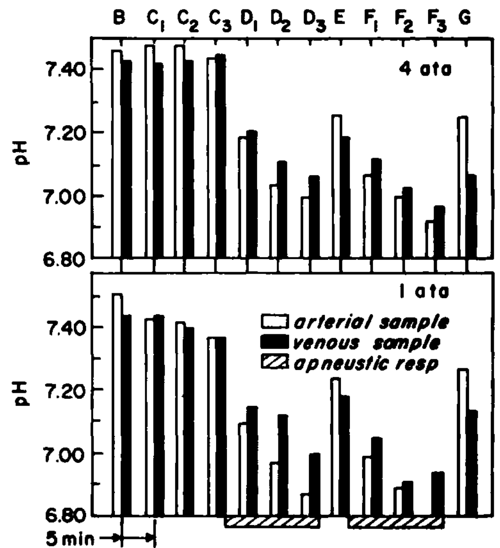

FIGURE 2. Arterial and venous blood pH levels in pigs subjected to repeated 15-min periods of apneustic respiration. Values between the diagonally striped bars were obtained on samples taken during periods of total expiratory obstruction but with inhalation of 100% O_2. All other samples were drawn during periods of unobstructed respirations.

ped to lower levels than venous pH in both groups. An arteriovenous pO_2 difference was maintained during the entire experiment with one exception (Figure 3), signifying O_2 uptake and CO_2 production. The lactic and pyruvic acid levels in the arterial and venous samples showed no definite differences between the pressurized and nonpressurized groups. At autopsy, the lungs were grossly normal in all of the animals.

In the normal-human series, during ventilation with a CO_2–O_2 mixture, venous blood values were highest when the subject was in air and lowest with water immersion (Figure 4). During the CO_2-breathing in air, a marked hyperpnea developed. The pH changes were consistent with the pCO_2 shifts, and the pO_2 was greatest at 2 atm, less at 1 atm in water, and least at 1 atm in air. When the subjects were asked to use the altered breathing pattern (inspiration every 15 sec, full expiration every 60 sec), the pCO_2 rose to far greater levels in air than

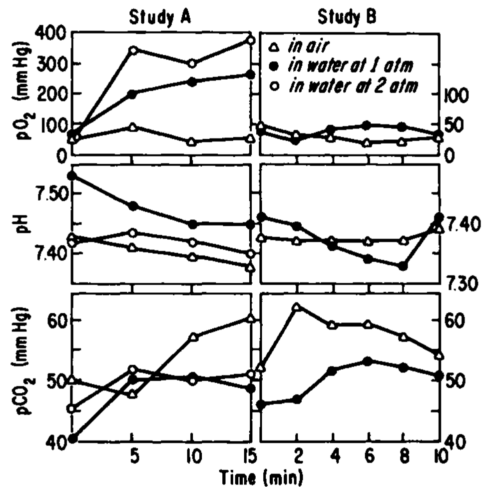

FIGURE 4. Blood gas levels in normal human subjects during maneuvers designed to cause an elevation of pCO_2 levels. Central venous samples were drawn during exposure of skin to air at 1 atm and during immersion of skin in water at 1 atm and at 2 atm. Study A: free breathing of a known CO_2–O_2 mixture. Study B: altered breathing with inspiration every 15 sec and expiration every 60 sec.

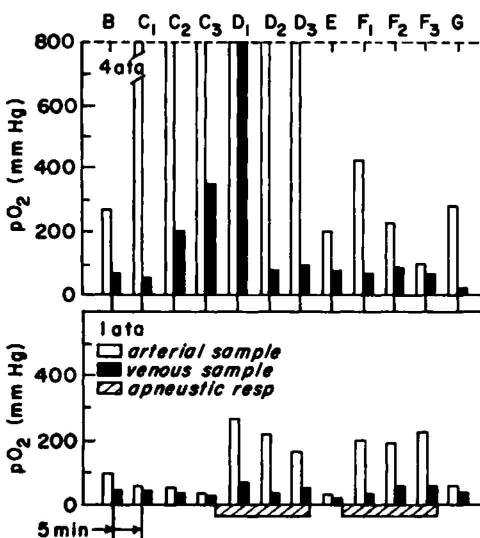

FIGURE 3. Arterial and venous blood pO_2 levels in pigs subjected to repeated 15-min periods of apneustic respiration. Values between the diagonally striped bars were obtained on samples taken during periods of total expiratory obstruction but with inhalation of 100% O_2. All other samples were drawn during periods of unobstructed respirations.

with the same subject in water. Finally, the highest end-expiratory pCO_2 levels occurred in subjects with dry skins, and the lowest levels occurred when the skin was in full contact with water (Figure 5). Each individual was a trained test subject. Each recorded that his ventilations were relatively constant. The significance of the changes depends, of course, upon the accuracy of their statements. Obvious hyperventilation was not present, although watched for.

In the patient study, O_2-breathing caused an increase in pCO_2 in the subject with a previously elevated pCO_2 level (Figure 6). This apparently linear rise was arrested with the skin in water, even though minute ventilation during the two phases remained constant (4.72 liters/min during O_2-breathing with skin in air, 4.83 liters/min during O_2-breathing with skin in water). In the patient with normal pCO_2 gradient, no significant changes were noted.

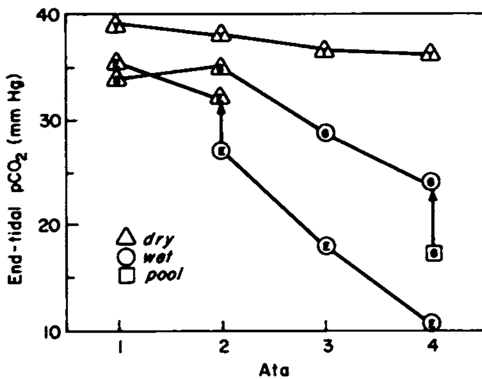

FIGURE 5. End-tidal pCO_2 levels in three normal individuals at 4 atm and at various levels of pressure during decompression. Chamber temperature was 46° C. Minute ventilations were relatively constant. The condition of the skin (dry, wet, or immersed) is correlated with pressure and CO_2 levels.

DISCUSSION

In three limited trials using animals, normal human subjects, and patients with pulmonary disease, evidence suggested that CO_2 must be escaping from the system by a route other than the respiratory tract. The assumption that excretion through the skin is the main secondary route is implied, however, rather than proven by the data. During total expiratory occlusion in the pig, the arterial pCO_2 levels became greater than (and pH lower than) the corresponding venous levels, which indicates a CO_2 loss during transit through the greater circulation. The quick return to normal levels in the recovery phase tends to rule out increased tissue storage as the only factor. Evidence that a healthy human can maintain nearer normal levels of CO_2 in water than in air, while engaged in maneuvers designed to elevate his blood CO_2 levels, is also significant. The probability that metabolic rate increased in the water phase, due to increased work of breathing (hydrostatic pressure) and to elevated temperature (water 37.5°C, ambient air 26°C), enhances the significance of the above finding. Finally, the patient study at 1 atm showed that any effect is likely to be obvious only with elevated gradients of pCO_2.

Obviously, more detailed experimental evidence will be necessary before the hypothesis of significant cutaneous excretion of CO_2 from skin into water can be accepted and quantitatively determined. Yet, it seems likely on the basis of the evidence presented above that loss of CO_2 through the skin–water interface has been an ever-present but unnoticed fact. The ability of divers to work under medium pressure for long intervals with low respiratory rates without encountering problems of severe CO_2 retention seems quite suggestive. Even more stimulating is Rudd's [13] recent report of the existence of a species of fish without any red blood cells, hemoglobin, or hemoglobin substitute. Marine biologists have postulated that the ice fish (*Chaenocephalus acera-*

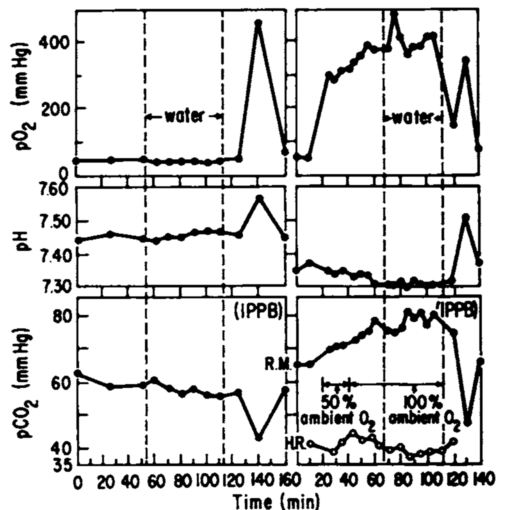

FIGURE 6. Blood gas and pH data from patients with pulmonary emphysema, breathing air or O_2 (in supine position). The data in the sections labeled water are values obtained while the patients' skins were in total contact with water (33–37° C). Data on the left are from patient R.M., that on the right are from R.M. and H.R. (pCO_2 only).

tus) must respire through the skin as well as the gills to survive. Thus, perhaps our enterprising ancestors of the nineteenth century not only anticipated us in the use of "compressed-air baths" but had an unsuspected physiologic rationale for the use of therapeutic mineral baths in certain illnesses.

ACKNOWLEDGMENT

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DISCUSSION

DR. R. C. GOODLIN (*Palo Alto, Calif.*): Using apneic oxygenation, we immersed rats and rabbits in warmed buffered salt solution baths, in an effort to measure cutaneous excretion of carbon dioxide. While technical problems have prevented us from making quantitative determination, cutaneous carbon dioxide excretion can occur in significant amounts, for such apneic animals may be maintained with a pCO_2 of less than 90 mm Hg. We have also been trying to convince our pediatric colleagues that they should use this "bath" method for the therapy of the respiratory distress syndrome in newborns. One such "immersed" infant seemed to be helped, as far as its carbon dioxide tension was concerned.

DR. A. R. BEHNKE, *Session Chairman (San Francisco, Calif.)*: Is ventilation rate increased in an individual breathing air but surrounded by carbon dioxide in a "bath" at 4 atmospheres?

DR. YANDA: I do not know. We have not gotten that far.

Determination of the Absolute Oxygen Tension in Tissues by a New Microelectrode Technique

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College of Physicians and Surgeons
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A technique has been developed in our laboratory for determining the absolute partial pressure of oxygen in tissue with a bare or open polarographic microelectrode. This technique relies upon analysis of the transient current response of an open polarographic microelectrode to a step change in electrical potential. Because of the importance of the determination of pO_2 in brain tissue as a function of the partial pressure of oxygen in inspired gas, we applied our method to studies on cat cortex.

Theoretical Development

An open electrode is composed of a suitable metal insulated from the medium to be measured except at one area, which is left completely exposed to the medium (Figure 1). The steady-state electric current [$I(\infty)$] in an open polarographic electrode is given by the equation:

$$I(\infty) = n \cdot D \cdot pO_2$$

where n is a constant determined by the number of electrons involved in the electrode reaction and electrode geometry, D is the diffusion coefficient of oxygen in the medium, and pO_2 is the oxygen tension. In order to ascertain pO_2 from $I(\infty)$, one must know D. The diffusion coefficient of oxygen in individual biological tissues is not known, nor can it be assumed to be constant. Thus, even relative values for pO_2 are difficult to interpret from measurements of $I(\infty)$.[1-3]

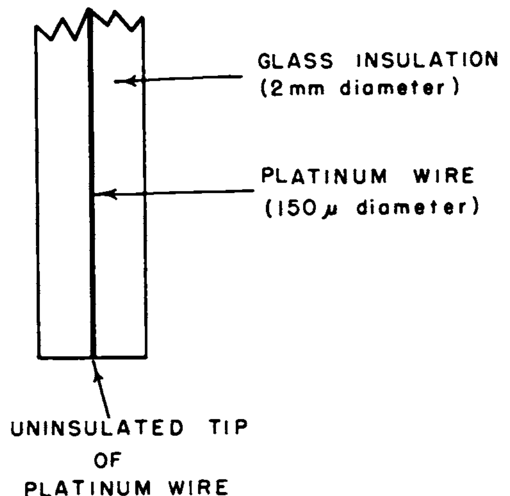

FIGURE 1. Diagram of the open polarographic microelectrode constructed in our laboratory.

Absolute measurements are possible with the circuit shown in Figure 2. The voltage source has a square-wave output, and a step change in electrical potential from 0.0 volts to −0.6 volts is applied to the platinum electrode. Simultaneous with the step change in potential is a spike in the electrode current, with a relatively slow decay occurring after the peak (Figure 3). This current response $I(t)$ to a step change in electrode potential is described by the equation:

$$I(t) = I(\infty) + Ae^{-\alpha t} + b_0e^{-\beta_0 t} + b_1e^{-\beta_1 t}$$

This equation is demonstrated graphically when $ln[I(t) - I(\infty)]$ is plotted against time (Figure 4). It is seen that as t increases, the logarithmic plot of $[I(t) - I(\infty)]$ becomes linear. This makes it apparent that $[I(t) - I(\infty)]$ is, for higher values of t, described by the equation $Ae^{-\alpha t}$. When $(lnA - \alpha t)$ is subtracted from $ln[I(t) - I(\infty)]$, another line $(lnb_0 - \beta_0 t)$ is obtained. Another subtraction in the logarithmic domain reveals a third exponential component, described by coefficients b_1 and β_1.

This equation for $I(t)$ was further supported when the output of an analog computer designed to generate curves approximating the equation was matched with the electrode current curves, and values for the equation coefficients were read from the computer directly.

The coefficients in the transient equation:

$$I(t) = I(\infty) + Ae^{-\alpha t} + b_0e^{-\beta_0 t} + b_1e^{-\beta_1 t}$$

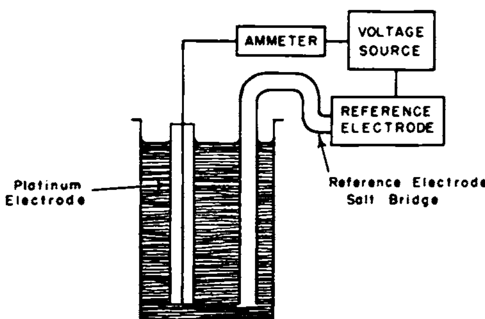

FIGURE 2. The electrical circuit used in the microelectrode technique.

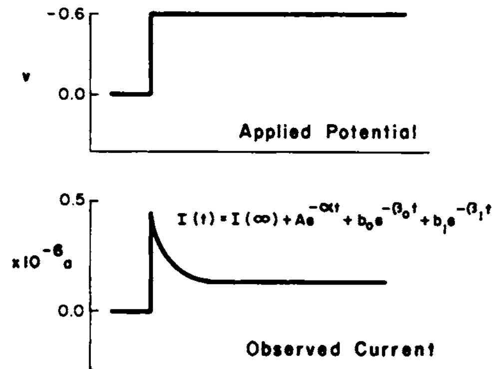

FIGURE 3. Voltage output from the square-wave source (upper curve) and the resultant simultaneous circuit transient current (lower curve).

are of interest and importance. The coefficients A and α are independent of the oxygen tension of the medium bathing the polarographic and reference electrodes. These coefficients may change, however, if the salt concentration of the medium changes.

As mentioned above, the term $I(\infty)$ is the steady-state current of the electrode

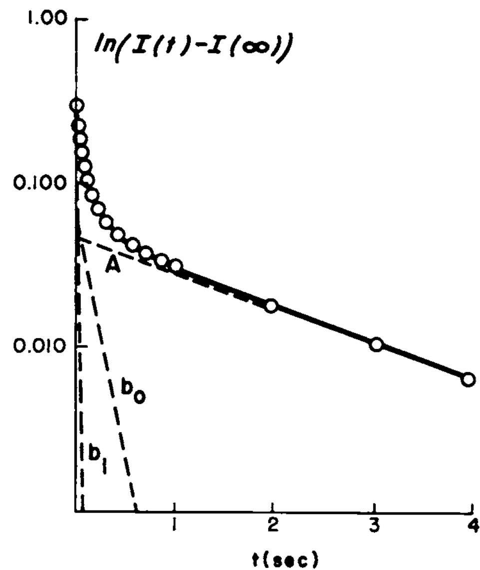

FIGURE 4. A typical logarithmic plot of $[I(t) - I(\infty)]$, showing the individual exponential components.

system occurring when a potential is applied. This variable is determined by the equation:

$$I(\infty) = n \cdot D \cdot pO_2$$

The ratio of the electrode steady-state current $[I(\infty)]$ to the oxygen tension (pO_2) is the product of $n \times D$. This product may be defined as the steady-state conductance of the system due to the electron transport by oxygen molecules, and it is given the symbol M, or:

$$I(\infty) = M \cdot pO_2$$

For the sake of brevity, M will be termed the oxygen conductance of the system.

The coefficients b and β are variables which also depend upon the oxygen tension and oxygen conductance of the electrode medium. Of greatest practical interest is the value B, which is defined by the equation:

$$B = b_0 + b_1$$

B varies linearly with changing oxygen conductance, or M values in the electrode system (Figure 5). Also, B varies linearly with changing partial pressures of oxygen

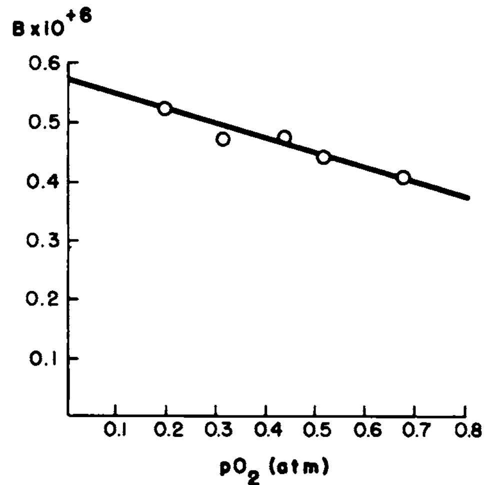

FIGURE 6. The linear relationship between the transient parameter (B) and the partial pressure of oxygen in the medium (pO_2).

in the medium (Figure 6). A family of curves describing the relationship between B and pO_2 for various values of M is shown in Figure 7, from which family of curves the following equation is derived:

$$B = (k_1 - k_2M) - (k_3 - k_4M)pO_2$$

Recalling that $I(\infty) = M \cdot pO_2$, one readily determines that:

$$pO_2 = \frac{-\Delta \pm \sqrt{\Delta^2 - 4k_2k_3I(\infty)}}{-2k_3}$$

where

$$\Delta = k_1 - B + k_4I(\infty)$$

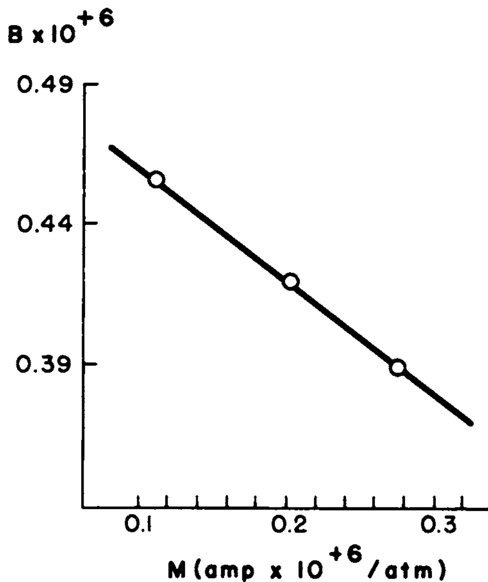

FIGURE 5. The linear relationship between the transient parameter (B) and oxygen conductance (M).

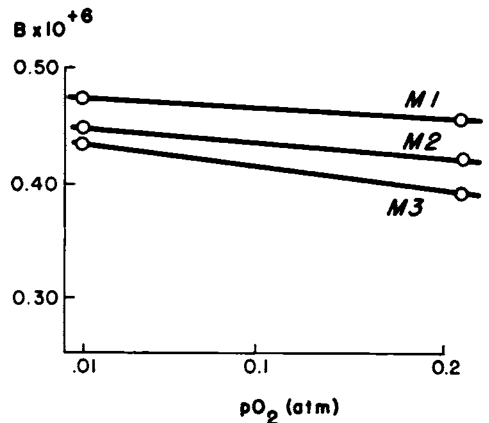

FIGURE 7. The transient parameter (B) versus the partial pressure of oxygen (pO_2) for various values of oxygen conductance (M).

It is this last derived equation which is used to obtain values for tissue oxygen tension from determinations of the steady-state electrode current, $I(\infty)$, and a parameter of the transient electrode current, B.

The validity of the technique is directly related to the constancy of the values of k_1, k_2, k_3, and k_4, which are determined by an initial electrode calibration. During operation of the electrode system following a calibration, the values of k_1, k_2, k_3, and k_4 have remained constant for at least 10 hours. Determinations must be made with careful temperature control, however. The explanation of the constancy of k_1, k_2, k_3, and k_4 may lie in the observation that alternating current, such as used in the present technique, diminishes the aging or poisoning of an oxygen electrode system.[2,4,5]

Apparatus

The apparatus used is schematically presented in Figure 2. The voltage source is the positive gate output of a 532 Tektronix oscilloscope. The ammeter function is performed by a Model 610B Keithley electrometer, which presents its current reading to a 565 Tektronix oscilloscope. The reference electrode is an Ag–AgCl type, and the oxygen-consuming electrode is the platinum electrode diagramed in Figure 1.

Application of the Technique on Cat Cortex

This microelectrode method was tested on the motor cortex of cat brain. A 6-mm burr hole was made through the cat's skull, with the animal under moderate pentobarbital anesthesia. The platinum electrode was placed on the cortex through the burr hole, as was the salt bridge of the reference electrode. Since compression of the cortex by electrode pressure alters the oxygen tension reading, the electrode was attached to a balanced lever arm and thus applied to the cortex, in much the same way that a phonograph needle is placed on a record. The electrode lever arm was arranged so as to exert a force of 1 gm on the cortical surface.

The animal and electrode system were prepared in a pressure chamber. The gas in the chamber was controllable as to pressure and composition, and the electrode was monitored outside the chamber. The gases in the chamber were varied, in the following order: normal air at 1 ata, 100% oxygen at 1 ata, and thence in steps to 100% oxygen at 3 ata. Cortical oxygen tension changes with these changes in inspired air are shown in Figure 8. These readings were obtained with the platinum electrode placed close to a cortical artery.

With the animal breathing air at atmospheric pressure, the oxygen tension in periarterial tissue was essentially 100 mm Hg. When the electrode was placed on cortex on which no vessels were seen with the unaided eye, oxygen tension readings were between 13 and 16 mm Hg. Future work will attempt to determine the in-

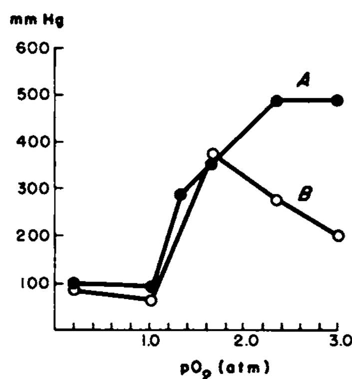

FIGURE 8. Typical curves relating cerebral cortical oxygen tension (vertical axis) to the pressure of inspired oxygen (horizontal axis) in the cat. Curve A was obtained from data on a cat which maintained its respiratory efforts during the entire experiment. Curve B was obtained from data on a deeply anesthetized cat whose respirations decreased in depth and frequency as the experiment progressed.

crease in oxygen tension in these areas with changing inspired gases.

This information is of practical and theoretical interest. Future work will involve objective monitoring of cardiorespiratory function simultaneously with measurement of cortical oxygen.

SUMMARY

By the technique described above, the absolute oxygen tension of animal tissue and aqueous solutions can be determined with an open polarographic microelectrode. An open electrode is preferred over a membrane-covered or recessed electrode because of its rapid response to changes in oxygen tension and its simple construction which allows considerable miniaturization.[2, 6-8] We hope that this study will aid research concerning the basic electrochemistry of the polarographic electrode and that this technique may help to elucidate the distribution and utilization of oxygen in the animal organism.

ACKNOWLEDGMENT

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DISCUSSION

DR. I. S. LONGMUIR (*Raleigh, N. C.*): There are two questions I would like to ask. First, what was the value of n with your electrodes, since this is known to vary between 2 and 4? Second, what sort of pictures did you get in your time curves with the use of nitrogen instead of oxygen?

DR. ALBANESE: As to the first question, we recognize the difficulty in calculating D from the product $n \times D$, which we have defined as the oxygen conductance of the system, M. We cannot determine D directly unless we assume n to be a constant, which is not the case, as shown by

Delahay (J. Electrochem. Soc. 97:198, 1950). It is because *n* is a variable and difficult to determine that we defined *M*. *M* is the steady-state conductance of the system due to electron transport by oxygen molecules in the system. Our technique allows absolute determination of pO_2 and *M*, but not of *n* or *D*. Using a solution saturated with nitrogen, we are able to place a point on the *B* versus pO_2 line at pO_2 equals zero atmospheres. Using such solutions, we find that the transient parameter *B* varies linearly with pO_2 between zero and 1 atmosphere. Quite recently, using a hyperbaric chamber, we have found this transient parameter relationship to hold between zero and 3 atmospheres pO_2.

DR. A. R. BEHNKE, *Session Chairman* (*San Francisco, Calif.*): If pure oxygen is inhaled, why should ventilatory rate affect the pressure of oxygen in brain tissue?

DR. ALBANESE: Without objective measurements of respiratory function and cardiac activity, I really cannot explain this.

DR. BEHNKE: How big is the electrode?

DR. ALBANESE: The actual active tip of the electrode is 150 microns. The diameter, including insulation, is as large as 2 mm.

DR. BEHNKE: Could you place this type of electrode in the thoracic duct and measure oxygen tension of lymph?

DR. ALBANESE: We would have to make the insulation much thinner. We have made small electrodes by putting antimony in glass and then drawing these materials out over a flame, but we have not used such an electrode in the thoracic duct. We have used small electrodes (to 10 microns) and tested them with our theory, however.

DR. BEHNKE: One would have anticipated that the oxygen pressure at 3 ata would be greater than 600 mm.

DR. ALBANESE: We hope in the future to have electrocardiographic monitoring of the animal, and to make objective measurements of respiratory effort.

DR. BEHNKE: Gersh, Davies, and Larrabee in 1945 (U. S. Navy Medical Research Institute, Project X-192, Rept. No. 6, May, 1945) found that the oxygen tension values of the cerebral cortex of cats rise early and then fall abruptly to a plateau which slopes to a slightly lower value prior to death.

Influence of Anesthetic Drugs on Dogs Subjected to Oxygen at 5 ata

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Oxygen at high pressure (OHP) has long been known to result in acute toxicity of the central nervous system (CNS). This toxicity is manifested by grand mal convulsions which rapidly terminate in death if OHP is not discontinued.[1] Bean and Siegfried[2] have shown that rats repeatedly subjected to nonfatal OHP exposures developed delayed or chronic signs of CNS toxicity consisting of irreversible ataxia, postural disturbances, and spastic paralysis.

The purpose of this study was threefold: (1) to determine the physiologic changes that occur during and after exposures to extreme OHP in the awake dog, (2) to ascertain how anesthetic drugs, potentially useful in the hyperbaric environment, may modify these responses, and (3) to determine any protective effects these drugs might have against the development of CNS oxygen toxicity.

METHODS

Thirty-two mongrel dogs were subjected to inspired oxygen tensions of 3860 mm Hg (60 psig). Seven control animals received only succinylcholine. The remaining 25 animals were divided into five equal groups. Each experimental animal of a group received succinylcholine and one of the following anesthetic drugs: thiopental, halothane, methoxyflurane, nitrous oxide (367 mm Hg inspired tension), or a neuroleptanalgesic combination of Droperidol and Fentanyl.

The following parameters were continually monitored: lead II of the electrocardiogram (ECG), frontal occipital electroencephalogram (EEG), arterial blood pressure, venous pressure, and, in most cases, the cerebrospinal fluid (CSF) pressure. Measurements of pO_2, pCO_2, and pH were made on arterial and venous blood and CSF in the chamber at various pressures. Respirations were controlled at a constant minute volume of 300–320 ml/kg/min by a mechanical ventilator in a non-rebreathing system. Hyperinflation (sighing) was performed periodically.

The animals were exposed to 3860 mm Hg inspired oxygen tension for 60 min or for 15 min after the development of convulsive activity was observed on the EEG. Animals not convulsing were decompressed over 10–15 min using oxygen ventilation; those convulsing were decom-

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pressed with the investigators in air according to the U.S. Navy decompression tables.

With the completion of decompression, ventilation with air was continued until the return of adequate spontaneous respirations or until death. Appropriate supportive care was given. Surviving animals were examined daily, then autopsied 5–7 days after exposure.

RESULTS

Table 1 lists the animals which convulsed in each anesthetic series, the mean time to onset of convulsions in those with seizure activity, and the highest rise of CSF pressure observed with the initial convulsion. As sufficiently large doses of succinylcholine were used to prevent all motor activity, the onset and nature of convulsions were determined from the EEG.

All control, halothane, and nitrous oxide animals developed seizure activity within a 1-hour period of exposure to oxygen at 3860 mm Hg. Four neuroleptanalgesic and three methoxyflurane dogs manifested convulsive activity. No thiopental animals showed evidence of convulsions at any time throughout the experiment.

The mean time to onset of seizure activity was shortest in the nitrous oxide series and longest in the neuroleptanalgesic series; marked rises in CSF pressure accompanied convulsive activity in the control, nitrous oxide, and neuroleptanalgesic groups despite the complete absence of skeletal muscular activity. CSF pressure increases were moderate in the methoxyflurane animals and completely absent in the halothane animals.

Table 2 summarizes changes in the cardiovascular system noted at the time of the first convulsion. Marked rises in arterial pressures, pulse rate increases, and severe cardiac arrhythmias were associated with seizure activity in the control, nitrous oxide, and neuroleptanalgesic series. The methoxyflurane animals showed only moderate rises in arterial blood pressure and a slowing of the pulse during convulsions. While all the halothane animals exhibited convulsions, cardiovascular changes were not associated with seizure activity. Figures 1–3 illustrate the cardiovascular changes accompanying convulsions in a control, a neuroleptanalgesic-treated, and a halothane-treated animal.

TABLE 1. Convulsion Data

Anesthetic series	Total animals in series	No. animals in series convulsing	Mean time to onset of convulsions [a] (min)	CSF pressure increase with initial convulsion (mm Hg) [a]
Control (succinylcholine alone)	7	7	14 ± 11	52 ± 11 [b]
Thiopental	5	0	—	—
Halothane	5	5	29 ± 9	No change
Methoxyflurane	5	3	27 ± 19	14 ± 2
Nitrous oxide	5	5	1.8 ± 3.1 [c]	24 ± 11
Neuroleptanalgesia	5	4	39 ± 16	27 ± 14
Total	32	24		

[a] Figure based on number of animals convulsing, listed in third column.
[b] Four observations only.
[c] Two animals convulsed before reaching 3860 mm Hg oxygen tension.

TABLE 2. Cardiovascular Reactions During Oxygen Convulsions

Anesthetic series	Total animals in series	No. animals in series convulsing	Vital signs at 3860 mm Hg oxygen tension before convulsions: Mean arterial blood pressure (mm Hg)	Vital signs at 3860 mm Hg oxygen tension before convulsions: Mean pulse rate	Vital signs during initial convulsions [a]: Mean arterial blood pressure (mm Hg)	Vital signs during initial convulsions [a]: Mean pulse rate	Cardiovascular reactions during convulsions
Control (succinylcholine alone)	7	7	190 ± 46 / 111 ± 30	97 ± 50	309 ± 64 / 198 ± 52	215 ± 99	Severe cardiac arrhythmias; ventricular tachycardia in four animals
Thiopental	5	0	157 ± 32 / 111 ± 26	129 ± 31	—	—	Cardiovascular system stable throughout entire exposure
Halothane	5	5	121 ± 28 / 81 ± 23	77 ± 16	121 ± 28 / 81 ± 23	71 ± 15	Cardiovascular system stable throughout convulsions and exposure
Methoxyflurane	5	3	171 ± 70 / 110 ± 51	94 ± 42	215 ± 47 / 99 ± 13	60 ± 21	Bradycardia, moderate hypertension, ECG stable
Nitrous oxide [b]	5	5	187 ± 14 / 130 ± 23	124 ± 26	285 ± 41 / 198 ± 27	143 ± 56	Severe cardiac arrhythmias; ventricular tachycardia in three animals
Neuroleptanalgesia	5	4	160 ± 19 / 85 ± 21	60 ± 10	302 ± 53 / 190 ± 56	203 ± 99	Severe arrhythmias, ventricular tachycardia in two animals, one proceeding to ventricular fibrillation

[a] Number of observations indicated by number of animals in drug series convulsing, shown in third column.
[b] Two of these animals developed grand mal convulsions before 3860 mm Hg oxygen tension was reached.

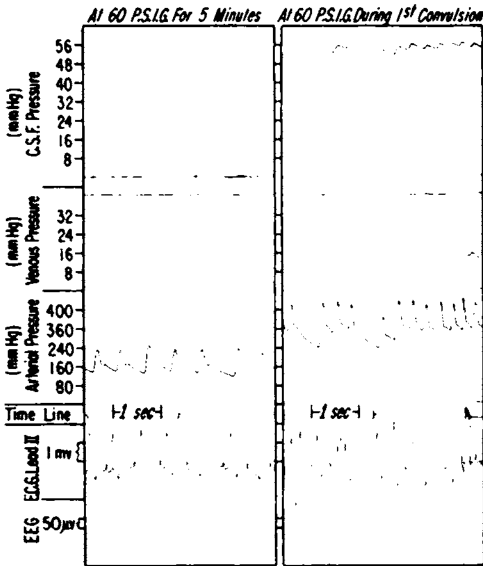

FIGURE 1. Cardiovascular parameters and CSF pressure recordings in a control succinylcholine-treated dog. Note the elevation of arterial and CSF pressure and the cardiac arrhythmias associated with convlusive activity. (Reproduced from Anesthesiology. In press.)

Table 3 lists the number of dogs from each series which had acute and/or chronic signs of oxygen toxicity. A total of 24 animals, consisting of all seven controls, all five halothane, all five nitrous oxide, four neuroleptanalgesic, and three methoxyflurane dogs, had acute signs of CNS oxygen toxicity consisting of EEG-documented grand mal convulsions. None of the five thiopental-anesthetized animals showed convulsive activity during or after exposure to OHP.

Eight of 15 surviving animals revealed a weakness or spasticity of the extremities following exposure and recovery from the anesthetic and relaxant drugs. Seven made a clinically complete recovery in 1–7 days. The eighth dog, while showing marked improvement, still had incoordination of the hind legs up to the time of sacrifice and autopsy on the seventh postexposure day. These chronic or delayed signs of CNS oxygen toxicity were observed in four animals which had acute signs of CNS oxygen toxicity and in four animals (three thiopental and one methoxyflurane) that

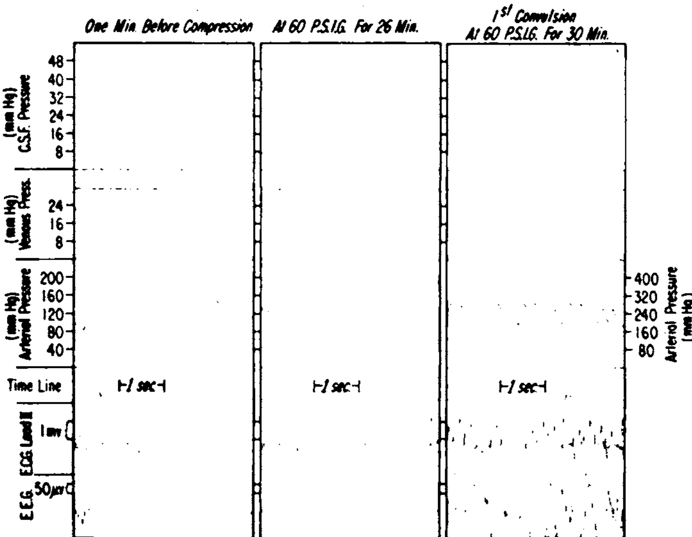

FIGURE 2. Cardiovascular parameters and CSF pressure recordings in a neuroleptanalgesic animal. The response to convulsive activity is similar to that seen in the control animal. (Reproduced from Anesthesiology. In press.)

TABLE 3. Acute and Delayed CNS Oxygen Toxicity

Anesthetic series	Total animals in series	No. with convulsions during exposure (acute)	No. with paralysis or spasticity (delayed)	No. without CNS involvement
Control (succinylcholine alone)	7	7	2	0
Thiopental	5	0	3	2
Halothane	5	5	1	0
Methoxyflurane	5	3	2	1
Nitrous oxide	5	5	0	0
Neuroleptanalgesia	5	4	0	1
Total	32	24	8	4

demonstrated no detectable signs of oxygen toxicity at any time during exposure.

Table 4 lists the causes of death in the 17 animals which succumbed. Death was found to result from two major causes: cardiovascular failure and a change in the CNS. (Three deaths were attributable to causes associated with the experimental procedure, but unrelated to oxygen toxicity.) Cardiovascular deaths were characterized by marked hypertension and ECG abnormalities associated with convulsive activity. The ECG rapidly deteriorated to ventricular tachycardia followed by a state of irreversible shock and death. Four controls and one neuroleptanalgesic animal died in this manner within 4 hours of exposure.

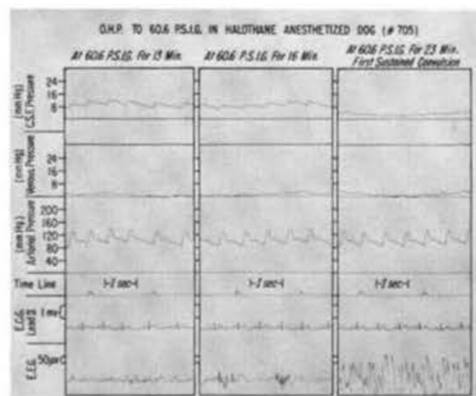

FIGURE 3. Cardiovascular parameters and CSF pressure recordings in a halothane-anesthetized dog. Note the stability of the cardiovascular system and the lack of increased CSF pressure in association with active convulsive activity. (Reproduced from Anesthesiology. In press.)

Nine animals (one control, four halothane, two methoxyflurane, and two nitrous oxide dogs) died from acute CNS effects. These animals all developed grand mal convulsions during exposure which continued as a state of status epilepticus, both clinically and by the EEG during and after decompression. The convulsive activity was unique in that the seizures were not continuous, but occurred during inspiration, either with spontaneous respirations or with controlled mechanical ventilation. The motor activity consisted of involuntary uncoordinated spastic movements confined primarily to the extremities in association with rapid high-voltage spikes on the EEG. Large doses of barbiturates stopped this motor and EEG activity only temporarily. Despite vigorous anticonvulsant and supportive therapy, nine of 10 animals showing convulsions after exposure died within 24–48 hours in a state of irreversible shock.

One nitrous oxide animal died during decompression with a tension pneumothorax. Another nitrous oxide and one neuroleptanalgesic dog died 48 hours after exposure with meningitis confirmed at autopsy examination.

TABLE 4. Principal Cause of Death

Anesthetic series	Total animals in series	Cardiovascular death	CNS death (convulsions)	Deaths unrelated to oxygen toxicity *	Total deaths
Control (succinylcholine alone)	7	4	1	0	5
Thiopental	5	0	0	0	0
Halothane	5	0	4	0	4
Methoxyflurane	5	0	2	0	2
Nitrous oxide	5	0	2	2	4
Neuroleptanalgesia	5	1	0	1	2
Total	32	5	9	3	17

* See text.

DISCUSSION

With the exception of nitrous oxide, all the anesthetic drugs studied delayed the onset of grand mal convulsions in dogs subjected to oxygen at 3860 mm Hg. The most effective drug was thiopental in that no animals of this series showed EEG evidence of convulsions. Next in effectiveness was a neuroleptanalgesic combination followed by halothane and methoxyflurane.

Associated with convulsions, serious cardiovascular abnormalities consisting of hypertension and cardiac arrhythmias were noted in the control, nitrous oxide, and neuroleptanalgesic series. Five animals died as a direct result of these alterations. The association of cardiovascular changes with grand mal convulsions induced by electroshock and drugs is well documented.[3-5] Apparently, rapid demise associated with oxygen toxicity or other forms of induced grand mal convulsions may be the result of cardiovascular malfunction. Oxygen seizures must be immediately controlled to prevent such deaths. The use of a muscle relaxant and ventilation is not adequate. Therapy must be aimed directly at the overactive CNS.

Halothane-anesthetized animals showed no alterations in cardiovascular function accompanying grand mal convulsions. Halothane has been stated to possess sympathetic blocking activity,[6,7] which might explain its apparent protection of the cardiovascular system. Cardiovascular changes associated with convulsions, while present in the methoxyflurane series, were minimal. Despite the protection afforded the cardiovascular system by both these drugs, death related to the CNS occurred within 48 hours of exposure in four halothane and two methoxyflurane dogs.

Eight surviving animals had transient neuromuscular disturbances thought to represent a delayed or chronic form of oxygen toxicity, first described in rats by Bean.[2] Four of these eight animals showed no evidence of acute oxygen toxicity which could be monitored during exposure to OHP. Suitable monitoring techniques to detect changes forecasting the onset of the delayed form of oxygen toxicity are not yet available.

Oxygen toxicity occurring in the CNS was manifested in awake and anesthetized dogs by convulsions frequently associated with cardiovascular changes and by delayed signs of chronic neuromuscular disturbances. Anesthetic drugs altered in varying degrees the onset and severity of

the acute aspects of CNS oxygen poisoning but did not ensure the absence of chronic or delayed aspects. No anesthetic technique tested in this study gave complete protection against all the aspects of CNS oxygen toxicity.

ACKNOWLEDGMENTS

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DISCUSSION

DR. A. R. BEHNKE, *Session Chairman* (*San Francisco, Calif.*): In the hundreds of convulsive seizures that have occurred in man, there have been no reports of gross neurologic impairment (Brit. Med. J. 1:667, 1947). The point here is that early symptoms of oxygen poisoning should not be masked by an anesthetic agent. From a paper presented yesterday, I gained the impression that barbiturates enhanced oxygen toxicity in rats.

DR. HARP: Yes, this may be so in the delayed form.

DR. BEHNKE: For a given exposure with and without an anesthetic, is there a difference in the histologic appearance of brain tissue?

DR. HARP: Dr. Balentine and Dr. Gutsche have investigated this in rats. With fairly large doses of barbiturates, there appears to be potentiation. However, rats given 37 mg/kg of pentobarbital are asleep, and they become somewhat hypercarbic. In other studies, Dr. Gutsche found that lower doses of barbiturates seem not to have this potentiating effect, and in fact may have an opposite effect (unpublished data). What we really need is a dose-response curve. In an earlier series (Anesthesiology, in press). working at 4 ata with halothane, dogs had no trouble throughout the 50-minute exposure, but they developed convulsions upon decompression. They continued to convulse in air, and died 48 hours later in status epilepticus. They never woke up, despite vigorous treatment.

DR. BEHNKE: At 3 ata, and within therapeutic levels of exposure, would you administer an anesthetic, and if so, what anesthetic?

DR. HARP: We always seem to end up at 4 ata when we operate, and that is why the earlier study was done at 45 psig. We feel we have to be very careful there.

Effects of Hydrogen Peroxide on the Cardiovascular System

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In an attempt to find a method for oxygenating tissues which would compare with hyperbaric oxygenation, we evaluated an intravascular method of administering oxygen in a regional or systemic system,[1,2] using dilute solutions of hydrogen peroxide (H_2O_2) given by a variety of routes. H_2O_2 is degraded to oxygen and water by catalase and peroxidases, enzyme systems present in excess quantities in the blood. Following H_2O_2 decomposition in biological fluids, it was noted that more oxygen was being recovered from such fluids than one would expect from 100% saturation with oxygen at 1 atm. Previously reported experiments showed this to be a simple supersaturation of a liquid with a gas (Figure 1).[3] H_2O_2 is able to release dissolved oxygen equivalent to that one would find in solutions under oxygen at 3-8 atm of pressure. Furthermore, H_2O_2 administration does not require lung transport. It can be given continuously over long periods of time, it can be administered by a single physician without expensive equipment and large teams, and it avoids compression–decompression hazards, as well as central nervous system and pulmonary toxicity.

Following is a report of some effects of H_2O_2 in dilute solution on the cardiovascular system, observed in our pilot studies.

Cardiovascular Effects of H_2O_2

Direct Tissue pO_2 Measurements (Myocardium)

A series of experiments was designed to clarify: (1) whether the intra-arterial infusion of dilute H_2O_2 increases the tissue oxygen content in the infused area, (2)

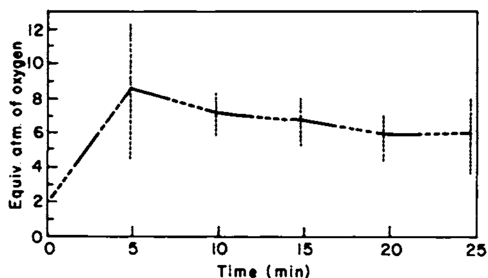

FIGURE 1. Oxygen content of human plasma 5–25 min after addition of 0.5% H_2O_2. The vertical lines represent 0.001 confidence intervals; the heavy dashed lines in the curve represent mean values.

whether dilute H_2O_2 applied directly to the tissue increases the oxygen content therein, and (3) the rate at which and extent to which oxygen thus supplied diffuses through a tissue medium.

Direct myocardial pO_2 measurements were taken in rabbits (13-kg giant New Zealand) during: (1) the intra-arterial infusion of H_2O_2 into the coronary arteries, and (2) the direct application of H_2O_2 to the epicardium. The animal was anesthetized with pentobarbital sodium, and a catheter was passed retrograde from the right carotid artery to the coronary ostia.[1] Intubation was then performed, the animal was placed on a respirator, and the left chest was opened. After the pericardium was split, a tissue needle probe attached to an IL-125A polarograph was inserted into the left ventricular wall. Figure 2 shows the relative increase in myocardial pO_2 during the intra-arterial infusion of H_2O_2 to the coronary arteries.

In the second series of studies, the rabbits were intubated and placed on the respirator, the left chest was opened and the pericardium similarly split, but the H_2O_2 was perfused over the epicardium through an inflow catheter and removed through an outflow catheter. Figure 3 shows the relative pO_2 increase in the myocardium by the direct diffusion.

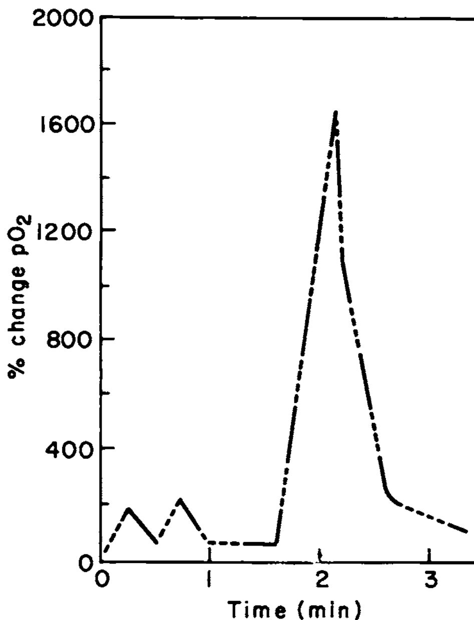

FIGURE 2. Percent change in myocardial pO_2 after infusion of 0.36% H_2O_2 into the coronary arteries.

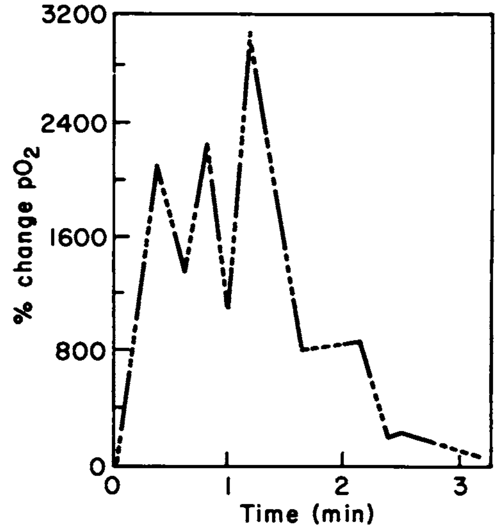

FIGURE 3. Percent change in the myocardial tissue pO_2 after direct epicardial application of 0.36% H_2O_2.

Oxygenation of the Ischemic or Anoxic Myocardium of Small Animals

Tracheal Occlusion. The myocardium of giant New Zealand rabbits (11–15 kg) was rendered anoxic by tracheal cross-clamp.[1] These animals were divided into three groups of 10 animals each: in Group 1, H_2O_2 was infused into the coronary arteries; in Group 2, it was perfused intrapericardially; in Group 3 (controls), the carrier solutions were administered by each of the two routes, but without H_2O_2. In all animals, arterial blood pressure, pH, and electrocardiogram (ECG) were monitored and solutions were kept normothermic.

In Group 1, tracheostomies were performed, and the animals were intubated and placed on the Bird respirator breathing 100% oxygen for 15 min. A small #90 polyethylene catheter was inserted

into the right common carotid artery and passed retrograde to the coronary ostia. The trachea was cross-clamped and 0.06-0.48% H_2O_2 in an electrolyte carrier solution (Ionosol T) was infused through the carotid catheter into the coronary ostia. Blood pressure fell to zero in an average of 15 min (Figure 4), and the blood pH declined to 7.0 in an average of 9.5 min (Figure 5). Neither nodal block nor cardiac arrest was observed during the 120-min period of monitoring. Occasionally, evidences of reversible ischemia appeared in the ECG.

In the Group 2 rabbits, tracheostomies were performed, and the animals were intubated and placed on the Bird respirator breathing 100% oxygen. The left side of the chest was opened, and two #5 French catheters were inserted and sutured into the pericardium. The pericardium and chest were closed and the animals taken off the respirator for 30 min before tracheal occlusion. After cross-clamping of the trachea, the pericardium was perfused in a closed system with 0.06-0.48% H_2O_2 in the carrier solution. Blood pressure fell to zero in an average of 12.5 min (Figure 4), and the pH reached 7.0 only after a prolonged period (Figure 5). Neither nodal block nor cardiac arrest occurred during the 120 min of monitoring.

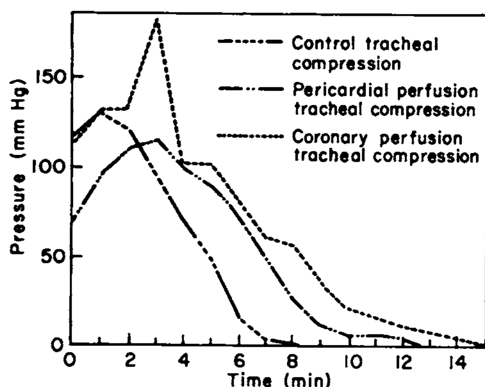

FIGURE 4. Blood pressure changes after tracheal occlusion in rabbits during the administration of dilute solutions of H_2O_2 directly to the epicardium or through the coronary arteries.

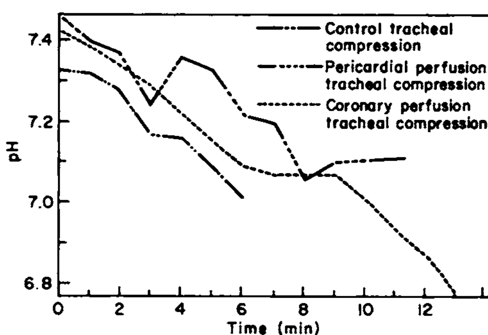

FIGURE 5. Changes in peripheral arterial pH after tracheal compression in rabbits during epicardial perfusion or coronary artery infusion with dilute solutions of H_2O_2.

Group 3 rabbits (controls) were equally divided, each half receiving the same treatment as Group 1 or 2 except that carrier solution alone (without H_2O_2) was administered. In both groups of control rabbits, the blood pressure dropped to zero in an average of 8 min and the pH declined to 7.0 in an average of 6 min (Figures 4, 5). In all control animals, ischemic changes noted in the ECG by nodal block or arrhythmias appeared in an average of 5 min (range 3.5–6.5 min). The average time of cardiac arrest was 13 min (range 10–16 min). All animals developed cardiac arrest, and, following failure of standard resuscitative methods, most could be revived by adding H_2O_2 to the heart by whichever route of administration of carrier solution was being employed.

Coronary Artery Ligation. In another group of giant New Zealand rabbits, the myocardium was rendered anoxic and ischemic by ligation of coronary artery branches singly or in combination.[1] As in the previous experiments, arterial blood pressure, pH, and ECG were monitored constantly. Because of the nature of rabbit myocardium, it was impossible in 20 animals to produce a standard ischemic lesion which was reproducible from animal to animal with regard to ECG or blood pressure changes. This made it necessary to evaluate the reversal of is-

chemic myocardial changes by regional applications of H_2O_2 within a given animal rather than by comparison of the effect on a standard lesion in a large number of animals.

In 10 rabbits, constituting Group 1, a catheter was inserted retrograde through the right carotid artery into the base of the aorta. The left chest was opened, the pericardium was opened, and a variety of coronary arteries were ligated, including the circumflex, the anterior descending, and the right coronary (or a combination of these). Dilute H_2O_2 (range 0.06–0.72%) was infused retrograde into the coronary arteries by a slow drip at the time of ligation, Ionosol T being used as the carrier solution. Specific ECG abnormalities associated with myocardial ischemia, such as ST segment elevation, elevation or depression of the T wave, nodal block, and ventricular tachycardia or fibrillation, could be readily and often repeatedly reversed in the animals by application of the H_2O_2. A blood pressure drop associated with cardiac arrhythmias could also be reversed, but less consistently (Figure 6).

In another group of 10 animals (Group 2), the left chest was opened, the pericardium was incised, and a similar group of coronary arteries and branches were ligated. Ventricular fibrillation usually followed in several minutes. After cardiac massage, which was carried on for 15 min with no reversal of fibrillation, 0.06–0.72% H_2O_2 in carrier solution at normal body temperature was added directly to the epicardium. Arrhythmia was reversed to regular sinus rhythm in seven of the 10 animals, with frequent simultaneous reversal of blood pressure (Figure 7).

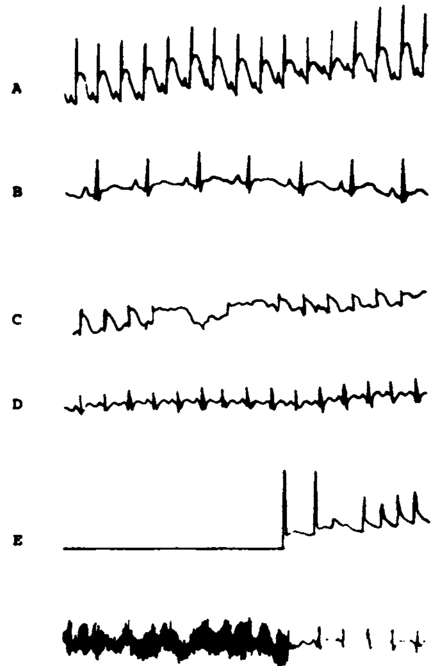

FIGURE 6. ECG changes in rabbits with ischemic myocardium during application of H_2O_2. A, 5 min after ligation of circumflex; note elevation of ST segment. B, 2 min after infusion of 0.48% H_2O_2 in 5% dextrose in water. C, 8 min after ligation of proximal and distal descending and circumflex branches of left coronary artery; note elevation of ST segment. D, 30 sec after direct application of 0.24% H_2O_2. E, fibrillation and arterial pressure drop to zero until 0.24% H_2O_2 was directly applied to the myocardium; at this point, beat resumed and arterial pressure rose to 50 mm Hg.

Myocardial Protection During Ischemia in Large Animals

To evaluate the effect of H_2O_2 in protecting a thicker ischemic myocardium, where diffusion factors better resemble those in humans, pigs weighing 40–80 kg were studied.[4,5] In a pilot series, the circumflex coronary artery, the circumflex in combination with the anterior descending coronary artery, or the anterior descending and right coronary arteries were ligated. Again, a standard lesion was difficult to establish, the most consistent ischemic lesion being produced by ligation of the right coronary artery with 50% of the anterior descending vessel.

Two groups of 10 pigs each were studied: Group 1 to evaluate the effect of H_2O_2 during coronary artery ligation and Group 2 to serve as controls. The animals were anesthetized with intravenous thiopental and curare. Tracheos-

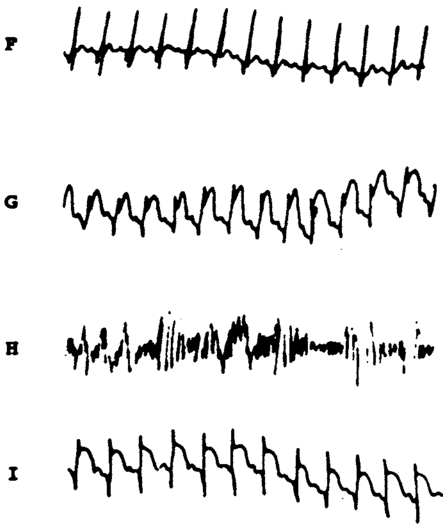

FIGURE 7. ECG changes in rabbits with ischemic myocardium during application of dilute H_2O_2. F, control ECG with chest open during 100% oxygen-breathing (blood pressure 140/110). G, 2 min after ligation of the distal descending circumflex branch of the left coronary artery (blood pressure 85/70). H, fibrillation 3.5 min after ligation (blood pressure zero). I, 5 min after direct application of 0.36% H_2O_2 and 20 min after onset of fibrillation (blood pressure 90/75).

tomies were performed and the pigs placed on a Bird respirator with 100% oxygen.

In Group 1, the sternum was split and pericardiotomy performed. Arterial cannulas were placed in the left femoral artery and vein for monitoring venous and arterial blood pressure. The ECG was constantly monitored, as was myocardial tissue pO_2 (by an IL-125 electrode inserted into the left ventricular myocardium). Two plastic #190 polyethylene catheters were sutured into the pericardium—one for inflow over the apex and the other for outflow near the atria anteriorly. The apical catheter was attached to a Harvard constant-infusion pump, and a steady flow of Hank's solution with 0.06% H_2O_2 at 37°C was constantly infused at the rate of approximately 400 ml/hour. After placement of the cannulas, the right coronary artery was ligated 1 cm from the ostia, and the anterior descending coronary artery was ligated at its midpoint on the anterior part of the heart. The hemiazygos vein was ligated, the pericardium was closed tightly, and infusion was begun. The apical catheter served as an outflow. Group 2 pigs (controls) were treated identically, except that Hank's solution without H_2O_2 was used.

In the 10 control animals (Group 2), ventricular fibrillation occurred between 2 and 32 min in all but one animal (average time 12 min). Resuscitation by cardiac massage and defibrillation was successful in seven animals, but failed in two. After return of regular sinus rhythm, mean blood pressure remained less than the original control value of 100 mm Hg in all but three animals, the average being 30–70 mm Hg mean arterial blood pressure. These animals developed ventricular fibrillation several times and could be resuscitated, but they never maintained a normal arterial blood pressure. In two animals which developed ventricular fibrillation and in one which did not, mean arterial blood pressure returned to 100 mm Hg. One of these animals survived for 2 hours before fibrillating and dying. The other two animals continued for 3 hours and were sacrificed. Survival time ranged from 2 min to 2 hours, the average being 20 min (excluding two animals which remained alive until sacrifice). Myocardial tissue pO_2 values were much lower than original control values in all cases, except for one animal which failed to fibrillate at all. Autopsy examination of all pigs revealed a large area of gross infarction over the lower right and left ventricles, with distal infarction in the septum.

In the Group 1 animals treated with 0.06% H_2O_2 in Hank's solution, five animals never developed ventricular fibrillation; in the other five, onset occurred between 5 and 30 min (average time 20 min). Resuscitation by cardiac massage and electrical defibrillation was successful in all cases. All but one maintained normal blood pressure (mean value 100 mm Hg) and normal to slightly elevated

tissue pO_2 in comparison to the control preligation values. The infarcted area was in a similar location to that of control animals, but it was much less extensive and limited primarily to the subendocardial area and the distal septum.

The serum sodium, potassium, calcium, and magnesium levels from the peripheral artery, vein, and coronary sinus (determined before, during, and near the end of each experiment) were too variable to permit any conclusions.

In all control animals, after a terminal episode when no blood pressure or cardiac activity was obtainable by standard resuscitative methods, dilute H_2O_2 solutions were added to the myocardium. In four animals, resuscitation was successful despite massive infarctions and systemic acidosis. Four animals failed to respond.

Although this method of application is limited by the diffusion of H_2O_2 through the thick myocardium, and although the septum does not appear to be protected by the epicardial application, the improved protection of the treated animals, when compared to controls, appeared significant. No toxicity was demonstrated during therapy.

Clinical Trial

Because of the apparent advantages in myocardial support during anoxia, H_2O_2 was used to treat a 60-year-old white woman for vascular collapse of unknown etiology, unresponsive to the conventional methods of resuscitation.[5] A cardiac catheter was passed through the right brachial artery to the route of the aorta. ECG and blood pressure were monitored. Within 1 min after the infusion of 0.12% H_2O_2, the ECG reverted from a nodal to a regular sinus rhythm, and the mean arterial pressure increased from 35 to 70 mm Hg within 3 min (Figure 8). Within 10 min after the infusion was stopped, the ECG again reverted to nodal rhythm and the blood pressure dropped. This sequence was repeated five times during a 12-hour period, reversal of ECG abnormalities and hypotension being achieved with each infusion of H_2O_2. The patient eventually died after H_2O_2 therapy was stopped.

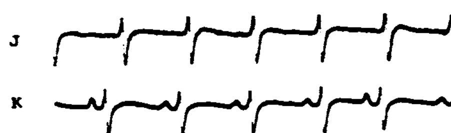

FIGURE 8. ECG tracings (lead II) in a hypotensive patient: J, before coronary infusion with 0.12% H_2O_2 (nodal rhythm) and K, 1 min after infusion (sinus rhythm).

Potentiation of the H_2O_2 Effect on the Ischemic Myocardium by Dimethyl Sulfoxide

Because of the diffusion limitation in humans due to the thick ventricle, and because of the vasoconstrictive effect when oxygen is given intra-arterially, we decided to try combining H_2O_2 with an agent such as dimethyl sulfoxide, which both increases diffusion and acts as a vasodilator in dilute solution.[5] The effectiveness of this combination was tested in rabbits.

Ten rabbits were anesthetized with sodium pentobarbital, intubated with an endotracheal tube, and placed on a Bird respirator with 100% oxygen. The left side of the chest was opened, the pericardium split, and the anterior descending coronary artery was ligated at its origin. Five different techniques were then applied, each on two rabbits: (1) Hank's carrier solution alone was applied directly to the epicardium, (2) 0.06% H_2O_2 in Hank's solution was applied to the epicardium, (3) 10% dimethyl sulfoxide was applied to the myocardium, (4) 0.03% H_2O_2 in Hank's solution was applied to the myocardium, and (5) 10% dimethyl sulfoxide with 0.03% H_2O_2 in Hank's solution was applied to the myocardium.

All 10 animals died by the end of a 2-hour period. The ventricle was then cut transversely, slides were made, and

the tissue was stained for 15 min with nitro-BT to detect the presence of lactic or succinic dehydrogenase (Figure 9). In the hearts where adequate oxygenation was maintained, the amount of lactic dehydrogenase was presumed normal in contrast to the ischemic hearts where this enzyme activity decreases.

These pilot experiments simply showed that 0.06% H_2O_2 could adequately maintain the rabbit myocardium, but a smaller percent could achieve almost the same protection when combined with dimethyl sulfoxide. Dimethyl sulfoxide alone was inadequate to maintain the ischemic myocardium.

Effect of H_2O_2 on Totally Ischemic Hearts During Aortic Cross-Clamping

In an effort to evaluate the effect of H_2O_2 on the totally ischemic heart of large animals with thick ventricles, dogs, calves, and goats were placed on cardiopulmonary bypass and the aorta was cross-clamped for 1–2 hours.[6] H_2O_2 was applied directly to the epicardium, perfused retrograde through the coronary sinus, infused through the coronary arteries, or instilled intraventricularly (or combinations of these methods were used). Experiments were carried out at normothermia to evaluate the effectiveness of H_2O_2 as the only source of oxygen for the heart. Twenty-five control animals (10 dogs, five goats, and 10 calves) were studied in similar fashion for comparative analysis.

All animals were anesthetized with endotracheal halothane and placed on total cardiopulmonary bypass, and the aortas were cross-clamped 60–120 min. The hemiazygos vein was ligated at its entry to the coronary sinus, providing a totally ischemic heart, except for the oxygen supplied by H_2O_2. The hearts were vented through the left and right ventricular apexes for decompression. After removal of the aortic cross-clamp, the coronary arteries were again perfused with

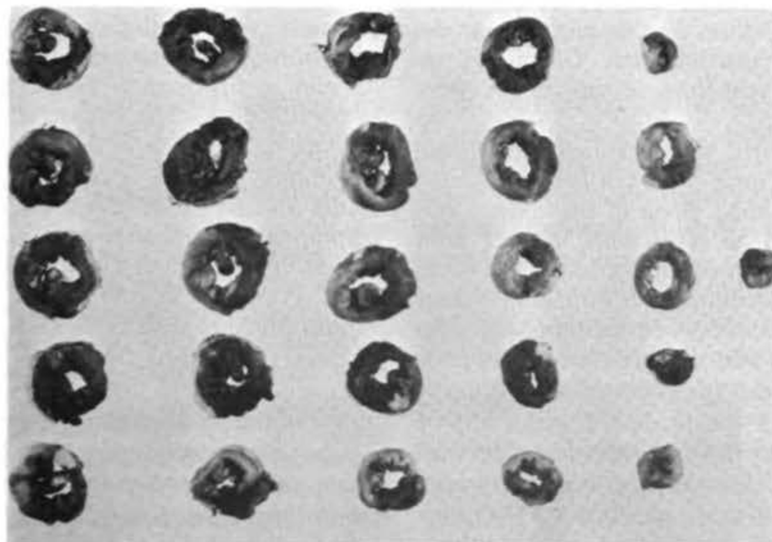

FIGURE 9. Changes in the ischemic rabbit myocardium with nitro-BT staining methods. Column 1 demonstrates treatment with the carrier solution alone, column 2 with 0.06% H_2O_2, column 3 with 10% dimethyl sulfoxide alone, column 4 with 0.03% H_2O_2 alone, and column 5 with a combination of 10% dimethyl sulfoxide and 0.03% H_2O_2. Dark-stained areas indicate the presence of lactic dehydrogenase.

blood, and sufficient time was allowed for normal cardiac rhythm to return without artificial resuscitation. H_2O_2 application (0.06–0.24%) began when the aortic cross-clamp was placed.

Initially, Ionosol T was the carrier solution, at pH 6.4, which is ideal for H_2O_2 decomposition into oxygen. This was changed to buffered 5% dextrose in saline solution, pH approximately 7.4, and finally to Ringer's lactate solution, pH 7.0, in the final experiments. The early experiments were conducted with intermittent lavage of the epicardium, the later ones with submersion of the heart in a constantly changing inflow and outflow solution of H_2O_2. Papaverine, 1 ampule/liter, was added to the coronary artery infusions.

Arterial and venous pressure and ECG were constantly monitored; venous pH was measured intermittently. Samples of the arterial, venous, and coronary sinus blood were drawn for sodium, potassium, and pH determinations before and after aortic cross-clamping. Relative myocardial and tissue pO_2 values were determined by a tissue pO_2 probe placed in the myocardium and monitored on the IL-125 oxygen recorder. Gross and microscopic pathologic examinations were performed.

The hearts of all animals fibrillated within 30 min, most fibrillating between 5 and 15 min. None of the control animals could be resuscitated after 1 hour of aortic cross-clamping, and most could not be resuscitated after 30 min. Average time of successful resuscitation in dogs was at 20 min of aortic cross-clamping; for calves and goats, it was approximately 40 min. In the treated animals, after 1 hour of aortic cross-clamping, reversal to regular sinus rhythm was fairly consistent with most methods of H_2O_2 application. Beyond this, however, protection was sporadic.

Most hearts required direct-current defibrillation and some required calcium and epinephrine injections into the aortic route to reverse ventricular fibrillation to regular sinus rhythm (Table 1). Coronary artery infusion for over 60 min was not protective when employed alone, and, when combined with epicardial application, the results were no better. Coronary sinus retrograde perfusion alone also failed to protect the heart. Epicardial application and coronary sinus perfusion protected 50% of the animals, some tolerating 100 min of aortic cross-clamping. The best protection was achieved with epicardial application, coronary sinus perfusion, and intraventricular instillation of H_2O_2. (Both of these animals were easily resuscitated, and their hearts reverted to regular sinus rhythm.)

Despite reversion to regular sinus rhythm, most of the animals could not maintain circulation off cardiopulmonary bypass, indicating only partial protection by the H_2O_2 and failure to support the whole heart during this length of ischemia at normothermia. Five calves maintained normal blood pressure when aortic cross-clamping lasted only 60 min, and these were long-term survivors of 2 weeks or more. Two dogs maintained normal blood pressure off cardiopulmonary bypass when aortic cross-clamping lasted less than 45 min, a significantly longer time than in control dogs. The long-term survivors showed no evidence of H_2O_2 toxicity.

Venous pressure during the experiments ranged from 2 to 20 cm H_2O before, during, and after cardiopulmonary bypass in the animals maintaining normal blood pressure. In those which did not, venous pressure rose to over 25 cm H_2O. Venous pH ranged from 7.50 to 7.38. Coronary sinus pH immediately after removal of the aortic clamp fell to as low as 7.0, but quickly returned to 7.2. Serum sodium, calcium, and potassium values in the coronary sinus and the systemic arteries and veins showed such wide fluctuations before and after aortic cross-clamping that no conclusions could be reached.

Myocardial tissue pO_2 levels in the H_2O_2-treated animals were equal to or

TABLE 1. Effects of Dilute H_2O_2 on Totally Ischemic Myocardium After Aortic Cross-Clamping and During Cardiopulmonary Bypass

Application Method	Animal	No.	Clamping time (min)	H_2O_2 conc. (%)	No. reversed to RSR	Circ. maint. off bypass
Direct to epicardium	Dog	1	60	0.24	1/1	1
	Calf	5	60	0.12	5/5*	5
	Calf	1	90	0.12	1/1	—
	Calf	1	120	0.12	1/1	—
Coronary artery infusion	Calf	3	90	0.06–0.12	1/3	—
	Dog	2	45	0.12	2/2	2
	Dog	1	60	0.12	0/1	—
Direct to epicardium+ coronary artery infusion	Calf	1	85	0.12–0.24	0/1	—
	Goat	1	75	0.06–0.12	0/1	—
Coronary sinus perfusion	Calf	1	100	0.24	0/1	—
Direct to epicardium+ coronary sinus perfusion	Calf	1	60	0.12–0.24	1/1	—
	Calf	1	100	0.12–0.24	1/1	—
	Goat	2	75	0.06–0.12	1/2	—
	Goat	1	110	0.06–0.12	0/1	—
Direct to epicardium+ coronary sinus perfusion+ intraventricular instillation	Goat	1	60	0.06–0.12	1/1	—
	Goat	1	78	0.06–0.12	1/1	—

RSR, regular sinus rhythm.
* All five calves survived > 2 weeks, the only animals in this study to do so.

greater than control levels during the ischemic phase. In the control groups, these values fell much below the original control values, in some animals reaching zero.

The epicardium appeared white after H_2O_2 oxygenation, but color returned on perfusion of the coronary arteries with blood. The opened hearts of the survivors had normal ventricular and atrial walls with occasional brownish areas in the septum. The hearts with prolonged ischemia, which failed to resume normal rhythm, showed moderate superficial edema, grossly normal atria, and normal ventricular walls two-thirds through their thickness from the epicardial surface. From there to the endocardial surface, the muscle appeared blue and ischemic except in the animals treated by intraventricular instillation of H_2O_2. In these hearts, the myocardium appeared normal. In the animals not treated by coronary artery or coronary sinus perfusion, the septum appeared bluish-black in areas, suggesting ischemia.

The microscopic findings were consistent with the gross pathologic appearance. Surviving animals demonstrated normal epicardial surfaces and ventricular walls, except for consistent minimal edema and hemorrhage limited to the subendocardial areas. The atria occasionally showed minimal hemorrhage. The hearts which could not be resuscitated demonstrated moderate edema and hemorrhage extending further from the endocardial surface. Control animals which received no H_2O_2 demonstrated marked edema and hemor-

rhage throughout both atrial and ventricular walls.

H_2O_2 appears to prolong the safe period of myocardial ischemia, but not consistently beyond 60 min. Survivors showed no evidence of H_2O_2 toxicity. The diffusion factors in the thick myocardium suggest that epicardial application or coronary artery infusion alone cannot protect the myocardium for long periods of time. Coronary artery infusion alone may carry the hazard of vasospasm despite administration of vasodilator agents such as papaverine. As the myocardium becomes thicker, problems of oxygen diffusion become more difficult. The conduction mechanism is not well protected by epicardial application alone, but H_2O_2 seems to have some value when given intra-arterially or retrograde by coronary sinus perfusion. The intraventricular H_2O_2 seems to aid diffusion through the thick-walled myocardium as well as through the septum.

The absence of blood flow through the heart to remove any waste products may be a problem in H_2O_2 application. Certainly other factors, such as carbon dioxide transport and acidosis, contribute to prolonged inadequate protection by H_2O_2 alone. This suggests that H_2O_2 may be an important adjunct in the treatment of myocardial ischemia and anoxia, but its application may be limited in total support of the bloodless heart.

Assisted Systemic Oxygenation with H_2O_2

Pilot attempts were made to administer dilute H_2O_2 to rabbits by three different techniques, to supply oxygen systemically: (1) by peritoneal perfusion, (2) by rectal perfusion, and (3) by ventilation with an H_2O_2 aerosol.

Peritoneal Perfusion of H_2O_2

For studies on peritoneal perfusion of H_2O_2, rabbits were anesthetized with sodium pentobarbital, intubated, and placed on an IPPB (intermittent positive-pressure breathing) apparatus with 100% oxygen in order to maintain a constant ventilation rate and 100% arterial saturation. A midline incision was then made to expose the portal vein, and a flanged PE-90 catheter was secured in the vein with a purse-string suture and withdrawn to the flange so as not to obstruct venous flow. A dialysis kit (Baxter K-128) was secured in the peritoneum and the wound closed. The peritoneum was perfused with warm dilute H_2O_2 solutions (0.12–0.48%), and blood samples were collected periodically from the portal vein.

The oxygen content of the portal vein blood during dialysis approximated that in the arterial blood. The carbon dioxide content of the venous blood could be adjusted by varying the pH of the buffer system of the H_2O_2. In the pilot studies, the carbon dioxide could not be adjusted to a degree necessary for systemic maintenance. We feel, however, that this may be possible after further investigation.

Rectal Perfusion of H_2O_2

Studies of rectal perfusion of H_2O_2 were conducted in dogs, and, as in rabbits, a catheter was placed in the portal vein for blood sampling. The large bowel was perfused via the rectum with dilute H_2O_2, and blood samples were collected from the portal vein during perfusion. As in peritoneal perfusion, the oxygen content of the portal vein blood approximated that of 100%-saturated arterial blood. No attempt has been made to date to adjust carbon dioxide content by this procedure.

Ventilation with an H_2O_2 Aerosol

Rabbits anesthetized with sodium pentobarbital were intubated and placed on 100% oxygen on a Bird respirator. The chest was opened between the fourth and fifth intercostal spaces, the pericardium slit, and a flanged PE-90 catheter secured

into the left atrium by a purse-string suture. The chest was closed and the animal allowed to breathe 100% oxygen for 30 min before aerosol H_2O_2 was given. Concentrations of H_2O_2 from 1% to 6% in normal saline were nebulized as an aerosol.

After nebulization therapy, the left atrial blood of these animals was found to be "supersaturated" with oxygen containing quantities equivalent to that expected with oxygen administration at 3 atm. When this value was exceeded, small bubbles began to appear in the samples. Figure 10 shows the effects of different H_2O_2 concentrations. An abrupt drop in atrial pO_2 occurred in the animal given 6% H_2O_2 by aerosol, but it was felt that this animal developed bronchospasm. The 1% aerosol, which was least irritating, provided as good arterial oxygen concentration as the higher concentrations.[7]

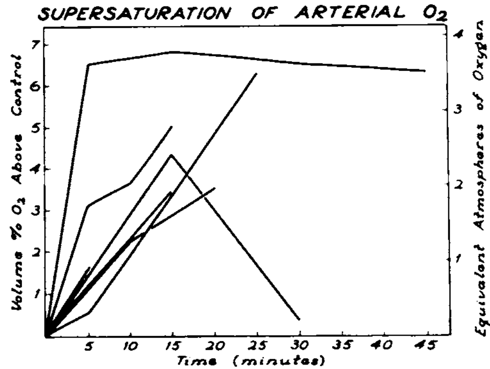

FIGURE 10. The increase in left atrial oxygen content after nebulization of 1–6% H_2O_2. Each line represents the response in one rabbit treated with an H_2O_2 concentration from 1% to 6%.

ACKNOWLEDGMENTS

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DISCUSSION

UNIDENTIFIED SPEAKER: Is there any rise in the bilirubin level during prolonged infusion of peroxide?

DR. URSCHEL: We have not seen hemolysis in any of the groups of patients which have been treated over a period of 6 weeks, or in animals that have been treated continuously for over 24 hours. We have seen one unexplained case in which hemoglobin changed affinity for oxygen—not methemoglobin, but a failure to pick up oxygen after prolonged massive peroxide therapy.

DR. MALLAMS: In a radiation series of nearly 200 patients, we have observed two cases of hemolysis. In both, the resulting low hemoglobin levels were reversed by transfusions. We completed their therapy in several weeks, and the hemolysis never recurred.

DR. A. R. BEHNKE, *Session Chairman (San Francisco, Calif.)*: What is the effect of intravascular peroxide on sickle cells?

DR. URSCHEL: We have had no experience with that.

DR. S. HUNTER (*St. Paul, Minn.*): We tried to repeat the work of the Dallas group in our laboratory in St. Paul, but after 3 or 4 weeks elapsed someone advised us that dogs have no catalase. I am reminded that Sauerbruch constructed a beautiful chamber in which to do chest surgery, and for a time it appeared that many of these tanks would be built throughout the world. Then an anesthesiologist came along with a hollow tube and obviated the need for the chambers. The Dallas group may be about to overcome the need for hyperbaric chambers with infusions of hydrogen peroxide.

DR. H. A. SALTZMAN (*Durham, N. C.*): There are obviously very exciting features in the use of hydrogen peroxide as an oxygen-delivery system. Oxygen is delivered in solution, and there is no problem of getting gas into solution—an intrinsic difficulty with many alternative delivery systems. In using hydrogen peroxide to improve oxygen transport, one must anticipate the formation of bubbles if the tension approaches or exceeds the unstable point of environmental pressure. Therefore, bends may be induced, even though these phenomena may not have been apparent in the systems employed thus far. Hydrogen peroxide may be very useful to the hyperbarists, however, as a convenient means of delivering oxygen to the blood, particularly when the lungs *per se* exchange gas poorly. At increased atmospheric pressure, one can maintain a stable solution of oxygen in blood without bubble formation at much higher tensions. Finally, the implication that oxygen diffuses well through a very thick tissue mass in the absence of a true capillary perfusion system deserves comment and challenge. Traditional concepts indicate that extravascular diffusion is severely limited. However, this new information requires that we change our present concepts concerning extravascular diffusion, or discover why Dr. Urschel obtained these very interesting findings.

DR. MALLAMS: I do not think the hyperbarists really need to worry, because we have our problems, too. For example, we are as yet unable to get sufficient circulation to the lungs to obtain the desired concentrations. Also, we are limited in our work to certain anatomical areas of the body. We are currently investigating the possibilities of combining hyperbaria and peroxide infusions in an effort to solve some of these problems.

Effect of Hyperbaric Oxygen on Gastric Secretion

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It is now known that when oxygen at high pressure is breathed, certain changes take place in the metabolism and blood supply to various tissues. Dickens,[1] Jacobson, Harper, and McDowall,[2] and Norman[3] have shown, for example, that oxygen consumption decreases in various tissues exposed to elevated oxygen pressures—probably, it is suggested, due to inactivation of thiol-containing enzymes. Following is a report of the effects of oxygen-breathing at pressures up to 2 ata on gastric secretion in rats.

METHODS

Male rats of the hooded Lister strain, weighing 175–225 gm, were divided into groups of 10 for experimental studies. Simultaneous control studies were always carried out on rats breathing room air. All rats were starved for 24 hours before the experiment was begun, as well as throughout the experiment itself, but water was freely available at all times. Gastric secretion was measured by the Shay pylorus ligation technique.[4] The test rats were placed in a small experimental pressure chamber, while the control rats were left on the bench outside. The chamber was flushed with oxygen until the effluent gas was shown to consist of 100% oxygen by measurement with a Beckman oxygen analyzer. The pressure in the chamber was then raised with more oxygen, and carbon dioxide was absorbed by means of a tray of soda lime. The highest carbon dioxide concentration reached in the chamber gas by the end of any experiment was 0.4%.

First, groups of 10 rats were exposed for 9-hour periods to oxygen pressures of 300, 760, 1314, and 1520 mm Hg; then, the gastric acid secretion of each rat during the 4 hours following exposure was measured and compared to that of its control counterpart which breathed room air. Previous work in this department has shown that exposure of rats to oxygen at 2 ata for 9 hours is close to the dose required to produce oxygen poisoning. Therefore, another group of rats was exposed to 2 ata of oxygen for 5.5 hours to detect possible changes in acid secretion following a shorter exposure.

In order to determine the duration of the depression in acid secretion, additional groups of rats were exposed to 2 ata of oxygen for 9 hours, and the total acid secretion was measured 1 hour, 24 hours, 48 hours, and 168 hours later. Finally, the effect of histamine on the acid secretion of rats exposed to 2 ata of oxygen for 9 hours was determined. Following pyloric ligation, histamine acid phosphate in a dose

of 3.6 mg/kg was given subcutaneously. This injection was repeated at hourly intervals for 3 hours, and the acid secretion was measured 1 hour after the last injection.

RESULTS

Figure 1 shows the total acid secretion of rats following exposure for 9 hours to various pressures of oxygen, compared in each case to the acid secretion found in the control groups breathing air at 1 ata. When the pressure of the inspired oxygen was 1314 or 1520 mm Hg, a highly significant difference was seen between test and control rats ($P < 0.001$ in each case). There was, however, no statistical difference in acid secretion between test and control rats when the pressure of inspired oxygen was 300 or 760 mm Hg. An oxygen tension of 300 mm Hg was obtained by pressurizing the chamber to 2 ata with air instead of oxygen. The absence of any difference in acid secretion between the test group and the controls indicates that compression to 2 ata alone did not alter acid secretion.

Table 1 shows the total acid secretion in a group of 10 rats exposed to oxygen at 2 ata for 5.5 hours, compared to that of control animals breathing air at atmospheric pressure. It can be seen that little difference in acid secretion occurred between the test and control groups when

TABLE 1. Mean Gastric Acid Secretion in 10 Rats Exposed to 2 ata Oxygen for 5 Hours Compared to That of Controls Breathing Air

Gas inspired	Total acid secretion (mEq/liter/100 gm weight)
Air at 1 ata	0.214
O_2 at 2 ata	0.191

oxygen was breathed for this period of time, and what difference did exist was not statistically significant.

In order to detect the duration of the depression in acid secretion, further groups of rats were exposed to 2 ata of oxygen for 9 hours, and the total acid secretion was measured 1 hour, 24 hours, and 168 hours later. The results may be seen in Figure 2, which shows the highly significant difference in acid secretion measured 1 hour and 24 hours following exposure ($P < 0.001$ in both cases). At 48 hours and 168 hours, no difference was seen between control and test animals.

In order to exclude the possibility that hyperbaric oxygen may have stimulated acid secretion during the early part of the exposure, so that the parietal cells were exhausted by the time secretion was measured, we ligated the pylorus in each of a group of rats before a 4-hour exposure to 2 ata of oxygen. The results indicated that no difference occurred between test and

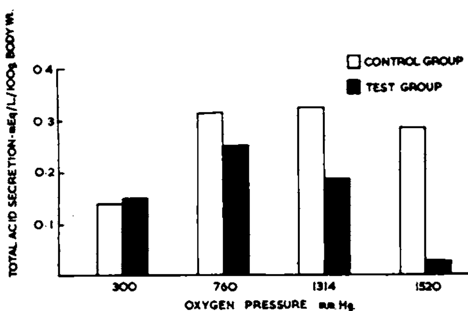

FIGURE 1. Gastric acid secretion in rats exposed to various oxygen pressures for 9 hours compared to that of controls breathing air.

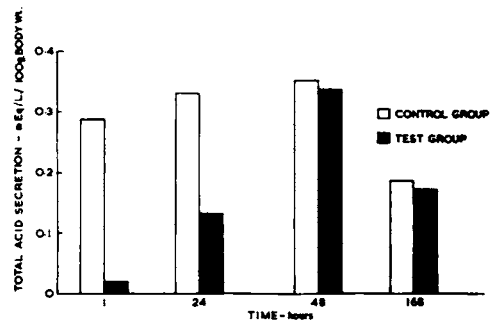

FIGURE 2. Gastric acid secretion in rats exposed to 2 ata oxygen for 9 hours compared to that of controls breathing air. Acid secretion was measured at various times after exposure.

TABLE 2. Mean Gastric Acid Secretion in 10 Rats Exposed to 2 ata Oxygen for 4 Hours Compared to That of Controls Breathing Air [a]

Gas inspired	Total acid secretion (mEq/liter/100 gm weight)
Air at 1 ata	0.268
O_2 at 2 ata	0.249

[a] Before treatment the pylorus was ligated.

control animals (Table 2). It was thus concluded that oxygen at 2 ata did not stimulate acid secretion.

Table 3 shows the effect of histamine on the acid secretion of rats exposed for 9 hours to oxygen at 2 ata. The table shows the total acid secretion in test rats given histamine, in control rats given histamine, and in control rats not given histamine. It can be seen that the administration of histamine to test animals resulted in an acid secretion which was even less than that found in the control rats not given histamine.

TABLE 3. Effect of Histamine Administration on Gastric Secretion in Rats After 9-Hour Exposure to Oxygen at 2 ata

Rats	Total acid secretion (mEq/liter/100 gm weight)
Test with histamine (after 9 hr of 2 ata O_2)	0.107
Controls with histamine (air-breathing at 1 ata)	0.504
Controls without histamine (air-breathing at 1 ata)	0.218

Finally, histologic studies were performed on the gastric mucosa from these animals, and no gross changes were found.

SUMMARY

Exposure of rats to 2 ata of oxygen for 9 hours depresses the total acid secretion. A lesser effect can be detected after exposure to 1.5 ata of oxygen for 9 hours, while oxygen at 1 ata causes no detectable effects following 9 hours of exposure. The effect is not found following a 5.5-hour exposure to 2 ata of oxygen; the depression in acid secretion persists for 24 hours but cannot be detected 48 hours after exposure.

Depression in acid secretion is due neither to the physical effects of compression nor to stimulation of acid secretion during exposure to oxygen followed by exhaustion at the time of measurement. Histamine does not restore the depressed secretion, and no gross histologic changes have been detected.

It seems likely that the decrease in acid secretion is due to a change at the level of the parietal cell in the gastric mucosa, possibly in the nature of enzymic depression.

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DISCUSSION

DR. A. R. BEHNKE, *Session Chairman* (*San Francisco, Calif.*): Has this been tried in man?

DR. CARIDIS: No, it has not.

DR. BEHNKE: It would be easy to do. At 4 ata, when respiration in the dog ceases, and carbon dioxide builds up in the tissues and lungs during the course of an hour, in addition to a decrease in gastric secretion, there is also anuria. Do you have any data which elucidate the mechanisms involved in these phenomena?

DR. CARIDIS: We have not yet determined the exact cause of this. This is what we are doing at the moment. It is a bit difficult to measure the acid–base balance adequately in rats, but we have measured the acid–base state of the heart blood in six of these animals, and found no gross abnormality. I feel that it will probably prove to be some basic change at the level of the parietal cell. We have looked at other constituents of gastric secretion and have found no change in the secretion of pepsin—only a reduction of acid and water. In a few preliminary experiments which we have carried out, neither bile nor pancreatic secretions seem to be altered in any way.

DR. H. C. URSCHEL (*Dallas, Texas*): We have run a pilot study on this same basis using peroxide in the stomach, and our data showed no effect on gastric ulcers.

DR. CARIDIS: It should be pointed out that this effect may not have therapeutic value in the long run as it can only be induced by near-toxic dose levels of oxygen. We are interested now in finding out the effect of short exposures to hyperbaric oxygen administered over several days to see whether gastric secretion can be reduced and maintained at a relatively low level.

Electroencephalographic Changes Induced by 100% Oxygen-Breathing at 3 ata in Awake Man

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Laboratory of Clinical Science, National Institute of Mental Health, and
Naval Medical Research Institute, Bethesda, Maryland

The cerebral circulation of man is controlled by such variables as arterial carbon dioxide and oxygen tensions, perfusion pressure, temperature, and the administration of drugs. It is well known that the diminished arterial pCO_2 produced by hyperventilation results in a decreased cerebral blood flow and jugular venous oxygen tension in both awake [1] and anesthetized [2-4] man. In addition, respiratory alkalosis occurring during hyperventilation shifts the hemoglobin dissociation curve to the left, making the transfer of oxygen to the tissues more difficult (Bohr effect). In considering these factors, many investigators [5-10] have suggested that hyperventilation may produce significant cerebral ischemia. Other workers,[11-13] however, are of the opinion that the cerebral effects of hyperventilation represent only the effects of diminished tissue pCO_2 and have largely discounted any role played by ischemia.

The goal of the present investigation was to separate the effects of low cerebral pO_2 from those resulting from low tissue pCO_2 in awake hyperventilating man. These studies have been conducted in the diving chamber at the Naval Medical Research Institute in Bethesda, Maryland.

METHODS

Eight normal male volunteers (20–49 years old) were studied under two experimental conditions. First, they were studied during hyperventilation at 3 ata breathing 7% oxygen in nitrogen (inspired pO_2 160 mm Hg). This served to lower jugular venous pO_2 to the same extent as would have occurred during hyperventilation in air at normal ambient pressure. Second, they hyperventilated at 3 ata breathing 100% oxygen (inspired pO_2 2280 mm Hg). This served to return jugular venous pO_2 to normal while hyperventilation continued and arterial pCO_2 remained at the same low level. Control measurements were also made during normal respiration after pressurization had been accomplished.

Following control measurements, voluntary hyperventilation was begun and continued for 9 min. Initially, either 7% or 100% oxygen was administered. After 3 min, the oxygen concentration was abruptly changed while hyperventilation continued. Following another 3 min of hyperventilation, the oxygen concentration used initially was again administered for a final 3 min. After a rest period of

several minutes, during which time control measurements were again obtained, the subject again hyperventilated for 9 min. During this time, a similar study was performed, but the order in which the gases were supplied was reversed.

During the entire study period, a standard eight-channel electroencephalogram (EEG) was continuously recorded and later interpreted by one of us (M. R.). The end-tidal carbon dioxide concentration was constantly monitored by infrared analysis. During hyperventilation, the end-tidal carbon dioxide concentration of each subject remained constant and ranged from 6 to 25 mm Hg.

Results

Two of the study subjects showed no alterations of the EEG at any time during hyperventilation. Five evidenced significant EEG changes (slowing to the theta and delta frequencies) *only* during hyperventilation with 7% oxygen (inspired pO_2 160 mm Hg). The EEG rapidly returned to normal upon administration of 100% oxygen, although the end-tidal carbon dioxide concentration remained unchanged (Figures 1–4). One subject (with the lowest recorded end-tidal pCO_2) showed slight abnormalities in his EEG during hyperventilation with 100% oxygen. The most pronounced slowing in all subjects, however, occurred after the administration of 7% oxygen (Figure 5).

No untoward events occurred during any phase of this study.

Summary

Significant EEG alterations were produced in awake man by hyperventilation with an air-equivalent gas mixture. A normal EEG pattern was consistently restored by administration of 100% oxygen at 3 ata while hyperventilation continued and end-tidal pCO_2 remained constant. We concluded, therefore, that the EEG changes during hyperventilation represented mild and reversible cerebral hypoxia rather than a direct effect of diminished tissue pCO_2.

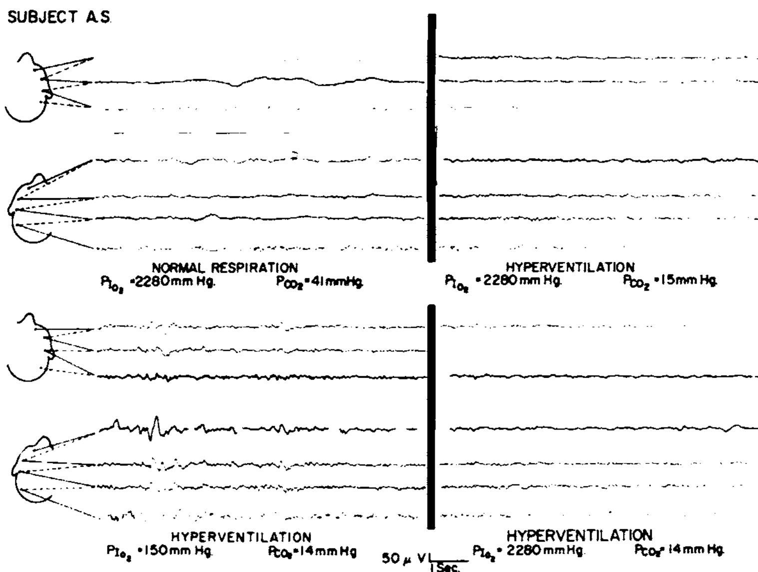

FIGURE 1. Effect of hyperventilation on the EEG of awake subject (A.S.). Note slowing only during hyperventilation with an air-equivalent gas mixture.

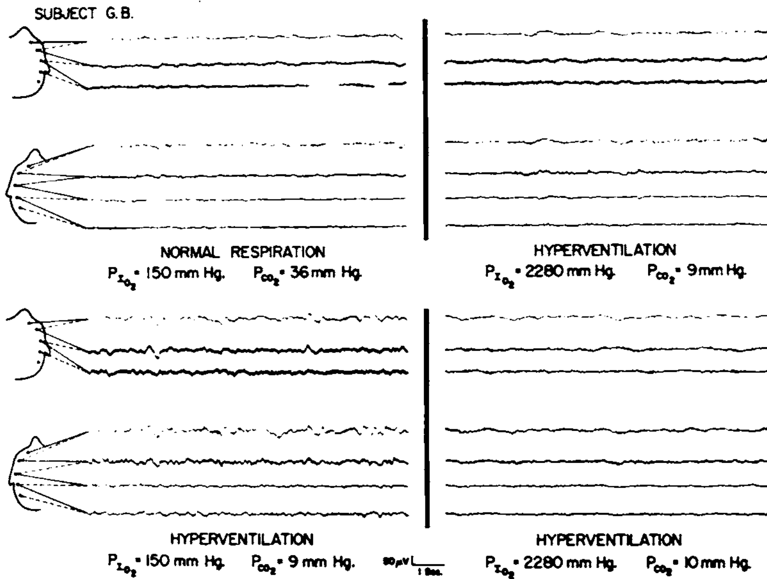

FIGURE 2. Effect of hyperventilation on the EEG of awake subject (G.B.). Slowing occurred only during hyperventilation with a normal inspired oxygen tension. No abnormalities occurred during hyperventilation with an elevated inspired oxygen tension.

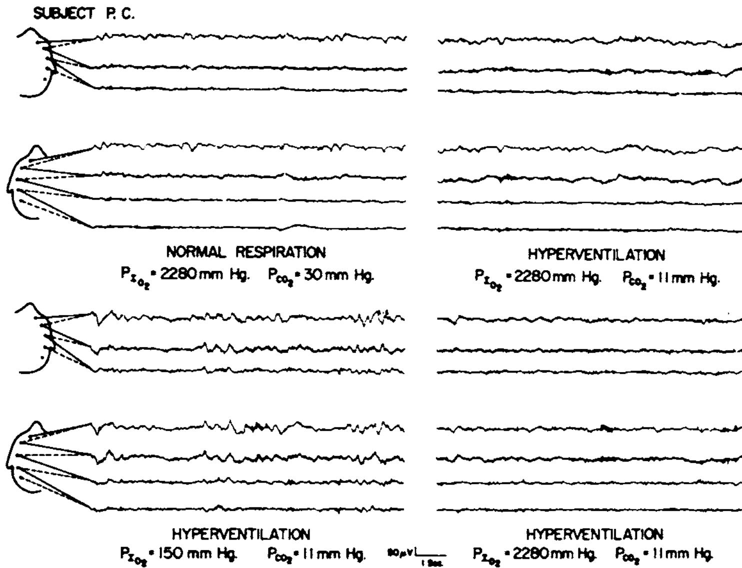

FIGURE 3. Effect of hyperventilation on the EEG of awake subject (P.C.). Slowing occurred only during hyperventilation with an air-equivalent gas mixture.

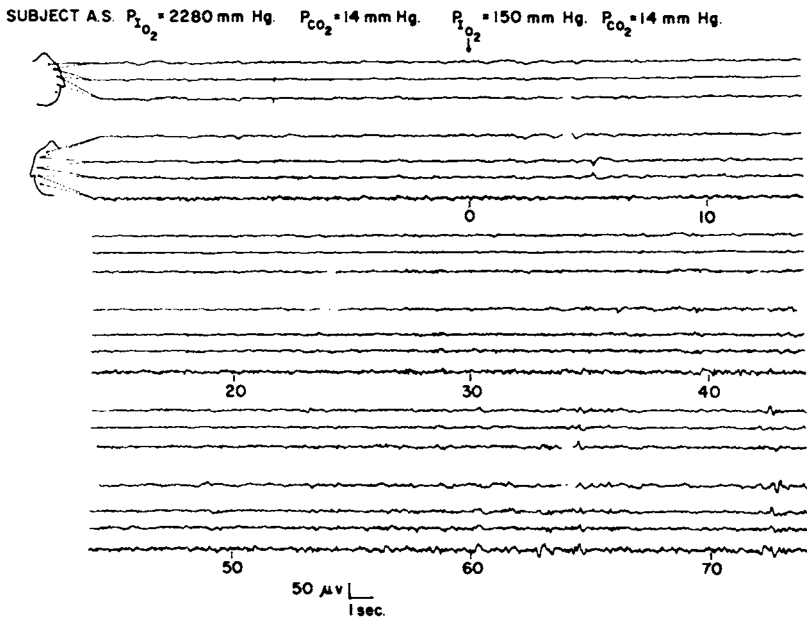

FIGURE 4. Continuous EEG record of awake subject (A.S.) showing the change during hyperventilation when the inspired oxygen tension was reduced to normal. The numbers below the record indicate the time in seconds following the transition from 100% to 7% inspired oxygen. Definite alteration of the EEG is seen between 50 and 60 sec after administration of the air-equivalent gas mixture.

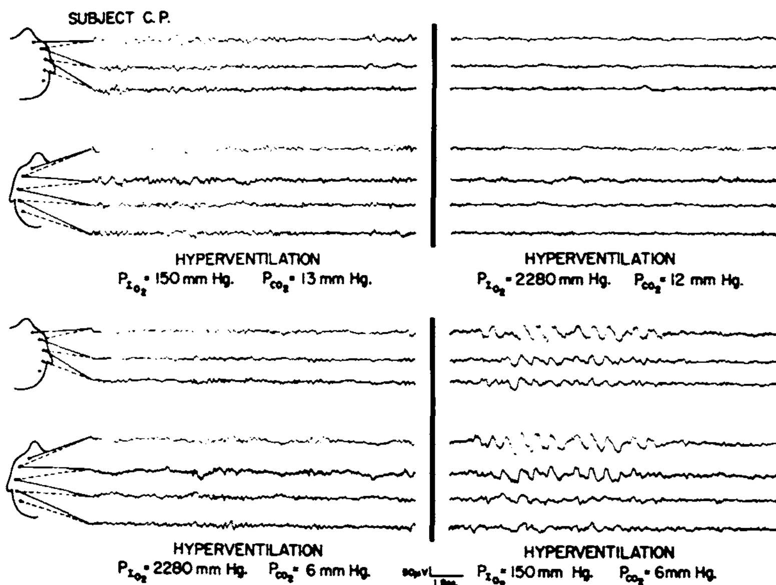

FIGURE 5. Effect of hyperventilation of the EEG of awake subject (C.P.). When end-tidal pCO_2 was 12–13 mm Hg, EEG abnormalities occurred only during hyperventilation with normal inspired oxygen tension. When end-tidal pCO_2 was 6 mm Hg, slight slowing took place during hyperventilation with 100% oxygen, while extreme slowing occurred during inhalation of a normal oxygen tension.

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DISCUSSION

DR. C. SEM-JACOBSEN (*Oslo, Norway*): I am very much interested in this problem, but I have attacked it from an entirely different point of view. This was a study I carried out about 10 years ago at the Mayo Clinic. We did not have a hyperbaric chamber, but we increased the supply of oxygen to the brain by increasing the blood sugar, and we showed in our studies that changes occurred in the EEG during hyperventilation. After having done an initial EEG record with hyperventilation, we gave the subjects glucose by mouth. There was a very temporary increase in their blood sugar to more than 200 for a short period. We found that as long as the blood sugar was down to a level of about 150 we still got the hyperventilation effect in the EEG, but at the peak with a blood sugar level of above 200, there was a complete disappearance of the changes seen earlier in response to hyperventilation. So, our findings at that time, although obtained by an entirely different method, are in complete agreement with your findings.

DR. COHEN: I think this is an interesting finding. The adequacy of cerebral metabolism, of course, depends basically on oxygen and glucose supply. I think we have seen two things: first, during the period of hyperventilation, when presumably oxygen availability is curtailed, one can supply the oxygen and restore an EEG to normal; and second, in the same situation when the brain perhaps requires an increased degree of anaerobic metabolism, a far less efficient form of metabolism requiring a good deal more

glucose per unit energy obtained, one can administer glucose and satisfy this increased substrate requirement. One of the authors of this paper has done similar experiments with the hypoxic activation of the EEG by breathing 5% oxygen. Also, if one gives glucose to these subjects, it takes a longer period to activate the EEG while they are breathing at hypoxic atmospheres.

DR. A. R. BEHNKE, *Session Chairman (San Francisco, Calif.)*: Oxygen at 4 ata will not raise blood sugar in the dog. Of possible interest is a patient whose petit mal seizures were regularly induced by hyperventilation, presumably as a result of decreased cerebral blood flow induced by hypocapnea. At the time it was anticipated that the administration at 4 ata would be beneficial. The results, however, were negative (Arch. Neurol. Psychiat. [Chicago] 35:782, 1936).

Effects of Increased Atmospheric Pressure During Acute Phase of Chronic Respiratory Failure

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Studies on healthy adults have established that an increased effort is required to ventilate the lungs at raised environmental pressures.[1-3] There is no reason to believe that the same increased effort will not be required for ventilation in patients with emphysema. However, some evidence is available to support the contention that the increased residual volume normally found in such individuals may be reduced after exposure to hyperbaric conditions. In contrast to the divisions of lung volume in the normal individual (Figure 1), in the emphysematous patient the residual volume is increased at the expense of the expiratory reserve volume, thus effectively reducing vital capacity. Yanda[4] has demonstrated that an increase in the expiratory reserve volume (and therefore in the vital capacity) takes place in such patients after exposure to hyperbaric air.

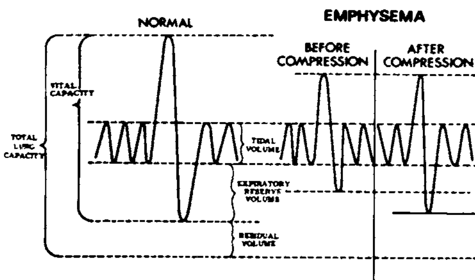

FIGURE 1. Divisions of lung volume in normal persons compared with those in patients with emphysema.

Our interest in the effects of increased atmospheric pressure upon such patients (reported in preliminary form at the Second International Conference[5]) concerns the acute phase of their disease. Rather than concentrating upon lung volume studies, which are virtually impossible to obtain at this stage of the disease, we have emphasized blood gas analysis and clinical observation.

Methods and Results

The effect of administration of 40% oxygen at normal pressure was compared with that of air-breathing at 2 atm, the two being, of course, equivalent in terms of inspired oxygen pressure. While we appreciated that 40% oxygen may exceed the indicated inspired concentration of oxygen in some patients because of the

depressed sensitivity of their respiratory center to a rise in arterial pCO_2 and their dependence on a hypoxic drive to respiration, we were restricted to a fixed pressure of 2 atm in the smaller of our two pressure vessels during the early phase of this study. The patients, moreover, were under the closest continuous observation throughout.

Figure 2 shows the blood gas data from one of the first few patients studied during the three situations of air-breathing at 1 atm, 40% oxygen-breathing at 1 atm, and finally air-breathing at 2 atm. During air-breathing at normal pressure, this patient had a low oxygen saturation and a high pCO_2. With 40% oxygen inhalation at normal pressure, the arterial oxygen saturation rose, but so did the arterial pCO_2 (to over 120 mm Hg). A subsequent exposure to 2 atm of air was

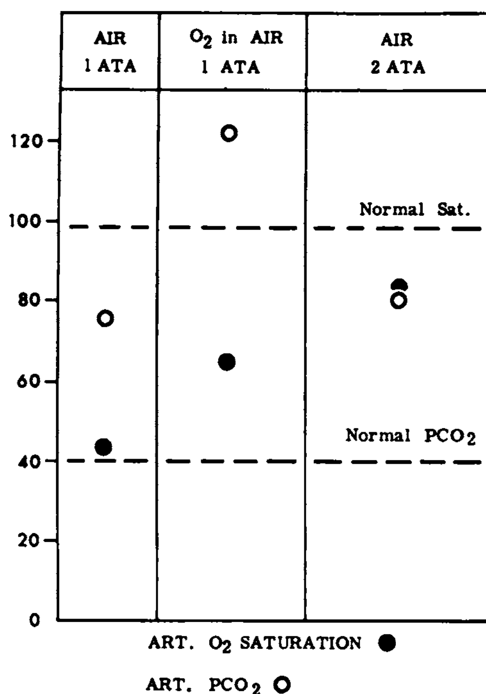

FIGURE 2. Arterial oxygen saturation and pCO_2 values in a patient with chronic respiratory failure during air-breathing at 1 atm, 40% oxygen-breathing at 1 atm, and air-breathing at 2 atm.

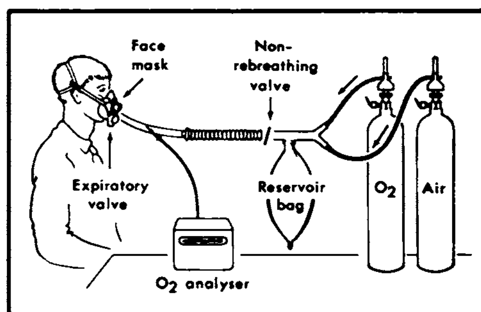

FIGURE 3. Mask circuit for oxygen-breathing and air-breathing.

associated with a greater rise in oxygen saturation. The arterial pCO_2, on the other hand, was not significantly altered in this situation.

Certain deficiencies emerged in this early work which prompted more careful planning of studies, including the use throughout of a mask circuit (Figure 3). Oxygen and air passed through flowmeters into a mixing reservoir bag, through a non-rebreathing valve and corrugated tubing to a mask from which expired air was totally exhausted through an expiratory valve. In this way, any concentration of inspired oxygen might be achieved and measured carefully with an oxygen analyzer. The total system was retained during air-breathing at 2 atm with the oxygen turned off, in order to maintain a constant circuit dead space.

Figure 4 shows the changes produced in the arterial pO_2 and pCO_2 of a patient,

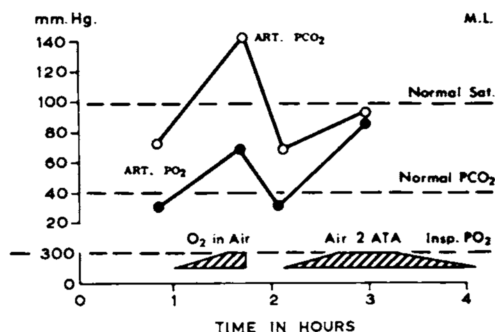

FIGURE 4. Blood gas changes in a patient with chronic respiratory failure in the two test situations compared with air control values.

first during oxygen-enriched air-breathing at 1 atm, and second during air-breathing at 2 atm. In both cases, the maximal inspired oxygen pressure reached was 300 mm Hg, and this level was attained slowly over a period of exactly 30 min. Between the two exposure periods, the blood gases were allowed to return to the base-line levels. In this patient, the arterial pO_2 rose from 30 to over 60 mm Hg in oxygen-enriched air at normal pressure, but the arterial pCO_2 rose to over 140 mm Hg. With air-breathing at 2 atm, despite a more obvious increase in the arterial pO_2 to over 80 mm Hg, the arterial pCO_2 did not exceed 100 mm Hg.

In another patient (Figure 5), the blood gases followed a slightly different pattern of events; the arterial pO_2 value with oxygen-enriched air at normal pressure was higher than the value obtained with air at 2 atm, although the latter was entirely satisfactory (saturation equivalent of over 90%). The arterial pCO_2, on the other hand, hardly altered with air at 2 atm, although it rose to very high levels with oxygen-enriched air at normal pressure. The other data indicated that the pCO_2 was very high about 1 hour after decompression with the patient breathing air at normal pressure. This was alarming at the time, but it has since been found that the addition of a small amount of oxygen during decompression or prolongation of the phase of decompression will prevent this feature. Figure 6 demonstrates the persistence of the im-

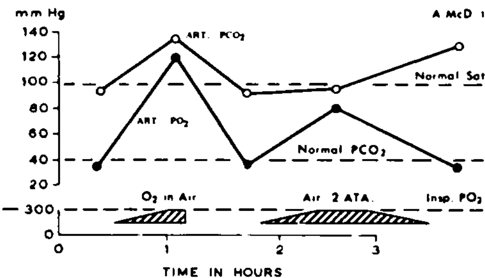

FIGURE 5. Blood gas changes in a patient with chronic respiratory failure in the two test situations compared with air control values.

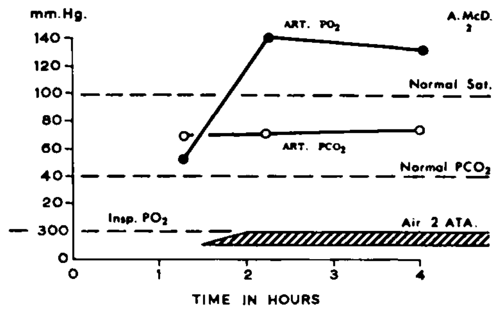

FIGURE 6. Evaluation of effect of time on blood gas values in a patient with chronic respiratory failure (see also Figure 5) during air-breathing at 2 atm.

proved arterial pO_2 obtained as a result of air-breathing at 2 atm; this value may be expected to remain stable for a period of time without a significant increase in arterial pCO_2 (in the case of this patient, over 2 hours).

Table 1 summarizes the arterial pO_2 and pCO_2 values during the three situations of air-breathing at 1 atm, 40% oxygen-breathing at 1 atm, and air-breathing at 2 atm in seven patients with moderate to severe chronic bronchitis and emphysema. During 40% oxygen-breathing at 1 atm, there was a mean rise in arterial pO_2 of 46 mm Hg associated with an increase of 30 mm Hg in arterial pCO_2, whereas during air-breathing at 2 atm there was a mean increase in arterial pO_2 of 60 mm Hg but a mean increase of only 6 mm Hg for pCO_2. Expressed as an arbitrary ratio of rise in pCO_2 to rise in pO_2, a value of 0.65 was obtained for 40% oxygen-breathing at 1 atm and a value of 0.12 for air-breathing at 2 atm.

Since the new, more versatile pressure chamber has become available, continued observations on such patients have revealed information of a more practical, clinically acceptable nature. In one patient (Figure 7) with moderate chronic bronchitis and emphysema, for example, exposure to gradually increasing concentrations of oxygen resulted in an arterial pO_2 increase to 100 mm Hg at a level of 30% inspired oxygen, with no concomi-

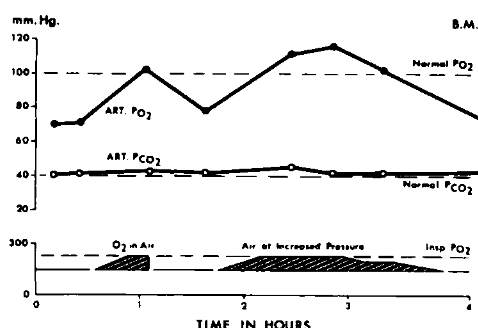

FIGURE 7. Manipulation of chamber pressure to produce arterial oxygen tension values in a patient with chronic respiratory failure identical to those during oxygen-enriched air-breathing at normal pressure.

tant increase in arterial pCO_2. At the equivalent air pressure which would give an identical inspired oxygen pressure (7.5 psig) the arterial pO_2 was 135 mm Hg—again without an elevation of the arterial pCO_2. An arterial pO_2 similar to that obtained with 30% oxygen was achieved later by slow decompression to 5 psig.

SUMMARY

1. Pulmonary function studies suggest that the increased residual volume associated with emphysema may be reduced following exposure to high pressures of air.

2. Measurements of blood gas responses to increased pressures of air in a carefully controlled manner indicate that ventilatory efficiency undergoes greater improvement during pressurization than in comparable inspired oxygen tensions at normal pressure. Studies are currently in progress to elucidate the mechanism of this apparent effect.

3. Any technique is advantageous which can produce effective and safe increases in arterial pO_2 and which does not at the same time necessitate the use of individual oxygen masks and humidification systems.

TABLE 1. Arterial Gas Tensions in Chronic Respiratory Failure

Patient	Air 1 ata		40% O_2 in air [a] 1 ata		Air [b] 2 ata	
	pO_2	pCO_2	pO_2	pCO_2	pO_2	pCO_2
A.M.	34	95	122	135	84	95
M.L.	31	75	68	145	88	100
J.R.	66	47	144	75	150	53
M.M.	72	53	108	59	172	53
C.A.	50	64	82	73	103	74
A.G.	40	43	69	57	83	45
M.C.	28	75	48	120	65	78
	Mean rise		46	30	60	7

[a] Rise in pCO_2/rise in $pO_2 = 0.65$.
[b] Rise in pCO_2/rise in $pO_2 = 0.12$.

ACKNOWLEDGMENTS

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DISCUSSION

DR. A. R. DOWELL (*Durham, N. C.*): In the light of Dr. Ledingham's interesting paper, I might add that Dr. Kylstra and I have studied eight patients with chronic obstructive lung disease at 1 and 3 ata. We determined the physiological dead space for oxygen and carbon dioxide, using the Bohr equation. We tried to keep the inspired pO_2 constant, as you have done at both 1 and 3 ata. We have had the patients on different breathing mixtures, including 20% oxygen–helium and 60% oxygen–nitrogen. We found that at increased pressure there was no significant increase in the physiological dead space for carbon dioxide, but there was a definite increase in the physiological dead space for oxygen. Five of the eight patients studied said there was definite improvement in their breathing at increased pressure, even though arterial pCO_2 values rose and the overall oxygen dead space increased.

DR. LEDINGHAM: The last comment that you made was of particular interest to us. I have kept myself strictly to the gas analysis figures because this is what we are looking for in the way of objective improvement. The patients themselves all agreed that they felt clinically better in the chamber. Of course, one can understand this in view of the difficulty and the discomfort that most people with this type of disease have in wearing any form of mask. However, we do not wish to stress this subjective improvement at the moment.

UNIDENTIFIED SPEAKER: Under hyperbaric conditions, when the pO_2 is increased and the pCO_2 remains the same, what is the state of consciousness of these patients?

DR. LEDINGHAM: This is very interesting, and it has a great bearing upon the phenomenon described as carbon dioxide narcosis. We can see that for an equivalent high pCO_2 level, if one can manage to raise the arterial oxygen tension, the patients are very obviously better oriented. Incidentally, the pH, which would normally be down because of the associated metabolic acidosis, tends to improve and we get an alteration in the metabolic changes toward the alkaline side.

Xenon as an Inhalation Contrast Medium Under Hyperbaric Conditions

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Xenon, an inert gas with an atomic number close to that of iodine and barium, offers promise for use as a radiographic contrast medium. In previous investigations of the radiopacity of xenon reported in the literature,[1-3] it was concluded that while xenon is slightly radiopaque with respect to air at atmospheric pressure, it is too faint to be of any practical use. We have shown, however, that when xenon molecules are concentrated (by compression in syringes) this gas is a good contrast medium with respect to air. Control experiments compressing air instead of xenon showed no detectable change in radiopacity of air with increasing pressure, but xenon at 3.3 ata had the same radiopacity as water or tissue. At this or higher pressures, xenon might indeed act as an effective inhalation contrast medium.[4]

For a more anatomic demonstration of the possibilities for xenon as an inhalation contrast medium, a small Lucite hyperbaric chamber for use with the excised lungs of dogs was constructed (Figure 1). A schematic diagram of operation of this chamber appears in Figure 2. The chamber is pressurized and the lung inflated

FIGURE 1. Hyperbaric chamber for excised dog lung.

with compressed air from a cylinder with valve A open. Chamber pressure is controlled by pressure gauge C and constant-pressure valve D. To allow xenon to flow into the lung, valve A is closed and valve B is open. The pressure of xenon inflow,

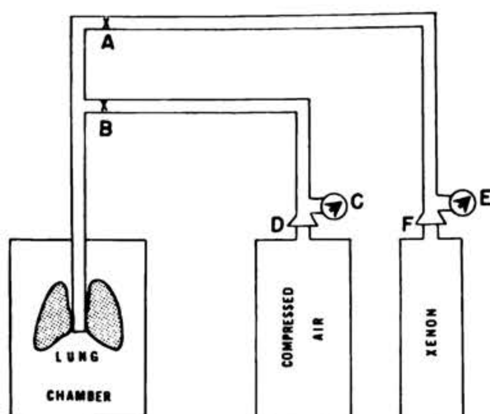

FIGURE 2. Schematic diagram of operation of hyperbaric chamber for excised dog lung. A and B are two-way open and close valves, D and F are constant-pressure valves, and C and E are pressure gauges.

which, of course, must be somewhat higher than chamber pressure, is set with pressure gauge E and constant-pressure valve F. Radiographs made during an experiment using this system appear in Figure 3.

DISCUSSION

Xenon is not a complete stranger to medicine. As a result of the considerable interest in the anesthetic properties of this gas, much is known of its pharmacology. At normal atmospheric pressure, xenon has about the same anesthetic potency as ethylene.[5] No untoward side effects have been noted from xenon anesthesia in either humans or animals.[6] Obviously, the anesthetic potency of this gas has implications for any radiographic applications.

The work of Pittinger *et al.*[7] has been of particular interest to us. These investigators anesthetized monkeys inside a hyperbaric chamber in order to investigate the anesthetic properties of xenon at increased partial pressures. The partial pressure of oxygen was kept constant at 150 mm Hg throughout the range of pressures used. Apnea was induced at a partial pressure of xenon of 1550 mm Hg, and at 2250 mm Hg the monkeys were described as "areflexic, apneic, and completely relaxed." Recovery from anesthesia was uneventful.

Following are some suggested possible applications of xenon as a contrast medium. In bronchography, a bolus of xenon could be inhaled at various phases of inspiration and its course followed through the bronchial tree with serial films or cineradiography. Since xenon is a gas, behaving much as the inhaled air, physiologic as well as anatomic information

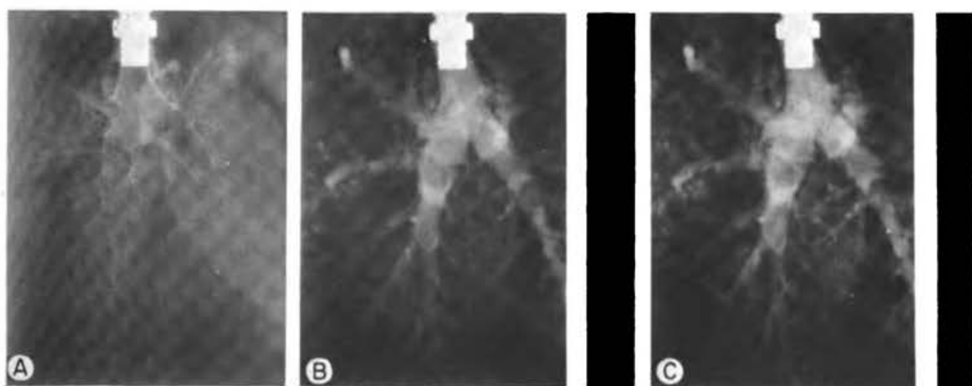

FIGURE 3. A, control film showing excised dog lung inflated in hyperbaric chamber at 30 psig. B, film exposed immediately after opening xenon inflow valve, with chamber pressure set at 30 psig and xenon inflow pressure adjusted to 38 psig. C, film exposed 1.5 sec later, showing progress of xenon from major bronchi to bronchiolar and alveolar spaces. Exposure factors in all cases: 58 kv, 10 ma, 40-in. target–film distance (TFD) in screen cassette in Sanchez-Perez automatic film changer.

might be gathered during this procedure. It is known, for example, that pulmonary ventilation is uneven in certain disease states such as emphysema, and several pulmonary function tests have been devised to measure the degree of this unevenness.[8] Xenon bronchography might allow anatomic localization of this unevenness to the segmental level. Bronchi with high flows might appear more radiopaque than bronchi with low flows, since there would be more dilution of xenon with air in the latter. In addition, the distribution of one full inhalation of xenon could be recorded on film at the end of inspiration, in order to define areas of trapping and bronchial obstruction.

Studies of pulmonary perfusion with xenon as a contrast medium might also yield important information. Since inhaled xenon comes to equilibrium with the bloodstream, its rate of appearance or disappearance in the lung might be used to localize differences in pulmonary perfusion. This might be done by anesthetizing the subject with xenon, then ventilating the lungs to wash out xenon, and finally taking serial films to record return of xenon from bloodstream to lungs.

ACKNOWLEDGMENTS

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Pulmonary and Systemic Arteriovenous Shunting in Clinical Septic Shock

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Failure of arterial blood oxygen to reach the partial pressures of oxygen achieved in the alveoli can be caused by three mechanisms: (1) right-to-left shunting, (2) ventilation perfusion defects, and (3) diffusion or equilibrium abnormalities.[1] We have witnessed the importance of intrapulmonary shunting as a cause of arterial hypoxemia in shock in a series of patients with portal hypertension.[2] In addition, hypoxic acidosis in shock patients with high cardiac outputs was shown by Udhoji and Weil [3] in a study of clinical bacteremic shock. They concluded that "inadequacy of effective blood flow and not necessarily a reduction in cardiac output is the essential feature of shock associated with bacteremia due to gram-negative enteric bacteria." Such inadequate blood flow leads to low oxygen consumption and hypoxic acidosis because of arterial hypoxemia and systemic arteriovenous shunting across capillary beds.

In an attempt to correlate the problems of oxygen uptake and delivery in clinical shock with the hemodynamic patterns observed, we undertook a prospective study at our institution.

Methods

Of the 75 gravely ill shock patients who have undergone serial hemodynamic determinations since 1961, 19 were chosen for this study. In these 19 patients, shock defined as hypotension, low oxygen consumption, and hypoxic acidosis was associated with sepsis or bacteremia. Patients with cirrhosis or other forms of liver disease were excluded, as were those in whom hypovolemia or myocardial infarction was known to be present. The clinical course in most cases was long and complicated. Multiple system disease was present in many cases.

In this investigation, indwelling catheters were introduced into the right side of the heart and the brachial artery by previously described techniques.[2,4,5] Mixed venous blood samples from the right side of the heart permitted calculation of pulmonary venoarterial admixture as percent of cardiac output, estimated by

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Berggren's principle.[6,7] This is based upon the fact that when 100% oxygen is breathed for at least 15 min, all pulmonary alveoli contain oxygen at a partial pressure of more than 650 mm Hg. In these circumstances, all blood leaving the pulmonary capillaries contains saturated hemoglobin and dissolved oxygen amounting to 0.0031 vol%/mm Hg of pO_2 in the alveoli. This is true even for poorly ventilated alveoli, but not for totally collapsed alveoli. With a lower pO_2 present in the arterial blood, there must be pulmonary arteriovenous admixture, since the high alveolar pO_2 eliminates the effects of ventilation perfusion defects and of impairment of diffuson. Oxygen was delivered by a tight hand-held face-mask, by a mouthpiece with a nose-clip, or through a cuffed tracheostomy tube and a pressure-regulated demand-type respirator.

The relationship between the percent of venoarterial admixture and other hemodynamic variables was evaluated by statistical analysis of regression and correlation coefficients.[8,9]

Results

Table 1 presents selected hemodynamic values obtained in the septic shock patients off and on 100% oxygen. The average values reveal a pattern typical of moderate hypotension and prolongation of the circulation time from right atrium to brachial artery. Central venous pressure was moderately elevated, as was total peripheral resistance. Considerable arterial hypoxemia was present, but lowered pCO_2 values ruled out hypoventilation as the cause. The surprisingly low arteriovenous oxygen difference, combined with the low cardiac output, resulted in decreased oxygen consumption. The resulting metabolic acidosis shown by the decrease in whole-blood buffer base was well compensated by the decrease in pCO_2. As shock progressed in many of the patients, serial studies showed that acidosis became more severe and decompensated eventually. Patients 9, 11, 12, and 13 were the only survivors. Some patients had high cardiac indexes, but inordinately low arteriovenous oxygen differences resulted in poor oxygen consumption by the tissues. In other words, the coefficient of oxygen utilization was low. Most of our patients received oxygen by nasal catheter before the test runs on 100% oxygen, accounting for those with arterial pO_2 values above 100 mm Hg.

The overall hemodynamic pattern of these shock patients did not change significantly while they were breathing 100% oxygen. Statistical analysis indicated that only the arterial and mixed venous pO_2 increases were not random phenomena ($P<0.01$). Values for venoarterial admixture in the lungs averaged 37.1% of the cardiac output, compared to 6% in normal individuals. Figure 1 summarizes the changes observed with 100% oxygen-breathing, compared to the normal values. The marked rise in mixed venous pO_2 without improvement in oxygen consumption is helpful in delineating the basic hemodynamic defect in these patients.

Figure 2 depicts oxygen consumption plotted against total peripheral resistance in individual patients. Although there was great variation in the peripheral resistance

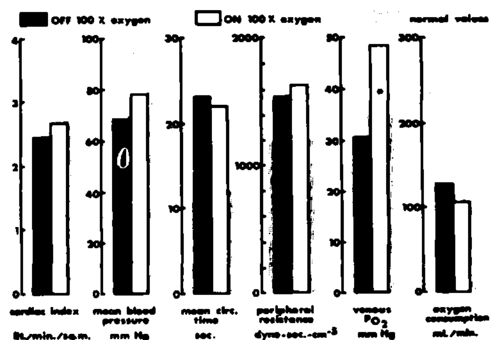

FIGURE 1. Hemodynamic parameters of severe septic shock, showing the response to 100% oxygen. Only the change in mixed venous pO_2 was statistically significant, indicating an inappropriately low coefficient of oxygen utilization in view of the low oxygen consumption.

TABLE 1. Selected Cardiorespiratory Variables in Septic Shock: Response to 100% Oxygen at Atmospheric Pressure

Patient	Age	Sex	Cardiac index (liters/ min/m²)	Stroke index (ml/ stroke/ m²)	Mean blood pressure (mm Hg)	Total peripheral resistance (dyne·sec·cm⁻⁵)	Stroke work (gm·meters)	Mean ejection rate (ml/ sec/m²)	Mean circulation time (sec)	Central blood vol. (liters)	Pulse rate (strokes/ min)	pH	Arterial pO_2 (mm Hg)	Arterial pCO_2 (mm Hg)	Arterial buffer base (mEq/ liter)	Arterio-venous difference (vol %)	Oxygen consumption (ml/ min)	Central venous pressure (mm Hg)	Venous admixture (% cardiac output)
Normal values:			3.20	40.00	96	1200	70.0	159.0	12.0	1.40	80	7.40	95.0	40.0	50	4.5	250	3	6
1	89	M	1.53	17.40	76	2811	25.4	82.9	42.29	1.52	88	7.46	55.0	29.3	44	—	—	—	—
			2.30[a]	27.52	59	1853	31.1	101.9	31.97	1.74	84	7.41	150	34.5	44	—	—	13	—
2	97	M	0.84	7.28	—	—	—	36.4	32.30	0.86	116	—	—	—	—	—	—	4	—
			0.93	8.03	71	3781	12.5	44.6	35.01	0.81	116	—	317	36.3	42	5.32	79.8	6	16.35
3	78	M	4.33	37.29	97	1007	87.6	155.4	18.65	2.39	116	7.51	54.0	30.9	50	—	—	16	—
			5.45	45.39	98	807	107.7	174.6	17.96	2.90	120	7.49	160	28.3	48	—	—	5	—
4	75	M	2.49[b]	24.90[c]	63	2022	21.3	—	35.77	1.48	100	7.15	56.0	38.1	—	—	—	—	—
			2.81[b]	28.10[c]	73	2076	27.9	—	30.88	1.45	100	7.37	86.5	42.6	—	—	—	—	—
5	73	F	1.43	15.86	87	2705	27.8	66.1	24.41	1.04	90	7.63	82.1	40.0	66	—	—	11	—
			3.20	30.40	115	1691	86.7	152.0	13.70	1.30	104	7.42	86.0	45.7	52	0.78	44.5	7	76.44
6	62	F	2.36	31.01	90	1816	63.8	129.2	13.00	0.86	76	7.47	43.5	34.7	46	3.32	131.5	2	—
			2.23	26.50	90	1278	54.6	110.4	17.65	1.10	84	7.45	150	36.0	46	—	—	4	35.94
7	71	M	1.23	8.86	69	2483	14.5	40.3	28.55	1.06	144	7.42	48.2	18.8	37	5.64	125.2	0	—
			1.00	8.93	51	2342	10.8	37.2	32.55	0.94	112	7.27	81.0	35.2	36	7.08	123.2	20	29.80
8	69	M	3.55	31.71	75	1011	54.0	151.0	14.03	1.39	112	—	—	—	—	—	—	—	—
9	62	M	1.40	15.19	56	1695	21.9	60.8	36.65	1.61	92	7.46	—	14.0	36	—	—	12	—
10	78	M	1.31	34.41	38	1624	25.4	137.6	45.42	1.42	38	7.14	97.6	55.0	36	8.77	164.0	7	—
11	52	M	3.36	34.94	63	833	53.9	139.8	13.89	1.40	96	7.58	58.0	13.0	38	4.15	250.7	4	—
12	38	F	2.30	21.33	55	1207	25.2	92.7	16.82	1.02	108	7.56	75.0	22.5	43	4.00	145.6	4	—
			2.35	22.59	60	1289	29.2	94.1	17.81	1.11	104	7.55	225	21.5	44	3.77	140.2	10	26.10
13	58	M	3.67	41.76	76	843	96.1	167.0	17.20	2.20	82	7.62	34.0	35.5	62	—	—	11	—
			2.97	35.32	76	979	76.3	153.6	17.96	1.86	84	7.62	75.0	36.0	62	—	—	6	—
14	47	F	4.11	57.08	54	550	80.1	203.9	17.91	2.34	72	7.43	31.5	35.4	46	2.04	160.1	12	—
			3.75	52.14	85	947	115.1	186.2	15.27	1.82	72	7.50	159	33.7	48	1.33	95.4	10	55.40
15	80	M	1.71	17.07	70	1997	26.7	61.0	16.63	0.78	70	7.47	91.5	24.6	42	2.32	65.0	0	—
16	69	F	2.27	24.65	55	1127	31.7	98.6	19.59	1.27	92	7.49	86.0	33.0	47	—	—	—	—
			1.97	20.98	77	1853	37.8	77.7	19.60	1.08	92	7.49	520	30.0	46	—	—	4	—
17	26	F	2.07	12.31	60	1440	16.2	51.3	18.20	1.01	168	7.23	200	51.0	42	—	—	2	—
18	69	F	3.77	34.94	68	813	57.2	151.9	9.40	1.05	108	7.33	124	21.5	32	5.91	394.7	10	—
			3.46	33.25	76	992	60.8	138.5	11.50	1.17	104	7.30	325	27.0	34	4.15	253.9	10	19.73
19	42	F	2.42	25.24	66	1588	31.0	148.5	18.04	1.00	96	7.17	22.0	35.6	32	3.83	127.2	15	—
Average value off 100% O_2			2.43	26.02	68	1532	42.2	109.7	23.09	1.35	98	7.42	72.4	31.4	44	4.44	173.8	7.8	—
Average value on 100% O_2			2.69	28.28	78	1623	54.1	115.5	21.82	1.44	98	7.40	191.2	33.9	46	3.74	122.8	11.9	37.10

[a] The second set of values, where present, are those determined after breathing 100% oxygen for 15 min.
[b] Cardiac output.
[c] Stroke volume.

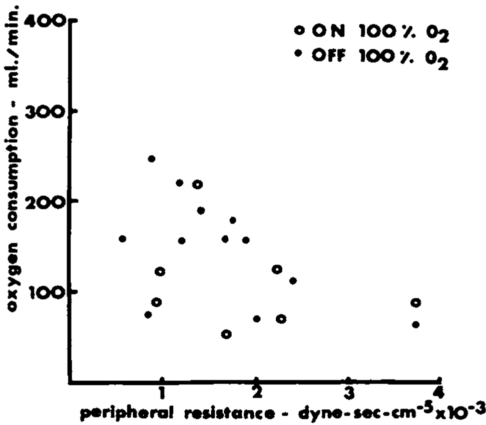

FIGURE 2. Oxygen consumption plotted against total peripheral resistance for individual patients, demonstrating wide variation in peripheral resistance and consistently low oxygen consumption.

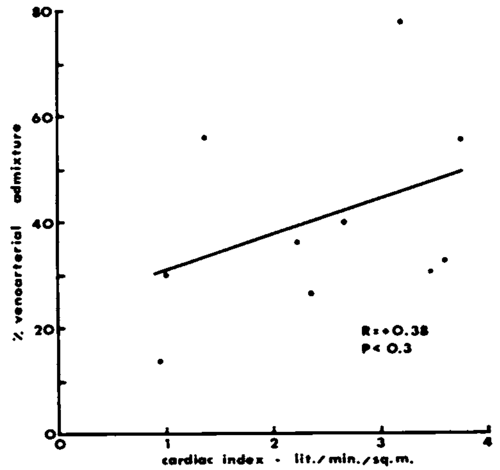

FIGURE 3. Venoarterial admixture in the lungs plotted against cardiac index. Although not statistically significant, it suggests that hypoxemia and venoarterial admixture are more likely in high-output shock states.

(expressed as the ratio of mean blood pressure to cardiac output), oxygen consumption was below normal in every instance. This corresponds to the findings of Hopkins and co-workers in 11 patients with septic shock.[10]

In an attempt to correlate pulmonary venoarterial admixture with other hemodynamic variables, numerous scatter diagrams were made, but only three showed patterns suggesting causal association. In these patients, there is a tendency to more right-to-left shunting with higher cardiac indexes and faster circulation (Figures 3, 4). Although the level of confidence is poor for these regression equations, further investigation is suggested. When pulmonary shunting was plotted in relation to mixed venous pO_2 levels, the relationship was statistically significant. The probability that the linear regression equation was due to chance was less than 2%. The high levels of oxygen tension in blood returning from the peripheral circulation corresponded with large volumes of shunting in the lungs.

DISCUSSION

In the presence of hypoxic acidosis and subnormal oxygen consumption, maximum extraction of oxygen from the arterial blood by the tissues would be expected. When this fails to occur, shunting across systemic capillary beds or inability of the cells to utilize available oxygen can be assumed. This was the major finding in our series of patients who were critically ill in septic shock. Increase in the arterial

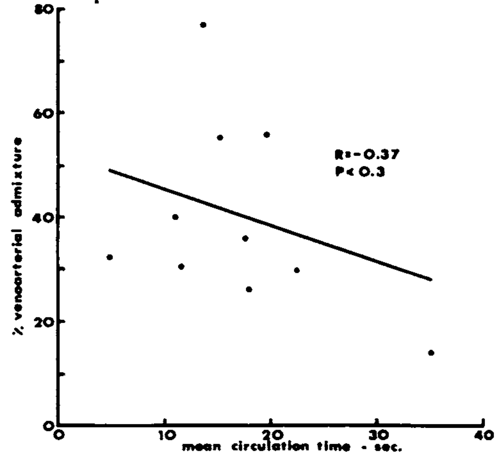

FIGURE 4. Venoarterial admixture in the lungs plotted against indicator-dilution mean circulation time. In these septic shock patients, a faster circulation between the right atrium and the brachial artery suggests higher levels of venoarterial admixture.

pO_2 significantly increased mixed venous pO_2 but failed to improve total oxygen consumption or other cardiovascular parameters. Myocardial performance, as judged by mean systolic ejection rate, mean circulation time, stroke work, stroke index, and central venous pressure, changed little with the administration of 100% oxygen at atmospheric pressure.

Arterial hypoxemia in these patients was accounted for by severe degrees of pulmonary venoarterial admixture. The linear relationship between this pulmonary shunting and systemic shunting, as indicated by the mixed venous pO_2 levels (Figure 5), suggests a common vasoactive factor. Reduced arterial oxygen content and an inappropriate coefficient of oxygen utilization resulted in decreased oxygen consumption and metabolic acidosis. The arterial hypoxemia was corrected by the breathing of oxygen in high concentrations, but the amount of oxygen extracted from that available in the systemic circulation did not increase. Clinical improvement could not be expected under these circumstances, and, in fact, without serial studies in the early stages of septic shock, we cannot say that this systemic shunting phenomenon is not due to terminal histotoxic anoxia.

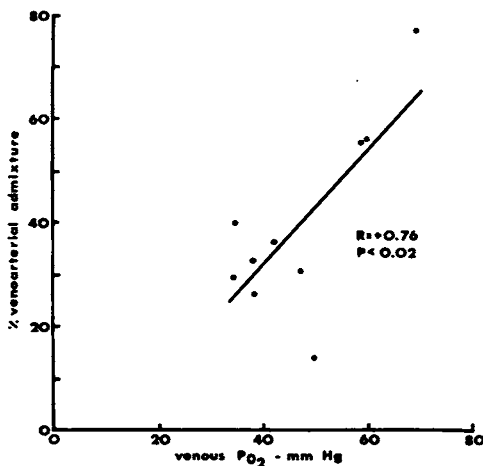

FIGURE 5. Venoarterial admixture in the lungs plotted against mixed venous oxygen tension. The direct correlation between intrapulmonary shunt and mixed venous pO_2 is statistically significant, suggesting that poor utilization of the venous oxygen reserve due to systemic arteriovenous shunting is related to pulmonary shunting in these septic shock patients.

SUMMARY

Hemodynamic parameters were measured in patients with septic shock. The response to 100% oxygen-breathing was determined and venoarterial admixture estimated by Berggren's principle. Only the arterial and mixed venous pO_2 increased significantly; the average venoarterial admixture was 37.1% of the cardiac output. There was direct correlation between pulmonary shunting and the mixed venous pO_2. The coefficient of oxygen utilization was low in these patients, resulting in inadequate oxygen consumption.

ACKNOWLEDGMENTS

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DISCUSSION

DR. I. MCA. LEDINGHAM (*Glasgow, Scotland*): In most patients in hemorrhagic shock, significant improvement in the oxygen consumption figures can be made by increasing the inspired oxygen tension, at least in the perfused areas. The areas which are not being perfused are not as well oxygenated.

DR. DEL GUERCIO: In hemorrhagic shock, if cardiac output and oxygen tension can be improved, oxygen consumption can also be improved in animals and in some humans as well. However, there seem to be certain differences in the type of hypoxic acidosis produced in septic shock. Recent studies, particularly one by Rosenberg (J.A.M.A. 192:563, 1965), have shown that the lactic acidosis and excess lactate do not correspond to so-called "oxygen debt" in animals with endotoxic shock. This correlates with our results. We find that septic shock is more complicated than hemorrhagic or other forms of shock. The latter forms basically involve inadequate delivery of oxygen to the tissues. In septic shock, however, although the oxygen is transported to the tissues, there remains an untapped venous oxygen reserve. The real question is why this is so. Possibly hyperbaric oxygen improves the general situation from the standpoint of antisepsis, but we cannot be certain of this since the blood cultures of these patients are already sterile when we see them. Yet, the basic hemodynamic defect, leading to inadequate oxygen consumption, persists.

SESSION IV

Hyperbaric Oxygenation in Cardiovascular Disease

Chairmen: R ADAMS COWLEY
Department of Surgery
University of Maryland School of Medicine
Baltimore, Maryland

EDWARD H. LANPHIER
Department of Physiology
State University of New York at Buffalo
Buffalo, New York

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Evaluation of Hyperbaric Surgery in Infants with Congenital Cardiac Defects

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A recent report from the National Office of Vital Statistics, U.S. Public Health Service,[1] indicates that more than 7000 babies under 1 year of age die of congenital cardiovascular defects in the United States each year. Approximately 60% of these infants die within 3 months after birth, with an additional 25% lost before the sixth month of life. Since experience has indicated that many critically ill infants may undergo successful palliative or corrective surgery if presented to the surgeon in time, early diagnosis of the existing defect and recognition of a failure in medical therapy becomes imperative. In many instances, surgical intervention in the deteriorating or moribund infant may be lifesaving.

In our experience, a variety of factors contribute to satisfactory surgical results in these patients: (1) an accurate anatomic or physiologic diagnosis, (2) skillful anesthetic management, (3) detection and treatment of severe alterations in acid–base equilibrium, and (4) an adequate technical surgical procedure. Recent investigations[2–4] suggest that a fifth factor might be added to this list: improved tissue oxygen availability during surgery, provided by hyperbaric oxygenation.

In application of this, we performed surgical correction or palliation of congenital cardiovascular abnormalities in a series of 135 infants in a hyperbaric environment. Our results corroborated the favorable role of OHP. Not only was death from "hypoxic cardiac arrest" completely absent in the series (compared with operative mortality in earlier series), but cardiac resuscitation, when necessary during surgery, appeared to be markedly facilitated by the increased quantity of oxygenated blood made available by the increased atmospheric pressure.

Clinical Results

Group I: Transposition of the Great Vessels

In 41 infants with transposition of the great vessels, open creation of an atrial septal defect, pulmonary artery banding, or a systemic–pulmonary arterial shunt procedure was performed at a chamber pressure of 30–44 psig (Table 1). Here,

TABLE 1. Ages of Infants at Time of Palliative Hyperbaric Surgery for Congenital Cardiovascular Defects

Diagnosis	Total no. patients	Age at operation					
		0–1 mo.	1–3 mos.	3–6 mos.	6–9 mos.	9–12 mos.	12 mos.
Transposition of the great vessels	41	23	10	1	2	2	3
Tricuspid or pulmonary valve atresia	26	13	5	4	2	1	1
Tetralogy of Fallot	22	4	7	3	3	3	2
Ventricular septal defect A-V canal	18	1	9	5	—	—	3
Anomalous left coronary artery from pulmonary artery	1	1	—	—	—	—	—

a large number of precompression arterial pO_2 values were less than 35 mm Hg, emphasizing the value of increased environmental pressure to achieve a small but significant improvement in blood oxygen tension (20–40 mm Hg). Preoperatively, 20 babies in this group had a metabolic acidosis (pH range 6.95–7.30), and 11 others had a mixed respiratory–metabolic acidosis (pCO_2 50–70 mm Hg, pH 7.10–7.30). Therefore, alterations in acid–base equilibrium occurred in 76% of the 41 patients.

Management of acute metabolic disturbances was achieved by the intravenous administration of tris buffer (THAM) and sodium bicarbonate.[5,6] Tris proved to be the most effective base available, since it was capable of modifying both intracellular and extracellular pH and, in addition, was not dependent for effect upon the presence of normal pulmonary diffusion (Figure 1).

All plasma and whole blood administered during operation was buffered to a normal pH (7.35–7.48) by adding 400 mg tris/500 ml infusate. In addition, the quantity of buffer administered to acidotic infants was 150 mg/kg (1.25 mmoles/kg), employing a 0.3 or 0.6 M solution. Additional tris or sodium bicarbonate was employed, when necessary, on the basis of repeated determinations of arterial pH, pCO_2, and pO_2.

Pulmonary artery banding was carried out in five of the 41 infants, each of whom had a large ventricular septal defect and severe pulmonary artery hypertension. The precompression arterial oxygen tension in these patients ranged between 55 and 70 mm Hg, with arterial carbon dioxide tension in the normal range (35–48 mm Hg). All pH values were above 7.30.

In two infants, a systemic–pulmonary artery shunt was carried out, in addition to creation of an atrial septal defect, because of the association of pulmonic stenosis and poor mixing (between the systemic and pulmonary circulations).

Thirty-five patients improved and were discharged from the hospital following surgery; the remaining six died from 1 day to 5 months postoperatively (Table 2). Two of the deaths occurred in infants subjected to pulmonary artery banding alone (pulmonary vascular disease), while the other four had creation of an atrial septal defect as the major operative procedure (two of these infants also had banding done).

Elimination of "hypoxic cardiac arrest" during operation was a striking feature of

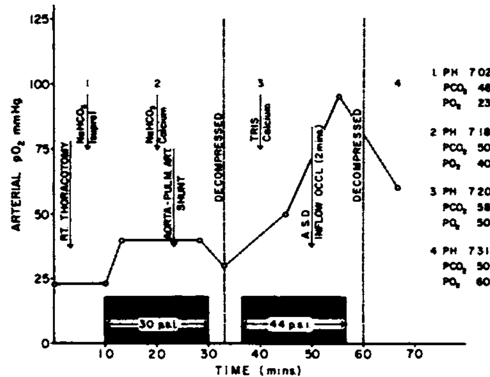

FIGURE 1. Arterial pH and blood gas data obtained in a 3-day-old infant with transposition of the great vessels and pulmonic stenosis. The patient was not catheterized preoperatively because he was considered too ill to withstand this procedure. A systemic–pulmonary artery shunt procedure seemed indicated on the basis of cyanosis and decreased pulmonary blood flow on x-ray examination. Transposition of the great vessels was discovered at the time of operation. A systemic–pulmonary artery shunt was carried out initially at a pressure of 30 psig. There was an increase in arterial oxygen tension of approximately 20 mm Hg at this pressure. Sodium bicarbonate, calcium gluconate, and Isuprel (isoproterenol hydrochloride) were administered during this part of the operation because of an increased hydrogen ion concentration and poor cardiac action. Following decompression, and in the presence of an open shunt, there was little improvement in arterial oxygen tension. Consequently, a second operative procedure was carried out to improve mixing between the systemic and pulmonary circulations (atrial septal defect creation). This was done at 44 psig and resulted in a prompt improvement in arterial oxygen tension after a second decompression. The total operating time at pressure was approximately 50 min. The final arterial pH was 7.31, with an arterial pO_2 of 60 mm Hg.

TABLE 2. Results of Palliative and Corrective Surgery in 135 Infants with Congenital Cardiovascular Defects

Diagnosis	No. patients	Survivors No.	Survivors Percent
Palliative surgery			
Transposition great vessels	41	35	86
Tetralogy of Fallot	22	19	86
Pulmonary atresia	17	10	59
Tricuspid atresia	9	5	56
Ventricular septal defect and pulmonary artery hypertension	13	13	100
Atrioventricularis communis and pulmonary artery hypertension	5	4	80
Anomalous left coronary artery	1	1	100
Total	108	87	81
Corrective surgery			
Aortic stenosis	9	7	78
Pulmonic stenosis	14	14	100
Coarctation of aorta and pulmonary artery hypertension	4	2	50
Total	27	23	85

the results in this group of seriously ill, cyanotic infants. To illustrate the significance of this aspect of the hyperbaric experience, review of a previous Children's Hospital series (1957–1962) was carried out. This study disclosed 60 deaths in a group of 130 patients who underwent palliative surgery for similar cardiac lesions. Twenty-six of the 60 fatalities (43%) occurred secondary to myocardial hypoxia and ventricular fibrillation, before the definitive operative procedure could be completed.

Group II: Pulmonary Atresia, Tricuspid Atresia, and Tetralogy of Fallot

Forty-eight infants with marked arterial unsaturation (arterial pO_2 15–40 mm Hg), with pulmonary atresia, tricuspid atresia, or tetralogy of Fallot, were operated upon at environmental pressures of 30–44 psig. After stabilization at the working pressure, small increments in oxygen tension were noted in all of them (20–60 mm Hg), and this was often accompanied by an improved systemic blood pressure.

Although hyperventilation with oxygen resulted in hypocapnia (pCO_2 18–40 mm Hg) and an elevated arterial pH (7.45–7.60), 35 infants were found to have a metabolic acidosis of varying severity immediately after surgery (pH range 6.90–7.31, pCO_2 range 28–50 mm Hg). Plasma lactate values before compression in these 35 patients ranged between 2.5 and 8.0 mmoles/liter.

Management of the acute disturbances in acid–base metabolism in Group II were carried out by the administration of tris buffer and sodium bicarbonate in the manner described for patients in Group I.

Since a major objective in this series was elimination of "hypoxic cardiac arrest" as a complicating factor during surgery, it is significant that *no operative deaths* occurred among the 48 patients who underwent systemic–pulmonary arterial shunts (Table 1). However, 14 patients died postoperatively, 12 hours to 5 months after surgery. Twelve of these infants, with pulmonary or tricuspid atresia, were found to have hypoplastic pulmonary and subclavian arteries, which contributed to an inadequate Blalock-Taussig shunt anastomosis. The other two

infants died of congestive heart failure because of excessively large Pott's shunts (aorticopulmonary anastomoses).

A recent change in the surgical method of performing systemic–pulmonary arterial shunts in infants gives promise of far superior results. In this operation, the surgical approach is made through the right chest (posterolateral incision in the fourth intercostal space). Dissection of the right pulmonary artery is carried out from the lobar branches at the hilum of the right lung to the origin of the right pulmonary artery. In all cases, the pericardium is opened for a distance of 4.0 cm, and the superior vena cava is mobilized from the level of the azygos vein to the caval–atrial junction.

In the presence of a right aortic arch (found in nine of our 12 cases), anastomosis between the posterior wall of the ascending aorta and the side of the right pulmonary artery is carried out in a retrocaval position. If a left aortic arch is present, a similar anastomosis is carried out with the superior vena cava retracted laterally. The anastomosis is created with a continuous row of 6–0 arterial silk sutures for the posterior layer, and interrupted 6–0 arterial silk sutures for the anterior row. Great care is taken to limit the size of the anastomosis to 3.0 mm in diameter.

This type of systemic–pulmonary arterial shunt has proved to be highly satisfactory in 12 consecutive patients, with no evidence of postoperative shunt thrombosis during a period of 6 months follow-up (Figure 2).

In all patients constituting Groups I and II, compression in the chamber was delayed (if the infant's condition under anesthesia remained stable) until the thoracotomy had been completed. This shortens the working time under pressure for the surgical personnel, which, in the last 100 cases, has not exceeded 60 min. In approximately one-third of the patients, however, a transient fall in systemic blood pressure, or the development of a bradycardia during thoracotomy (or upon retraction of the right lung) makes early compression mandatory. In all cases, once an improvement in oxygenation has been achieved under hyperbaric conditions, the surgical procedure can be carried out without further difficulties.

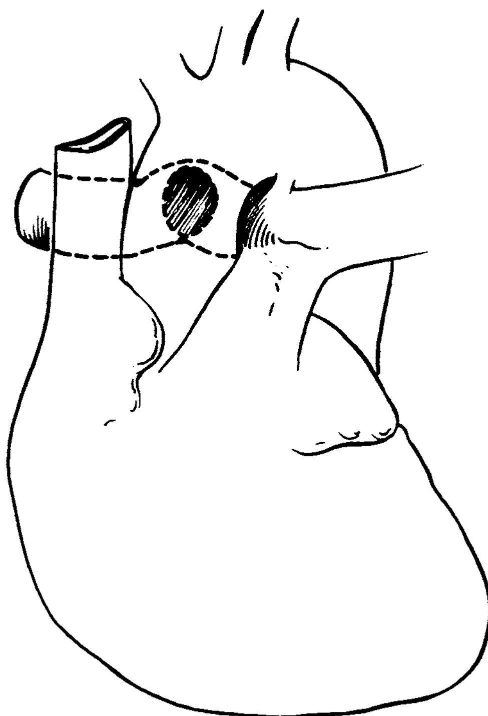

FIGURE 2. Systemic–pulmonary arterial shunt created between the ascending aorta and right pulmonary artery. This side-to-side anastomosis is carried out posterior to the superior vena cava in the presence of a right aortic arch, and medial to the superior vena cava in the presence of a left aortic arch. Exposure through a right lateral thoracotomy provides access to the base of the right atrium so that an atrial septal defect can be created at the same operation if necessary.

Group III: Aortic and Pulmonic Stenosis

Studies in our surgical laboratory indicated that interruption of the circulation, under normothermic conditions, was possible for short intervals of time in the presence of markedly elevated arterial oxygen tensions (1500–2000 mm Hg). Since open aortic or pulmonic valvulot-

omy can be carried out very satisfactorily (during inflow occlusion) in a maximum period of 4 min, considerable protection from hypoxia is obtained with the hyperbaric technique.

In the current series of surgical cases, direct valvulotomy was undertaken in nine infants with aortic stenosis and 14 with valvular pulmonic stenosis. Chamber pressures, ranging between 30 and 44 psig, were used in these patients depending upon the level of arterial oxygen tension achieved at the first stop (30 psig). A minimum value of 1300 mm Hg was accepted as the lowest base-line level permitting safe inflow occlusion for periods up to 4.0 min.

All of the patients subjected to pulmonic valvulotomy (14 infants) are alive and well (Table 2). However, two of the nine infants with aortic stenosis died postoperatively. Both were found to have associated cardiac defects which contributed to death: subendocardial fibroelastosis, mitral stenosis, and subvalvular muscular aortic stenosis. It is perhaps noteworthy that the last seven (consecutive) aortic valvulotomies have been performed with no fatalities.

Ventricular fibrillation developed during operation at pressure in four patients, before and after definitive operative procedure. In each instance, cardiac resuscitation (and electrical defibrillation) was apparently facilitated by the increased oxygenation of the myocardium, provided by the supersaturated blood.

Group IV: Pulmonary Artery Hypertension and Left Ventricular Failure

Twenty-three patients with ventricular septal defect, atrioventricularis communis, complicated coarctation of the aorta (ventricular septal defect plus pulmonary ductus arteriosus), and anomalous left coronary artery draining into the pulmonary artery comprised this group. In 14 infants, inadequate diffusion of carbon dioxide and oxygen across damaged alveolar membranes resulted in moderate hypercarbia (pCO_2 45–55 mm Hg) and slight arterial unsaturation (pO_2 55–70 mm Hg). However, in nine patients, a significant respiratory acidosis was detected at the time of thoracotomy, with pH values of 7.05–7.30 and markedly elevated carbon dioxide tensions (55–70 mm Hg). All infants with acid–base disturbances responded satisfactorily to treatment with tris buffer and small quantities of sodium bicarbonate (2.0 mEq/kg body weight).

Surgery under hyperbaric conditions was carried out in these critically ill infants for the following reasons: (1) to maintain a normal arterial oxygen tension in patients with compromised pulmonary diffusion and arterial unsaturation, (2) to reduce the incidence of acute ventricular arrhythmias frequently noted in infants with severe pulmonary artery hypertension, and (3) to facilitate cardiac resuscitation and defibrillation if arrhythmias developed.

Three deaths occurred postoperatively. Two infants (with complicated coarctation of the aorta) died during the first week after surgery (digitalis intoxication and aspiration pneumonia), and a third patient with atrioventricularis communis died suddenly 24 hours after an uneventful pulmonary artery banding operation. Thirteen infants with large ventricular septal defects and pulmonary artery hypertension (pulmonary artery mean pressure greater than two-thirds of systemic mean pressure) were operated upon (pulmonary artery banding) without a fatality.

It is interesting to note the results of the previous Children's Hospital series (1957–1962), in which 35 infants (less than 1 year old) underwent pulmonary artery banding. There were eight deaths in this series, four of which occurred during operation (and before banding), secondary to irreversible ventricular fibrillation.

Summary

1. One hundred thirty-five infants with severe forms of congenital heart disease

were operated upon under hyperbaric conditions during 1963–1965. There were 110 survivors (81%). Twenty-five patients died in the postoperative period 12 hours to 5 months after surgery.

2. Of 108 patients subjected to a palliative operation for a variety of congenital cardiac defects (transposition of great vessels, tetralogy of Fallot, pulmonary and tricuspid atresia, ventricular septal defect, atrioventricularis communis, and anomalous left coronary artery), 87 are alive and well postoperatively (81%).

3. Corrective surgical procedures were undertaken in 27 infants, with 23 survivors (85%).

4. In an analysis of this series of patients, a number of factors appeared to underlie a satisfactory surgical result: determination of an accurate anatomic or physiologic diagnosis by means of cardiac catheterization and cineangiography, skillful anesthetic management, repeated determinations of arterial blood gases and pH values to detect alterations in acid–base equilibrium, and improved surgical operations to palliate or correct the existing cardiovascular defects. Experience from this hospital indicates that an increased tissue oxygen availability provided by a hyperbaric environment is also highly desirable.

5. That a hyperbaric environment is beneficial is testified to by the fact that "hypoxic cardiac arrest" resulting in death during surgery was completely absent in this series of 135 cases. In addition, cardiac resuscitation, when necessary at the time of operation, was facilitated by the improved arterial oxygen saturation.

6. Severe alterations in acid–base equilibrium occurred in 88 patients (65% of the series). Acidosis was managed in these patients by the administration of tris buffer (THAM) and sodium bicarbonate.

7. An improved type of systemic–pulmonary arterial shunt procedure, carried out through a right thoracotomy and involving an anastomosis between the ascending aorta and right pulmonary artery, has been described. Results with this procedure, to date, indicate the possibility of obtaining a far superior result in infants with pulmonary and tricuspid atresia than that previously obtained.

ACKNOWLEDGMENTS

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DISCUSSION

DR. E. A. STEAD (*Durham, N. C.*): What other variables changed when hyperoxia was started? Did the operating time change? Were the problems of acid–base balance handled in the same way during the two periods which are compared? I know these are not easy questions to answer from records, but I wondered what information you might have.

DR. BERNHARD: These questions are almost impossible to answer, because two series of cases are never exactly comparable. As far as the surgical procedures are concerned, the first series was done by at least three different surgeons over a period of 5 years, and the last hyperbaric series has been done exclusively by one surgeon. With increasing experience, the results will obviously improve. The detection of acid–base changes in infants was not attempted in the previous series at all; therefore, the periods are not comparable.

DR. R A. COWLEY, *Session Chairman* (*Baltimore, Md.*): I would like to ask Dr. Bernhard two questions that might be of interest. First, what security does hyperbaric oxygenation offer you, as a surgeon, with these children? The second question is, has it been necessary to treat any of them postoperatively for a period of time?

DR. BERNHARD: First of all, if the surgical procedure is done properly, it is amazing how rapidly these infants improve. We have not been forced to return any patients to the compression chamber because of severe cyanosis. I think one of the reasons why these patients are so appealing is their great ability to recover from severe hypoxia if something is done surgically to correct the problem. Even small increments in arterial oxygen tensions are very important in these blue babies. Operating on patients with arterial saturations of 30 to 40%, despite a high concentration of alveolar oxygen administered by the anesthetist, leaves the surgeon with a very brittle patient. This tends to make you hurry even more than you would ordinarily, and often leads to a poor technical operation. In the shunt surgery where vessels are small, the anastomosis must be made accurately. This 15 or 20 mm Hg increase in arterial oxygen tension permits you to take your time. You do not have to worry about hypoxic bradycardia and cardiac arrest, which occurred in some 30 or 40% of our control series.

DR. R. L. FUSON (*Durham, N. C.*): A few years ago, we attempted a technique called gastric freezing, because the initial results looked extremely good to those who were doing it at the time. However, when a control series was done, the early data proved questionable. Do you plan a randomized control study to evaluate the efficacy of hyperbaric oxygenation in clinical cardiac surgery?

DR. BERNHARD: I do not plan to do a randomized control series. We will continue to manage patients as we see them. This is an unselected series, and time will tell whether beneficial effects really are present. Certainly our results are encouraging to date.

Hyperbaric Oxygen in Cardiac Surgery

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In January, 1956, Boerema initiated a series of experiments at our institution in order to determine the possible value of oxygen under increased atmospheric pressure in cardiac surgery. These experiments were conducted in a pressure chamber of the Royal Dutch Navy until 1959, when a more sophisticated pressure chamber was completed close to the hospital. Not until the end of 1960, however, was the first blue baby operation performed under increased atmospheric pressure.[1]

During that period and since then, we have also been exploring the possibilities of hyperbaric oxygenation in the management of other disorders. We, like others, are interested in determining whether the increased storage of oxygen under hyperbaric conditions is sufficient to allow significant extension of the time during which circulatory arrest is feasible. Although we have had no clinical experience thus far, we are also interested in the possibilities afforded by the increased efficiency of extracorporeal oxygenators in a hyperbaric environment. This should permit the use of an extracorporeal device of small priming volume, which would be particularly valuable in operations on small children.

Following is a report of our results in blue baby surgery performed under OHP and in hyperbaric treatment of postoperative cardiac failure with pulmonary complications in adult patients.

OHP in the Surgical Management of Blue Babies

In recent years, children undergoing palliative surgical correction of cyanotic cardiac anomalies at our institution have been operated upon in the hyperbaric chamber. The anomalies treated have included cyanotic tetralogies, severe valvular pulmonary stenosis with atrial septal defect or patent foramen ovale, and complete transpositions of the great vessels. The corrections have been carried out using Blalock-Taussig or Pott's anastomoses, relief of valvular pulmonary stenosis, and creation of atrial septal defects.

Ventilation with oxygen at 3 ata was expected to reduce the incidence and severity of hypoxia, prevent bradycardia and cardiac arrest, and permit tissue oxygenation (after a period of inflow occlusion) to reach the desired level more rapidly than at 1 ata.

Technique

Between December 1960 and November 1965, 18 infants and children were operated upon under hyperbaric conditions. The patients were anesthetized at normal atmosphere pressure. Premedication usually consisted of pentothal sodium and atropine, and anesthesia was induced by kemithal. In order to prevent rupture of the tympanic membrane, before compression myringotomy was performed on both sides. Usually kemithal, curare, and pethidine hydrochloride were used to maintain an adequate anesthetic level. The chest was opened at 1 ata through a right- or left-sided thoracotomy and a part of the intrathoracic dissection was carried out at the same pressure.

When it was clear that the planned procedure was possible, compression to 3 ata was started. The procedure was carried out at 3 ata after at least 5 min of hyperventilation with oxygen.

When the condition of the patient had been stabilized after surgical correction, decompression was started. Blood samples were drawn at various stages during ventilation with oxygen (except in the first four cases, in which some samples were drawn during ventilation with an oxygen–air mixture). Always a sample was drawn before compression, at 3 ata after 5 min of oxygen ventilation and before decompression, and after decompression.

Electrocardiogram, electroencephalogram, and blood pressure were monitored outside the chamber. In most cases, blood samples were drawn during compression for determination of pH, bicarbonate, pCO_2, potassium, hematocrit, glucose, lactic acid, and pyruvic acid. Determinations were performed outside the chamber at normal atmospheric pressure. When, in a few cases, control determinations were carried out at 3 ata as well, no significant differences were noted between analyses performed inside and outside the chamber, if determinations were done immediately.

Results

Patients' ages ranged from 3½ months to 6 years (Table 1). Of the 18 patients treated, three died: one from cardiac failure during surgery and two from respiratory complications, one on the first postoperative day and one 7 days after surgery. All three patients had complete transpositions of the great vessels. The average oxygen tension before compression was 51 mm Hg; at 3 ata this value rose to 175 mm Hg; before decompression, 427 mm Hg was measured; and, at the end of the procedure, during spontaneous oxygen-breathing, oxygen tension was 57 mm Hg. The values measured in the pulmonary artery or right atrium before compression, after 5 min of oxygen ventilation at 3 ata, and before decompression were 42, 58, and 232 mm Hg, respectively (Table 2). The average pCO_2 value in the radial artery before compression was 38 mm Hg; during compression there was usually no rise. In most cases, hyperventilation with oxygen was carried out during compression; at 3 ata the average values were 37 mm Hg in the beginning and 43 mm Hg before decompression; after decompression, an average of 41 mm Hg

TABLE 1. Average Values for 18 Blue Babies Operated upon Under Increased Atmospheric Pressure

	Age	Hemoglobin (gm%)	Weight (kg)	Time at 3 ata (min)
Average	2.9 yr	18.6	10.3	49
Ranges	3.5 mo–6 yr	15.0–22.1	4.6–20.0	31–83

TABLE 2. Oxygen Tensions (mm Hg) in Blue Babies at 1 and 3 ata

Pt. no.	Before compression		After 5 min at 3 ata		Before decompression		After decompression
	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial
5	47	—	155	—	265	—	—
6	41	44	45	83	710	1000	66
7	58	42	100	65	1400	250	67
8	50	40	170	63	86	64	22
9	84	54	—	—	760	360	77
10	47	32	64	47	75	75	40
11	75	55	680	70	400	300	70
12	45	32	100	53	900	400	70
13	46	43	130	58	480	78	56
14	21	24	56	38	52	38	41
15	39	30	52	42	62	54	42
16	32	22	47	39	48	48	32
17	55	53	135	85	175	92	77
18	75	70	540	50	560	260	82
Average	51	42	175	58	427	232	57

was found (Table 3). The respective averages of pH values of these four samples were 7.32, 7.31, 7.30, and 7.35. The average blood glucose level before compression was 114 mg%; at 3 ata the value rose to an average of 161 mg%; after decompression 131 mg% was measured.

The potassium values remained relatively stable: 4.7, 4.6, 4.3, and 4.2 mEq/liter in the respective samples. Oxygen saturations were 75, 93, 95, and 81%, respectively. The average lactic acid level before compression was 13.6 mg%; at 3 ata there was a slight decrease to 12.0 mg%; before decompression 15.0 mg% was measured and after decompression 18.3 mg% (Table 4).

In six cases, excess lactate could be calculated according to the method of Huckabee. At 3 ata, less hypoxia was found. (Biochemical values appear in Tables 4–7.) A typical picture of the im-

TABLE 3. CO_2 Tensions (mm Hg) in Blue Babies at 1 and 3 ata

Pt. no.	Before compression		After 5 min at 3 ata		Before decompression		After decompression
	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial
5	39	—	33	—	74	—	40
6	36	36	34	34	47	47	36
7	42	42	34	42	34	40	37
8	30	31	40	42	71	75	52
9	45	37	32	40	44	44	34
10	41	39	34	45	37	38	45
11	51	50	58	60	49	60	44
12	26	30	38	51	40	50	47
13	33	40	31	38	30	38	48
14	32	32	32	42	37	34	37
15	29	28	28	29	27	26	37
16	39	40	35	35	38	39	37
17	43	48	44	46	34	35	36
18	42	51	38	42	43	47	37
Average	38	39	37	42	43	44	41

TABLE 4. Biochemical Values in 18 Blue Babies Operated upon Under Increased Atmospheric Pressure

Arterial		Before compression	After 5 min at 3 ata	Before decompression	After decompression
pH	Average	7.32	7.31	7.30	7.35
	Range	(7.20 to 7.60)	(7.17 to 7.45)	(7.17 to 7.43)	(7.26 to 7.43)
pCO_2 (mm Hg)	Average	38	37	43	41
	Range	(26 to 51)	(28 to 58)	(27 to 74)	(36 to 52)
Bicarbonate (mEq/liter)	Average	17.7	17.8	20.5	21.9
	Range	(13.0 to 22.8)	(13.4 to 24.5)	(12.6 to 26.6)	(15.7 to 26.6)
Base excess (mEq/liter)	Average	−8.0	−7.5	−7.0	−3.5
	Range	(−1 to −13.5)	(−2 to −13.5)	(+1 to −14)	(+2 to −10.5)
pO_2 (mm Hg)	Average	51	175	427	57
	Range	(32 to 84)	(45 to 680)	(62 to 1400)	(22 to 82)
HbO_2 (%)	Average	75	93	95	81
	Range	(34 to 92)	(73 to 100)	(75 to 100)	(35 to 96)
Lactic acid (mg%)	Average	13.6	12.0	15.0	18.3
	Range	(8.3 to 24.0)	(8.0 to 18.3)	(7.5 to 28.2)	(9.0 to 32.0)
Glucose (mg%)	Average	114	135	161	131
	Range	(75 to 189)	(78 to 240)	(73 to 300)	(91 to 220)
Potassium (mEq/liter)	Average	4.7	4.6	4.3	4.2
	Range	(3.6 to 6.0)	(3.4 to 6.0)	(3.3 to 6.0)	(3.0 to 6.7)

TABLE 5. Lactic Acid Levels (mg%) in Blue Babies at 1 and 3 ata

Pt. no.	Start operation	Before compression		After 5 min at 3 ata		Before decompression		After decompression
	Arterial	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial
6	5.5	7.5	—	10.5	—	11.5	—	13.0
7	—	8.3	13.1	11.1	11.8	25.0	21.5	18.7
8	—	8.7	9.5	9.0	10.0	7.0	6.5	9.0
9	—	16.5	14.5	18.3	17.3	20.0	21.4	28.7
10	22.0	24.0	36.0	11.0	11.0	12.0	13.0	10.5
11	6.5	11.5	11.0	10.5	10.0	10.5	11.5	13.0
12	11.0	15.5	16.0	16.0	17.0	15.0	16.0	17.5
13	6.1	11.5	—	11.0	—	28.2	—	28.5
14	10.6	19.2	21.6	10.7	12.1	19.4	18.5	32.0
15	—	9.3	10.0	10.2	9.2	22.0	20.6	11.9
16	12.0	10.5	11.0	8.5	8.5	7.5	10.5	14.5
17	9.5	14.5	12.5	12.0	14.0	12.0	11.5	16.5
18	8.5	15.3	14.5	14.8	14.1	12.3	12.3	18.0
Average	10.2	13.3	15.4	11.8	12.3	15.6	14.8	17.8

TABLE 6. Pyruvic Acid Levels (mg%) in Blue Babies at 1 and 3 ata

Pt.	Start operation	Before compression		After 5 min at 3 ata		Before decompression		After decompression
no.	Arterial	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial
9	0.35	1.45	1.05	1.15	1.20	1.30	1.45	1.45
12	0.86	0.86	0.77	1.14	0.49	0.77	0.49	0.49
14	0.85	0.70	0.85	0.70	0.65	1.10	1.15	1.05
16	0.70	0.70	0.85	0.75	0.70	0.45	0.45	0.80
17	0.73	1.04	0.98	0.73	1.15	1.01	1.01	1.23
18	0.45	0.40	0.50	0.40	0.40	0.40	0.55	0.60
Average	0.66	0.86	0.84	0.81	0.77	0.84	0.85	0.94

provement of pO_2 is shown in Figures 1 and 2; both cases had severe pulmonary stenosis and a patent foramen ovale or atrial septal defect.

Discussion

The main advantage of performing these operations at 3 ata is the decrease in risk of hypoxic bradycardia and cardiac arrest. The great improvement of oxygen tension in radial artery and pulmonary artery demonstrates a greater oxygen availability. The removal of CO_2 has not been a serious problem in our cases. Experimentally, we usually see some rise of CO_2 during compression when the ventilation stays at a constant level. In this series, all patients were hyperventilated before samples were drawn. Most of the patients already had a more-or-less serious metabolic acidosis on admission to the hospital, as, for example, Case 17 with a base deficit of 13 mEq. These acidoses most likely are related to the poor condition of the patients and the insufficient oxygen transport.

During surgery this acidosis probably increases despite the high pressure. Although a tendency undoubtedly exists toward carbonic acidosis under increased pressure, in the most gravely cyanotic children at 3 ata a great amount of reduced hemoglobin is still present and the loss of isohydric buffering capacity is small; in our cases, hyperventilation could compensate for it. In less cyanotic patients, the veno-arterial shunt is smaller, which may result in increased loss of isohydric buffering capacity. However, in

TABLE 7. Excess Lactate in Blue Babies at 1 and 3 ata

Pt.	Start operation	Before compression		After 5 min at 3 ata		Before decompression		After decompression	
no.	LA/PA	LA/PA	XL	LA/PA	XL	LA/PA	XL	LA/PA	XL
9	27.8	11.3	− 5.7	15.9	− 4.2	15.4	− 5.7	19.6	− 2.6
12	12.8	18.1	+ 4.5	14.0	+ 1.4	19.5	+ 5.2	35.7	+11.2
14	12.5	27.5	+10.5	15.3	+ 2.0	17.7	+ 5.7	30.5	+18.9
16	17.3	15.0	− 1.5	11.3	− 4.4	16.7	− 0.2	18.2	+ 0.8
17	13.0	14.0	+ 1.0	16.4	+ 2.5	11.9	− 1.1	13.4	+ 0.5
18	18.9	38.3	+ 7.8	37.0	+ 7.3	30.8	+ 4.8	30.0	+ 6.7
Average	17.0	20.7	+ 2.8	18.3	+ 0.8	18.7	+ 1.5	24.6	+ 5.9

Excess lactate (XL) formula: $(LA_n - LA_o) - (PA_n - PA_o) \times \frac{LA_o}{PA_o}$, where LA_o = lactic acid at start of operation, PA_o = pyruvic acid at start of operation, LA_n = lactic acid in studied sample, and PA_n = pyruvic acid in studied sample.

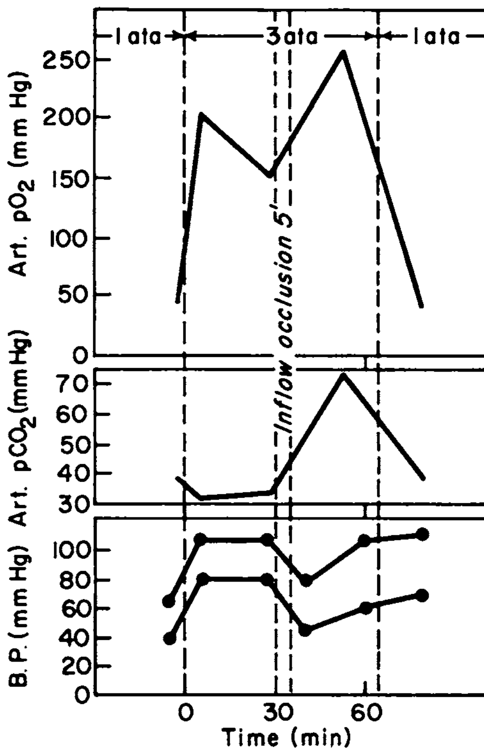

FIGURE 1. Blood gas and blood pressure data from a 4-year-old boy during operation under hyperbaric oxygenation (3 ata) for relief of severe pulmonary valvular stenosis associated with an atrial septal defect. Note that arterial pCO_2 did not rise before inflow occlusion because of removal of CO_2 by hyperventilation. The pCO_2 rose during the period of inflow occlusion and while ventilation was diminished before decompression, reaching a level of 74 mm Hg.

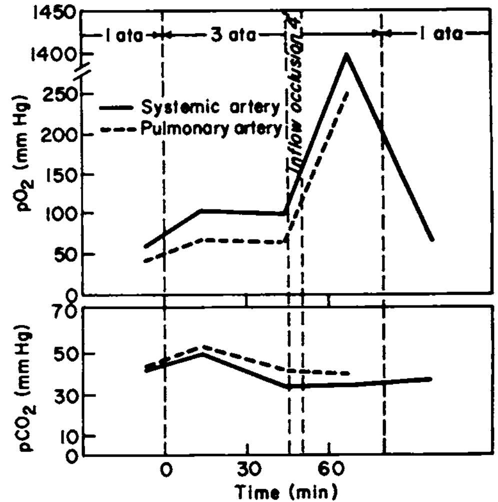

FIGURE 2. Blood gas data from a 4-year-old girl during operation under hyperbaric oxygenation (3 ata) for relief of pulmonary stenosis. Note the improvement in arterial pO_2 with oxygen ventilation at 3 ata. Note also that in this case the pCO_2 did not rise during the period of increased pressure.

these infants, hyperventilation is more effective in its compensatory effect.

We believe that the main problem in these children is the metabolic acidosis, which should be treated with sodium bicarbonate or tris buffer. Bernhard's figures[2] also indicate no serious rise in pCO_2, and the carbonic acidosis problem seems to us to be less of a practical problem than Fuson's figures suggest.[3]

The improved results obtained with these children can be attributed not only to the increase in oxygen tension, but also to the consequent decrease in the ratio of lactic acid to pyruvic acid and the decrease of excess lactate.

Conclusions

Hyperbaric oxygenation can decrease the risk of cardiac surgery in blue babies; however, the metabolic acidosis associated with their disease is a much more serious problem than the tendency toward carbonic acidosis, which can be controlled by hyperventilation.

Oxygen Under Increased Pressure in Postoperative Cardiac Failure

Experimental evidence has indicated that a diffusely damaged myocardium can be improved by the administration of oxygen under increased pressure, as seen in dogs with myocardial damage inflicted by injection of microspheres.[4,5] Furthermore, during various animal experiments of our own, at critical levels of cardiac failure, oxygen under increased pressure resulted in a significant improvement. At the same time, we got the impression that the ranges of cardiac damage wherein improvement could be expected were rather small. It is

probable that this is at least partly related to the fact that the coronary artery flow decreases during rise of oxygen tension.[6] This would result in less of a rise of pO_2 in the coronary sinus blood than of venous pO_2 in blood from other organs.

A great drawback of the oxygen treatment at 3 ata is the fact that oxygen itself produces lung damage after some time; oxygen tensions in the alveoli at the level of 2000 mm Hg are tolerated for only a few hours. Thereafter, lung damage causes inadequate oxygenation.

Oxygen at 1 ata is tolerated for 3 to 4 days. Six cases of postoperative cardiac failure were treated with oxygen under increased pressure. Two of these were ventilated with oxygen or oxygen-rich gas mixtures; four had a partial bypass at the same time. One case of each group, typical for the technique, is discussed in detail below.

Case 1. C. V., a 20-year-old woman, underwent surgical correction of mitral regurgitation on November 5, 1964. While a cleft in the septal leaflet of the mitral valve was sutured, the patient remained on cardiopulmonary bypass for 43 min. Before surgery, she had been in cardiac failure several times; the heart was greatly enlarged. The pulmonary artery pressure was high (75 mm Hg), and slight desaturation of the arterial blood (86%) was noted, caused by an impaired diffusion capacity of the lungs. Although a good repair was possible, blood pressure became depressed a few hours after surgery. A tracheostomy was performed and the patient was placed on artificial ventilation.

Notwithstanding optimal ventilation, the arterial oxygen tension remained around 73 mm Hg with maximal arterial hemoglobin saturation of 94%. To maintain blood pressure at an adequate level, a small amount of Metaraminol [1-(*m*-hydroxyphenyl)-2-aminopropanol] was necessary. The amount was gradually increased, and eventually levarterenol was substituted, Ten hours after surgery, the patient's condition gradually deteriorated, with venous pressure ranging between 24 and 30 cm H_2O. Consequently, it was decided to treat the patient with oxygen at 3 ata; after compression the arterial oxygen tension rose from 73 to 750 mm Hg. Ventilation at 3 ata was continued for 1 hour, after which the pressure in the chamber was lowered to 2 ata, because oxygen tensions of 750 to 800 mm Hg were not deemed necessary and it seemed more advantageous to stay for a longer time at 2 ata. At this pressure, the arterial pO_2 ranged from 105 to 210 mm Hg. The condition of the patient improved considerably and the levarterenol was stopped for some time. After 2.5 hours at 2 ata, decompression was started in order to determine whether 1 ata would provide sufficient oxygenation. After decompression, the pO_2 remained at approximately 85 mm Hg. Later the pO_2 dropped again, and 18 hours after surgery the patient was recompressed to 3 ata (Figure 3). The pO_2 rose to 500 mm Hg. Levarterenol administration was again stopped, and the patient was decompressed first to 2.5 ata, then to 2 ata. However, the pO_2 also fell to approximately 85 mm Hg with some decline in blood pressure. Once more, the pressure in the chamber was raised to 3 ata, and at that level the patient was ventilated intermittently with oxygen and oxygen–air mixtures.

Again there was a definite improvement and levarterenol administration was stopped. Gradually, however, the condition of the patient deteriorated, and after the treatment

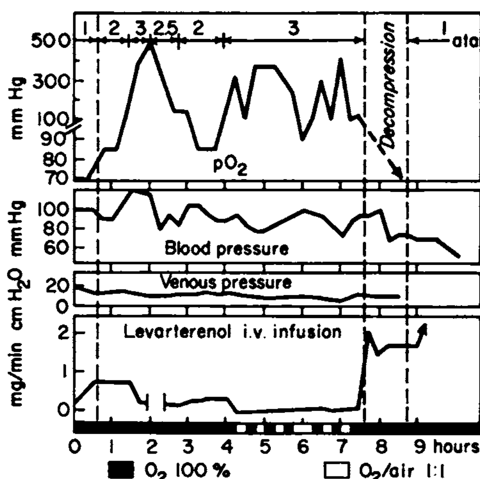

FIGURE 3. Case 1, arterial pO_2, blood pressure, and venous pressure responses during hyperbaric oxygen therapy in a patient with postoperative cardiac failure following surgery for mitral insufficiency.

at increased pressure had lasted for almost 7 hours, decompression was started. The patient died within a few hours after decompression. At autopsy examination, signs of pulmonary edema were apparent.

Case 2. J.d.V., a 38-year-old man, had a Starr-Edwards valve implanted on October 26, 1965, because of mitral stenosis and mitral regurgitation. After surgery, his respiration was inadequate and he was put on artificial ventilation. The blood pressure had to be kept up with a small amount of Metaraminol and levarterenol. Gradually, the cardiac condition improved, and on the third postoperative day levarterenol administration could be stopped. On the fourth day, the blood pressure ranged between 120 and 130 mm Hg. Valve function was excellent and urinary output was good. Cultures of bronchial and tracheal secretions showed pseudomonas infection, so antibiotic treatment was started. Despite this treatment, pneumonitis increased, so that by the fifth postoperative day the arterial pO_2 had dropped to 70 mm Hg and it was necessary to increase the pressure of the respirator. Because of these factors, the arterial blood pressure dropped and levarterenol administration had to be restarted.

Progressive difficulties in oxygenating the patient led to the decision to initiate hyperbaric oxygen therapy (Figures 4,5). At 3 ata with the patient breathing pure oxygen, however, the pO_2 rose no higher than 90 mm Hg. Because the patient had already been ventilated with pure oxygen for 4 days at normal atmospheric pressure, it was decided to start a partial bypass in order to oxygenate the patient and to ventilate with oxygen or oxygen–air mixtures. At the same time, antibiotic therapy was intensified and other treatment to improve ventilation was instituted. Via the right saphenous vein, a #24 Couvelaire catheter was introduced into the inferior vena cava to the level of the diaphragm; blood was drawn into a Rygg-Kyvs-gaard bubble oxygenator primed with 2 pints of blood and 1 pint of Rheomacrodex; a heat exchanger in the arterial line kept the temperature at a constant normal level. The blood was pumped back to the body through a cannula in the right femoral artery. Using a flow of 1000 ml/min (body weight 75 kg) and oxygen ventilation, the arterial pO_2 was maximally 760 mm Hg. The pO_2 in the arterial line of the oxygenator was about 1700 mm Hg. When the patient was ventilated with an oxygen–air mixture, the values in the radial artery were about 250 mm Hg.

In the first hours, gradual improvement occurred in the patient's condition. Levarterenol administration was gradually decreased and the condition of the lungs improved. However, after 8 hours of partial

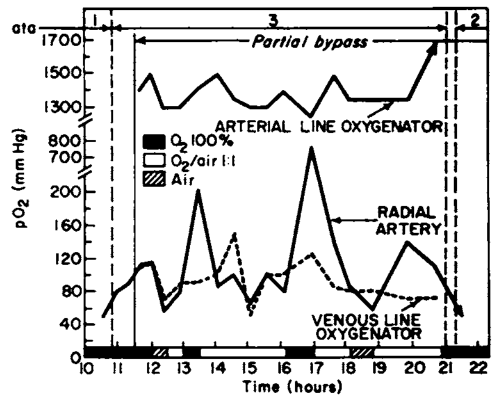

FIGURE 4. Case 2, blood gas data during partial bypass support with an extracorporeal pump oxygenator under increased atmospheric pressure (3 ata). Ventilation of the patient's lungs during the 9 hours 38 min of compression was alternated among pure oxygen, oxygen–air mixture (1:1), and air alone. The period of extracorporeal partial bypass support at 3 ata was 7 hours 57 min in duration.

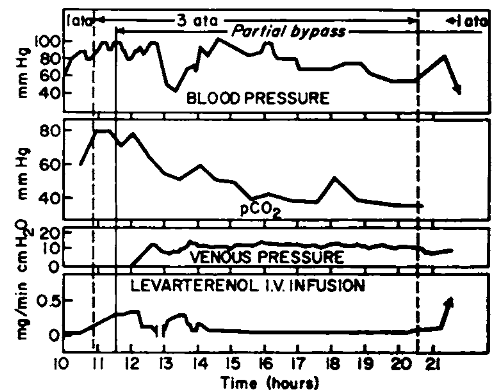

FIGURE 5. Case 2 (see also Figure 4), showing blood pressure, arterial pCO_2, and venous pressure responses during partial bypass support at 3 ata.

bypass at 3 ata, the total improvement of the patient was such to indicate a hopeless prognosis. The hemolysis at the end of 8 hours amounted to only 20 mg%. Decompression was started and the bypass was stopped. One hour after decompression, the patient died from hypoxia.

Three other patients have been treated in this way, and all died after an initial improvement (Table 8).

Discussion

Another patient was treated by the same method used for Case 1 described above. In both cases, the arterial tension increased significantly but treatment could not be continued without producing lung damage by oxygen itself. For this reason, we consider partial bypass a better method for utilizing oxygen under increased pressure. At 3 ata, the amount of flow through the bypass resulting in a given rise in arterial oxygen tension can be much less than at 1 ata. The treatment can therefore be continued much longer, because only the level of plasma hemoglobin imposes its limits. Neurologic disturbances from high oxygen tension need not be feared, for a high arterial pO_2 in the brain circulation is extremely unlikely. The purpose is to deliver as much oxygen to the body as necessary for adequate oxygenation of the myocardium. At the same time, the lungs can be ventilated with air or intermittently with oxygen and air.

Usually, patients after cardiac surgery do not tolerate depression in arterial pO_2 values. A normal heart reacts to a moderate oxygen tension drop with increase of cardiac output; when the pO_2 drop is severe, the cardiac output decreases. Diseased hearts, particularly after cardiac surgery, are frequently unable to increase their output. These hearts react to each pO_2 drop with a drop in cardiac output, and in this way a vicious circle is set in motion, the drop in cardiac output resulting in even worse myocardial anoxia.

The method described above for interrupting this vicious circle seems promising. We have applied it four times thus far (always as a last resort), and in each instance initial improvement was very clear. We are convinced that the treatment is a valuable one and, if started before irreversible myocardial degeneration occurs, will be successful, but our cases have also taught us that the ranges within which success may be attainable are prob-

TABLE 8. Treatment of Postoperative Cardiac Failure with Oxygen at 3 ata[a]

Patient	Artificial ventilation		
	Time at 3 (or 2) ata	Precompression pO_2	Maximal pO_2 at 3 ata
T.B.	35 min	85	300
C.V.	3 hr 25 min	90	800
	6 hr 55 min	70	500

Patient	Artificial ventilation + partial bypass				
				Maximal pO_2 at 3 ata	
	Total time at 3 ata	Bypass time at 3 ata	Precompression pO_2	Before bypass	During bypass
H.v.d.S.-W.	7 hr 3 min	4 hr 55 min	66	160	650
K.V.	7 hr 21 min	5 hr 51 min	65	420	900
J.J.	10 hr 5 min	9 hr 33 min	200	1000	1250
J.d.V.	9 hr 38 min	7 hr 57 min	49	90	760

[a] All pO_2 values measured in arterial blood (mm Hg).

ably rather small. Also, an improvement of our pumping technique will be necessary in order to synchronize cardiac activity with the speed of the pump.

Conclusions

1. Treatment with oxygen under increased atmospheric pressure should not be used if oxygen treatment at 1 ata can produce the same effect.

2. In cases of cardiac failure and pulmonary complications after cardiac surgery, very little can probably be expected from ventilation with oxygen at 3 ata only.

3. In our opinion, the best method to oxygenate a patient under increased pressure in these cases is to use a partial bypass with an extracorporeal oxygenator. Even here, however, the number of patients who would benefit is probably small.

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Protective Effect of Hyperbaric Oxygenation on the Central Nervous System During Circulatory Arrest*

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One of the theoretical advantages of hyperbaric oxygenation that has attracted the attention of cardiovascular surgeons is the elevation of oxygen (O_2) content in the central nervous system during exposure. Applied practically, this might permit a prolongation of the safe period of circulatory arrest. The studies of Smith *et al.*,[1] Richards *et al.*,[2] and Edwards *et al.*,[3] using the dog, have all indicated that hyperbaric oxygenation up to 3 ata, particularly when combined with moderate hypothermia, considerably extends the safe period of circulatory arrest for the central nervous system.

Most of these past studies, however, have been conducted without benefit of detailed neuropathologic examinations, and results have been interpreted principally on the basis of survival or of clinical behavior of the animals after the experiments. Unfortunately, the dog is known to be capable of masking rather severe neurologic damage, especially if given sufficient time to recover after episodes of cerebral anoxia.[4] Feeling that such experiments should include evaluation of histopathologic damage to the central nervous system, we undertook the following studies.

Experimental Design and Methods

Healthy adult mongrel dogs were subjected to periods of total circulatory arrest of 5, 10, and 15 min under the following conditions: (1) normothermia and ventilation with 100% O_2 at 3 ata, (2) normothermia and ventilation with 98% O_2+2% CO_2 at 3 ata, (3) moderate hypothermia (28–30°C) and ventilation with 100% O_2 at 3 ata, and (4) moderate hypothermia (28–30°C) and ventilation with 98% O_2+2% CO_2 at 3 ata. Thus, there were 12 experimental groups in all, no dog being used for more than one circulatory-arrest period. Six to 12 animals constituted each of the 12 groups.

Corresponding control experiments

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were carried out under identical conditions (6–12 dogs in each group), except that the atmospheric pressure was normal (1 ata). In the course of the investigation, two of the 12 control groups were eliminated, however: (1) control normothermic animals breathing 100% O_2 at 1 ata prior to 15-min circulatory arrest, and (2) control normothermic animals breathing 94% O_2+6% CO_2 at 1 ata prior to 15-min circulatory arrest. Since 100% of control normothermic animals were damaged after a 10-min period of total circulatory arrest, there was no reason to conduct control normothermic studies for the longer 15-min arrest period.

A total of 152 separate experiments were carried out in both hyperbaric and control groups. All animals were lightly anesthetized with methohexital (Brevital) and succinylcholine, intubated, and then ventilated with a Bird or Harvard respirator. The normothermic animals were placed on a circulating water blanket to maintain normal body temperature during the experiment, as monitored by thermistor readings from the rectum and midesophagus. The hypothermic groups of animals, after being anesthetized, were cooled by immersion in ice water. When their midesophageal temperatures reached 33°C they were removed from the ice-water bath and their temperature was allowed to drift down to 28–30°C, where it was maintained by a temperature-regulated circulating water blanket.

Arterial blood pressure, central venous pressure, electrocardiogram, and electroencephalogram were monitored on each animal studied. Both arterial and venous blood samples for determination of pO_2, pCO_2, and pH were obtained before and immediately after the periods of circulatory arrest. These determinations were made at 37°C and always at the ambient atmospheric pressure of the experiment.

By aseptic technique, the left side of the chest was entered and the pericardium opened. For the hyperbaric experiments, the chamber was then pressurized to 3 ata. The respirator gas was shifted from air to the ventilating gas of the experiment, and the animal was equilibrated with this gas by ventilation for 10 min before circulatory arrest. In the animals exposed to OHP, the prearrest ventilating gas was either 100% O_2 or a mixture of 98% O_2+2%CO_2. In order to render the control experiments at 1 ata comparable to those conducted at 3 ata, the control animals were ventilated during the 10-min prearrest period with either 100% O_2 or 94% O_2+6% CO_2, the latter mixture at 1 ata being equivalent in CO_2 partial pressure to the 98% O_2+ 2% CO_2 mixture used at 3 ata.

At the end of the 10-min equilibration period with the ventilating gas of the experiment, total circulatory arrest was induced in all animals by delivery of a 20-volt 3-ma AC shock to the heart for 1 sec through a needle electrode inserted in the left ventricle. With ventricular fibrillation, the arterial blood pressure fell to 0–5 mm Hg in 3–5 sec. During the period of circulatory arrest, the respirator was stopped. Fifteen seconds before the end of the arrest period, 2.0 ml of epinephrine (1:10,000) and 2.0 ml of calcium chloride solution (100 mg/ml) were injected into the left ventricular cavity, and the respirator was restarted with 100% O_2. At the end of the arrest period, the heart was massaged until it became pink and tone returned (15–30 sec). The heart was then defibrillated by an external countershock of 200–300 watt-sec, and a second set of blood samples was drawn immediately for determination of blood gases and pH. In the hypothermic animals, rewarming was begun using electric or recirculating water blankets as the chest incision was closed. In the hyperbaric groups, decompression was begun as soon as the heart was restarted and circulation re-established.

After the experiments, all animals were observed daily for clinical neurologic deficits and were given antibiotics and intravenous fluids if necessary. The ani-

mals were sacrificed and autopsied 5–7 days following arrest, since this had been found to be the ideal time for full development of any neurohistologic lesions. As shown by Cammermeyer [5] and Malm,[6] the methods for autopsy and fixation of central nervous system tissue are critical in experiments of this sort to obtain artifact-free histologic sections of the nervous system for detailed neuropathologic study. Consequently, animals were sacrificed by administration of a lethal dose of sodium thiopental (Pentothal). The chest was immediately opened and the aorta ligated above the aortic valve and at the midportion of the descending thoracic aorta. After ligation of the right subclavian artery, this isolated segment of the aorta was perfused through the left subclavian artery. Thus, the arterial supply to the brain and spinal cord was isolated for perfusion by 500 ml of Ringer's lactate solution followed immediately with 2000 ml of Heidenhain's Susa solution [5] or with 2000 ml of 0.6 M *p*-toluenesulfonic acid (PTSA).[6]

Results

In those animals exhibiting neurologic deficits in the 5- to 7-day observation period after circulatory arrest, the clinical picture ranged from mild hind-leg weakness with or without ataxia to a spastic or a comatose paralytic state. Regardless of the initial severity of the neurologic injury observed in affected surviving animals, most animals had considerable recovery of normal neurologic function by the end of the 5–7 days before autopsy. Frequently, animals with mild neurologic deficits noted on the first postarrest day appeared completely normal by the time of sacrifice 5–7 days later. The lesions found on neuropathologic examination ranged from overt injury typical of anoxic damage (Figure 1) involving the cerebral cortex and cerebellum to more subtle cryptic injury involving selective neuronal necrosis and mild glial response (Figure 2).

The results of the clinical and pathologic examinations on surviving animals in the 5-min total circulatory arrest groups exposed to 3 ata of O_2 with or without hypothermia and with or without added CO_2 appear in Table 1, along with results of control studies of 5-min circulatory arrest carried out under the same conditions, but at 1 ata. Note that hypothermic animals in both the hyperbaric and control groups with or without added CO_2 had no clinical signs of neurologic injury nor any evidence of injury by neuropathologic examination. In comparison, normothermic animals had both clinically and neuropathologically assessable damage in the 5-min-arrest groups. It is to be noted that slightly less neurologic injury resulted if the CO_2 tension was increased during hyperbaric oxygenation prior to arrest. However, from the control data on 5-min arrest in normothermic animals, it would appear that increasing the pCO_2 offered considerably less protection than prearrest O_2-breathing alone. It is of interest that in this latter control group over 60% of the animals showed only cryptic neuropathologic injury (Figure 2A, 2B).

The results with animals surviving 10 min of circulatory arrest also appear in Table 1. All normothermic control animals were damaged both by clinical and neuropathologic criteria whether or not the pCO_2 was increased during the prearrest period. In the normothermic hyperbaric arrest groups, there appeared to be less injury by clinical examination, but, as with the controls in the 10-min-arrest group, all of the normothermic animals subjected to hyperbaric oxygenation with or without added CO_2 showed definite neuropathologic damage. Thus, other than the occurrence of a higher survival rate in the 10-min-arrest normothermic hyperbaric group, there was little difference in the results between these groups of animals and their controls. While far less neurologic injury (by both clinical and

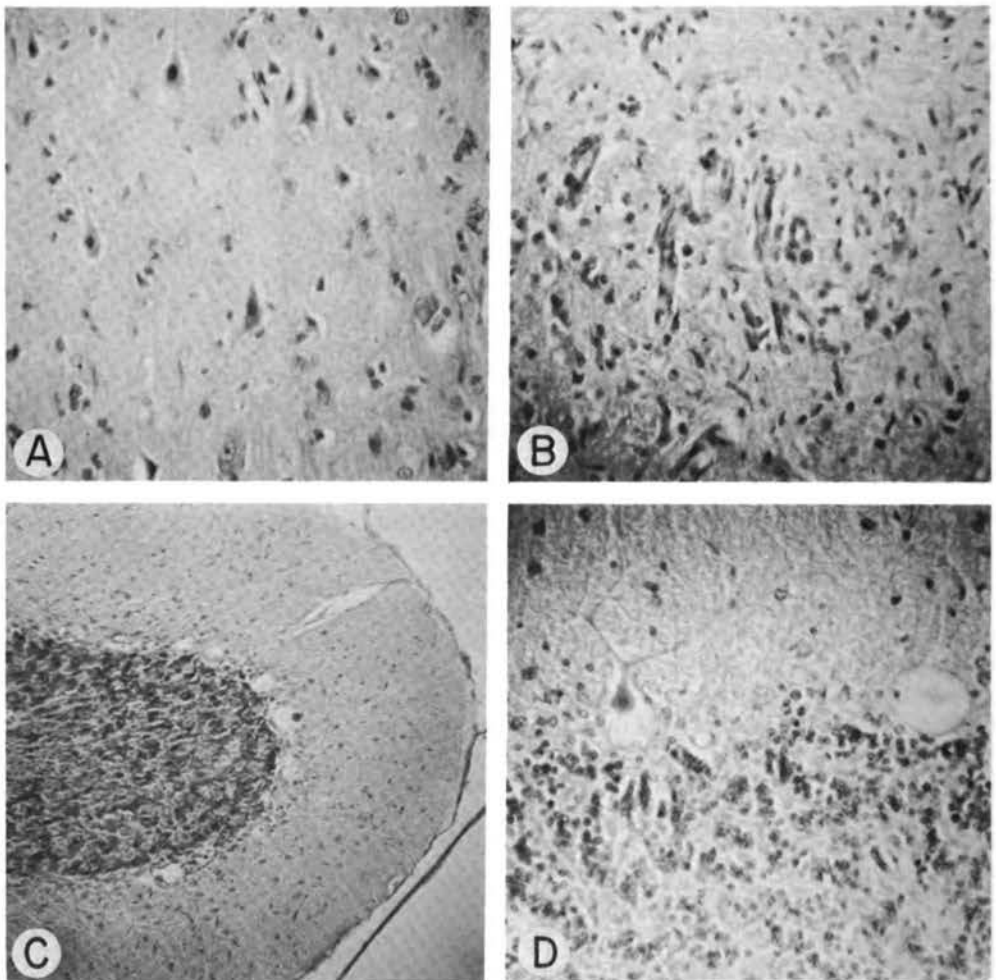

FIGURE 1. A, overt injury to cerebral cortex. Selective neuronal necrosis occurring in hippocampus of a dog subjected to a 10-min period of cardiac arrest while breathing a mixture of 94% $O_2 + 6\%$ CO_2 at 1 ata during normothermia; a severe neurologic deficit was observed. The shrunken eosinophilic neurones contrast sharply with the unaffected neurone population with which they are intermingled. This lesion is a characteristic sequela of temporary anoxia or circulatory arrest (hematoxylin and eosin, ×60). B, overt injury to cerebral cortex. Laminar cortical necrosis occurring in a dog subjected to a 10-min period of cardiac arrest while breathing a mixture of 98% $O_2 + 2\%$ CO_2 at 3 ata during normothermia. A severe neurologic deficit was observed. At this magnification, the profound degree of neuronal necrosis is not visible, but the zone of tissue injury is readily delineated by the reactive vascular endothelial hyperplasia (hematoxylin and eosin, ×60). C, overt injury to cerebellum. Selective neuronal necrosis occurring in same animal. This section shows a selective injury of the Purkinje cells in the cerebellum and sparing of all other tissue elements. Purkinje cells either have "dropped out" or remain as necrotic shrunken eosinophilic structures, in pools of fluid (hematoxylin and eosin, ×60). D, overt injury to cerebellum. Selective neuronal necrosis, cerebellum. Enlargement of C, showing dead Purkinje cells in pools of fluid or empty zones where these cells have "dropped out" (hematoxylin and eosin, ×140).

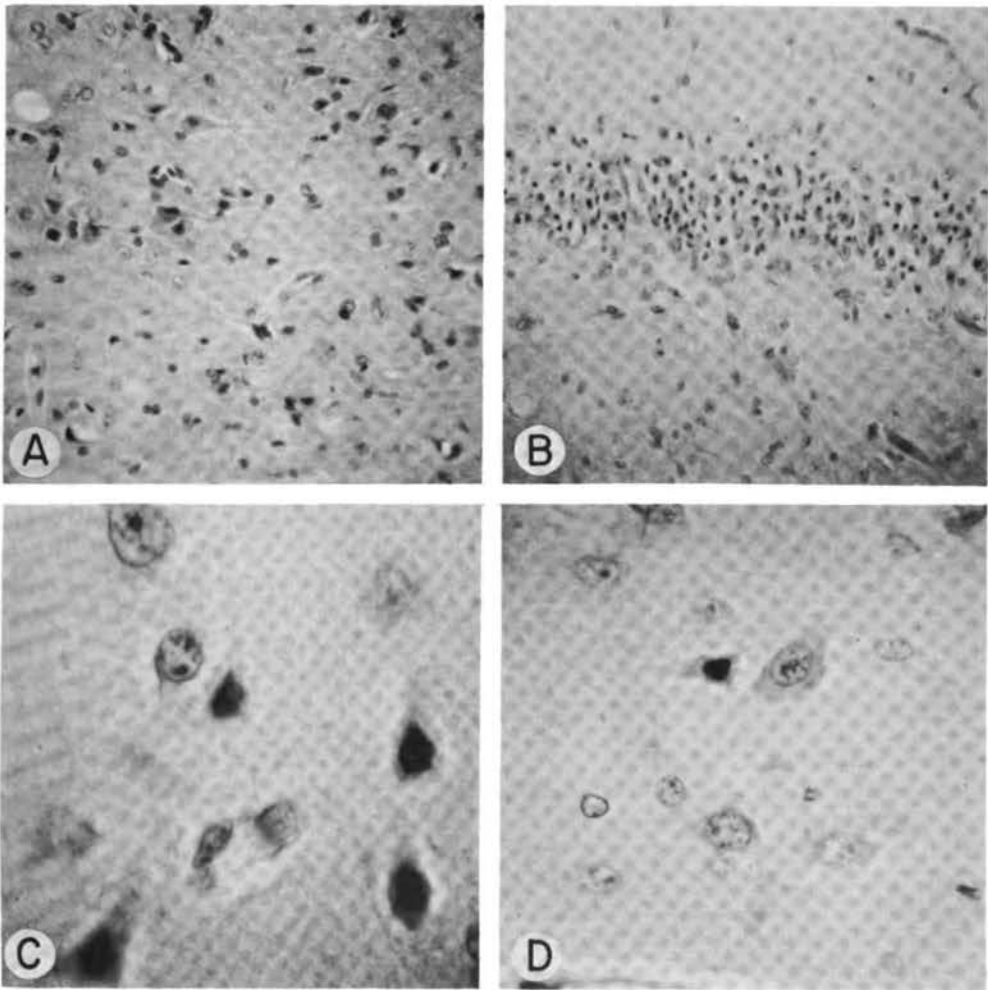

FIGURE 2. A, cryptic injury to cerebral cortex. Mild hyperplasia of microglia (rod cells). A subtle response to neuronal injury occurring in a dog subjected to 5 min of circulatory arrest at 1 ata during normothermia while breathing a mixture of 94% O_2 + 6% CO_2. This animal had minimal temporary neurologic signs, which cleared completely by 2 days (hematoxylin and eosin, ×140). B, cryptic injury to cerebral cortex. Selective neuronal necrosis in same animal. The several dead neurons stand out because of nuclear pyknosis, shrinkage of cell cytoplasm, and pericellular edema (cresyl fast violet, ×400). C, cryptic injury to cerebral cortex. Selective neuronal necrosis observed in brain of dog subjected to 10 min of circulatory arrest at 3 ata under mild hypothermia (29°C) while breathing a mixture of 98% O_2 + 2% CO_2. This animal appeared clinically normal after the procedure. Four necrotic neurones exhibit characteristic nuclear pyknosis and cell shrinkage (cresyl fast violet, ×630). D, cryptic injury to cerebral cortex. Selective neuronal necrosis in cortex of dog subjected to circulatory arrest for 10 min at 3 ata under mild hypothermia (29°C) while breathing a mixture of 98% O_2 + 2% CO_2. A single necrotic neurone is shown. The minimal residual glial response likely to represent the only trace of this injury would be difficult to recognize if postmortem study were delayed beyond 3 weeks (cresyl fast violet, ×400).

TABLE 1. Protective Effects of OHP, Hypothermia, and CO_2 During Total Circulatory Arrest in Dogs

Arrest period	Temperature	Gas mixture	Pressure (ata)	Survival (%)[a]	Clinical injury (%)[b]	Pathologic injury (%)[c]
5 min	38°C (normothermia)	O_2	1	55	0	20
			3	71	20	40
		$O_2 + CO_2$	1	83	20	80
			3	100	0	16
	28–30°C (hypothermia)	O_2	1	50	0	0
			3	71	0	0
		$O_2 + CO_2$	1	83	0	0
			3	50	0	0
10 min	38°C (normothermia)	O_2	1	16	100	100
			3	57	50	100
		$O_2 + CO_2$	1	83	100	100
			3	83	75	100
	28–30°C (hypothermia)	O_2	1	40	0	50
			3	37	0	67
		$O_2 + CO_2$	1	100	0	33
			3	53	25	37
15 min	38°C (normothermia)	O_2	1	—	—	—
			3	0	—	—
		$O_2 + CO_2$	1	—	—	—
			3	50	100	100
	28–30°C (hypothermia)	O_2	1	33	50	100
			3	25	0	50
		$O_2 + CO_2$	1	17	0	100
			3	50	33	67

[a] Percent of animals surviving 5–7 days to autopsy.
[b] Percent of animals surviving to autopsy with clinical signs and symptoms of neurologic injury.
[c] Percent of animals surviving to autopsy with injury apparent by neuropathologic examination.

neuropathologic examination) was noted in the 10-min-arrest hypothermic groups of animals, there appeared to be no significant difference between the experimental groups subjected to circulatory arrest at 3 ata and the controls subjected to arrest at 1 ata. Furthermore, there was no evidence that increasing the pCO_2 afforded any additional protection. Since all normothermic control animals showed neurologic damage by both criteria after 10 min of circulatory arrest, no normothermic control studies were conducted for longer periods of arrest.

In the 15-min total circulatory arrest groups shown in Table 1, there were no survivors of the hyperbaric normothermic 15-min arrest experiments when O_2 alone was used as a prearrest ventilating gas. In this group of animals, consciousness was not regained after the arrest period, and most of these comatose animals succumbed* to massive neurologic injury within 24 hours after arrest. In those normothermic animals with circulatory arrest for 15 min under hyperbaric conditions after ventilation with the O_2 + CO_2 mixture, 50% survived the experi-

* It should be noted here and elsewhere that the designation "surviving animal" refers only to animals surviving to autopsy at 5–7 days following the arrest. Animals that did not survive for at least 5 days following arrest were considered nonsurvivors and unsuitable for interpretation by our neuropathologic studies.

ment to the time of the autopsy, but all were injured both by clinical and neuropathologic criteria. Under moderate hypothermic conditions, all control animals showed injury by neuropathologic examination, whereas hyperbaric oxygenation under the same conditions resulted in greater survival and less neuropathologic injury. The addition of CO_2 to the prearrest ventilating gas appeared to have little or no effect on the results.

Since all available evidence indicates that the most favorable conditions (of those in our experiments) for safely prolonging circulatory arrest should be OHP, increased pCO_2, and moderate hypothermia, these groups of animals treated at 3 ata were compared with their controls treated at 1 ata for 5-, 10- and 15-min arrest periods (data summarized in Figure 3). The results showed little difference for any of the three arrest periods studied between these hypothermic animals exposed to 3 ata and their controls, particularly if neuropathologic evidence of brain injury was taken as the criterion for comparison.

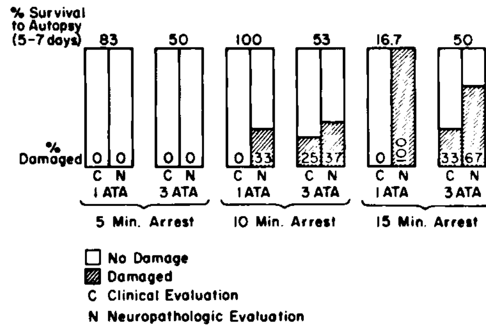

FIGURE 3. Comparison of the protective effects of OHP (3 ata) under conditions of hypothermia (28–30°C) and $O_2 + CO_2$ with the effects of exposure at 1 ata (controls) to these conditions.

Average blood gas and pH values determined just before circulatory arrest for the animals exposed to four experimental conditions, as well as for their control counterparts, appear in Table 2. Both the experimental and control animals respirated with oxygen alone showed the usual respiratory alkalosis due to hyperventilation by the mechanical respirator. The animals exposed to hyperbaric conditions and the control animals studied at normal atmospheric pressure in which the $O_2 + CO_2$ gas mixtures were administered had very similar levels of hypercarbia due to the equivalent alveolar pCO_2 tensions at the two environmental pressures studied.

DISCUSSION

Extension of the safe period of circulatory arrest by hyperbaric oxygenation has been previously studied by several techniques, including various means of assessing the safe end-point.[1,2,7-10] With one exception,[3] most of these previous studies have assumed that the highly vulnerable central nervous system was the limiting tissue in determining the safe period of circulatory arrest. However, none of these

TABLE 2. Analysis of Arterial Blood [a]

Respiratory gas	Pressure (ata)	Temperature (°C)	pH	pCO_2 (mm Hg)	pO_2 (mm Hg)
100% O_2	1	37	7.55	21.7	399.9
94% O_2 + 6% CO_2	1	37	7.26	53.1	451.2
100% O_2	1	28	7.56	23.3	504.0
94% O_2 + 6% CO_2	1	28	7.12	67.0	536.3
100% O_2	3	37	7.41	31.7	1338.0
98% O_2 + 2% CO_2	3	37	7.19	66.7	1330.0
100% O_2	3	28	7.51	23.5	1841.0
98% O_2 + 2% CO_2	3	28	7.14	69.1	1864.2

[a] Average values of arterial blood samples drawn just before arrest period.

studies have considered cryptic neurologic injury, which can be determined only by pathologic study. We consider that this type of injury, although subclinical, should be avoided, and we have therefore based our conclusions on neuropathologic findings.

Our data make it apparent that very little additional safe time can be gained by hyperbaric oxygenation at 3 ata before circulatory arrest, even when it is combined with moderate hypothermia (28–30°C) and CO_2 is added to the ventilating gas mixture to enhance cerebral circulation and thus cerebral O_2 content. This might be expected, however, when one considers that pure O_2-breathing at 3 ata can result in a maximum increase in blood oxygen content of only 27%. If one logically assumes that no significant storage volume of O_2 above normal levels can be achieved in the extravascular water of the central nervous system due to its limited volume with relation to its rapidly metabolizing mass, then the most optimistic prediction for the extension of the safe period for circulatory arrest would be only about 25% by prior hyperbaric oxygenation at 3 ata. Thus, if the safe period of circulatory arrest under normothermic conditions at 1 ata is approximately 4 min, one would expect OHP at 3 ata to extend this period by no more than an additional minute or so. Although we have not studied circulatory arrest periods terminated at minute intervals between 5 and 10 min to determine the exact safe end-point, our data indicate that it is well under 10 min at 3 ata under normothermic conditions.

Similarly, if modest hypothermia (28–30°C) under normal atmospheric conditions permits extension of the safe arrest period to 8–10 min one could then expect only 2–3 additional safe minutes under moderate hypothermia combined with hyperbaric oxygenation at 3 ata.

SUMMARY

In the dog, hyperbaric oxygenation at 3 ata appears to extend the safe period for total circulatory arrest by about 1–2 min under normothermic conditions and 2–3 min under moderate hypothermia (28–30°C), as judged by careful neuropathologic study. The addition of CO_2 to the prearrest ventilating O_2 — not only to counteract any OHP-induced cerebrovasoconstriction but also to achieve maximum cerebrovasodilatation (and hence maximum O_2 delivery)—seemed to have little effect upon the results obtained with hyperbaric oxygenation. Our data, based upon neuropathologic study, are consistent with the theoretical prediction that the O_2 available to the brain under OHP at 3 ata will be only about 27% more than that normally available with air-breathing at 1 ata, and only 20% more than that available with 100% O_2-breathing at 1 ata.

ACKNOWLEDGMENTS

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We wish to thank Miss Laura Ann Ballard for her invaluable assistance in the performance of these studies.

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DISCUSSION

Dr. A. C. Guyton (*Jackson, Miss.*): About 10 years ago, Dr. Crowell (Surgery 38:696, 1955) found that if animals are protected against blood clotting, circulation can be suspended for prolonged periods of time without significant clinical signs of brain damage. He was able, after heavy heparinization, to stop circulation in dogs for 10 minutes, and all the animals survived. Without heparin, however, he observed the same effect that you did, *i.e.*, approximately 50% nonsurvival, or severe brain damage after 5 minutes' suspension of the circulation. He also found that, even with heparin, the pH decreases so much in 10 minutes that the blood begins to sludge markedly. With the addition of streptokinase, however, circulation could be stopped for 20 minutes, and almost all of the animals were walking around the next day. Recently, Dr. Neeley informed me that he had compressed the brain by increasing the cerebrospinal fluid pressure to several hundred mm Hg and thereby pushed all the blood out of the brain. He then caused circulatory arrest for as long as 30 minutes, and the dogs survived. The question here is whether hyperbaric oxygen might not be utilized to better advantage after the period of circulatory arrest than before, because if it is intravascular clotting that causes brain damage, high pressure oxygen might allow better diffusion of oxygen to the ischemic tissues.

Dr. Moor: These animals were not heparinized. In regard to clinical signs, we realize that this is a very stringent evaluation of circulatory arrest by a histoneuropathologic examination. Many of our animals looked quite normal at the end of 5 to 7 days, although they did have extensive neuropathologic lesions. We also did 20-minute circulatory arrests. Of three survivors, two looked perfectly normal, and you might take them home as house pets. However, microscopically, they were severely damaged.

Dr. R A. Cowley, *Session Chairman (Baltimore, Md.)*: Dr. Moor, were you able to see thrombosis in the pathological sections?

Dr. Moor: Perhaps Dr. Margolis could comment a little more extensively on this. During the autopsy procedure, the blood was washed from the cerebral circulation prior to perfusion with fixative solution, in order to prevent agglutination of the blood and protein elements in the cerebral vasculature which would prevent adequate fixation.

Dr. Margolis: Dr. Guyton's critical comments are very welcome because they bring

to focus some points which should be made about a study like this. I have been quite interested in the work quoted by Dr. Guyton and have been extremely enthusiastic about it on first reading. But, when I look for the autopsy findings in such studies, I find none. The reports are, in fact, labeled "survival studies." It might be worthwhile to emphasize the difference between survival and morbidity with a few illustrations. First, I want to differentiate between overt damage and cryptic damage. Figure 1C (p. 366) shows an example of overt damage, a section of the cerebellum in which you see absolutely no Purkinje cells. Figure 1A (p. 366) illustrates another example of an overt injury in which you see many eosinophilic necrotic neurones and many pale nuclei which belong to hypertrophied astrocytes. The point I wish to make here is the value of Cammermeyer's perfusion fixation technique. Those of us who go to neuropathology meetings often see a rather ascetic-looking man stand up and criticize the work of his fellow pathologists because they have not used suitable methods for eliminating various common artifacts. As he states, whenever you see perivascular spaces about capillaries and perineuronal spaces about normal neurones, the evaluation of the neuronal changes is most difficult. Figure 2C (p. 367) shows an example of the degree of neuronal damage that we have seen in dogs which have survived experimental procedures and are walking around, wagging their tails, and, as you said, would make good house pets. This contrasts with the structural features of normal neurones, and the absence of perineuronal spaces in well-perfused normal tissue. The striking contrast between these cells and the shrunken, eosinophilic neurones with pyknotic nuclear changes is readily appreciated. Dead cells such as these can be easily picked out in the brains of these so-called "normal" dogs. The other point concerns the concept of delayed neuronal damage. Any of us attempting from this picture to gauge the age of neuronal death would say that this happened within a day or two of death. Yet, this animal might have been autopsied a week following the experimental procedure. Does this mean that at some time during the postoperative period, this animal which got up and walked around still had cardiopulmonary difficulties because of the hyperbaric procedure, or because of other surgical procedures? I do not know how to evaluate critically all the factors here. For example, what would have been gained by intensive postoperative heparinization, periodic assistance or stimulation of respiration, and careful control of blood volume, pH, *etc?*

DR. J. D. BALENTINE (*Durham, N. C.*): Dr. Guyton's criticism has raised the question whether we, as neuropathologists, can tell the difference in distribution between lesions produced by focal ischemia, as seen in cerebral thrombosis and embolization, and those produced by hypoxemia and generalized cerebral ischemia, as seen in circulatory arrest. If there were thrombi, I would not expect to see what has been identified as selective neuronal necrosis. I think you have to underline "selective," because I believe the embolic or thrombotic phenomenon would bring about more widespread damage, damaging white matter perhaps more than grey, or at least, in addition to grey. I would like to ask Dr. Margolis if he feels that the distribution of the lesion helps him to distinguish between ischemia produced by thrombi or emboli and that produced by circulatory arrest.

DR. MARGOLIS: These lesions were seen in the absence of any evidence of thrombi at the time of the autopsy. The question of sludging and the question of disappearing thrombi cannot be answered, of course, except that with perfect perfusion one can see the vessels standing open. Another change in the blood vessels which is visible by electron microscopy, though perhaps not visible in light microscopy, was recently described by Dr. Majno of Harvard (unpublished data). This is a peculiar bubbling of the endothelium which he feels may be an obstructive factor. I do not know whether this is an irreversible or a reversible change. We saw no microinfarcts and no thrombi, and I would agree with you that what we have is a selective neuronal necrosis which is cryptic in the sense that it is not clinically recognized, and delayed in that its onset occurred sometime after the experimental procedure.

DR. R. H. PATTERSON (*New York, N.Y.*): Should neuropathological damage be the only yardstick of the safe period of circula-

tory arrest? If the early microscopic changes induced by ischemia are reversible, an additional criterion may be the length of time required for the animal to recover sufficiently to meet the demands of his environment. Certainly, cardiopulmonary bypass is responsible for neurological deficits in a substantial number of patients, yet the technique is employed because there is usually functional recovery, and because the necessity for extracorporeal circulation in cardiac surgery is unquestioned.

Another question is prompted by our experience that, in animals in which circulatory arrest is obtained by ventriculai fibrillation, starting the heart was sometimes more difficult than protecting the brain. Could you reliably start the heart in all the animals?

Dr. Moor: I can confirm Turnbull's observation (J. Thorac. Cardiov. Surg. 50:842, 1965) that defibrillation of the heart under hyperbaric conditions is extremely easy in comparison to 1 ata. Frequently, one external countershock of 100 watt-seconds is sufficient to restart the heart immediately and to sustain good blood pressure throughout the remainder of the procedure. In regard to the first part of your question, we feel that the neuropathologic endpoint is the true endpoint if you are producing these lesions electively. We do not like to voluntarily produce a lesion of this nature in patients.

Effect of Hyperbaric Oxygen and Hypothermia on Cerebral Ischemia

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The ultimate value of hyperbaric oxygenation will depend largely on whether it can sustain hypoxic tissue while reparative processes or surgical intervention restore an adequate circulation. Possibly pretreatment with hyperbaric oxygen will allow prolonged interruption of the cerebral circulation during operations on the heart, brain, aortic arch, and brachiocephalic vessels. The clinical application of OHP under these circumstances depends, of course, on adequate preliminary evaluation in the laboratory, but this has been difficult because complete cerebral ischemia is not easily attained in animals. Indirect approaches to the problem such as inflow–outflow cardiac occlusion,[1] electrically induced ventricular fibrillation, and inhalation of 100% nitrogen[2] have been relatively unsuccessful due to effects on the cardiovascular system which may cause death, even during adequate protection of the brain from hypoxia. Raising intraspinal pressure above systemic blood pressure has been tried, but whether the central nervous system effects are wholly due to ischemia is dubious.

Interruption of the arteries supplying the brain is a logical way to attain cerebral ischemia. In 1961, Smith *et al.*[3] reported that when bilateral occlusion of the carotid and vertebral arteries was performed on dogs, electroencephalographic (EEG) activity persisted only 15 sec in dogs breathing air, but continued indefinitely in dogs breathing 100% oxygen at 2 atm. We could not duplicate this experiment; the carotid and vertebral arteries were occluded for 30 min in five dogs breathing air at 1 atm without any change in the EEG pattern (Figure 1). Radiographs of the cerebral circulation obtained by retrograde injection of a barium solution into the aortic arch revealed extensive collateral circulation which bypassed the ligated arteries to supply the brain (Figure 2).

In 1938, Kabat and Dennis[4] described a technique for producing cerebral ischemia in dogs, which has recently been used by Boyd and Connolly.[5] By this method, most of the lamina of the second cervical vertebra is excised to expose the dura enclosing the spinal

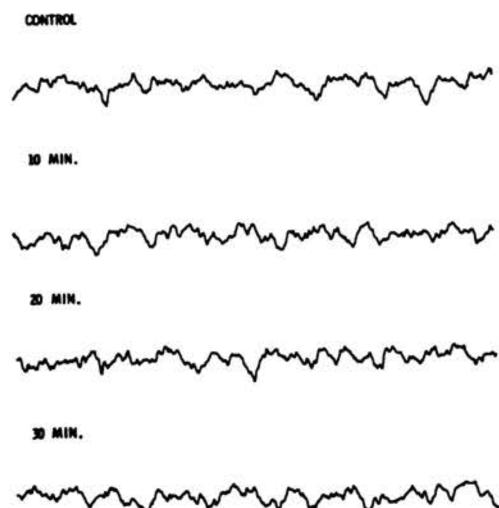
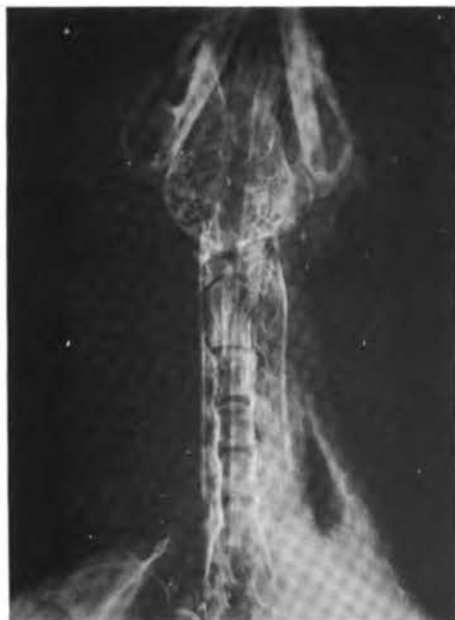


FIGURE 1. EEG activity in a dog breathing air at 1 atm with both carotid and vertebral arteries ligated.

cord. After the animal recovers from the operation, a pneumatic cuff is placed around the neck over the laminectomy. Rapid inflation of the cuff occludes both extraspinal and intraspinal arteries, and EEG activity stops within 15–25 sec. Although dogs rarely survive more than 7 min of ischemia by this method, cerebral angiograms have shown that the intraspinal circulation is not completely arrested. The few small intraspinal arteries which remain open assume importance in a hyperbaric environment, as even a small blood flow may adequately supply oxygen to the brain. More extensive laminectomy permits the cuff to occlude all the intraspinal arteries, but tetraplegia as a result of compression of the spinal cord also occurs, even after a brief period of ischemia. Survival is therefore difficult to evaluate in these animals, although they can be used to study the persistence of EEG activity after interruption of the cerebral blood supply.

Figure 3 illustrates the length of time the EEG persisted after inflation of the cuff in 11 animals. The average duration of EEG activity was 15.1 sec when the animals breathed air at 1 atm. With oxy-



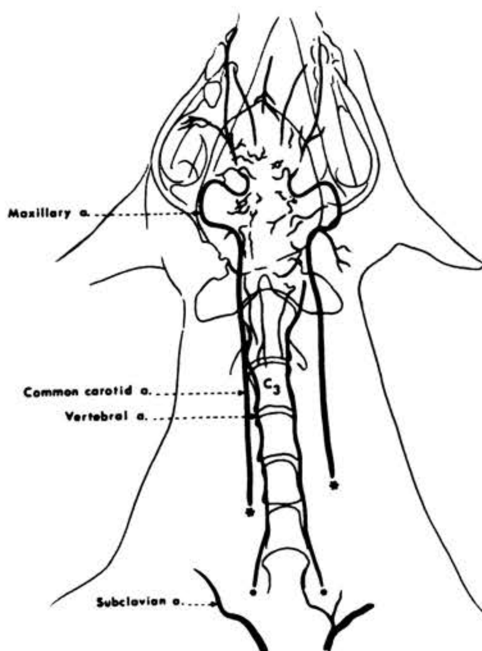


FIGURE 2. Cerebral angiogram of dog with carotid and vertebral arteries ligated, demonstrating extensive collateral circulation.

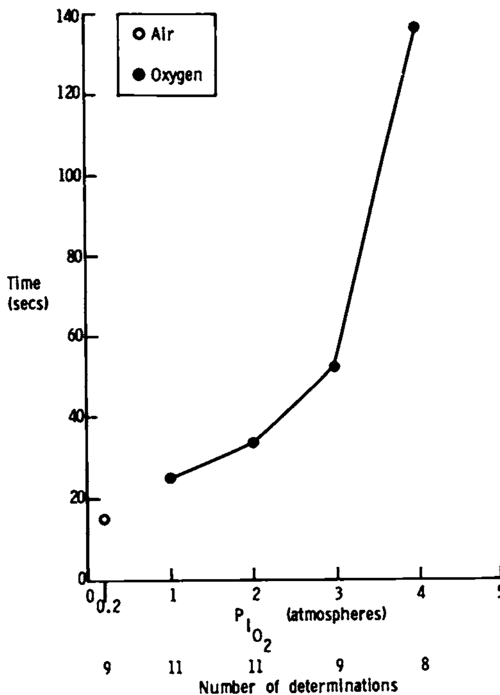


FIGURE 3. The effect of hyperbaric oxygen on EEG persistence time (method of Kabat and Dennis[4]).

gen at 1 atm, EEG activity was prolonged to 25.1 sec. By raising alveolar oxygen tension to 4 atm, the duration of EEG activity was increased to 136.6 sec. The mean oxygen tension of arterial blood measured in six of these dogs at 4 atm was 2771 mm Hg. Aveolar oxygen tension was calculated to be 2972 mm Hg.

To study survival after total arrest of cerebral blood flow under hyperbaric oxygenation and moderate hypothermia, a new technique for producing complete cerebral ischemia in dogs was developed.

MATERIALS AND METHODS

Operative Procedure

Under general endotracheal anesthesia (methoxyflurane), the dogs were supported on a wooden frame in prone position, and a 10-cm midline incision was made over the second cervical vertebra. The paraspinal muscles were reflected subperiosteally from the spine and lamina of C-2, and both vertebral arteries were coagulated and divided at their exit from the vertebral canal. The midportion of the vertebral spine was removed and the lamina thinned by a motor-driven burr. A laminectomy 2 cm wide, extending from one vertebral foramen to the other, was performed. The dura was opened and the small arteries on the posterior and lateral surfaces of the spinal cord coagulated with bipolar forceps submerged in saline to minimize heat spread.

After wound closure, the animal was turned to the supine position, and a 12-cm linear incision was made centering over the notch in the thyroid cartilage. The dissection was carried around the strap muscles and esophagus, which were retracted laterally. The body of the first cervical vertebra could be easily identified by palpating its prominent ventral spine, and the muscles were reflected to expose the C-1 vertebra and the foramen magnum. The ventral arch of C-1 and the underlying odontoid process with its heavy transverse ligament were then removed. The dura was opened to reveal the upper cervical cord and the large anterior spinal artery. The artery was coagulated with the bipolar forceps and divided. Other small arteries on the surface of the cord were simply coagulated. A pledget of absorbable gelatin sponge was placed over the dural defect and the wound closed. An occasional dog, left paraplegic by the operation, was unfit for further study, but as we gained experience most of the animals sustained little or no weakness.

Pressurization

After satisfactory recovery from the operative procedure (usually 4–10 days),

the dogs were lightly anesthetized with sodium thiopental, a noncompressible cuffed endotracheal tube was inserted, and respirations were controlled with a Bird respirator. Gallamine triethiodide, 2–3 mg/kg, was given intravenously to prevent muscular activity from interfering with the EEG, and 0.4 mg of atropine was administered to reduce tracheobronchial secretions. The neck of the animal was then wrapped with two 4-in. inflatable cuffs. An Offner oscillograph recorded the electrocardiogram (ECG) and two channels of an EEG from electrodes in the scalp. When hypothermia was used, body temperature was reduced to 28°C by immersion of the animal in a tub of ice water.

The animal was then placed in a one-man hyperbaric chamber 9.5 ft long, with an internal diameter of 28 in. (Figure 4). For occlusion of the cerebral circulation, the cuffs were inflated to 20 psig above ambient pressure in the chamber from a reducing valve on a cylinder of oxygen (Figure 5). Since inflation of the cervical cuffs was regularly followed in 3–4 min by ventricular fibrillation, fibrillation was prevented in animals used for survival studies by the prior administration of 200 ml of low molecular weight dextran.

At the conclusion of the investigations, cerebral angiograms were obtained in all

FIGURE 4. One-man hyperbaric chamber (Bethlehem Corporation) used at Cornell University Medical College.

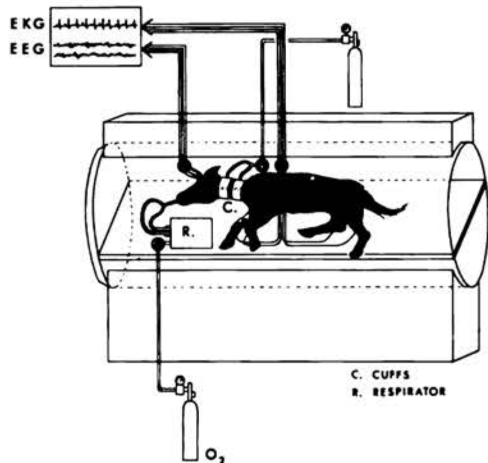


FIGURE 5. Graphic representation of experimental method.

animals with the pneumatic cuffs inflated. Barium was perfused into the aortic arch at a pressure of 275 mm Hg after ligation of the ascending and descending aorta. Unless radiographs of the head and neck in the anteroposterior and lateral projections confirmed the complete absence of barium in the cerebral blood vessels, the animal was discarded from the study.

EEG Persistence Time

The duration of EEG activity was measured on nine mongrel dogs weighing 10–18 kg. No animal was subjected to more than one episode of cerebral circulatory arrest per day to eliminate the possibility that reactive hyperemia or a cumulative oxygen debt might influence the disappearance of EEG activity. Data were gathered with the dogs breathing air at 1 atm and oxygen at ambient pressures from 1 to 5 atm both at normal body temperature and at 28°C.

Survival Studies

Each dog was subjected to increasing periods of complete cerebral ischemia until it died or became decerebrate at 24

hours. In one group, cerebral circulation was arrested in five dogs breathing air at 1 atm for 4, 6, and 8 min. In a second group, the dogs breathed oxygen at 3 atm for arrest periods of 10 and 12 min. A third group also breathed oxygen at 3 atm, but the animals were cooled to 28°C before occlusion of cerebral circulation for 14–50 min.

Results

EEG Persistence Time

As alveolar oxygen tension was raised in increments of 1 to 5 atm, EEG activity persisted after arrest of the cerebral circulation for progressively longer intervals (Figure 6). The rate of increase was not linearly related to ambient pressure and varied from 2.0 sec between 2 and 3 atm to 12.2 sec between 4 and 5 atm. EEG activity was prolonged an average of 46% at 3 atm and 156% at 5 atm.

Moderate hypothermia (28°C) with hyperbaric oxygenation further prolonged the duration of EEG activity at any given oxygen tension. The increased EEG persistence time that could be attributed to hypothermia varied from 2.8 sec at 4 atm to 6.2 sec at 5 atm (Figure 7).

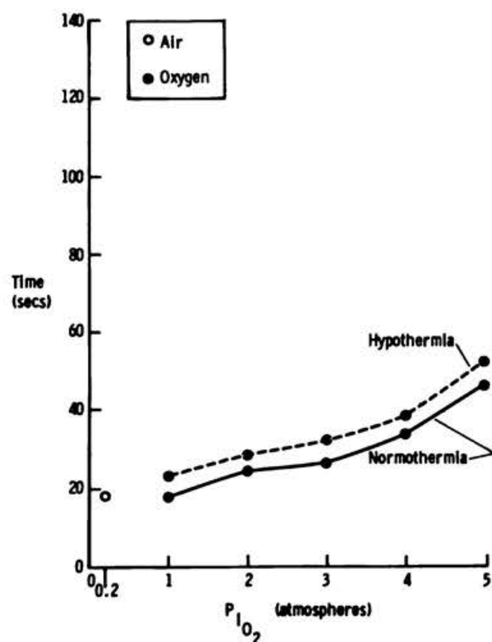


FIGURE 6. The effect of hyperbaric oxygen and hypothermia on EEG persistence time after complete cerebral circulatory arrest.

Survival Studies

All five dogs breathing air at 1 atm survived 4 min of cerebral anoxia. Two died after 6 min of anoxia, and the other three died after 8 min. Of the dogs breathing 100% oxygen at 3 atm, two died after 10 min of cerebral anoxia and the other three died after 12 min. In the third group breathing 100% oxygen at 3 atm during hypothermia, death or severe neurologic damage was delayed until after 30 min of cerebral circulatory arrest, when two of the five dogs died. One more died after 40 min, and the last two not until after 50 min of interrupted cerebral blood flow.

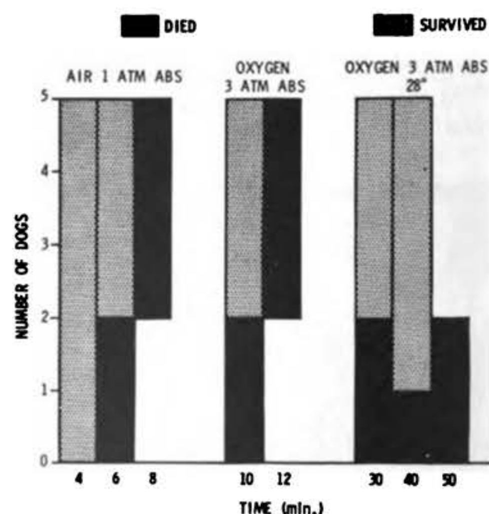


FIGURE 7. The effect of hyperbaric oxygen compared to hypothermia plus hyperbaric oxygen on survival of dogs after complete cerebral ischemia.

DISCUSSION

EEG activity persisted in our studies for an average of 18.2 sec after interruption of the cerebral circulation when the dogs breathed air. When oxygen at 1 atm was administered, cerebral electrical activity still continued for 18 sec. At 3 and 5 atm of oxygen-breathing, however, EEG activity persisted for 26.5 and 46.6 sec, respectively. These data for 1 atm air, 1 atm oxygen, and 3 atm oxygen are almost identical with those reported by Fuson *et al.*,[6] who studied animals in which cerebral ischemia was obtained by the injection of saline in the subarachnoid space to a pressure higher than that of arterial blood.

When Heyman *et al.*[2] arrested the circulation by inducing ventricular fibrillation, EEG activity was recorded for 40 sec in animals breathing oxygen at 1 atm —more than twice as long as EEG persistence in our study. Increasing the ambient pressure to 3 atm sustained electrical activity for 60 sec, which was also double the time observed in our animals.

Carlisle *et al.*,[7] in studies on the visual loss which follows retinal ischemia induced by pressure on the globe, reported that scotomata occurred after an average of 4 sec in subjects breathing air at 1 atm, after 6 sec during oxygen-breathing at 1 atm, and after 30 sec during oxygen-breathing at 3 atm. The length of time vision was preserved was proportional to the arterial oxygen tension. Anderson and Saltzman,[8] using a modified technique, reported similar data. It appears that visual persistence in humans is slightly longer at 3 atm than EEG persistence in the animals in our study.

We found that all dogs breathing air at 1 atm survived cerebral circulatory arrest for 4 min, but some died after 6 min and the rest after 8 min. Pretreatment with hyperbaric oxygen lengthened the time that the animals tolerated cerebral ischemia in about the same proportion as it lengthened the duration of EEG activity. Some dogs withstood 10 min of arrest at 3 atm during oxygen-breathing, but none survived after 12 min.

Hyperbaric oxygenation supplemented by moderate hypothermia only slightly increased the time that electrical activity continued after the cerebral circulation was stopped. Survival was much enhanced by the combination, however. Serious neurologic damage or death did not occur in dogs with less than 30 min of ischemia when the dogs breathed oxygen at 3 atm at a body temperature of 28°C.

Our data suggest that hypothermia and hyperbaric oxygenation combined may find clinical application in operations in which the arterial supply to the brain is likely to be compromised. Further studies in dogs and in other species are advisable, and the value of adding carbon dioxide to the respiratory gas needs investigation.

ACKNOWLEDGMENTS

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DISCUSSION

DR. R. L. FUSON (*Durham, N. C.*): We have produced selective cerebral ischemia by inserting a needle in the cisterna magna and rapidly increasing the cerebral spinal fluid pressure over arterial pressure with a saline infusion. Once the cerebral spinal fluid pressure is greater than arterial pressure, there certainly is no cerebral blood flow. This technique achieves absolute cerebral ischemia without the need for the extensive surgical dissection required in your preparation. EEG flattening time or persistence was taken as the endpoint of each ischemic episode. Dogs ventilated with air at 1 atmosphere had EEG persistence of 17 seconds. There was no significant change with 100% oxygen or with 2% carbon dioxide, but with the addition of 6% carbon dioxide at 1 atmosphere to the oxygen mixture, a significant increase of the EEG flattening time occurs. We believe this effect is due to cerebral vasodilatation in response to carbon dioxide and increased oxygen storage in the brain. The EEG persistence of these normothermic animals was also studied during successive respirations of air, 100% oxygen, and a carbon dioxide–oxygen mixture at 1, 3, and again at 1 atmosphere absolute. Thus, these animals all served as their own controls. Since there was a very quick return to base-line values on the return to 1 ata, we felt that no damage had been produced by the occlusion *per se*. At 3 atmospheres, ventilation by air or 100% oxygen resulted in only a slight increase in EEG flattening time over ambient values. However, when we ventilated the animal with 2% carbon dioxide in oxygen at 3 atmospheres we obtained a striking fivefold increase in EEG flattening time. These results indicate increased brain oxygen storage during hyperbaric oxygenation at 3 ata when carbon dioxide is used to avoid cerebral vasoconstriction. However, this 70-second prolongation of EEG activity would seem to have little clinical value if circulation is absolutely halted.

DR. R. WHALEN (*Durham, N. C.*): We, too, have had some experience in trying to solve this problem (Arch. Neurol. 14:15, 1966). Dr. Heyman, Dr. Saltzman, and I did a similar study of EEG flattening time and found essentially what you did. We chose not to use your surgical isolation technique but used ventricular fibrillation, which produced complete cessation of circulation to the brain, as indicated by xenon blood flow studies. We were able to fibrillate the animals, wait until the EEG flattened, and then defibrillate them repetitively, thus using each animal as its own control. We found that there was a small, but statistically significant, percentage increase in EEG flattening times under normothermic conditions. Hypothermia and hyperbaria were additive in prolonging EEG activity. It was interesting that hypothermia at 1 atmosphere was as effective as hyperbaria at 3 atmospheres. The increases in EEG activity could be expressed in seconds, rather than minutes or hours. This made us less willing to accept hyper-

baria as a miraculous way of protecting the brain.

DR. R A. COWLEY, *Session Chairman* (*Baltimore, Md.*): Dr. McSherry should be congratulated on his persistence in using dogs to complete his studies. However, these animals are very difficult to work with in such experiments, and I believe that many problems could be solved by using an animal closer to us phylogenetically, *i.e.*, the primate.

Survival After Prolonged Induced Circulatory Arrest, Hyperbaric Oxygenation, and Hypothermia

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The use of hyperbaric oxygen in ischemic conditions is based on the premise that tissue survival is jeopardized more by hypoxia than by failure of circulation to maintain homeostasis (*i.e.*, supply nutrients, remove catabolites, or maintain pH). Consequently, we designed some experiments on dogs in which a large reservoir of oxygen was made available to the tissues by combined hypothermia and hyperbaric oxygenation, but circulation was stopped for long periods. The effect of adequate oxygen without perfusion upon an intact animal was then evaluated by determining survival after prolonged circulatory arrest.

The amount of available oxygen can be increased both relatively and absolutely by combining hypothermia and hyperbaric oxygenation. Figure 1 shows the exponential decline of oxygen requirements as temperature is reduced. Several studies [1-3] on the relationship of oxygen utilization to temperature variation are in agreement with van't Hoff's rule of the velocity of chemical reactions. Thus, when the body temperature is 3°C, oxygen consumption is approximately 10 ml/kg/hour, or about 0.3% of normal requirements.

In addition to raising the ratio of the availability of oxygen to utilization of oxygen, hypothermia also increases the supply of dissolved oxygen, according to Henry's law. At 3°C the solubility of oxygen in blood is increased to 5.1 vol% from its value of 2.3 vol% at 37°C. The solubility is directly proportional to the oxygen pressure, so that for blood at 3°C it increases from 5.1 vol% at 1 atm to 15.3 vol% at 3 atm. The number of hours of oxygen supply available can, of course, only be estimated and depends on many details of oxygen storage that will not be discussed here. Furthermore, the total amount of oxygen stored within the body is not of much account when one considers the fate of a specific organ. The body contains tissues that diffuse gases at low and high rates. Some tissues, such as fat, myoglobin, and hemoglobin, have greater affinity for oxygen than others. Many distribution and diffusion factors at the cellular level are unknown. Nevertheless, it would seem that available oxygen could be increased to meet metabolic

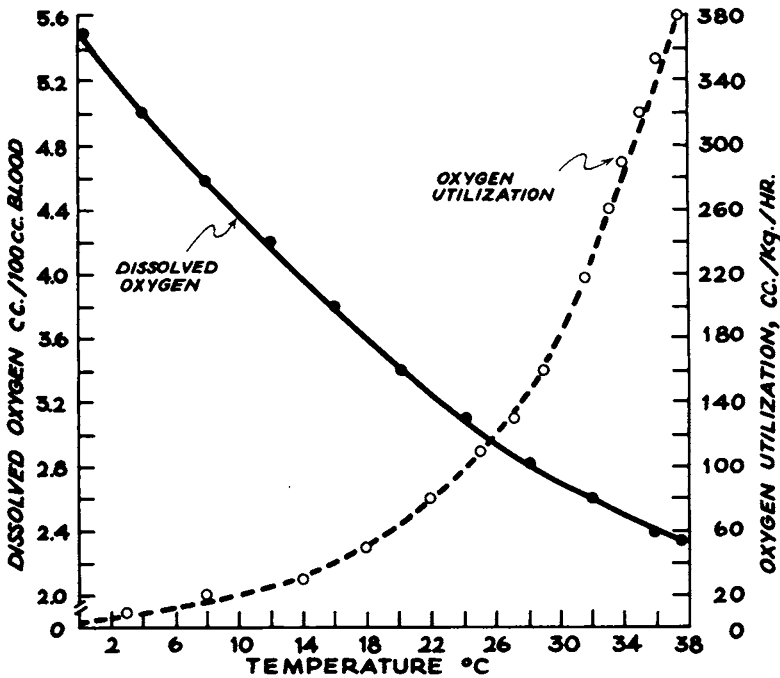

FIGURE 1. Effect of temperature on amount of oxygen dissolved in blood and rate of oxygen utilization (according to van't Hoff's law). (Modified from J. Sendroy, Jr., *et al.*: J. Biol. Chem. 105:597, 1934.)

needs for several hours at 3°C and 3 atm (Figure 2).

Materials and Methods

Twenty-five dogs (10-30 kg) were lightly anesthetized with thiamylal sodium (Surital) and an endotracheal tube was inserted. External bypass was begun so that venous return taken from the right jugular and femoral veins flowed by gravity to a reservoir and disc oxygenator (Figure 3). Blood was then circulated by a roller pump through a heat exchanger and bubble trap and was returned to the animal through the femoral artery. The left atrium was drained by gravity into the reservoir in nine of the dogs.

After preparation for bypass was completed, the animals were placed in the hyperbaric chamber and pressure was increased to 10, 15, or 30 psig. After the desired pressure was reached, a test bypass was begun and (in the absence of problems) cooling was started. Perfusion rates varied from 50 to 100 ml/kg/min and were determined by the rate of venous return. The perfusion rate decreased with temperature decrease, being 20–35 ml/kg/min as 3°C was approached. Temperature fell precipitously during the first 10 min of perfusion; esophageal temperature during this period was 15–18°C. Rectal temperature decreased much more slowly, and the more rapid the perfusion, the greater the disparity between esophageal and rectal temperatures. The time required for cooling the esophagus to 3°C and the rectum to 4–7°C was 30–75 min.

Carbon dioxide was mixed with the oxygen supplying the oxygenator to give a partial pressure equivalent to 5% carbon dioxide at the three pressure levels used. The priming volume of the bypass system was 1500 ml; another 1000 ml

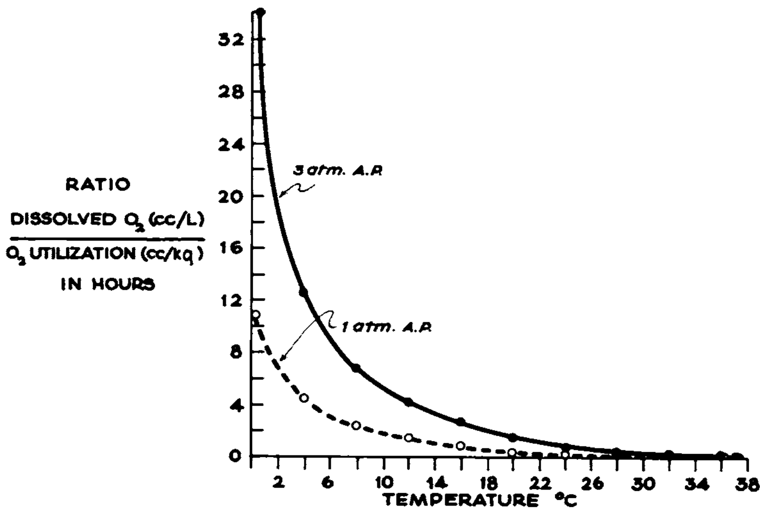

FIGURE 2. Available oxygen relative to metabolic requirement during hypothermia at 1 and 3 ata.

was placed in the reservoir. The priming solution was composed of freshly obtained heparinized blood, 500 ml of Ringer's lactate solution, and low molecular weight dextran (Rheomacrodex), 10 ml/kg body weight. Heparin (3 mg/kg) was given before the bypass, and half of the initial dose was given again just before circulatory arrest. Sodium bicarbonate was used to control acidosis.

Circulatory arrest was accomplished by turning off the arterial pump. The venous return was left open so that the dogs were partially exsanguinated. Ice water was pumped through coils of tubing (arranged to form a jacket) to sustain the low temperature of the dogs. After 3.5 hours of circulatory arrest, esophageal temperature rose to only 8–9°C. After circulatory arrest was terminated, rewarming was performed by reversing the cooling process. Initially, blood was slowly transfused

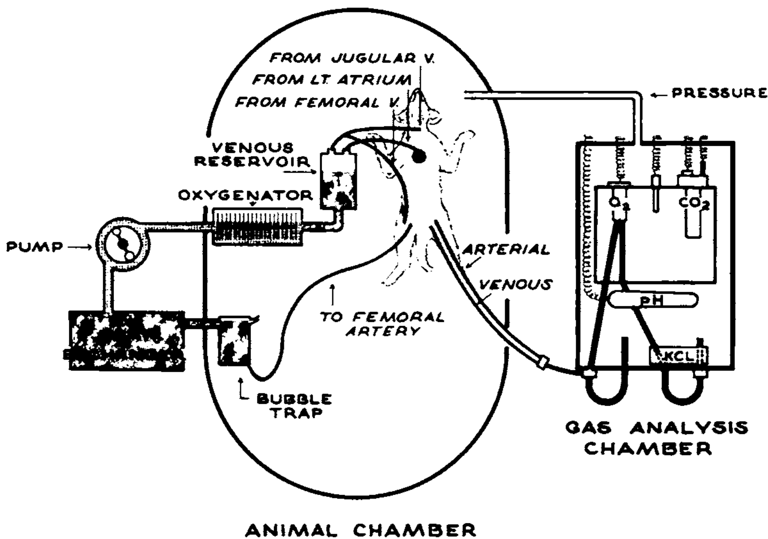

FIGURE 3. Schematic diagram of cardiopulmonary bypass circuit and hyperbaric chamber.

through the arterial cannula. Blood did not flow on the venous side of the bypass during the first few minutes. When venous return did begin, the perfusion rate was gradually increased. After the dogs were warmed to 30°C esophageal temperature and the chamber was flushed of oxygen, defibrillation (if needed) was performed by an external defibrillator. Circulation was assisted by pump bypass to insure gradual rewarming to 38–39°C. During rewarming, sodium bicarbonate was administered in amounts dependent upon blood pH. Total rewarming time was 35–75 min, but many dogs required assisted circulation with the pump for an indefinite period. Sometimes the heartbeat was strengthened by small intravenous doses of isoproterenol (Isuprel). Excessive irritability was usually treated by intravenous lidocaine. After complete rewarming, slow decompression was started.

During these experiments, venous and arterial blood pressures, electrocardiogram, electroencephalogram, and rectal and esophageal temperatures were monitored continuously. The blood pH, pO_2, and pCO_2 were measured at regular intervals. The experiments were performed in an animal chamber 3 ft in diameter and 8 ft long. Once the chamber pressure had been elevated, the animal was inaccessible to direct control.

Results

During circulatory arrest the animals did not move. The electroencephalographic tracing was flat. Wave activity was usually absent in the electrocardiogram as well, but four dogs intermittently had a slow rhythmic QRS complex during circulatory arrest for brief periods.

At the end of circulatory arrest, slow rewarming and transfusion were begun. The heartbeat returned at esophageal temperatures as low as 8°C. Fibrillation usually occurred at temperatures of 17–18°C, increasing in amplitude as warming continued. Respiration was usually first detected by slight rhythmical muscular movements in the throat, which gradually progressed to involve abdominal and thoracic musculature and produced an increasing tidal volume. While respiration was observed as early as 17°C esophageal temperature, it usually did not occur until 25°C was reached. Shivering began only when esophageal temperature reached 32–34°C.

Several signs, including failure to breathe, difficulty in defibrillation, and bulging of the eyes, indicated that the animal was unlikely to recover. Later, a poor venous return and decreased blood pressure made transfusion necessary, and this was followed by pulmonary edema or hemorrhage from the tracheobronchial tree. The earlier respiration occurred, the better was the prognosis. When spontaneous respiration did not occur by the time an esophageal temperature of 30°C was reached, the animal did not survive.

The events important to recovery of the dogs after circulatory arrest are summarized in Figures 4 and 5. In most dogs with impaired cardiovascular function, perfusion had been technically unsatisfactory or resuscitation was difficult because monitoring equipment had failed and blood pressure and other vital signs could not be measured. Other technical problems included kinking of venous return lines and partial withdrawal of a cannula during defibrillation. On one occasion, the depleted oxygen supply to the oxygenator was not recognized.

Six dogs spontaneously developed sinus rhythm during rewarming. Defibrillation was accomplished in all but two of the remaining dogs. The central nervous system was more susceptible to failure than the cardiovascular system. On seven occasions, dogs that had normal sinus rhythm did not develop spontaneous respiration. Figure 4 indicates that the duration of circulatory arrest did not determine whether injury to either the cardiovascular

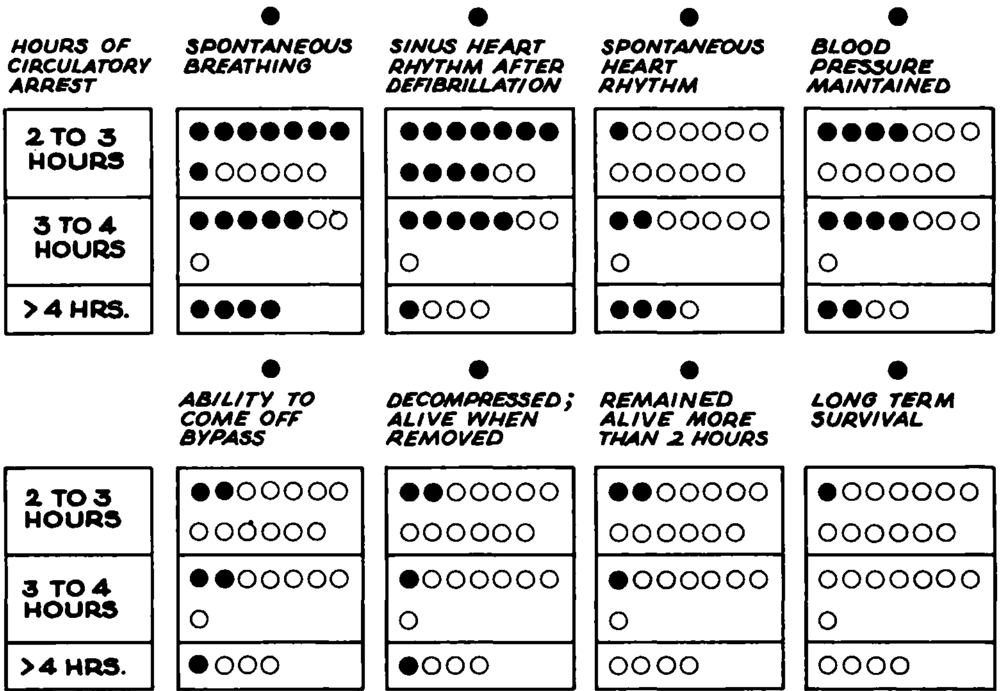

Figure 4. Effect of duration of circulatory arrest upon survival (during OHP and hypothermia).

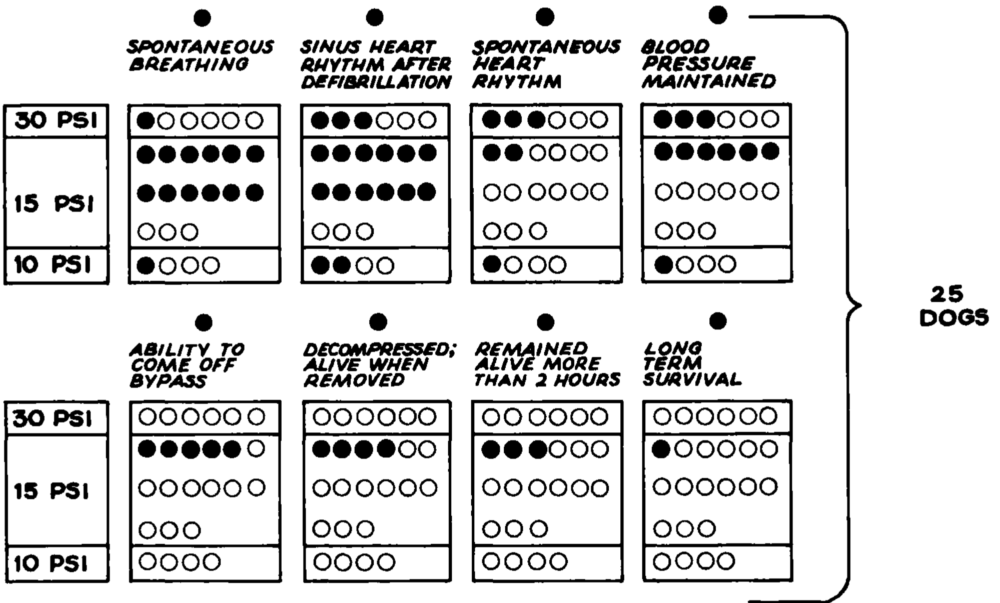

Figure 5. Effect of pressure upon survival after circulatory arrest (during OHP and hypothermia).

system or central nervous system would occur. The chamber pressure did influence ability to recover, however (Figure 5). Dogs subjected to 10 psig and to 30 psig had weaker responses than those subjected to 15 psig. None of the dogs exposed to 10 or 30 psig recovered sufficiently to permit termination of bypass. It was remarkable how often dogs exposed to 30 psig did not breathe.

Typically, the dogs in this experiment appeared to be recovering, as indicated by sinus rhythm, rising blood pressure, and regular breathing. But the animal then gradually deteriorated as warming and decompression were continued. Respiration became slow, shallow, and irregular. Blood pressure fell and would not respond to transfusion. The voltage of the electrocardiogram decreased, and myocardial irritability appeared. Soon afterward, respiration stopped. Blood pressure fell further, and additional assistance from the pump oxygenator was required. The eyes bulged, and watery-pink then frankly bloody froth exuded from the tracheobronchial tree. As the animal became progressively less responsive, the experiment was terminated.

Four of five animals recovered enough to be taken off bypass but later died. The one animal that survived 2.25 hours of circulatory arrest first appeared weaker during rewarming and decompression than the other two animals which survived 2 and 3.5 hours after decompression. These two animals appeared alert and had brisk pupil reaction to light. One of these dogs died, apparently from hypovolemia and hypotension, after being outside the chamber for 2 hours. The animal that died 3.5 hours after removal from the chamber appeared to have an intact nervous system, but frothy fluid flowed from the tracheobronchial tree, and this later became grossly bloody. Respiratory excursions decreased, hypoxia occurred, and the animal died. The dog surviving until sacrifice at 10 months was lethargic, the pupils reacted slowly to light, and there were few spontaneous movements during the first 8 hours, although all four extremities did move. After this, the animal's responses gradually improved. On the second day, the dog was extremely irritable, growled when approached, and resented movement. Disposition became friendly and appetite improved on the sixth day. The dog could remember its cage position when returned from the yard. Behavior was then normal until sacrifice.

Pathology

Postmortem examination was performed on all animals and specimens were examined microscopically. The gross findings were usually dependent congestive atelectasis and pulmonary edema with accumulations of blood-tinged or bloody tracheobronchial fluid. The animals often had edema and congestion of the liver and mediastinum with fluid in the peritoneal cavity. The heart usually had patchy areas of ecchymosis but was grossly similar to the hearts of animals subjected to 2.5 hours of heart-lung bypass and hypothermia. One animal subjected to 4 hours of bypass seemed certain to survive, but its condition deteriorated and death ensued. Autopsy examination showed a diffuse infestation with heart worms.

The prominent bulging of the eyes noted in many dogs was thought to be due to increased intracranial pressure, but gross cerebral edema was not seen in any of the four dogs in which the brain was examined. The dura mater was loose over the brain and the cerebrospinal fluid appeared normal. Retrobulbar edema was the only explanation. A striking finding was that a few animals had bubbles in the vena cava, and in several dogs the myocardium and liver exuded small, barely visible bubbles when squeezed.

The microscopic findings on animals that died during or shortly after the experiment were similar. Brain sections showed minimal histologic changes, but

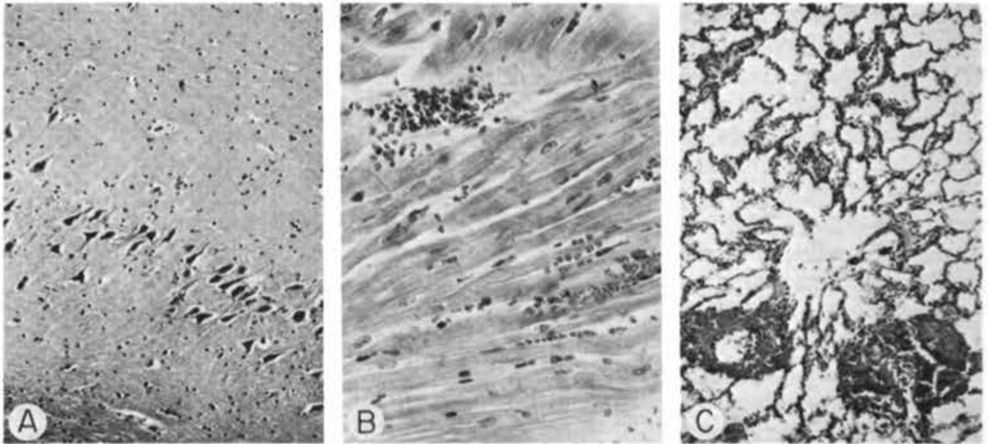

FIGURE 6. A, section of normal-appearing cerebral cortex from dog not surviving circulatory arrest. B, section of myocardium from dog not surviving circulatory arrest, showing patchy areas of red blood cell extravasation. C, section of lung from dog not surviving circulatory arrest, showing interstitial edema and luminal secretions.

since specimens were taken so soon after injury, there may have been no opportunity for morphologic changes to occur (Figure 6A). The myocardium showed mainly patchy areas of red blood cell extravasation (Figure 6B). Congestion, interstitial edema, and luminal secretions were present in the lungs (Figure 6C). The kidneys and adrenal glands were unremarkable.

Gross examination of the surviving dog at sacrifice after 10 months revealed the brain, meninges, heart, lungs, liver, and adrenal glands to be normal. Microscopically, the brain showed gliosis and a diminished number of cortical neurons (Figure 7A) compared to the brains of animals surviving briefly (Figure 6A); the number of Purkinje cells in the cerebellum was reduced. The heart appeared normal. The lungs, if they had been damaged from the procedure, seemed to have recovered well except for occasional peribronchial fibrosis (Figure 7B). The

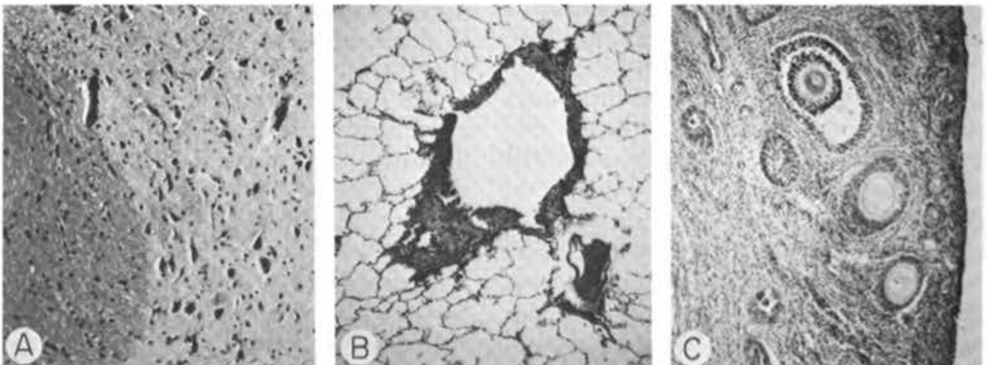

FIGURE 7. A, section of cerebral cortex from dog sacrificed 10 months after circulatory arrest, showing gliosis and diminished number of cortical neurons. B, section of lung from dog sacrificed 10 months after circulatory arrest, showing peribronchial fibrosis. C, section of normal-appearing ovary from dog sacrificed 10 months after circulatory arrest.

remainder of the microscopic examination was normal, and there was evidence in the ovaries that ovulation had occurred (Figure 7C).

Discussion

The duration of successful circulatory arrest was extended by combining hypothermia and hyperbaric oxygen in these experiments. As far as we can determine, 2.25 hours of successful (in terms of survival) circulatory arrest is the longest reported in the literature. No significant differences were noted between animals with brief periods of circulatory arrest and those with circulatory arrest for 4 or 5 hours. We estimated that adequate oxygen was available at 3°C and 3 atm for several hours.

The combination of hypothermia and hyperbaric oxygen did cause serious problems with decompression, however. Hypothermia not only decreases the metabolic requirement for oxygen, it raises the concentration of dissolved oxygen in the blood due to increased gas solubility at lower temperatures. Thus, the amount of dissolved oxygen at 3°C in the blood at 1 atm is approximately 5.0 vol%, compared with about 2 vol% at 37°C. At 3 atm, hypothermia to 3°C makes possible an oxygen concentration of 15 vol%. It would require 7.5 atm of pressure at 37°C to obtain a concentration of dissolved oxygen equal to that present in the animals studied during hypothermia at 30 psig. Total compression time would include 1.25 hours during cooling and another 1.25 hours during rewarming. In addition, the period of circulatory arrest from 2 to 5 hours should be considered, giving a total compression time of 4.5–7.5 hours.

How long decompression should last is open to question. Most of the dissolved gas is composed of oxygen and carbon dioxide. In our remote-control circumstances, decompression periods over 2 hours were impractical, because we usually required access to the animal. Two hours of decompression did not prevent bubble formation in the heart and liver of dogs which underwent circulatory arrest at 15 psig. In dogs allowed only 30–60 min for decompression after 3–4 hours of exposure at 30 psig, gas bubbles were withdrawn from the vena cava.

Several factors may be involved here, the first being that no attempt was made to keep the peripheral circulation open by drugs or other means except heparinization and reduction of viscosity with low molecular weight dextran. Possibly some of these dormant vascular beds did not reopen until late in rewarming or decompression. Blood contained in these vessels upon entering the circulation would be rapidly warmed, and its capacity to keep gas dissolved would be reduced. There may also be some rapid decompression when this blood that had not been allowed to equilibrate in the oxygenator or lungs during decompression was exposed. This would explain the appearance of bubbles despite attempts to thoroughly warm dogs before starting decompression. Gas embolism may explain deterioration of dogs which initially looked as though they would recover. It may also be responsible for the behavior of the surviving dog during the first 2–3 days and closely resembled the clinical course seen in patients suffering from air embolism after bypass.

Another source of gas embolism is the heat exchanger during rewarming. If, during rewarming, blood is abruptly warmed from 8°C to 40°C, bubbling can occur, and this is seen in the tubing leaving the heat exchanger. These bubbles could be prevented, however, by raising the temperature in the water bath of the heat exchanger slowly, keeping the gradient between water and blood to less than 10°C. In the few instances where bubbles were noted, they were probably prevented from reaching the animal by the bubble trap. Miller *et al.*[4] have reviewed some of the problems concerning deep hypothermia and circulatory arrest.

The dogs subjected to circulatory arrest at 30 psig frequently showed no attempt to breathe, despite an initially strong cardiovascular response. If their oxygen exposure was equivalent to 95% oxygen-breathing at 7.5 atm, oxygen toxicity to the central nervous system may have occurred. This, if true, may limit the duration of circulatory arrest unless there exists a differential exposure of the central nervous system to oxygen. The ability of the heart to withstand high pressures of oxygen for several days seems to be established.[5]

The value of the left atrial drain is unclear. When a left atrial drain was not used, pulmonary edema and congestion sometimes did not occur, but usually edema fluid and blood issued from the tracheobronchial tree after rewarming. However, thoracotomy weakened the animals' ability to ventilate strongly.

Summary

Twenty-five dogs subjected to hypothermia at 3°C and hyperbaric oxygen at 10, 15, and 30 psig underwent circulatory arrest for 2–5 hours. One dog survived a 2.25-hour period of circulatory arrest and behaved normally until sacrifice at 10 months.

We conclude that the combination of hypothermia and hyperbaric oxygen increased the duration of circulatory arrest that could be survived. Problems of decompression and oxygen toxicity to the central nervous system were discussed.

ACKNOWLEDGMENTS

These studies were supported in part by the Sonoma, the San Francisco, and the Nevada Heart Associations, and by USPHS Grant HE-08657. We are grateful for the technical assistance of Mr. Charles E. Michielsen during these studies.

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A Small Pneumatic Pump Oxygenator for Use in a Hyperbaric Environment

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Animal studies in our laboratory have revealed that a hyperbaric environment (3 ata of air-breathing) fails to protect dogs subjected to 10 min of total circulatory arrest against central nervous system damage. Careful pathologic studies of these animals have shown central nervous system lesions even under modest hypothermia (28–30°C) and with carbon dioxide added to the respiratory gas mixture during the prearrest period. In all animals, the prearrest arterial pO_2 was 1700 mm Hg or more.

Since the safe period of total circulatory arrest cannot be extended much beyond 8–9 min in humans under these conditions, particularly in cyanotic infants with a right-to-left shunt, operations involving simple inflow occlusion under hyperbaric oxygenation are limited to palliative procedures or to hurried corrective procedures. Because of these problems, we designed a miniature pneumatic pump oxygenator to take advantage of the rapid blood oxygen uptake in a hyperbaric environment. This apparatus is intended for experimental use during extracorporeal circulation in the hyperbaric chamber and as a prototype for the development of miniaturized pump oxygenators which could be used with infants and small children.

Mechanical Design

The oxygenator consists of a small stainless steel trough covered by a Lucite hemicylinder (Figure 1). One rotating double-concentric Teflon-coated perforated stainless-steel cylinder (Senning type) exposes the blood to oxygen. The spindle is turned at 90–100 rpm by an air motor. As the blood flows longitudinally down the trough, it films across all the perforated holes on the cylinders (due to surface tension); thus, both sides of a blood film of minimum thickness are exposed to oxygen. The oxygenated blood then flows over a weir into a small reservoir. The bottom of the stainless-steel trough of the oxygenator contains a water jacket through which water at the required temperature is circulated from a mixing valve and supply line located outside the chamber. This simple heat exchanger permits maintenance of normothermia or hypothermia without adding to the small

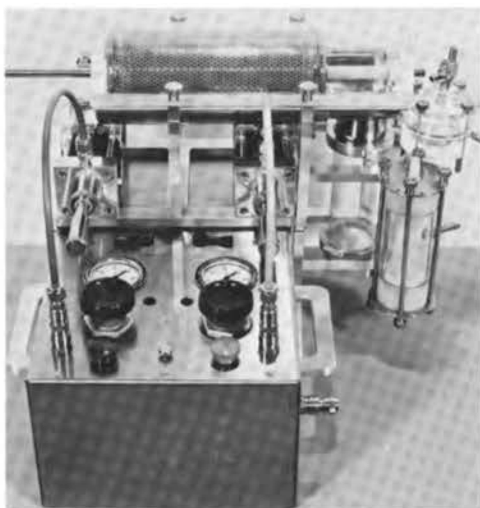

FIGURE 1. Miniature pneumatic pump oxygenator for use in a hyperbaric environment. The entire unit, as shown, is capable of gas sterilization and measures approximately 15 in. × 14 in. × 12 in.

blood priming volume required in the circuit.

The arterial ventricle is immediately adjacent to the reservoir and consists of a 4-in. segment of ¾-in. (internal diameter) Silastic tubing housed in a Lucite cylinder. Silastic ball valves of 0.455-in. diameter are housed in cages at both ends of the ventricle.

Pulses of air under pressure, transmitted through a rate-controlled switching valve, drive a bellows diaphragm which transmits the air pressure to a saline-filled hydraulic system. This hydraulic system is then connected to the Lucite cylinder surrounding the ventricle. The pulsatile pressure applied to the ventricle is set by a pressure-regulating valve, and the rate of pulsation is regulated by a needle valve controlling a switch valve. A vernier scale on the bellows diaphragm plunger can be set to deliver any desired stroke volume between 3 and 10 ml. The bellows diaphragm of the hydraulic system to the ventricle housing assures an accurate and constant stroke volume regardless of the perfusion resistance or changes in atmospheric pressure.

The blood priming volume of the system using ¼-in. inflow lines and 5/16-in. outflow lines is 275 ml. The oxygenator is primed with 100 ml of blood, the ventricle with 20 ml, and the bubble trap with 40 ml. Any additional priming volume is dependent upon the amount of blood desired in the reservoir (capacity 225 ml). A volume change of 10 ml in the reservoir is easily seen and quantitated.

There is, of course, no electrical hazard associated with this pump oxygenator since pneumatic power drives both the ventricles and the oxygenator drum.

FUNCTION

Oxygen Uptake. The oxygen tension in the blood line leaving the pump oxygenator is dependent upon: (1) the venous oxygen tension entering the oxygenator, (2) the blood flow rate through the oxygenator, and (3) the partial pressure of oxygen in the oxygenator housing. Since the priming volume of the oxygenator is fixed at 100 ml, blood transit time is short, particularly at the higher flow rates. At a flow rate of 600 ml/min, for example, the mean blood transit time through the oxygenator is 10 sec.

Figure 2 shows the arterial pO_2 values obtained in relation to flow and pressure at 1, 2, and 3 ata. At normal atmospheric pressure, the blood does not reach a satisfactory pO_2 level at the higher flow rates. At 2 and 3 ata, the oxygenating capacity is significantly higher. At blood flows of 300 ml/min at 3 ata, the arterial oxygen tension reaches 1467 mm Hg. With a single ventricle, the maximum pump output is 600 ml/min. For larger output requirements, a second ventricle can be added.

Carbon Dioxide Exchange. The amount of carbon dioxide eliminated as blood passes through the oxygenator depends upon the blood flow rate through the oxygenator and upon the carbon dioxide gradient between the blood and the gas

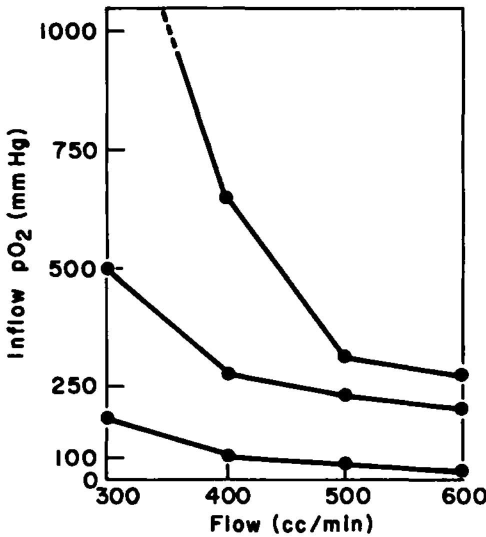

FIGURE 2. The arterial pO_2 values reached in one pass through the oxygenator with relation to flow and environmental pressure.

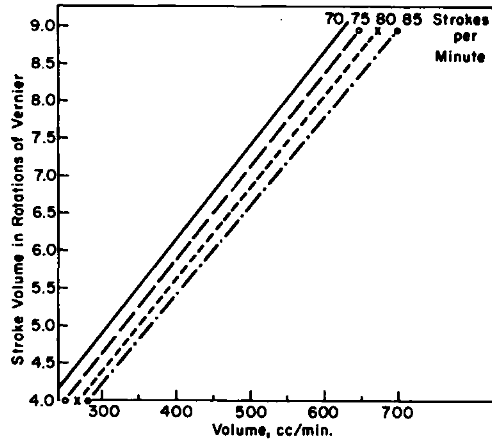

FIGURE 3. Range of pump output with single ventricle.

in the oxygenator. The pCO_2 in the oxygenator housing is negligible. Carbon dioxide elimination is independent of the chamber (or ambient) pressure. Table 1 shows the mean values for the decrease in blood pCO_2 in one pass through the oxygenator at various ranges of venous outflow pCO_2 from the subject and at various flow rates (*i.e.*, pCO_2 difference between values taken before and after passage through the oxygenator obtained during 25 experimental perfusions). These values are plotted against the variables of flow rate and initial pCO_2. As flow increases, there is less of a fall in blood pCO_2 values; as the initial pCO_2 rises, there is a greater fall in blood pCO_2. Carbon dioxide elimination is adequate with this single-cylinder oxygenator for flow rates over 600 ml/min.

Flow. The flow rate has been calibrated using stroke volume and pulse rate as independent variables. The stroke volume is determined by the number of rotations, measured in tenths of a rotation on the micrometer-type plunger adjustment. The flow rate was calibrated with stroke rates of 70, 75, 80, and 85 strokes per minute. The flow can be selected to deliver 250–700 ± 5 ml/min (Figure 3).

Hemolysis. A circuit was assembled to determine the rate of hemolysis produced by the pump oxygenator. The test cir-

TABLE 1. Mean Decrease in Blood pCO_2 in One Pass Through Oxygenator[a]

Venous pCO_2 entering oxygenator (mm Hg)	Mean decrease in pCO_2 (mm Hg) at flow rates of:			
	300 ml/min	400 ml/min	500 ml/min	600 ml/min
20–30	3.0	3.4	2.9	3.6
30–40	10.3	8.5	7.2	7.6
40–50	17.5	—	18.5	9.8
>50	32.3	17.2	17.8	16.0

[a] Twenty-five experiments.

cuit consisted of the oxygenator, reservoir, ventricle, heat exchanger, bubble trap, a 3.0-mm cannula, and an additional reservoir. This circuit was primed with fresh undiluted heparinized dog blood. The blood was recirculated continuously at 600 ml/min for 2 hours. Samples were taken before and every 10 min during recirculation and were analyzed for free plasma hemoglobin by the method of Crosby *et al.*[1] Hemolysis in the test circuit was 17 mg/100 ml of blood pumped per hour.

SUMMARY

A small pneumatic-hydraulic pump oxygenator has been designed and constructed for use in a hyperbaric environment. Thus far, its use has been limited to experimental extracorporeal circulation in puppies weighing 4–8 kg. Hyperbaric oxygenation should allow further miniaturization of this pump oxygenator and perhaps make extracorporeal circulation in infants more practical.

ACKNOWLEDGMENT

Silastic ball valves were kindly supplied by Mr. Astle of the Edwards Laboratory.

REFERENCE

1. Crosby, W. H., F. W. Furth, and C. E. Thibeault. A modification of the benzidine method for measurement of hemoglobin in plasma and urine. Blood 11:380, 1956.

Effect of Hyperbaric Oxygenation on Coronary Artery Occlusion in Pigs

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Hyperbaric oxygenation has been used to treat myocardial infarction on the rationale that when sufficient oxygen is physically dissolved in the blood entering the coronary circulation, the ischemic areas surrounding the damaged myocardium may be significantly limited. This, it has been suggested, may eliminate the foci of ventricular arrhythmias which commonly precipitate sudden death after myocardial infarction.

The previous animal studies evaluating the possible protective effect of hyperbaric oxygenation in acute myocardial infarction bear little relationship to the course of events preceding myocardial infarction in man. Experimental coronary occlusions have been produced either by acute ligation of a coronary artery or by embolization of the coronary vessels with microspheres. Furthermore, the dog has been used exclusively for these studies, and canine coronary circulation distinctly differs from that of man. There is also often a wide variation in pattern of the coronary vessels among individual dogs. In addition, the studies have used general anesthesia, and OHP exposure has been for 2 hours or less, at pressures up to 4 ata.

Our study was designed to determine the effects of longer OHP treatment, up to 32 hours. The experimental animal chosen was the common farm pig, whose pattern of coronary circulation is uniform and is similar to that of man. In addition, the coronary occlusion was induced gradually by external compression while the pigs were conscious and carrying out their usual activities.

METHODS

Twenty-four farm pigs (5–6 weeks old, 11–16 kg) were anesthetized with halothane. By sterile surgical technique, a left thoracotomy was performed. After the heart was exposed through pericardiotomy, a 6-mm segment of the left anterior descending coronary artery beginning at its origin from the left main coronary artery was freed by blunt dissection (Figure 1). An Ameroid constrictor with a 1.5-mm central lumen was then placed around the exposed vessel. Although the blood flow was not initially interrupted, because of the hygroscopic nature of the Ameroid, the coronary artery was completely occluded within 48 hours. Follow-

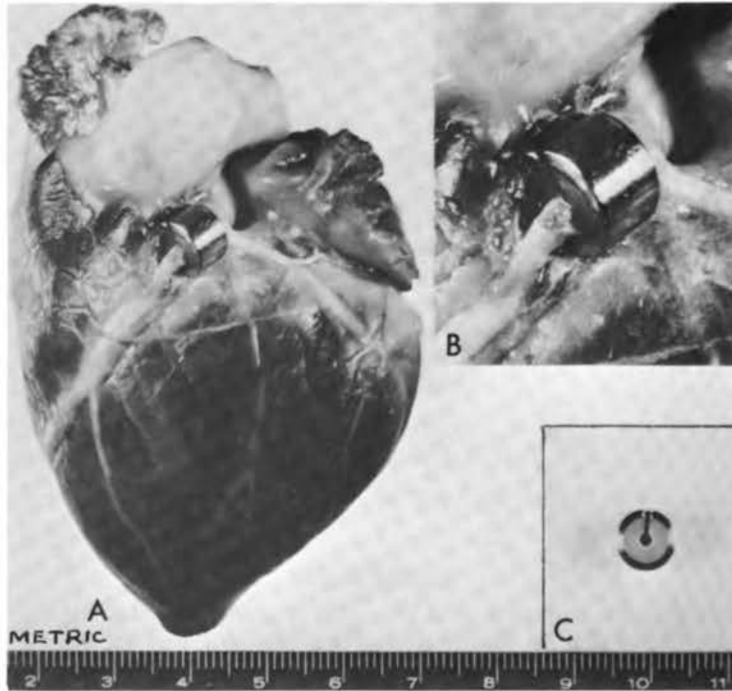


FIGURE 1. A, photograph of Ameroid constrictor placed on the left anterior descending coronary artery just distal to the bifurcation of the left coronary artery. B, enlarged (×4) section of the Ameroid constrictor in place. C, Ameroid constrictor showing 1.0-mm slit and 1.5-mm central lumen.

ing placement of the constrictor and closure of the thoracotomy incision, the pigs were returned to their cages.

An arbitrary period of 16 hours was chosen to allow recovery from the thoracotomy and elimination of any animals which might die as a result of the surgery. All animals surviving this period were ambulatory, eating, and drinking. The pigs were then divided into two equal groups: experimental animals (Group A) and controls (Group B). Group A pigs were placed in a hyperbaric chamber which permitted freedom of movement to the animals and allowed continuous observation by personnel through portholes. The animals were maintained for 32 hours at 1.25 ata while breathing 100% oxygen. The temperature and moisture in the chamber were approximately the same as those of the room. The partial pressure of oxygen in the chamber was over 800 mm Hg by measurement and approximated 950 mm Hg by calculation.

This length of hyperbaric exposure, gas mixture, and atmospheric pressure were selected in a preliminary study. The reason for selecting only 1.25 ata pressure was that pigs could be exposed to 100% oxygen under these conditions for at least 32 hours without toxic effects or the need for a decompression schedule.

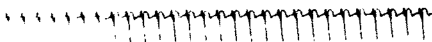
The control animals in Group B were kept outside the chamber breathing room air at normal atmospheric pressure. Several of the Group B animals were monitored throughout the study with a transistorized electrocardiographic telemeter. This type of monitoring device could not be used on Group A pigs because the electrocardiographic signal could not be consistently received outside the chamber.

All animals were continuously observed from the sixteenth to the forty-eighth hour

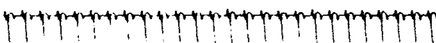
after placement of the constrictor. Animals remaining alive in the hyperbaric chamber during this period (a total of 32 hours) were returned to their cages. When death occurred it was sudden and without forewarning in both groups of animals. Analysis of the telemetered electrocardiograms in Group B indicated that death was due to ventricular fibrillation (Figure 2). Since the animals in Group A died in the same manner as those in Group B, it is presumed that they also died with ventricular fibrillation. All animals surviving for 72 hours were sacrificed.

Complete gross and microscopic pathologic examinations of the heart and lungs were carried out on all animals in both groups within 2 hours after death. Each Ameroid constrictor was found sufficiently closed to completely occlude the left anterior descending coronary artery.

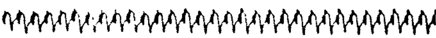
A. IMMEDIATE



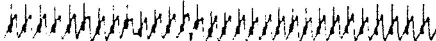
B. 15 HOURS



C. 17 HOURS



D. 17.5 HOURS



E. 18.5 HOURS

F. 18.6 HOURS

FIGURE 2. Electrocardiographic changes following placement of Ameroid constrictor. A V_1 electrocardiographic lead was used in all animals monitored. This representative electrocardiogram shows early T-wave changes at 15 hours, which progressed to severe changes 2 hours later. One premature ventricular contraction not shown occurred 1 min prior to the terminal unremittent arrhythmia.

RESULTS

Pigs in Group A survived for periods of 25 to 72 hours after surgery, the mean survival time for the group being 46.8 hours. Two animals in this group lived 72 hours and were sacrificed. Pigs in Group B lived 16.5 to 32 hours, with a mean survival time of 21.4 hours. The one animal in Group A that survived for only 25 hours was found to have had complete atelectasis of the left lung, which was thought to account for the early death despite treatment with hyperbaric oxygenation. The next shortest survival time in the treated group was 32.5 hours (Figure 3). The majority of pigs in Group A had significant myocardial damage.

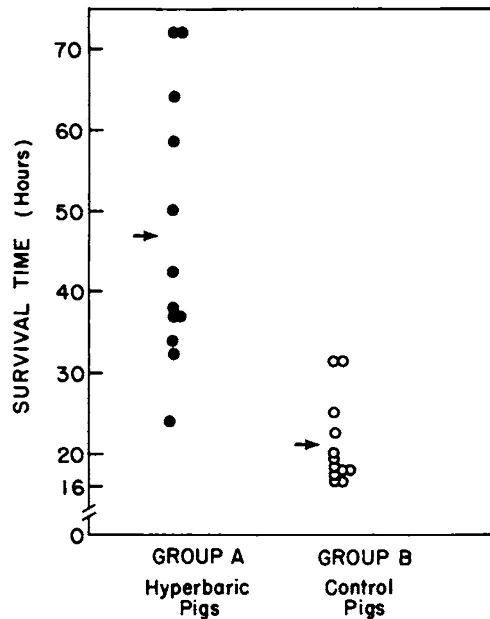


FIGURE 3. Comparative survival times in both groups of pigs. Mean survival time is designated by arrows: Group A, 46.8 hours; Group B, 21.4 hours.

The pathologic grading of the myocardial tissue taken from the anterior wall and septum using succinic dehydrogenase stain for early detection was as follows: Grade 0, no pathologic diagnosis; Grade I, minimal spotty areas of necrosis in the subendocardium only; Grade II, scattered subendocardial infarction; Grade III, uniform subendocardial infarction; and Grade IV, large transmural infarction.

The pathologic examinations of the hearts demonstrated that myocardial infarctions could not be found in 10 of the 12 animals, all of which died early after the placement of the Ameroid constrictor. However, in 9 of the 11 animals treated with hyperbaric oxygenation and surviving for a longer period of time, varying degrees of myocardial infarction were discovered. Furthermore, no animals in Group B had Grade III or IV infarcts while eight pigs in Group A developed lesions of this degree (Figure 4). In general, the longer the animal survived, the larger the myocardial infarction found at autopsy (Figure 5).

No animals in the study were found to have pulmonary changes suggestive of oxygen intoxication. One animal in Group A surviving 60 hours was found to have a Grade IV myocardial infarct and lung changes compatible with mild congestive heart failure.

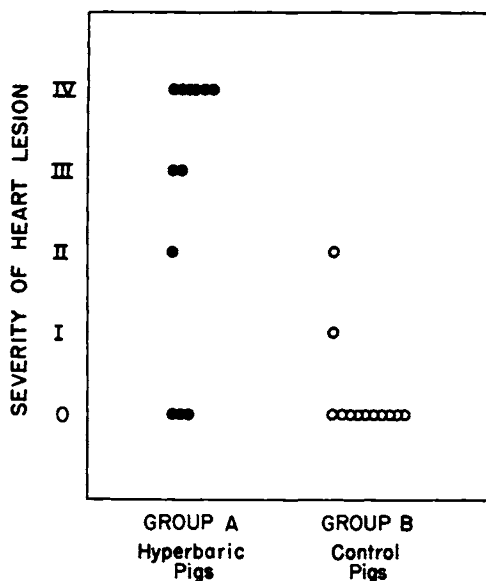


FIGURE 4. Severity of heart lesions in both groups. Note the low incidence of myocardial infarction in the control animals.

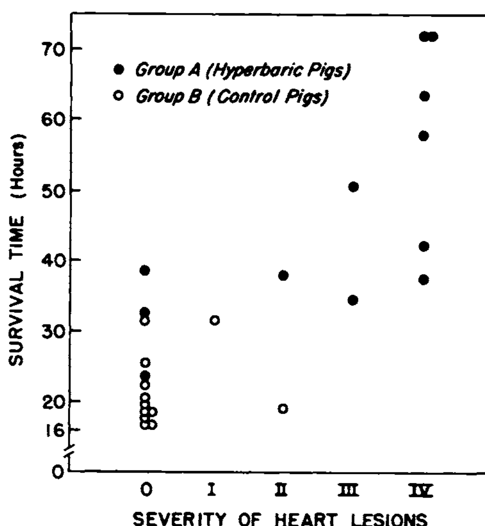


FIGURE 5. Comparison of survival time and severity of heart lesions in both groups. The OHP-treated animals lived the longest and had the larger myocardial infarctions.

SUMMARY

This study was designed to evaluate the possible protective effect of longer periods of treatment with hyperbaric oxygenation in myocardial infarction. Infarction was experimentally induced in pigs by gradual occlusion of the left anterior descending coronary artery. The striking survival time differences found between animals exposed to OHP and controls left in room air were highly significant, and were presumably due to the hyperbaric oxygenation. Except for one animal with complete atelectasis and infarction of one lung, all pigs treated with hyperbaric oxygenation outlived untreated pigs. These findings suggest that modest but prolonged hyperbaric oxygenation prolongs life following coronary occlusion, probably by

preventing the fatal ventricular arrhythmia or by increasing the time before onset of the arrhythmia. This prolongation of life then results in the full development of pathologically detectable myocardial infarctions.

DISCUSSION

DR. K. H. SMITH (*Ft. Collins, Colo.*): I am glad to see that you use swine. We have been using swine for some time and have found that they are very nice to work with. However, you mentioned several EKG aberrations. We have had a great deal of difficulty in establishing a base-line EKG on swine, and, having a large SPF colony, we have been able to do a great number of them. We have yet to find good indices for an EKG base-line on swine. I wondered if you had run normals previous to your experiment.

DR. PETER: We took 12-lead electrocardiograms on many of the animals, and the QRS axes did vary considerably. However, since we were using the left anterior descending coronary vessel, and since the coronary vessels in the pig are analogous to those in the human, we placed the positive electrode about the midsternum, and the negative electrode on the back attached to a battery-operated telemeter simulating lead V_1. The V_1 electrode picked up an anterior myocardial infarct on every animal that we monitored. I cannot say what would happen if we used a lead III or lead AVF to pick up a right coronary artery lesion or diaphragmatic infarct. We were able to pick up an anteroseptal, or an anterior wall, infarct in the left anterior descending coronary vessel with no trouble. We demonstrated this repeatedly.

DR. G. MARGOLIS (*Hanover, N. H.*): Dr. Peter, do you agree with the following interpretation? You have not prevented, in fact you have allowed to become manifest, a massive myocardial infarct by prolonging the survival of your animals. The gains that you have made, however, have been in preventing the vicious circle we see in any coronary occlusion, *i.e.*, a deficiency in cardiac output, a deficiency in coronary perfusion, more myocardial ischemia, more myocardial trouble, *etc.* Is this a correct interpretation?

DR. PETER: If we tried to allude to what happens in the human, we can look at any series of myocardial infarcts. Most of the deaths due to myocardial infarcts in humans occur in the first 24 to 48 hours, and are presumably caused by arrhythmia. In fact, a study done by Yater (Ann. Intern. Med. 34:352, 1951) soon after the war, on several hundred soldiers who died suddenly and presumably of myocardial infarction, a significant number had no evidence of infarction, though coronary occlusion was invariably present. I am sure if they had lived long enough, they would have developed a pathologically detectable infarct. We found the same thing in our animals. When the constrictive lesion limited the coronary blood flow sufficiently to cause anoxia, these animals died. We could not detect the infarcts, even with the best techniques available (the succinic dehydrogenase stain, for example). On the other hand, in those animals that lived longest, the infarct was evident. I would dare say that someone who has had an acute coronary occlusion and lived several days should have an infarct demonstrable by pathologic staining. If we give the patient those first 48 hours, we can usually get him through. This is what we have demonstrated here in the two animals that did survive the full 72 hours. We did have a few who survived 48 hours in the treated group, but none in the nontreated group.

DR. J. M. CLARK (*Philadelphia, Pa.*): In a human breathing 100% oxygen for 24 hours there is chest pain, cough, and a 5 or 10% decrease in vital capacity. You mentioned

that you looked at the lungs of pigs that were exposed to 1.25 atmospheres for 32 hours. Did you see any pulmonary pathology?

DR. PETER: We found no changes in these lungs with either the conventional technique of hematoxylin and eosin staining or other stains. We did test a variety of pressures in order to find a level that the pig could withstand without toxicity to the lungs. At 2 atmospheres, the animals died of severe oxygen toxicity in about 16 hours. At 2 atmospheres, alternating 100% oxygen for 2 hours with air for 30 minutes without varying the pressure, the animal survived about 22 hours. There were, of course, severe pulmonary lesions in these animals. I do not know about the intermediate atmospheric pressures. We did not test, for example, 1 or 2 ata intermittently with a lower percent of oxygen. I am sure there would be fewer degrees of change if we did do this on a graded scale.

DR. W. TRAPP (*Vancouver, Canada*): Does the pig have a septal branch that occurs within the area around which you placed the Ameroid constrictor? In the dog, this proved to be a crucial point. If you did include the septal branch, it immediately changed your mortality.

DR. PETER: The dog has a distinctly different coronary circulation, as shown by Donald and Essex (Amer. J. Physiol. 176:143, 1954) among others. I do not know whether you mean the posterior septal branch which, in the dog, comes from the left coronary. It comes from the right in swine, as it does in humans.

DR. S. HUNTER (*St. Paul, Minn.*): The septal branch does come from the anterior descending in the dog. We did similar work in 1958 in our laboratory in St. Paul (*Clinical Application of Hyperbaric Oxygen*, Elsevier, 1964, pp. 105–110). We found no difference in results when the coronaries of dogs were ligated under hyperbaric conditions or at 1 atmosphere of air. Both groups fibrillated at exactly the same time. I am glad that Dr. Peter and his group have turned to a more realistic approach using the Ameroid constrictor. I am sure that his results are closer to the human situation. I believe that we have now gone far enough in the animal experimentation. This is analogous to our initial problems with open-heart surgery and the pump, where had we persisted with the dogs, we would still be there and getting unfortunate results. Have you done any humans yet?

DR. PETER: We have used hyperbaric oxygenation on a few patients in critical condition following myocardial infarct and not responding to medical treatment.

DR. S. ATTAR (*Baltimore, Md.*): Our colleagues from Glasgow have demonstrated that there is no significant improvement in myocardial infarction with OHP. They have shown that if two groups of patients are treated under strictly comparable conditions, the incidence of arrhythmias, fibrillation, and mortality is equal. Have you observed any beneficial effect, and, in view of the Glasgow experience, would you still advise OHP treatment of patients with myocardial infarction, if they do not respond to standard treatment?

DR. PETER: We have put some patients in the chamber, with varying results. One problem with human studies is that they cannot really be controlled in the way that animal studies can be. For example, it is difficult to know how many vessels were involved, or whether the infarct was in a diaphragmatic wall or a lateral wall, *etc.* The study presented here was a controlled experiment. These animals were of the same age and weight, the same vessels were used, the constrictor was closed in the same amount of time, and so on.

DR. R A. COWLEY, *Session Chairman* (*Baltimore, Md.*): In reference to Dr. Attar's comment, 20 randomized myocardial infarctions were present, and it was agreed that the series was not large enough to show a clear result. It was thought then that the work should continue until a larger series of randomized patients was obtained. I recall, for instance, of the group that went in the hyperbaric chamber, three were in cardiogenic shock, but there were none in the control. This would be a tremendous factor to consider statistically.

DR. R. WHALEN (*Durham, N. C.*): In all fairness to the Glasgow group, we should note that they feel that their series did not settle this question. Perhaps one of the most perceptive articles written on the problems of this series appeared in the Proceedings of the Second International Hyperbaric Conference (*Hyperbaric Oxygenation*, E. & S. Livingstone, Ltd., 1965, p. 277). Dr. Cameron and his group clearly outlined where their series leaves many questions unanswered. I am sure they would not want to be quoted as saying that their studies have eliminated the possibility of treating myocardial infarction with hyperbaria. We at Duke have not felt that we could put the routine myocardial infarction in the chamber because of the newness of the technique and the relatively good prognosis in these patients. We have felt, however, that any patient who has had severe heart failure or arrhythmias that do not respond to the usual medical regimen, or who has shock which has not responded very promptly, can be considered a candidate for hyperbaric therapy. We have had two patients whom hyperbaria may have brought through the critical period which Dr. Peter has mentioned with the pig study. We feel that our experience lends some direction, but it is neither pro nor con, nor is the series from Scotland that definitive at the moment.

Hemodynamic Responses to "Life Without Blood"

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In 1960, Boerema and his associates[1] demonstrated that enough oxygen could be dissolved in plasma to meet the tissue oxygen needs of pigs in which red blood cells had been eliminated. A study of the acute hemodynamic responses in such an animal preparation might extend our understanding of the mechanisms regulating cardiac output and the mechanical role that red cells play in the circulation. The present studies were designed to observe the acute hemodynamic responses of dogs in which dextran was substituted for blood during hyperbaric oxygenation.

Materials and Methods

Five mongrel dogs weighing 10–20 kg were studied several weeks after splenectomy, performed to minimize red-cell sequestration. Hemodynamic and blood gas studies were performed serially in each dog under four conditions: (1) at 1 ata during air inhalation, (2) at 1 ata during inhalation of 100% oxygen, (3) at 3.5 ata during inhalation of 100% oxygen, with the normal number of red cells circulating, and (4) at 3.5 ata during inhalation of 100% oxygen after blood had been replaced with dextran.

Figure 1 depicts details of the animal preparation. Anesthesia was induced with intravenous pentobarbital sodium (30 mg/kg body weight). Respirations were controlled by means of an endotracheal tube and a positive-pressure respirator. Central venous pressure (right atrial) and femoral artery pressure were measured with #8 Lehman catheters connected to Statham P-23 Db strain gauges. The arterial and venous pressures, as well as the electrocardiogram, were continuously recorded on a Gilson direct-writing recorder. Cardiac output was estimated in duplicate by indicator-dilution techniques described previously.[2] Separate densitometer cali-

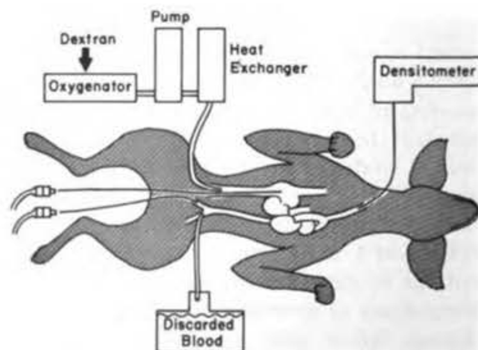

FIGURE 1. Diagram of the animal preparation (see text).

brations were made with blood and blood–dextran solutions.

Blood samples were collected periodically from the arterial and venous catheters for determination of pH, pCO_2, and pO_2. Arterial samples were also checked for osmolality, electrolyte content, hemoglobin, hematocrit, and levels of pyruvate and lactate.[3,4]

The exchange transfusion was accomplished at 3.5 ata by infusing 6% dextran (molecular weight approximately 75,000) in 5% dextrose–water solution through an oxygenator, a pump, and a heat exchanger into the inferior vena cava. During the infusion, the animal was simultaneously bled from the femoral artery. The exchange transfusion was continued until the hematocrit was less than 0.5%. An oxygenator was incorporated into the infusion circuit, since previous experience had indicated that preoxygenation of the dextran protected the animal from unpredictable hemodynamic alterations associated with sudden changes in hemoglobin mass. The heat exchanger was utilized to prevent the animal's temperature from falling below 34°C. When the exchange transfusion was terminated, the volume of dextran remaining in the dog was equal to or slightly greater than the orginal blood volume, as determined by intake and output balance records as well as maintenance of a comparable central venous pressure. The animals required an exchange transfusion of approximately 6 liters to reduce the hematocrit to less than 0.5%. Throughout the exchange transfusion, the arterial pH and pCO_2 were maintained within physiologic limits by the periodic addition of tris buffer.

After completion of the study, the stroke volume was calculated utilizing the cardiac output and heart rate present during the inscription of the indicator-dilution curves; the peripheral resistance was calculated by dividing the mean arterial pressure (mm Hg) by the cardiac output (liters/min) and was expressed as arbitrary units; the oxygen content was calculated from hemoglobin and blood pO_2 values by previously described formulas.[5]

In vitro determination of blood viscosity was carried out with a Brookfield cone-plate viscometer as described by Wells and his associates.[6] Dog blood and mixtures of dog blood and dextran with varying hematocrits were studied at different shear rates. All samples were anticoagulated with a balanced oxalate solution.

Results

Figures 2 and 3 illustrate the mean values for arterial and venous pO_2 and oxygen content under the four conditions of this study. The arterial pO_2 was increased from 107 to 588 mm Hg by oxygen-breathing at 1 ata and was further elevated to over 2000 mm Hg during oxygen-breathing at 3.5 ata. The arterial pO_2 at 3.5 ata was essentially the same whether blood or dextran was circulating (2155 *vs.* 2021 mm Hg). However, the arterial oxygen content at 3.5 ata fell from 24.9 vol% to 6.6 vol% when dextran was substituted for blood. The venous pO_2 was increased slightly from 53 to 61 mm Hg when oxygen was substituted for air at 1 ata. At 3.5 ata, the venous pO_2 rose

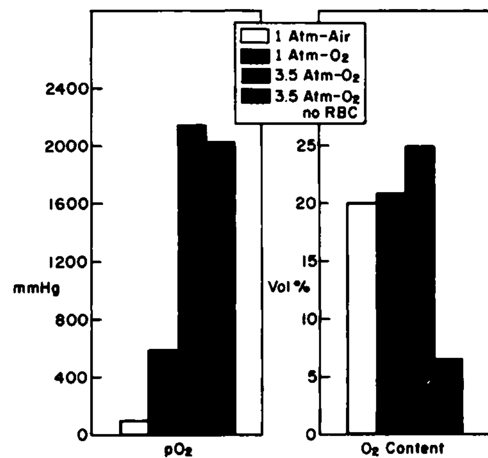

FIGURE 2. Mean arterial pO_2 and oxygen content values under the four conditions of this study.

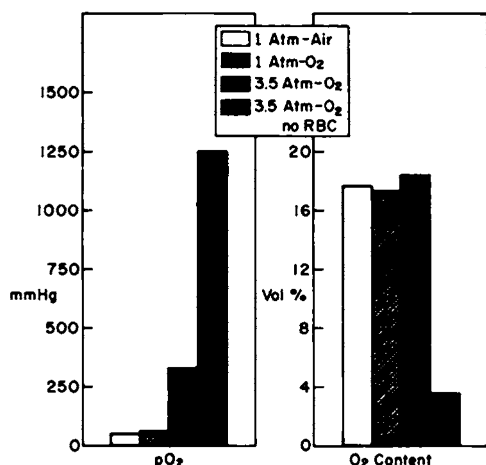

FIGURE 3. Mean venous pO_2 and oxygen content values under the four conditions of this study.

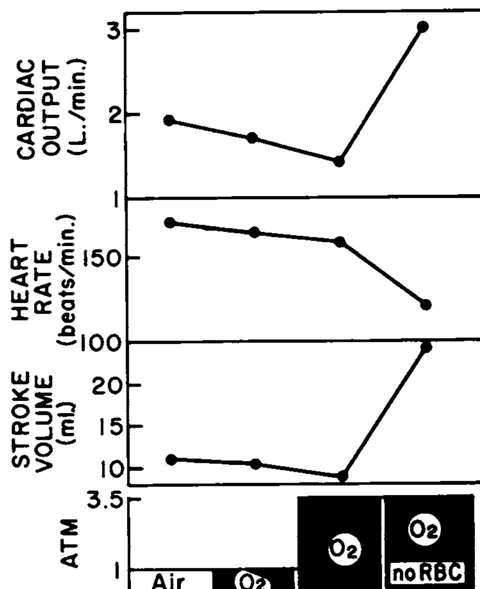

FIGURE 4. Hemodynamic responses to life without blood. The blocks on the lower border of the figure show the conditions under which the measurements were made (no RBC indicates dextran circulation). Note that there is a small decrease in mean cardiac output and heart rate with little change in stroke volume at 1 and 3.5 ata during the circulation of blood. Cardiac output and stroke volume rise and heart rate falls markedly when dextran is substituted for blood.

from 333 mm Hg during the circulation of blood to 1251 mm Hg during the circulation of dextran. The venous oxygen content, however, fell from 18.4 vol% to 3.5 vol%. Despite the low venous oxygen content when the dextran was substituted for blood, the arteriovenous oxygen difference narrowed from 6.5 vol% to 3.1 vol%.

Figures 4 and 5 summarize the hemodynamic observations made during this study. There was a small decrease in cardiac output during oxygen inhalation at 1 and 3.5 ata while blood was circulating. The substitution of dextran for blood at 3.5 ata more than doubled the mean cardiac output (from 1.43 liters/min to 3.11 liters/min). The heart rate, which had decreased in a parallel manner with the cardiac output during the circulation of blood, fell even further when dextran was exchanged for blood. This increase in cardiac output and concomitant decrease in heart rate is emphasized by the striking increase in stroke volume from 8.9 to 26.2 ml/beat following the exchange of dextran for blood at 3.5 ata. This response was noted in all dogs.

The arterial pressure showed little overall change until the exchange transfusion (Figure 5). The substitution of dextran for blood was associated with a decrease in mean arterial pressure from 131 to 91 mm Hg. This decrease in mean pressure, despite an increase in cardiac output, accounts for the striking fall in calculated peripheral resistance from 106 to 33 units.

The results of multiple viscosity studies appear in Figure 6. At low shear rates, derived from slow rotation of the plate in the cone-plate viscometer, the viscosity of blood was greater than that of dextran with 1% red cells. As the shear rate was increased by increasing the rate of rotation of the plate, the viscosity of blood decreased, while the viscosity of dextran showed little change despite alterations in shear rate.

Figure 7 illustrates changes in arterial lactate, pyruvate, and lactate/pyruvate ratios. Little overall change occurred in

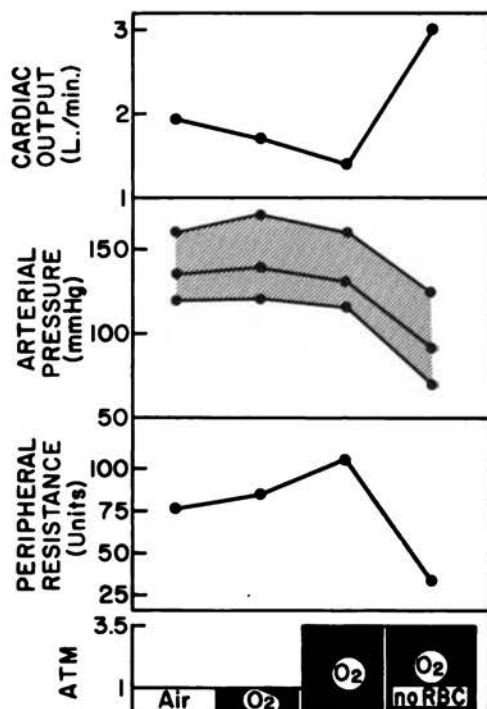

FIGURE 5. Hemodynamic responses to life without blood. Little overall change in blood pressure occurs until dextran is exchanged for blood. The marked decrease in mean arterial pressure (middle panel) coupled with the increase in cardiac output (top panel) during dextran circulation accounts for the large decrease in calculated peripheral resistance (bottom panel).

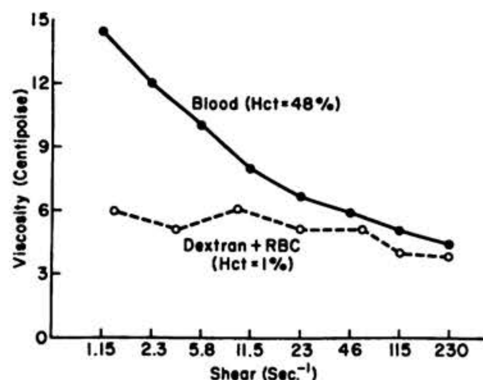

FIGURE 6. Viscosity, as determined by a cone-plate viscometer, for dog blood with a hematocrit of 48% and dextran with a hematocrit of 1% is plotted against shear rate. Note that blood has a consistently greater viscosity than dextran and that this discrepancy is most marked at low shear rates.

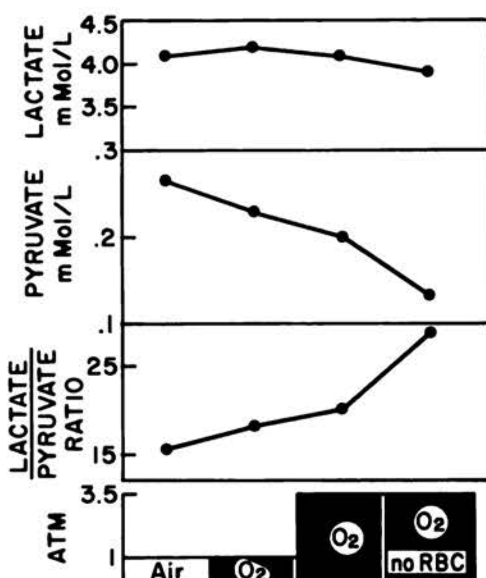

FIGURE 7. Lactate and pyruvate values during life without blood. Note that mean blood lactate levels (top panel) remain stable throughout the study, while mean pyruvate levels (middle panel) fall progressively with the greatest decrease occurring during the circulation of dextran. The decrease in pyruvate accounts for the increase in lactate/pyruvate ratio in the bottom panel.

lactate levels but pyruvate levels fell at 3.5 ata during the circulation of dextran. This accounts for the increase in lactate/pyruvate ratio indicated in the lower panel of this figure. Blood electrolytes and blood osmolalities remained within physiologic limits despite the substitution of dextran for whole blood.

DISCUSSION

Our results showed that hyperbaric oxygenation led to the expected rise in arterial and venous pO_2 (Figures 2, 3). The near equality of arterial pO_2 in both blood and dextran and the markedly lower oxygen content in dextran when compared to blood at 3.5 ata is an excellent demonstration of Henry's law and the impor-

tance of the hemoglobin-oxygen transport system. Henry's law would indicate that the partial pressure of oxygen physically dissolved in blood and that dissolved in dextran should be the same if these solutions were both exposed to the same partial pressure of oxygen—which was indeed the case in our studies. The oxygen carried by hemoglobin is not governed by Henry's law since it is chemically bound to hemoglobin. The 18 vol% difference found between the oxygen content of blood and that of dextran at 3.5 ata is a manifestation of the loss of hemoglobin as an oxygen carrier following substitution of dextran for blood.

Despite the low arterial and venous oxygen content in dextran, the arteriovenous oxygen difference at 3.5 ata during dextran circulation was approximately half that noted when blood was circulating (6.5 vol% *vs.* 3.1 vol%). This narrowing of the arteriovenous oxygen difference despite the elimination of hemoglobin as an oxygen carrier was a manifestation of marked increases in cardiac output (Figure 4).

Although oxygen consumption was not measured in these studies, it is interesting to note that the calculated mean oxygen consumption of the dogs at 3.5 ata was essentially the same during the circulation of blood (93 ml/min) and the circulation of dextran (96 ml/min). Thus, apparently at 3.5 ata total body oxygen consumption during the circulation of blood was maintained with an increased oxygen extraction and a decreased cardiac output, whereas during the circulation of dextran, the same oxygen consumption was maintained with an increased cardiac output and a decreased oxygen extraction. Figure 7 suggests that there may have been some deficiency in meeting tissue oxygen demands during the circulation of dextran since there was an increase in lactate/pyruvate ratio. However, these increases were due to a decrease in pyruvate rather than an increase in lactate. The maintenance of a stable lactate level suggests that there was little change in tissue oxygenation during the circulation of dextran.

The increased flow during dextran circulation was associated with a fall in arterial pressure and thus a decrease in calculated peripheral resistance (Figure 5). Local vascular beds were not studied, so that their status could not be characterized. However, injection of vasopressors on several occasions produced prompt and marked increases in arterial pressure. This suggests that the arterial system was certainly not in a state of maximal constriction.

The mechanisms responsible for these hemodynamic responses are undoubtedly complex and our studies do not pinpoint any one mechanism to the exclusion of others. It would appear that the cardiac output and peripheral resistance act in concert to maintain total oxygen consumption at a stable level whether blood or dextran is circulating. This may well be accomplished through a series of local autoregulatory actions which serve to produce vasodilatation and thus lower peripheral resistance.[7] A series of reflex responses which would involve chemoreceptors and baroreceptors acting through the central nervous system cannot be excluded either.

Two factors associated with dextran circulation serve to either initiate or enhance the increase in cardiac output and decrease in peripheral resistance. The elimination of red cells, and probably large amounts of plasma protein, from the circulating medium lowers the viscosity of the medium. Wells and his associates[8] have emphasized that whole blood is not a Newtonian fluid and the viscosity of whole blood at low shear rates, as might occur in the microcirculation, is significantly greater than that at high shear rates. This is not true for solutions without red cells. Figure 6 illustrates the dif-

ference in viscosity at low and high shear rates between blood with a hematocrit of 48% and dextran solution with a hematocrit of 1%. The differences in the viscosities of the two fluids are primarily due to the presence of large numbers of red cells in the blood. Furthermore, the elimination of red cells, and thus the elimination of red-cell aggregation, should decrease peripheral resistance and increase flow in the microcirculation.

No matter which factor (*i.e.*, increase in cardiac output, decrease in peripheral resistance, decrease in viscosity, or elimination of red-cell aggregation) is primary, they all serve to maintain adequate tissue oxygenation.

SUMMARY

Five mongrel dogs were studied at 1 atm while breathing room air and 100% oxygen and then later at 3.5 atm while breathing 100% oxygen before and after an exchange transfusion of dextran for blood. Substitution of dextran for blood was associated with an increase in cardiac output and a decrease in heart rate, peripheral resistance, and arteriovenous oxygen difference. *In vitro* studies confirmed the previously noted decrease in viscosity of dextran when compared to whole blood. These hemodynamic responses are probably a function of many regulatory mechanisms and the decrease in viscosity of the circulating dextran.

ACKNOWLEDGMENTS

This study was supported in part by Grants HE-07563, HE-07896, and HE-04702 from the National Heart Institute of the National Institutes of Health, and by grants-in-aid from the American Heart Association, the North Carolina Heart Association, the Council for Tobacco Research–U.S.A., and the Life Insurance Medical Research Foundation. Dr. Moor's work as a Postdoctoral Fellow was supported by a fellowship from the National Heart Institute, USPHS.

Dr. George H. Berryman of Abbott Laboratories, North Chicago, Illinois, kindly supplied us with dextran.

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DISCUSSION

DR. J. MEREDITH (*Winston-Salem, N. C.*): I would like to ask what the venous pO_2's were when you used the dextran.

DR. WHALEN: The venous pO_2's went up markedly. We were dealing with a mean venous pO_2 of close to 1000, whereas the previous mean venous pO_2 when the red cells were circulating was in the range of 300.

DR. G. G. NAHAS (*New York, N. Y.*): What were the pH and pCO_2 values in venous and arterial blood when you used dextran?

DR. WHALEN: As I pointed out in the introduction, we regulated these fairly closely by adding tris buffer. The pH in the arterial dextran ranged from 7.27 to as high as 7.55 with a mean of 7.38. However, there was no correlation between alterations in cardiac output and variations in pH or pCO_2.

Endoscopic Visualization of the Intact Canine Heart Under Hyperbaric Conditions

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Internal visualization of the live heart *in situ* was first attempted by Cutler *et al.*[1] in 1924. Since then, several investigators have reported cardiac endoscopic procedures for experimental as well as for therapeutic purposes.[2-14] These procedures were initially used on hearts in open chests or hearts in which the normal circulation had been disrupted. Sakakibara *et al.*[15] employed the chamber isolation technique with patients on extracorporeal circulation. Others [6] used a jet of fluid under pressure to displace the blood away from the viewing lens. Carlens and Silander[16-18] were able to get limited views of the interior of the heart in a closed chest without disrupting the circulation. Their cardioscope was introduced into the right side of the heart through the jugular vein, but the views were restricted to the surface contact of a transparent saline-distended balloon that surrounded the lens.

These methods gave only limited views or disrupted the normal circulation during visualization of the inside of the heart. In order to get an unimpeded view of the internal movements of the functioning heart, we modified the exchange-infusion method of Boerema *et al.*,[19] using Ringer's dextran solution to replace the blood in the circulatory system under hyperbaric conditions.

Materials and Methods

Healthy mongrel dogs weighing 10–15 kg were anesthetized with intravenous pentobarbital (Nembutal), 25 mg/kg; cyclic positive-pressure ventilation with unhumidified 100% oxygen was maintained through a cuffed endotracheal tube at 15 respirations/min, using a unidirectional flutter valve. The pressure chamber has been described previously.[20]

The experimental method consisted essentially of the rapid exchange of blood with a large amount of clear Ringer's dextran solution infused into the inferior vena cava and left jugular vein (Figure 1). The inferior vena cava was cannulated with a polyethylene catheter (internal diameter 7 mm), and its tip was advanced to the estimated diaphragmatic level. The left jugular vein was cannulated with a similar catheter (4 mm internal diameter). Both catheters were

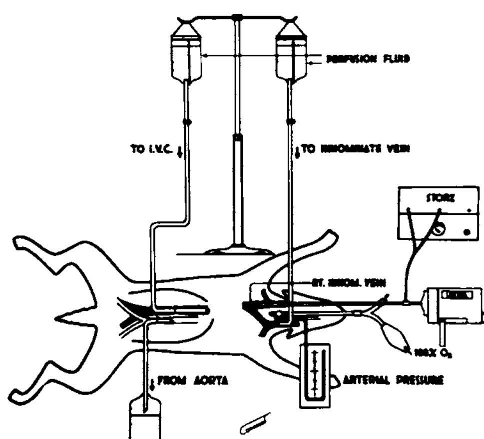

FIGURE 1. Diagram of the experimental model.

connected to a gravity infusion system. Bleeding and blood sampling were accomplished through a 4-mm catheter introduced into the abdominal aorta. The right jugular vein was isolated and prepared to receive a Carlens-Silander cardioscope without a balloon at its tip. A mercury manometer connected to the left carotid artery was used to monitor the blood pressure.

The electrocardiogram (ECG) and electroencephalogram (EEG) were monitored with needle electrodes and were recorded on an EFM multichannel recorder. The ECG lead selection was determined by the best pattern available. A modification of the solution described by Neely *et al.*,[21] containing 4.5% low molecular weight dextran, was used (Table 1). The final pH of this solution

TABLE 1.

Composition of Perfusion Fluid

KCl	4 mEq
$NaHCO_3$	27 mEq
NaCl	100 mEq
Na acetate	2 mEq
THAM ($C_4H_{11}NO_3$)*	10 mEq
$MgCl_2 \cdot 6H_2O$	2 mEq
$CaCl_2 \cdot 2H_2O$	4 mEq
Dextran "40"	45 gm
Glucose	10 gm

* Kindly provided by Abbott Laboratories, North Chicago, Illinois.

was 7.45–7.48 and its osmolarity was approximately 345 milliosmols. The solution was not sterilized. It was warmed to 33–37°C before use.

The chamber was pressurized to 45 psig in 12–22 min, and the exchange infusion was begun. The infusion was delivered through the inferior vena caval catheter at the rate of approximately 500 ml/min. Bleeding and blood sampling were accomplished through the aortic catheter. Initially, a careful balance was kept between the rates of infusion and bleeding to maintain a satisfactory blood pressure. At an arbitrary point when the bled fluid became lightly blood-tinged, the cardioscope was introduced into the right side of the heart through the right jugular vein. The light source was provided by a Storz light fountain, and light was delivered through a flexible optical light guide connected to the cardioscope. At this time, visual inspection of the interior of the right atrium was carried out.

When definitive structures could be clearly visualized, an 8-mm Leicina 8S movie camera was connected to the eyepiece of the cardioscope with a special adapter, and infusion was started through the superior vena cava by means of the catheter in the left jugular vein. Through the reflex eyepiece of the camera, the interior of the heart was viewed during cinematography. The cardioscope was advanced through the tricuspid valve into and out of the right ventricle while pictures were being taken. Two types of movie films were used: black and white (Perutz Perkine, 400 ASA) and color (Ektachrome ER daylight film, 160 ASA).

In some cases, isoproterenol (80 μg/100 ml of 5% dextrose in water) was administered through the cardioscope at the rate of approximately 15 drops/min. The Astrup apparatus with the Clark oxygen macroelectrode[22] was used to determine the oxygen tensions of the arterial samples. No attempt was made to revive the animals after termination of the procedure. Compression time lasted up to

120 min, and decompression was accomplished according to the standard U. S. Navy decompression tables.[23]

Results and Discussion

We were able to clear the blood from the circulatory system so successfully that the effusate from the aorta differed from the infusate only in that it had a slight yellow tinge. After adequate exchange, the fluid in the circulatory system was completely transparent, permitting clear viewing of the movements in the intracardiac structures. About 40–50 liters of rapidly infused solution was necessary to accomplish this. The oxygen tension of the arterial fluid ranged from 2500 to 2800 mm Hg (Figure 2), decreasing when heart failure ensued. The heart rate slowed to less than 40 beats/min and the blood pressure fell below 30 mm Hg systolic pressure. There was a good response to the infusion of isoproterenol.

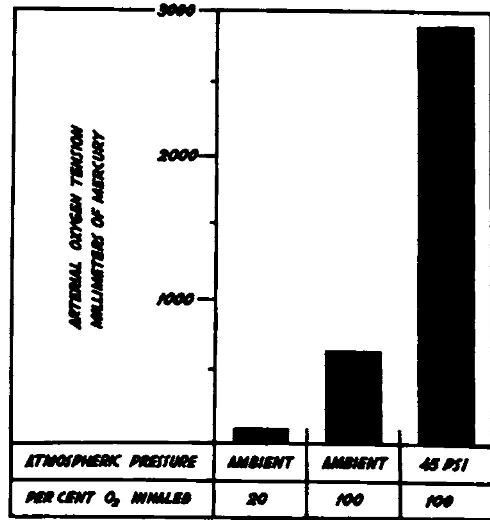

Figure 2. Arterial oxygen tensions of dogs breathing room air and 100% oxygen at 1 atm and 45 psig.

Direct viewing of the heart permitted the observation of the papillary muscle; it relaxed during the major period of diastole but contracted, with its chordae tendinae becoming taut, just before closure of the tricuspid valve. The papillary muscle seemed to be involved in the initiation of the closure of the tricuspid valve. The valve closure was extremely rapid, so that our system did not permit study of the detailed events leading from its open to its closed position. All three leaflets, however, could be seen to contribute to closure of the valve. During ineffective premature systoles, the valve leaflets could be seen to move toward the closed position. The tricuspid annulus appeared to constrict during systole. This constriction of the annulus appears significant enough to be an important factor in the closure of the tricuspid valve.

ACKNOWLEDGMENT

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DISCUSSION

DR. R A. COWLEY, *Session Chairman (Baltimore, Md.)*: To some of you, a paper such as this might seem unusual, but for many who are doing open-heart surgery a procedure such as this may assist the surgeon in evaluating where to put his sutures rather than the usual technique of closing the valve annulus to prevent regurgitation. It would be particularly useful when putting in two valves and working with the tricuspid valve as well.

Hyperbaric Oxygen in the Treatment of Cardiac Arrhythmias

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Royal Victoria Hospital and
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Cardiac output and coronary perfusion are compromised by cardiac arrhythmias, and normal sinus rhythm must be restored for efficient function. Administration of digitalis, procaine amide, and quinidine is not always successful and is attended by a high incidence of side effects and even risk of sudden death.[1]

Interest in electroversion as an alternative was stimulated by Zoll *et al.*[2] and Paul and Miller,[3] who first used alternating-current countershock successfully in 1956. In 1960, Kowenhoven *et al.*[4] demonstrated that external cardiac massage could maintain circulation to vital organs after cardiac arrest, thereby allowing enough time for defibrillation techniques to be implemented. The complications of severe postshock arrhythmias and myocardial damage led to the introduction of direct-current countershock by Lown *et al.*[5] in 1962. This proved to be safer and more effective, with a high conversion rate and little risk of morbidity or death. Nevertheless, about 10% of patients with atrial fibrillation, and an undetermined proportion with ventricular fibrillation, resist all attempts at conversion.

In previous experiments demonstrating the lack of relationship between the threshold of ventricular fibrillation and varying arterial oxygen tension, we noted that defibrillation of dogs with persistent coronary occlusion was strikingly facilitated by hyperbaric oxygenation at 3 atm.[6] Accordingly, we designed further experiments (reported herein) to obtain data on the effects of OHP in defibrillation of the canine heart.

Materials and Methods

Ten adult mongrel dogs of both sexes, weighing 10–22 kg, were anesthetized with 30 mg/kg of intravenous pentobarbital (Nembutal), ventilated mechanically with 100% oxygen, and paralyzed with succinylcholine (Anectine). A left fifth interspace thoracotomy exposed the pericardium, which was opened and suspended as a cradle to support the heart without impeding lung excursion. Temperature was measured with an esopha-

geal probe and maintained between 37°C and 39°C by an electric heating pad. Arterial blood pressure was monitored continuously, and arterial pO_2, pCO_2, and pH were determined intermittently throughout each experiment.

In five dogs, the anterior descending coronary artery alone was clamped just below the takeoff of the left circumflex artery. Both of these arteries were occluded in the remaining animals. Ventricular fibrillation was electrically induced in less than 30 sec from the time of occlusion and allowed to persist until the fibrillatory movements became very weak. With one or more coronary arteries occluded, defibrillation was attempted by single shocks of 0.15-sec duration from an alternating-current (AC) defibrillator. If fibrillation persisted after one shock of 100 volts, cardiac massage was begun and the voltage progressively increased through 170, 240, 310, and 380 volts until defibrillation occurred. The procedure was then repeated once the vital signs had returned to normal.

Each of these same animals was then placed in a hyperbaric chamber at 3 atm pressure and the procedure duplicated, with observations made of the pO_2 and blood pressure. If the heart appeared weak after the shock sequence, cardiac massage was used until tone and rhythm returned to normal.

In all experiments, an AC defibrillator and large heart-shaped paddles were used, the latter ensuring uniform contact with minimal thermal injury to the myocardium.

Results

Single 100-volt shocks were consistently successful at 3 atm in restoring normal sinus rhythm (mean pO_2 1513 mm Hg), whereas defibrillation required higher voltages and multiple shocks at normal atmospheric pressure (mean pO_2 355 mm Hg) (Figure 1). Furthermore, myocardial tone and forceful contraction returned immediately, even after 10–12 min of persistent fibrillation. At 1 atm, fibrillatory movements became extremely weak within 2–3 min, and cardiac massage was required to maintain blood pressure after reversion.

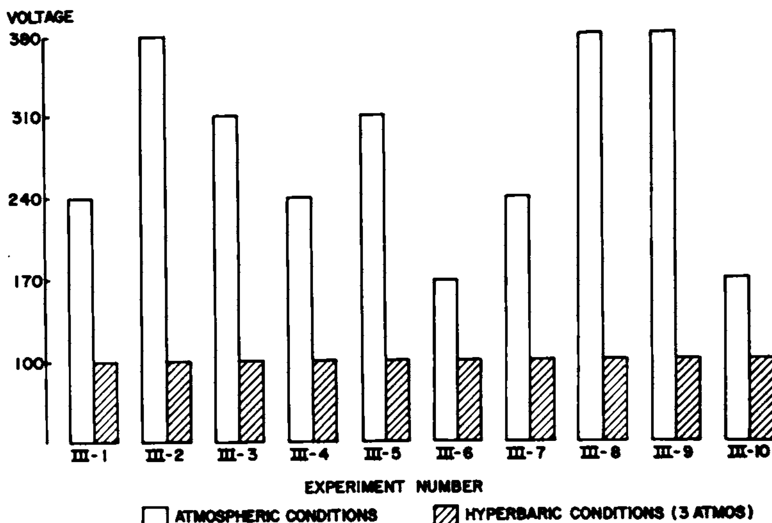

FIGURE 1. Voltage required to defibrillate 10 canine hearts, first under normal atmospheric conditions and then during pressurization to 3 atm in the hyperbaric chamber.

Discussion

As mentioned above, the marked facilitation of conversion to normal cardiac rhythm by the supplementary use of OHP became apparent during our earlier experiments demonstrating the inability of hyperbaric oxygen to offer any protection against ventricular fibrillation induced in the presence of coronary artery occlusion.[6] In 1940, Wiggers *et al.*[7] stated that defibrillation of the dog heart in the presence of persistent coronary artery occlusion was impossible. Others have found that sinus rhythm can be restored under these circumstances, but only after repeated or serial shocks of high voltage and cardiac massage.

Although the contrast between defibrillation attempts made at normal atmospheric pressure and those made during OHP is certainly clear (Figure 1), it is minimized by the fact that the lowest setting of our defibrillator was 100 volts. A single defibrillation attempt was uniformly successful in all 10 dogs at this setting under hyperbaric conditions, whereas multiple shocks of high voltage with cardiac massage were required at atmospheric pressure. Probably, reduced shocks would have been equally effective under hyperbaric conditions. Furthermore, each heart had already been fibrillated and defibrillated several times at normal atmospheric pressure before undergoing a further trial at 3 atm. Certainly these traumatic maneuvers could only reduce the ease of defibrillation.

This finding is in accordance with observations reported by Meijne and Boerema,[8] who noted that fibrillating ventricles under 3 atm of pressure easily resumed tonus by manual massage and occasionally reverted spontaneously. Hyperbaric oxygen did not cause coronary vasoconstriction but significantly reduced arteriovenous potassium differences and blood lactic acid, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) levels. They attributed this to a reduction in anaerobic metabolism in the fibrillating heart due to better tissue oxygenation.

It may be that the subendocardial position of the conducting mechanism protects it from hypoxia[9] when one or two major coronary arteries are occluded at 3 atm. This would facilitate a return to normal sinus rhythm following defibrillation. Accordingly, other arrhythmias of ischemic origin may be found more susceptible to electroversion under hyperbaric conditions.

We have had one clinical experience involving a 50-year-old man with refractory ventricular fibrillation following coronary occlusion. The fibrillation could not be converted despite repeated attempts with a direct-current (DC) defibrillator by our cardiac arrest team. The patient was rapidly moved to the hyperbaric chamber while external massage was continued. After 5 min of exposure to 3 atm of pressure, defibrillation was successfully accomplished with a single shock of 100 volts from an AC defibrillator. Although sinus rhythm returned, however, the patient died from irreversible hypotension. Autopsy revealed a massive anterior infarction and severe pulmonary disease.

Certain safety precautions are mandatory if this technique is to be used without mishap. Following are several measures which we consider essential and apply in our unit: (1) The patient is placed on a nonconductive rubber sheet, with a thin fireproof mattress and a second nonconductive sheet between it and the metal floor. In this manner, electrical grounding is prevented. (2) Oxygen (100%) is delivered by intubation or with a specially constructed leakproof mask to prevent oxygen spill in the vicinity of the external defibrillator paddles. (3) Fresh air is circulated in the chamber at the rate of 50 ft^3/min, eliminating dangerous concentrations of oxygen. (4) Personnel are provided with fire-resistant scrub suits,

and two 5-lb Pyrene potassium bicarbonate air-expellent fire extinguishers are kept in the chamber. (A water-sprinkling system is preferable, if available.)

In addition, the defibrillator unit (including ECG ocilloscope, pacemaker, and defibrillator) is kept outside the chamber, thus not only conserving space but also protecting against sparking from the contact relays. Hermetic feed-through connectors are provided to carry the ECG to the defibrillator unit. Also, specially designed high-voltage connectors with a minimum 5000-volt (DC) voltage breakdown were tested for pressure and air leak by the manufacturer. Standard defibrillator paddles are considered adequate.

Conclusions

Hyperbaric oxygen markedly facilitates electrical defibrillation of the canine heart in the presence of persistent coronary occlusion. Our findings suggest that cardioversion of refractory arrhythmias, particularly ventricular fibrillation, may be clinically practicable under hyperbaric oxygen. We hope that other hyperbaric units will find our observations worthy of a clinical trial.

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DISCUSSION

DR. R. WHALEN (*Durham, N. C.*): I would like to compliment the authors on their study and to confirm several of their observations. Hyperbaria does not change electrically determined ventricular fibrillation thresholds, according to studies completed by Mr. C. Frank Starmer from our group. We have had one case which corroborates your observations. A patient developed ventricular tachycardia 2 weeks after myocardial infarction and was successfully cardioverted at 1 atmosphere, but he then reverted to ventricular tachycardia. Another attempt at cardioversion using a DC cardioverter at 1 atmosphere was unsuccessful. He was then placed in the chamber, taken to 3 atmospheres, and, after a period of oxygenation, successfully cardioverted with DC countershock. He showed a progressive decrease in the number of PVC's during the next 4 hours and was eventually decompressed after a 6-hour stay in the chamber. He had no recurrence of his arrhythmia and was discharged.

Further Evaluation of Hyperbaric Oxygen in Hemorrhagic Shock

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In 1962 we reported on the beneficial effects of hyperbaric oxygen in experimental hemorrhagic shock.[1] The mortality rate in dogs subjected to hemorrhagic shock (mean arterial blood pressure 30 mm Hg) for 2.5 hours and treated with 3 ata of oxygen was reduced from 74% in the control group to 17% in the OHP-treated group. The effectiveness of OHP was demonstrated not only in the survival rate of the treated animals, but also in the physiologic and biochemical determinations, reflecting the salutary effect at the cellular level.

This finding differs from that of Frank and Fine,[2] who found OHP of doubtful value in hemorrhagic shock. Elliott and Paton [3] found that administration of oxygen at 3 ata to dogs subjected to hemorrhagic hypotension increased the oxygen availability to the tissues, decreased buffer–base depletion, and increased the survival rates, as compared with the effect in dogs breathing air or oxygen at atmospheric pressure. Clark and Young [4] reported a 25% increase in the survival rate of dogs in hemorrhagic shock treated with OHP at 2 ata. Finally, Jacobson *et al.*[5] reported that OHP administered after hemorrhagic shock was ineffective in reversing the survival pattern or associated metabolic changes.

In order to evaluate further the proper role of hyperbaric oxygen in hemorrhagic shock, the present studies were undertaken to determine the effect of the following variables: (1) systemic vasoconstriction associated with OHP therapy, (2) duration of shock, (3) time of administration of OHP in relation to shock, and (4) chamber pressure.

Materials and Methods

Mongrel dogs were sedated with morphine sulfate (1.5 mg/kg body weight) 1 hour before induction of bleeding. Aseptic technique was observed throughout the procedure. Both femoral arteries were cannulated under local procaine (1%) anesthesia; one was connected to the elevated siliconized glass reservoir, and the other to a mercury manometer. All animals were given aqueous heparin, 2 mg/kg body weight. Bleeding was accomplished by the elevated reservoir technique previously described.[1]

OHP was administered in a hyperbaric chamber after reinfusion of the shed blood

(unless otherwise specified). The pressure chamber used in these experiments was a converted autoclave, described elsewhere.[1] Arterial and venous blood samples were drawn before hemorrhage, just before pressurization (with the dog in shock), at the end of 1 hour of OHP administration, immediately after decompression, and again 1 hour later. The samples were examined for pH, pCO_2, and base deficit using the Astrup method.[6] Blood lactates and pyruvates were determined enzymatically.[7] In some dogs, tissue oxygen tensions were determined and recorded continuously by means of a Clark polarographic needle electrode inserted in the muscle.

In both control and treated groups, only those animals surviving the experiment beyond reinfusion of the shed blood were included for evaluation. Animals surviving 48 hours or longer were considered long-term survivors, since most of the deaths occurred within the first 24 hours after termination of the experiment.

Experimental Groups I–IV

Group I dogs were studied to determine the effect of systemic vasoconstriction during OHP administration on the course of shock. In our original series mentioned above, shocked dogs treated with OHP at 3 ata showed an average rise of 25 mm Hg in mean arterial blood pressure during pressurization. In order to investigate the possible role this rise in blood pressure might have on the survival rate, 30 dogs were bled until 30 mm Hg mean arterial blood pressure was reached, and they were maintained at this pressure during OHP by further bleeding. Sixteen dogs showed a rise in pressure which necessitated additional bleeding to keep the pressure down to 30 mm Hg. The extra blood removed averaged 6.76 ml/kg.

Group II animals were studied to determine the effect of duration of shock on the response to OHP therapy. Four subgroups were studied, with varying durations of shock of 1.75, 2, 2.5, and 4 hours. The shock level was maintained at 30 mm Hg mean arterial pressure in all subgroups, except the 4-hour shock subgroup, which was kept at 45 mm Hg to give an LD_{50} preparation. A shock level of 30 mm Hg maintained for 4 hours would have resulted in too many deaths to demonstrate the therapeutic effectiveness of OHP.

Group III animals were studied to determine the effect of time of hyperbaric oxygenation in relation to shock. OHP was administered at 0.5 and 2.5 hours after the onset of shock.

Group IV dogs were studied to determine the effect of chamber pressure. This was done at 2 and 3 ata.

Bleeding Volume and Uptake

Although the severity of shock in these experimental animals was controlled by the level of blood pressure and duration of shock, their maximum bleed-out volumes were generally comparable (Figure 1). The bleed-out volume of the

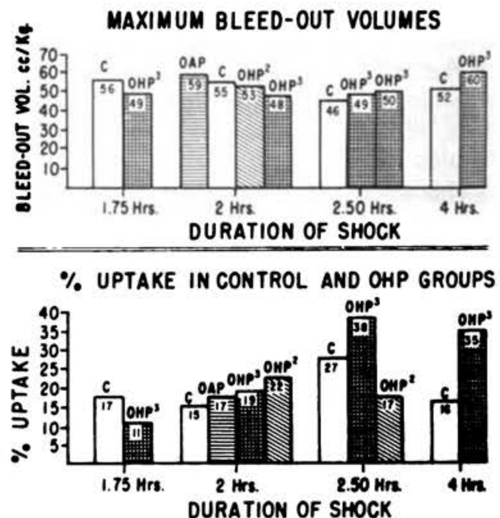

FIGURE 1. Bar graphs illustrating the maximum bleed-out volumes and percent uptake of the shed blood in the control (C) and hyperbaric (OHP) groups. OHP was given at 2 ata (OHP^2) and at 3 ata (OHP^3).

4-hour shock + OHP preparation, however, was significantly larger than that of the control group, by 8 ml/kg body weight. This was also associated with a significant uptake amounting to 35% of the maximum bleed-out ("uptake" meaning the amount of blood the animal took up spontaneously or had to be reinfused with to maintain 30 mm Hg mean arterial blood pressure before reinfusion of the total shed blood). These two factors made the 4-hour shock + OHP a more lethal preparation, although the blood pressure and duration of shock were comparable to that of the control group. The same observations applied to the 2.5-hour shock preparation, where the OHP group took up 36% of the shed blood in comparison to 27% in the control group.

The uptake of shed blood by the shocked animals reflects deterioration of the vasomotor compensatory mechanisms to such a degree that a spontaneous uptake of more than 40% of the shed blood is always lethal. This finding was first reported by Fine [8] and was confirmed by our experience. Therefore, the extent of the uptake by the 2.5- and 4-hour preparations reflects the severity of the shock studied, which has significant bearing on the results.

Biochemical Changes

We reported previously [1] on the effects of hyperbaric oxygen on the biochemical changes in normal and shocked dogs treated with OHP prior to reinfusion of the shed blood. In the present investigation, we limited our biochemical studies to blood samples obtained from dogs subjected to 2 hours of hemorrhagic shock at 30 mm Hg mean arterial blood pressure and then compressed with OHP at 3 ata for 2 hours, simultaneously with reinfusion of the shed blood. Figures 2–5 represent the average biochemical changes in five dogs which survived and seven which died in shock. The changes were similar in both groups, reflecting severe metabolic acidosis associated with shock.

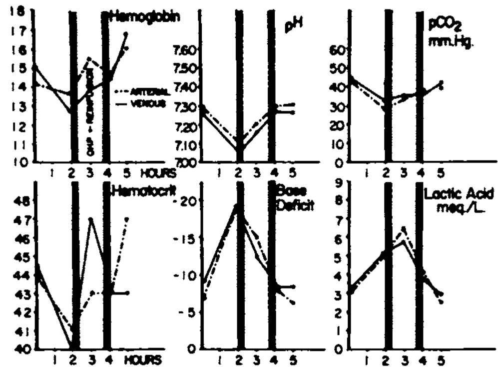

FIGURE 2. Graphs showing changes in hemoglobin, hematocrit, and acid–base balance in dogs which survived hemorrhagic shock.

There was a significant decrease in arterial and venous pH, an increase in base deficit after shock, associated with a marked rise in lactic acid and lactate/pyruvate ratio. A decrease in both arterial and venous pCO_2 occurred after shock, probably due to hyperventilation. All these changes returned to normal values after reinfusion and OHP therapy in both surviving and nonsurviving animals. Among the nonsurvivors, an increase in base deficit occurred after decompression, which was associated with a lowering of the pH, reflecting metabolic acidosis. Another major difference between the two groups was the level of lactic acid and lactate/pyruvate ratio before reinfusion and OHP therapy. The mean lactic acid level in the surviving group at the end of shock was 5 mEq/liter compared to an average of 9 mEq/liter in the nonsurvivors. This was also reflected by the lactate/pyruvate ratio, which was twice as high in the fatal group, compared to the survivors. Although reinfusion and

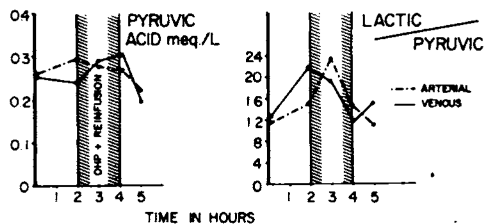

FIGURE 3. Graphs showing changes in pyruvic acid and pyruvate/lactate ratio in dogs which survived hemorrhagic shock, treated with OHP.

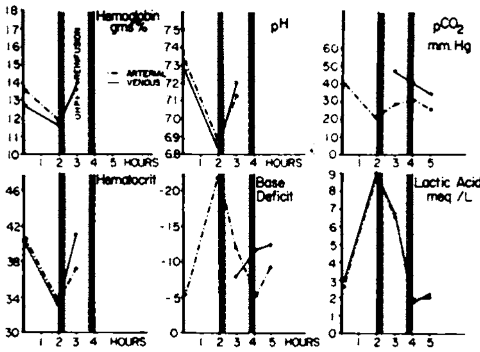

FIGURE 4. Graphs showing changes in hemoglobin, hematocrit, and acid–base balance in dogs which died in hemorrhagic shock, treated with OHP.

OHP returned these values to the normal levels as in the surviving group, the damage to the anoxic tissues was irreversible.

Tissue Oxygen Tensions

Realizing the inadequacies of the Clark needle electrode for measurement of tissue oxygen tensions, we considered these measurements of only relative value. Figure 6 shows the average percent change of tensions in four shocked animals treated with OHP. There was an average decrease of 50% in the tissue oxygen tensions after hemorrhagic shock. This increased to nine times the original tissue oxygen tension upon exposure to 3 ata. The tensions returned to lower levels than preshock values upon decompression.

RESULTS

Group I

The effect of vasoconstriction, studied in Group I animals, is illustrated in Figure 7.

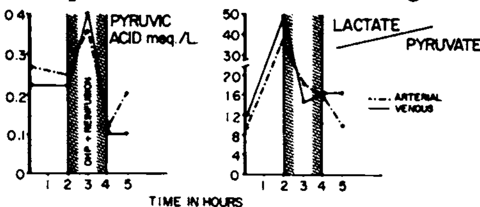

FIGURE 5. Graphs showing changes in pyruvic acid and pyruvate/lactate ratio in dogs which died in hemorrhagic shock, treated with OHP.

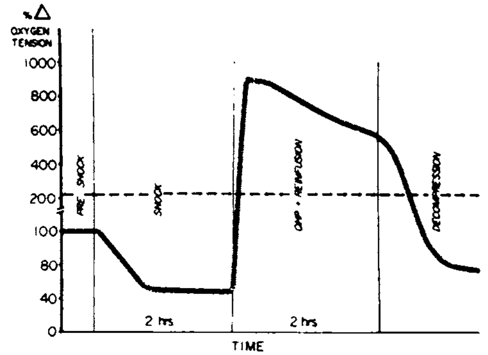

FIGURE 6. Graph illustrating changes in tissue oxygen tension of the front leg muscles of dogs in hemorrhagic shock, treated with OHP.

In the control group of 25 dogs subjected to hemorrhagic shock for 2.5 hours, only five were long-term survivors, giving a mortality rate of 80%. In the hyperbaric group of 19 dogs treated at 3 ata, but with the mean arterial blood pressure allowed to rise an average of 25 mm Hg during OHP therapy, five dogs died (mortality rate 26%). When the mean arterial pressure was kept at 30 mm Hg by further bleeding during OHP therapy, 17 dogs survived in a group of 29 (mortality rate 59%). However, this number was still significantly lower than in the control group (80% mortality).

Group II

The effect of duration of shock on the four subgroups of Group II, kept in shock at

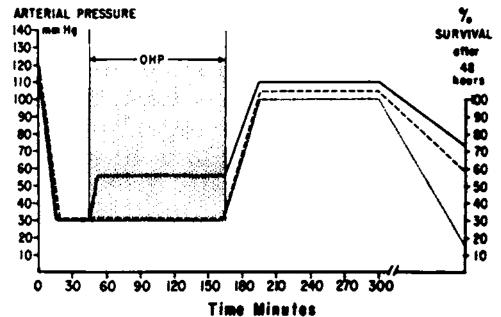

FIGURE 7. Graph illustrating the effect of vasoconstriction due to OHP on survival rate of dogs in hemorrhagic shock.

1.75, 2, 2.5, and 4 hours, is shown in Figure 8.

Subgroup A: 1.75 hours. The control group comprised 30 dogs. The mean maximum bleed-out was 56 ml/kg body weight, and the uptake was 17% of the maximum bleed-out. Ten dogs died after completion of the study. The hyperbaric group initially comprised 26 dogs, but two died before reinfusion and were excluded. There were 13 deaths among the 24 dogs that received OHP therapy (mortality rate 54%). The maximum bleed-out volume in this group was 49.87 ml/kg body weight, and the percentage uptake was 20%. Because of the lack of any demonstrable beneficial effects of OHP in this preparation, another series of 23 dogs was tested in a similar manner. The maximum bleed-out volume was 49 ml/kg body weight and the percent uptake 11%. There were 12 deaths in this group (mortality rate 52%). The results in both hyperbaric groups were similar and showed no significant difference from the control group.

Subgroup B: 2 hours. The control group consisted of 10 dogs subjected to shock of 30 mm Hg mean arterial pressure; three died before reinfusion of shed blood and were excluded. There were five deaths in

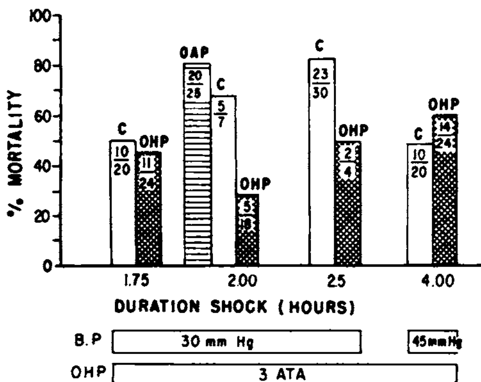

FIGURE 8. Graphs illustrating the effect of duration of shock on survival of dogs treated with OHP, compared with control animals (C) and animals given 100% oxygen at ambient atmospheric pressure (OAP).

the remaining seven, giving a mortality rate of 71%. The maximum bleed-out was 55 ml/kg body weight, and the uptake was 15% of the shed blood. Because of the small number of dogs included in this control group, another series of 11 dogs was added, with eight deaths, confirming the average mortality rate of 70% in this 2-hour shock preparation.

The value of 100% oxygen at ambient atmospheric pressure was tested in 25 dogs. The maximum bleed-out volume was 59 ml/kg body weight and the percent uptake was 17%. There were 20 deaths in this group (mortality rate 80%).

In the OHP group, 28 animals were included. Five died before receiving the OHP therapy and were excluded. The average maximum bleed-out volume was 48 ml/kg body weight, and the percent uptake 19%. There were five deaths in this group, giving a mortality rate of 28% —which was significantly better than in the 2-hour control group.

Subgroup C: 2.5 hours. The control group consisted of 30 animals subjected to hemorrhagic shock of 30 mm Hg mean arterial pressure for 2.5 hours. The maximum bleed-out volume was 46 ml/kg body weight with an uptake of 27%. There were 23 deaths (mortality rate 83%). The hyperbaric group, which was treated with OHP after reinfusion of the shed blood, consisted of 13 animals. Nine animals died before receiving OHP therapy and were excluded. Only four dogs completed the experiment; two were long-term survivors (50%). Too few animals survived this preparation to make the results statistically significant.

Subgroup D: 4 hours. The control group consisted of 20 dogs. The maximum bleed-out was 52 ml/kg body weight and the uptake 16% of the shed blood. There were 10 deaths in this group (mortality rate 50%). The OHP group comprised 24 animals. The maximum bleed-out volume was 60 ml/kg body weight and the

uptake 35% of the shed blood. These two factors illustrate the severe conditions imposed by this preparation and the inefficacy of OHP in deep shock states. Fourteen dogs died within 48 hours of completion of the experiment (mortality rate 58%).

Group III

The effect of time lapse in the shock state before administration of OHP was tested in two groups of animals after 0.5 and 2.5 hours of shock. The mortality rate increased from 26% in the 0.5-hour group to 50% when OHP was administered 2.5 hours after shock. Further work is needed to test the effects of more intervals between the two time limits.

Group IV

Since oxygen toxicity increases with higher pressures, it was decided to investigate the effect of varying chamber pressures on the survival rate of dogs in hemorrhagic shock (Figure 9). Pressures of 2 and 3 ata were tested. The 2-hour shock preparation was best suited for this investigation. As mentioned above, the control group had a mortality rate of 71% and the OHP group treated at 3 ata had a mortality of 28%. Another series of 12 dogs was subjected to 2-hour hypotension at 30 mm Hg mean arterial pressure, after which the dogs were compressed to 2 ata simultaneously with reinfusion. One dog was excluded; the remaining 11 dogs completed the experiment and only two died within 48 hours (mortality rate 18%). In the 2.5-hour shock preparation, no significant difference occurred between the control group, with a mortality rate of 83%, and the group given OHP at 2 ata, with a mortality rate of 70%. The small group of four dogs which completed the experiment at 3 ata had a 50% mortality rate. While this may appear superior to the 2 ata pressure, the preparation is so lethal that the number of animals surviving the whole experiment is too small to yield significant results.

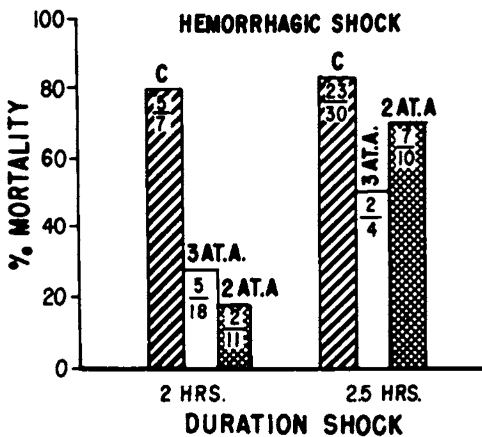

FIGURE 9. Bar graphs illustrating the effect of chamber pressure on the mortality rate of dogs in hemorrhagic shock treated with OHP.

DISCUSSION

The basic problem in hemorrhagic shock is impairment and eventual failure of cellular oxygenation. The various changes associated with refractory hypotension and irreversible damage follow the development of a tissue oxygen debt.[9] The most important factor in the management of hemorrhagic shock is the avoidance of tissue hypoxia. A logical solution to this problem is the administration of oxygen, in addition to blood replacement. However, the literature is full of conflicting reports on the value of oxygen in hemorrhagic shock. The same controversy seems to exist with regard to hyperbaric oxygen. This difficulty arises from the lack of a standardized shock model with known behavior and predictable mortality, that takes into consideration the multiple variables inherent in the shock preparation. In experimental hemorrhagic shock, suitably controlled conditions are difficult to achieve and frequently the experimental shock preparation is so drastic that no therapy can be effective.

Assessment of any treatment is possible only when a sensitive experimental prepa-

ration is tested. This is best achieved by a shock preparation of about 70% mortality.[9] Since this is difficult to achieve, various shock preparations were tested in the experiments presented above. It became clear that the same method of therapy gave different results in the various preparations. Hyperbaric oxygen was tested in animals in shock from 1.75 to 4 hours. Experimental shock preparations of 2.5 hours at 30 mm Hg mean arterial pressure and 4 hours at 45 mm Hg proved highly lethal. In the 2.5-hour preparation, very few animals survived the shock experiment to undergo the hyperbaric therapy. The 4-hour preparation was even more severe, as demonstrated by the maximum bleed-out of the hyperbaric group before undergoing OHP therapy (60 ml/kg body weight), compared to 52 ml/kg in the control group. This was also manifested by the 35% uptake of the shed blood, compared to 16% in the control group. These same observations apply to the 2.5-hour preparation.

Two series of animals were tested at 30 mm Hg mean arterial pressure for 1.75 hours to determine the effectiveness of OHP, without any demonstrable effect. This led us to investigate the time factor with respect to OHP administration, and we concluded that the earlier OHP was administered, the better were the results. We believe that our best experimental shock preparation was the 2-hour preparation, with a mortality rate of 70%. Although we have not demonstrated any beneficial effects of oxygen at ambient pressure in hemorrhagic shock, significant improvement in the survival rate was demonstrated with OHP. The beneficial results were obtained at 2 and 3 ata and were reflected in the biochemical changes and tissue oxygen tensions discussed previously. This improvement was related partly to the rise in systemic arterial pressure secondary to vasoconstriction, but mostly to better tissue oxygenation.

Further work is in progress to improve the beneficial effects of hyperbaric oxygenation in hemorrhagic shock by the use of plasma expanders.

SUMMARY

1. The best experimental hemorrhagic shock preparation to test the efficacy of OHP therapy was that of 30 mm Hg mean arterial blood pressure maintained for 2 hours. The mortality rate from this preparation was 70%.

2. OHP therapy reduced the mortality rate of shocked dogs from 70% in the control group to 28% in the treated group.

3. The beneficial effects of OHP were not exclusively related to the concomitant rise in blood pressure. Improved tissue oxygenation was a major factor.

4. A pressure of 2 ata was as effective as 3 ata, provided it was given early in shock, before the occurrence of irreversible cellular damage.

ACKNOWLEDGMENTS

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Hyperbaric Oxygen Therapy in Experimental Hemorrhagic Shock*

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The basic mechanism in the pathophysiology of irreversible hemorrhagic shock is thought to be defective tissue metabolite and gas exchange resulting from poor tissue perfusion in the period following hypotension. Theoretically, hyperbaric oxygen therapy may be able to meet the oxygen requirements of tissues struggling against an overwhelming burden of hypoxia. The ability of tissues to recover by extracting more oxygen than usual might extend the benefits of OHP to permit tissue survival at the extremely subnormal blood flows occurring in the period after shock.

In 1943, Frank and Fine[1] reported that 3 hours of OHP treatment during and after the induction of hemorrhagic shock in dogs did not alter survival rate (compared with controls). They did not pursue their study further, but concluded that "the course of events in hemorrhagic shock is in no way altered when venous anoxemia is prevented by administering oxygen at high pressure. Oxygen as a therapeutic agent in hemorrhagic shock is therefore of doubtful value." More recently, however, Attar *et al.*[2] and Elliott and Paton [3] reported increased survival in dogs in hemorrhagic shock treated with oxygen at 3 ata for 2 hours—although Attar's OHP-treated dogs were not continually bled to maintain a constant level of hypotension, and his control dogs were apparently not in the same weight range as the treated animals.

Since none of these studies has attempted to simulate the clinical situation (*i.e.*, treatment of the shocked victim after replacement of the blood loss), we undertook a study to evaluate the effectiveness of oxygen therapy in a stage of shock considered irreversible because of the severity of the experimental preparation.

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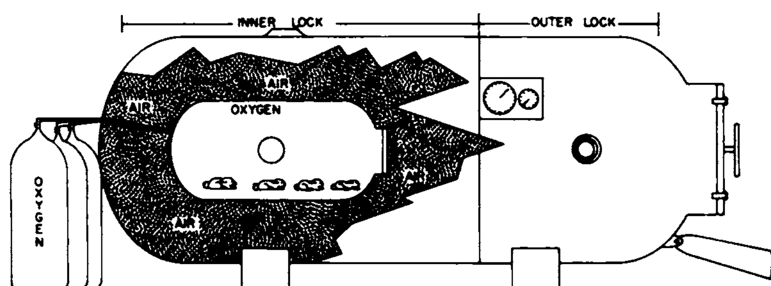

FIGURE 1. Experimental pressure chamber at the Naval Medical Research Institute.

MATERIALS AND METHODS

New Zealand albino male rabbits (2.4–3.7 kg) underwent splenectomy 3 weeks before the definitive experiment. Streptomycin and penicillin were given before and after operation. All animals had normal intestinal flora. Heparin (2 mg/kg) was given before induction of shock and when the shed blood was reinfused.

Under open-drop ether anesthesia and supplemental local anesthesia, the femoral artery was cannulated. When fully reactive, the animals were bled into citrated Fenwal plastic bags in graded increments over a 15-min period until a mean arterial pressure of 45 mm Hg was recorded. This pressure was maintained by subsequent blood removal and sustained for 5 hours, during which time the animals were alert and responsive. At the end of 5 hours, the blood was warmed to 37°C and was returned slowly intra-arterially over a 1-hour period. An additional 40 ml of citrated cross-matched blood was given to replace that taken for samples. Randomized blood cultures were obtained to make certain that contamination had not occurred during the shock period.

Three groups of animals (10 rabbits in each group) were then studied: Group I animals were kept in a constant-temperature tank at 25°C breathing room air and were observed until death occurred; Group II animals were kept in a constant-temperature tank at 25°C breathing 100% oxygen at ambient pressure (except for removal to air during sampling) for 36 hours, or until death or survival was imminent; Group III animals were placed in a small accessory tank compressed with oxygen, contained in a standard Navy decompression chamber compressed with air (Figure 1). These last 10 rabbits were kept at 25°C under 2 ata of pressure breathing 100% oxygen (except for removal to compressed air during sampling) until death occurred or 12 hours of treatment had been completed.

In Groups II and III, normal control splenectomized animals were placed in the tanks with the experimental animals and were observed for signs of oxygen toxicity (which did not occur). The oxygen concentration in the tanks was measured by gas chromatography and was maintained between 99% and 100%. Arterial pH, pO_2, and pCO_2 were measured on an Instrumentation Laboratory blood gas analysis apparatus and converted to the *in vivo* temperature by the nomogram of Severinghaus.[4] Arterial lactic acid was measured by the method of Barker and Summerson[5] and pyruvic acid by the technique of Friedemann and Haugen.[6] Base bicarbonate was determined from the Singer-Hastings[7] nomogram and converted to pH 7.4 to determine the bicarbonate deficit, as described by Brown.[8] Excess lactate was determined by the formula of Sendroy.[9]

Blood volumes were measured using ^{131}I serum albumin (RISA) and found to be about 8% of the body weight. Blood samples were analyzed before shock, after 2.5 hours of shock, after 5 hours of shock, and every 3 hours thereafter until death occurred (up to 18 hours after

shock). Survival over 48 hours was considered permanent. Complete autopsy examinations were performed immediately on all animals that died.

RESULTS

The amount of blood which had to be removed to produce shock varied between 27 and 38 ml/kg, with no significant differences present in any group. All randomized blood cultures showed no growth. Rectal temperature varied between 29°C and 31°C, and no attempt was made to correct this. Several rabbits died during the shock period or while receiving their shed blood, and were therefore not included.

Autopsy examinations showed the following changes in all groups: (1) the liver showed central vacuolization and congestion with various degrees of necrosis; (2) the kidneys had tubular vacuolization, congestion, and focal necrosis with arteriovenous shunting (as evidenced by india ink and fluorescein studies;[10] (3) the adrenals showed congestion and hemorrhage; (4) the heart had congestion and focal areas of fibrosis and necrosis; (5) the small and large bowel had variable congestion, ranging from moderate to severe, with no necrosis; and (6) the lungs almost always showed evidence of patchy atelectasis and congestion and frequently had a pattern of hypoaeration, with some emphysema. Only randomized study was made of brain specimens, and no pathology was evident.

When statistical analysis of all the data was performed for unpaired samples to a confidence limit of 95%, there were no differences in any of the three groups when pCO_2 (Table 1), excess lactate (Table 2), and pH and bicarbonate deficit (Table 3) were determined. Survival time (Table 4) for Group I (kept in air) averaged 11.6 ± 0.7 hours, with no long-term survivors. Animals in Group II (oxygen at 1 atm) survived an average of 11.3 ± 0.8 hours, and there was one permanent survivor. Group III (oxygen at 2 ata) survived 15.2 ± 0.8 hours ($P < 0.05$), with one permanent survivor.

DISCUSSION

The results of this study indicate that hyperbaric oxygen therapy at 2 ata instituted after a period of profound oligemic hypotension is ineffective in enhancing tissue oxygenation. This was evidenced by lack of reversal of the metabolic acidosis in the OHP-treated group compared with controls. A possible explanation for the failure of OHP to increase tissue oxygen extraction in the postictal state is that tissue microcirculatory perfusion is markedly decreased and prevents the increased oxygen in solution from reaching the tissues. Schumer,[11] Schoemaker,[12] and others have suggested that tissue microcirculatory perfusion changes develop

TABLE 1. Blood Gas Determinations

	Base line	End of 5-hr shock	3 hr after reinfusion	6 hr after reinfusion	9 hr after reinfusion
Air at 1 atm					
pO_2 (mm Hg)	95±1.4	99±2.0	87±1.8	88±2.4	59±5.0
pCO_2 (mm Hg)	26.6±1.7	15.2±3.2	21.9±1.8	23.6±1.5	21.5±1.3
100% O_2 at 1 atm					
pO_2 (mm Hg)	96±1.8	88±1.2	79±1.2	81±1.4	68±1.2
pCO_2 (mm Hg)	27.2±1.0	15.5±1.2	18.4±1.4	20.1±1.3	19.6±1.1
100% O_2 at 2 atm					
pO_2 (mm Hg)	90±1.0	91±1.2	268±8.0	297±11.0	326±13.0
pCO_2 (mm Hg)	26.5±0.9	15.7±0.9	24.8±1.8	23.9±1.2	24.0±1.0

TABLE 2. Lactate, Pyruvate, and Excess Lactate Values

	Base line	End of 5-hr shock	3 hr after reinfusion	6 hr after reinfusion	9 hr after reinfusion
Air at 1 atm					
Lactate (mmoles/liter)	7.10±0.7	18.2±1.4	12.3±0.7	9.2±0.8	7.2±0.6
Pyruvate (mmoles/liter)	0.35±0.03	0.49±0.03	0.47±0.03	0.45±0.04	0.40±0.05
Excess lactate (mmoles/liter)	—	6.4±0.6	1.7±0.4	—	—
100% O_2 at 1 atm					
Lactate (mmoles/liter)	6.90±1.0	16.7±1.6	13.7±1.3	8.9±1.1	8.2±1.0
Pyruvate (mmoles/liter)	0.4±0.04	0.49±0.03	0.48±0.04	0.43±0.07	0.37±0.04
Excess lactate (mmoles/liter)	—	6.4±0.8	2.8±0.2	—	—
100% O_2 at 2 atm					
Lactate (mmoles/liter)	6.40±0.6	17.6±1.4	12.1±1.4	10.0±1.2	7.8±1.1
Pyruvate (mmoles/liter)	0.36±0.03	0.55±0.02	0.41±0.03	0.38±0.03	0.32±0.03
Excess lactate (mmoles/liter)	—	6.9±0.9	1.9±0.3	—	—

during hypotension and persist even after the blood volume is restored. This could explain the observation of Attar, Elliott, and others that hyperbaric oxygen improves survival at the initiation of and during oligemic shock. In these situations, the increased tissue oxygenation provided by OHP in the basal state and early in the hypotensive period may well improve survival after hemorrhagic shock.

Although tissue pO_2 was not measured in this study, its determination would be

TABLE 3. Bicarbonate Values Corrected to pH 7.4, Bicarbonate Deficit, and pH

	Base line	End of 5-hr shock	3 hr after reinfusion	6 hr after reinfusion	9 hr after reinfusion
Air at 1 atm					
HCO_3^- at pH 7.4 (mEq/liter)	14.9±1.5	4.5±1.1	10.5±1.5	12.2±1.8	12.2±2.1
HCO_3^- deficit (mEq/liter)	—	10.4±1.8	4.60±1.5	3.30±1.6	3.70±0.8
pH	7.37±0.01	7.12±0.04	7.34±0.03	7.36±0.03	7.40±0.04
100% O_2 at 1 atm					
HCO_3^- at pH 7.4 (mEq/liter)	15.1±1.3	5.6±0.5	9.0±1.1	11.0±1.1	11.6±0.8
HCO_3^- deficit (mEq/liter)	—	10.5±1.7	6.7±0.9	5.1±1.1	2.8±0.4
pH	7.40±0.01	7.30±0.05	7.32±0.06	7.34±0.06	7.36±0.09
100% O_2 at 2 atm					
HCO_3^- at pH 7.4 (mEq/liter)	15.4±1.3	5.1±0.7	10.2±1.4	10.3±1.4	12.2±1.1
HCO_3^- deficit (mEq/liter)	—	12.3±1.8	6.3±1.7	4.2±1.6	2.5±1.0
pH	7.45±0.01	7.24±0.04	7.32±0.03	7.32±0.05	7.35±0.03

TABLE 4. Mortality Rate and Survival Time

	Mortality	Survival time (hours)
Air at 1 atm	100%	11.6±0.7
100% O_2 at 1 atm	90%	11.3±0.8
100% O_2 at 2 atm	90%	15.2±0.8 ($P<0.05$)

extremely worthwhile under different experimental conditions, whereby tissue oxygen availability could be quantitatively assessed and the efficacy of hyperbaric oxygen ascertained more directly. Similarly, it would be of considerable interest to measure cardiac output, which is known to be reduced in oligemic shock and after reinfusion, to ascertain any effect of OHP.

We conclude that altered circulatory dynamics are the primary pathophysiologic mechanism in hemorrhagic shock and that hyperbaric oxygen administered *post facto* is ineffective in reversing the severe metabolic changes associated with hemorrhagic shock.

ACKNOWLEDGMENTS

We are grateful to the men of Experimental Surgery and Experimental Diving at the Naval Medical Research Institute and to Dr. Janice Mendelson of the Army Chemical Center, Maryland, for their help in this study. Dr. Jude Hayes examined and interpreted the pathologic specimens.

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DISCUSSION

Discussion of papers by Attar et al. *(pp. 417–424) and Jacobson* et al. *(pp. 425–429).*

DR. C. HITCHCOCK (*Minneapolis, Minn.*): Did I understand correctly that in the control animals there was 100% mortality?

DR. M. KELLER: In the ones which were not treated with oxygen at ambient pressures or oxygen at higher pressure?

DR. HITCHCOCK: Your base control.

DR. KELLER: Yes sir, 100% mortality.

DR. HITCHCOCK: I think when there is 100% mortality in a control group, there is really no valid base line. Therefore, your last remark about the severity of the preparation is very important. I believe you should immediately redo this study with a fairly large number of animals and try to achieve a 70% or 80% mortality in your basic control group. Then you would know what you are really accomplishing.

DR. KELLER: I agree with you absolutely. At the beginning of the project we tried to think of more sophisticated ways to determine when the animal was entering the stage of irreversibility, for example, direct visualization of the microcirculation for sludging, and examination of the fatty envelope of the RBC which is said to change at the time of sludging. All of these, however, were too difficult to do in the chamber, and we decided we would follow the old standby established by Fine and Lamson (J. Pharmacol. Exp. Ther. 83:250, 1945, and J. Clin. Invest. 24:435, 1945).

DR. H. A. SALTZMAN (*Durham, N. C.*): The area of shock has a long and honorable history in hyperbaric experimentation. Studies have reported either no benefit, or varying degrees of improvement, according to the particular experimental conditions. The important test will be to study shock under conditions seen in man. My guess would be that as one approaches the biology of the sick patient, oxygen will assume lesser importance as compared with circulation in restoring or protecting blood levels.

DR. S. F. SEELEY (*Washington, D. C.*): I would like to suggest that since your model is such a severe one, it might be useful, with controls, as a test model for evaluation of vasodilators in shock. I agree with Dr. Hitchcock that you must achieve 70%, or perhaps 50%, mortality. I am impressed with a reproducible shock model which is in final stages of refinement in the laboratories of Dr. Cowley at the University of Maryland. He can apply the model to a number of mortality rates. Your present model would be very valuable in vasodilatation studies. You could go on from there to a 70 or 50% mortality model.

DR. S. ATTAR: I am not going to defend the value of hyperbaric oxygen in shock. I have shown that hyperbaric oxygen does have a place in the management of hemorrhagic shock. However, this place is limited. Had we used only the 1 hour and 45 minute shock preparation, or the 4-hour preparation, we would have come up with the conclusion that hyperbaric oxygen was useless. In our best preparation, we used oxygen at 1 atmosphere which gave a 70% mortality and did not produce any improvement. However, there are certain reasons accounting for the lack of beneficial effect of 100% oxygen in the 2-hour shock preparation. Dr. Nahas has demonstrated that unless you correct the acid–base balance of the anoxic tissues, they will not be able to utilize the increased oxygen made available to them. This is what I indicated at the beginning of my presentation. We do not advocate the exclusive use of hyperbaric oxygen for the treatment of hemorrhagic shock. Still, we believe that it does have a place, and our investigations are carried

further by the administration of plasma expanders and studies of microcirculation to show that if the microcirculation is improved, probably the increased hyperbaric oxygen will get to the tissues, and if the acid–base balance is corrected simultaneously, much more significant improvement will be demonstrated.

DR. S. W. HANDFORD (*Bethesda, Md.*): Dr. Attar, were the animals that were held at a mean arterial pressure of 30 mm Hg for 2 hours held by the Lampson bottle technique, and, if so, did they take up? Were they reinfusing themselves during that period?

DR. ATTAR: I assume you are referring to the group that we kept for 2 hours in shock and then treated with OHP.

DR. HANDFORD: I am speaking of your better group.

DR. ATTAR: Yes, the Lampson bottle technique was used, and there was an uptake.

Oxygen Tension Influence on Blood Flow from Splanchnic, Renal, and Infrarenal Areas

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Evidence suggests that high oxygen tensions in humans and animals decrease cardiac output, an effect which is apparently mediated by an adaptational system geared to the amount of oxygen available. The fact of increased cardiac output in the lowering of oxygen tension is well established; less information is available about what happens under conditions of high oxygen tension. In animals verging on irreversible shock, oxygen is useful in improving the general condition by increasing the cardiac output. In addition to changes in cardiac output, however, local changes occur that result in distributional changes of blood flow to various organs, the best established example being the decrease of coronary flow which occurs with the increase of oxygen tension. Furthermore, the splanchnic flow decreases in cases of hypoxia.

Following is a report of our study of blood flow to splanchnic, renal, and infrarenal areas. We investigated this problem from several angles: (1) Flow measurements were taken from hepatic, renal, and infrarenal veins under conditions where the cardiac output could be altered by the dog's own physiologic responses. The flows were measured in a graduated cylinder, and this blood was pumped back into the right atrium. (2) Flow measurements were made from the same areas while cardiac output was regulated by a heart–lung machine during total heart–lung bypass. (3) Flow measurements were performed by electromagnetic flow probes around the celiac, renal, and iliac arteries.

Below are described primarily the experiments mentioned in (1), although relevant information from studies (2) and (3) is presented for comparative purposes.

Methods

Seventeen mongrel dogs weighing 18–30 kg were anesthetized with pentobarbital sodium (30 mg/kg body weight). After administration of 0.5 mg atropine and 50 mg suxamethonium, artificial ventilation was started using a semiclosed system with a respiratory rate of 20/min; inspiration phase was 1.3 sec, and expiration phase was 1.7 sec. The chest was opened via a right-sided thoracotomy. The pericardium was opened anterior to the phrenic nerve, and the inferior vena cava was doubly looped extrapericardially.

After administration of heparin (2 mg/kg body weight) a cannula was introduced into the inferior vena cava via the right atrium. The tip of the cannula was at the level of the diaphragm (Figure 1). Another cannula was introduced into the right atrium via the right auricular appendage. Via a paramedian incision in the upper abdomen, the inferior vena cava was doubly looped above and below the renal veins. Via the left femoral vein, a cannula was introduced into the inferior vena cava; the tip with sideholes was placed at the level of the renal veins. Another cannula was introduced via the right femoral vein; the tip was positioned at the level of the bifurcation of the inferior vena cava. A polyethylene cannula was introduced into the abdominal aorta via the right femoral artery. The inferior vena cava was occluded in three places (at the level of the diaphragm and above and below the renal veins) and the left femoral vein was occluded in the groin. By this means, the venous drainage from the liver, the renal and infrarenal areas, and the right hind leg was diverted into separate graduated reservoirs for flow measurement. This blood then emptied into a common reservoir from which it was returned by a De Bakey roller pump to the right atrium (Figure 1). The collecting reservoir was primed with 500 ml of low molecular weight dextran in isotonic saline solution. To this solution was added 20 ml of 1% $CaCl_2$ solution, 17 ml of 5% $NaHCO_3$ solution, and 0.6 ml of KCl solution (1.5 mg/10 ml). Flows from the separate areas were measured every 5 min. The effect of changes in arterial blood oxygen tension on these flows was studied.

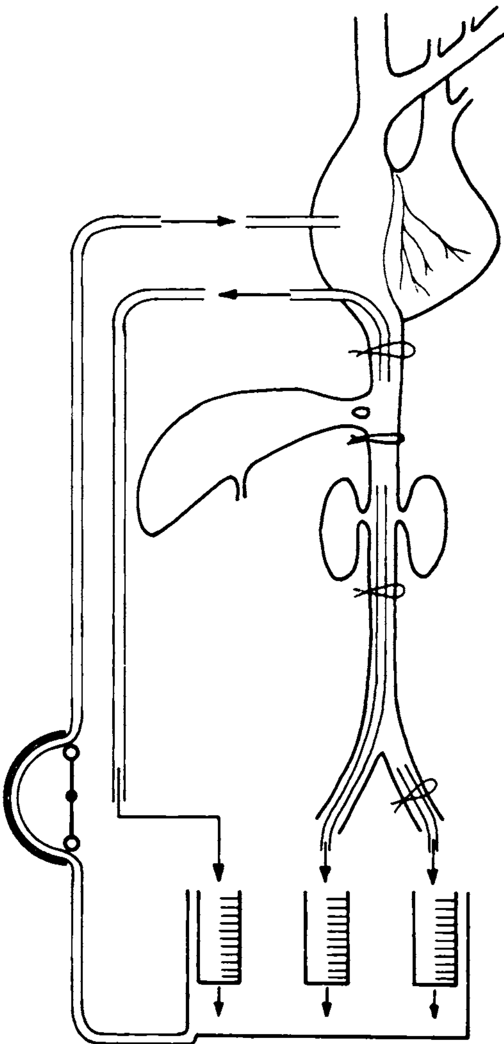

FIGURE 1. Schematic representation of the method for venous drainage measurement from liver, renal, and infrarenal areas and distal vena cava exclusive of the left hind leg.

In the first seven experiments (Group I), partial bypass was started at 1 ata. Ventilation with air was continued for 15 min, and the animals were subsequently ventilated with oxygen for 15 min, after which compression to 3 ata was started. At 3 ata, oxygen and air were administered.

In the last 10 experiments (Group II), partial bypass was started at 3 ata, and changes in oxygen tension were carried out more frequently. Ventilation with a specific gas was always continued for 15 min. In three of these experiments (Table 2), the effect of high concentration of CO_2 was studied, and in three additional animals (Group III), the effect of oxygen tension on the ratio of superior vena cava

flow to inferior vena cava flow was investigated (Table 1, Figure 2).

All measurements were performed at normal body temperature. At the end of each 15-min period, blood samples were drawn from the aorta, hepatic veins, renal veins, and infrarenal area for determination of pH, bicarbonate, pCO_2, pO_2, potassium, lactic acid, and blood glucose values. All determinations were performed on decompressed samples.

Results

Table 1 shows the averages of data obtained at 1 ata from the seven dogs in Group I. (Three measurements, expressed in percentages of vena cava flow, were taken on each dog, then averaged to arrive at a single value for that animal; these single values were then averaged to obtain a value for the total group.) The average values for hepatic, renal, and femoral vein flow measurements during air ventilation were 46.5, 34.3, and 20.0%, respectively. During oxygen ventilation, 46.2, 34.3, and 20.3% were found. The average data on flow measurements in Group II during oxygen and air ventilation at 3 ata also appear in Table 1. During oxygen ventilation, the flow values for hepatic, renal, and femoral veins were 40.4, 35.1, and 24.5%, respectively; during air ventilation, they were 42.2, 36.1, and 21.8%. This indicated a slightly increased femoral flow during oxygen ventilation. When the whole group was divided into a series with inferior vena cava flows of 50 ml/kg or more and a series with less than 50 ml/kg, in both series the values were at essentially the same level during oxygen and air ventilation (Table 1). Figure 3 shows the data obtained in one experiment.

In three dogs from Group II, the effect of O_2 and $O_2 + CO_2$ was studied (Table 2). During CO_2 ventilation, the hepatic flow increased significantly, regardless of increase in total flow.

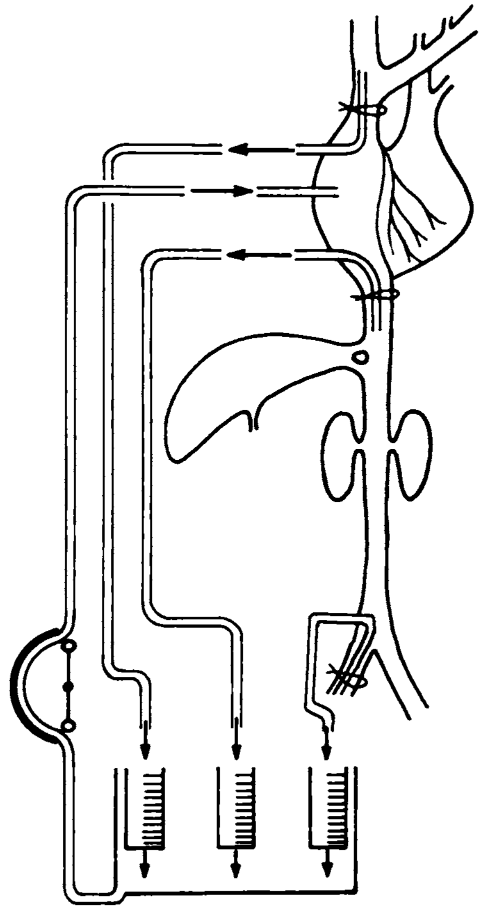

FIGURE 2. Schematic representation of the method for venous flow measurements from superior vena cava and inferior vena cava of an animal from Group III.

In three additional animals constituting Group III, the effect of O_2 on distribution of flow to superior vena cava and inferior vena cava was studied (Table 3). The average superior vena cava flow was 35.7% of the total during oxygen ventilation and 35.6% during air ventilation. Thus, neither oxygen nor air ventilation at 3 ata influenced the superior to inferior vena cava flow distribution. Figures 4 and 5 depict the data for these two cases.

TABLE 1. Average Venous Flow Measurements in Percent of Inferior Vena Cava Flow [a]

	Pure oxygen					Air				
	H.V.	R.V.	F.V.	Flow	B.P.	H.V.	R.V.	F.V.	Flow	B.P.
1 ata Group I (7 expts)	46.2	34.3	20.3	—	131/93	46.5	34.3	20.0	—	122/82
3 ata Group IIA[b] (13 expts)	39.9	36.6	23.5	62	134/97	41.0	36.2	22.8	62	121/88
3 ata Group IIB[c] (17 expts)	40.8	33.9	25.3	39	99/76	43.4	36.0	20.7	43	95/71
3 ata Group II (30 expts total)	40.4	35.1	24.5	49	114/85	42.2	36.1	21.8	52	107/79

H.V., hepatic veins.
R.V., renal veins.
F.V., total flow from inferior vena cava below the infrarenal snare, exclusive of flow from the left leg.
B.P., blood pressure.
[a] Flow measured in ml/kg body weight.
[b] Group IIA dogs (eight animals) had an inferior vena cava flow of greater than 50 ml/kg body weight.
[c] Group IIB dogs (nine animals) had an inferior vena cava flow of less than 50 ml/kg body weight.

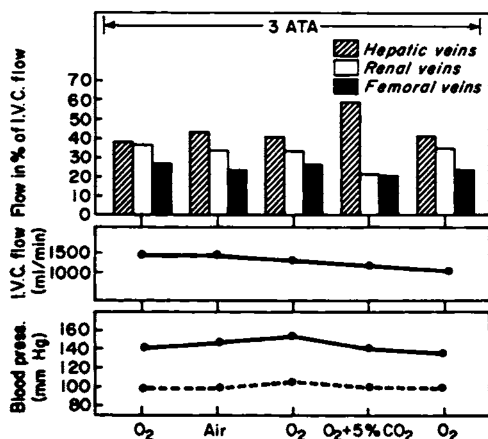

FIGURE 3. Venous flow measurements in one dog (21.5 kg) from Group II during ventilation with O_2, air, and O_2 + 5% CO_2. Flow differences between oxygen and air ventilation were not significant, but ventilation with a mixture of O_2 + 5% CO_2 caused a great increase in hepatic flow at the expense of renal and femoral flow, without change in inferior vena cava flow. Blood pressure remained constant.

TABLE 2. Average Venous Flow Measurements at 3 ata to Determine the Effect of High CO_2 Concentration [a]

Ventilating gas	H.V.	R.V	F.V	Flow	B.P.
O_2	40.2	34.8	25.0	51	117/82
O_2 + 5% CO_2	56.4	25.5	18.1	54	112/75
O_2	43.7	30.4	25.9	44	113/82

H.V., hepatic veins.
R.V., renal veins.
F.V., total flow from inferior vena cava below the infrarenal snare, exclusive of flow from the left leg.
B.P., blood pressure.
[a] These experiments were performed on three animals from Group II, with flow measured in ml/kg body weight and expressed as percent of inferior vena cava flow. Values represent averaged data from the three animals. Dogs were ventilated first with O_2, then with O_2 + 5% CO_2, and, finally, once again with O_2.

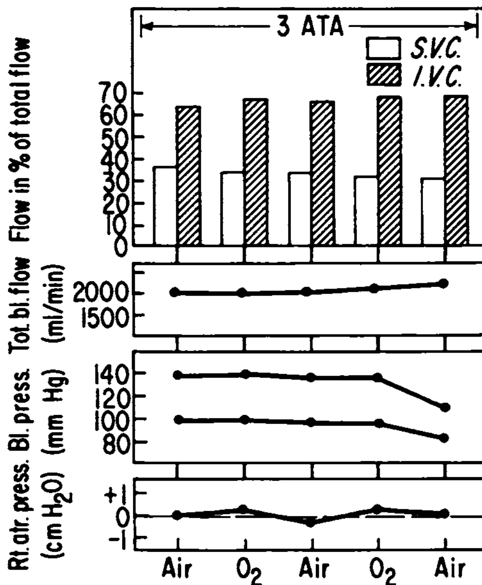

FIGURE 4. Venous flow measurements in one dog (29.5 kg) from Group III during ventilation with oxygen and air. The differences in blood flow from superior vena cava and inferior vena cava were not significant. Total blood flow and right atrial pressure remained at approximately the same level, but blood pressure dropped slightly at the end of the experiment.

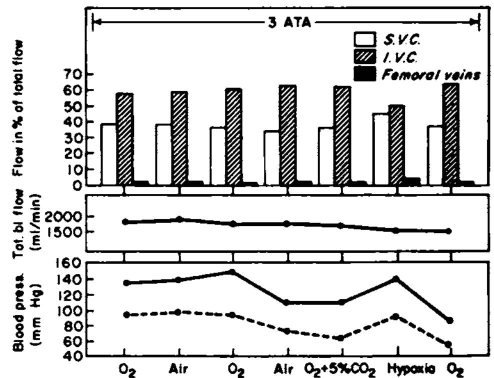

FIGURE 5. Venous flow measurements in one dog (30 kg) from Group III during ventilation with O_2, air, and O_2 + 5% CO_2 and during hypoxia. The differences in blood flow from the superior vena cava and inferior vena cava were not significant. The gradual decrease in superior vena cava flow reversed to a slight increase with O_2 + 5% CO_2 ventilation. Hypoxia caused a decrease in inferior vena cava flow and an increase in superior vena cava flow. Total flow remained constant during the experiment. When the blood from a small femoral vein was collected separately, the flow was lowest during ventilation with O_2 + 5% CO_2 and highest during hypoxia.

Biochemical Values

Oxygen administered at 1 ata as well as at 3 ata resulted in greater oxygen availability (Tables 4–8). The highest venous oxygen tensions were measured in the renal veins. In hepatic and femoral veins, the oxygen tension sometimes rose well over 100 mm Hg, but if the flow was low (Table 5) these values were considerably lower, although still much higher than during high flow with air ventilation. All pCO_2 levels during air ventilation were lower than during oxygen ventilation, especially at 3 ata. The results in Table 5 demonstrate how the highest pCO_2 values were found during low flow and oxygen ventilation at 3 ata. During air ventilation, the pCO_2 values in high and low flow were at the same level. The lactic acid levels were lowest during oxygen ventilation (Tables 6, 7).

TABLE 3. Average Venous Flow Measurements at 3 ata in Percent of Total Flow [a]

Ventilating gas	S.V.C.	I.V.C.	Flow	B.P.
O_2	35.7	64.3	61	120/86
Air	35.6	64.4	65	111/81

S.V.C., superior vena cava.
I.V.C., inferior vena cava.
B.P., blood pressure.
[a] A total of eight experiments were performed on the three dogs in Group III (used for this study), with flow measured in ml/kg body weight. Values represent averaged data from the eight experiments.

DISCUSSION

In most experiments, gradual decrease of inferior vena cava flow occurred without change in flow distribution during the progress of the experiments. In the beginning of the series, where we started taking measurements at 1 ata, the effect of

TABLE 4. Average Levels of pO_2, pCO_2, and Lactic Acid During Oxygen and Air Ventilation at 1 ata [a]

	Ventilating gas	Aorta	H.V.	R.V.	F.V.
pO_2 (mm Hg)	O_2	316	39	101	54
	Air	82	32	48	38
pCO_2 (mm Hg)	O_2	36	43	43	43
	Air	31	38	42	41
Lactic acid (mg%)	O_2	21.4	20.1	21.3	20.5
	Air	22.2	23.1	23.0	23.2

H.V., hepatic veins.
R.V., renal veins.
F.V., total flow from inferior vena cava below the infrarenal snare, exclusive of flow from the left leg.
[a] Group I dogs were used for this experiment, with flow measured in ml/kg body weight.

TABLE 5. Average Levels of pO_2, pCO_2, and Lactic Acid During Oxygen and Air Ventilation at 1 ata [a]

	Ventilating gas	Aorta	H.V.	R.V.	F.V.
pO_2 (mm Hg)	O_2	1525	172	1028	217
	Air	278	37	129	42
pCO_2 (mm Hg)	O_2	37	49	40	54
	Air	34	41	35	49
Lactic acid (mg%)	O_2	25.8	25.0	25.0	27.2
	Air	27.2	27.0	26.6	27.5

H.V., hepatic veins.
R.V., renal veins.
F.V., total flow from inferior vena cava below the infrarenal snare, exclusive of flow from the left leg.
[a] Group II dogs were used for this experiment, with flow measured in ml/kg body weight.

TABLE 6. Levels of pO_2 and pCO_2 During Oxygen and Air Ventilation at 3 ata, in High and Low Flow Groups [a]

	Ventilating gas	Aorta	H.V.	R.V.	F.V.
pO_2 (mm Hg)	O_2—High flow	1765	251	1224	332
	O_2—Low flow	1231	66	788	64
	Air—High flow	321	38	156	44
	Air—Low flow	201	35	87	40
pCO_2 (mm Hg)	O_2—High flow	35	47	38	49
	O_2—Low flow	40	53	42	60
	Air—High flow	34	42	35	48
	Air—Low flow	34	40	36	50

H.V., hepatic veins.
R.V., renal veins.
F.V., total flow from inferior vena cava below the infrarenal snare, exclusive of flow from the left leg.
[a] See also Table 1.

TABLE 7. Effect of High CO_2 Concentration on pO_2, pCO_2, and Lactic Acid Levels During Ventilation at 3 ata [a]

	Ventilating gas	Aorta	H.V.	R.V.	F.V.
pO_2 (mm Hg)	O_2	1640	211	1227	405
	O_2 + 5% CO_2	1510	515	1043	188
	O_2	1603	88	1187	351
pCO_2 (mm Hg)	O_2	36	50	39	53
	O_2 + 5% CO_2	90	110	106	102
	O_2	54	73	56	74
Lactic acid (mg%)	O_2	31.5	31.7	30.8	32.6
	O_2 + 5% CO_2	26.5	26.0	24.9	27.8
	O_2	23.9	24.3	22.9	24.9

H.V., hepatic veins.
R.V., renal veins.
F.V., total flow from inferior vena cava below the infrarenal snare, exclusive of flow from the left leg.
[a] These experiments were performed on three animals from Group II (see also Table 2).

compression could not be excluded as the cause of this decrease. Later, when we started making measurements at 3 ata, the oxygen tension was the only variable factor; then it appeared that the same decrease of flow occurred without pressure changes. The measurements of superior vena cava and inferior vena cava flow were performed in order to rule out that decrease of inferior vena cava flow was accompanied by increase of superior vena cava flow. It appears that no change in the ratio of superior vena cava to inferior vena cava flow occurred under the influence of oxygen tensions.

TABLE 8. Average Levels of pO_2, pCO_2, and Lactic Acid During Oxygen and Air Ventilation at 3 ata [a]

	Ventilating gas	Aorta	S.V.C.	I.V.C.
pO_2 (mm Hg)	O_2	1526	135	240
	Air	186	47	50
pCO_2 (mmHg)	O_2	46	60	58
	Air	42	49	50
Lactic acid (mg%)	O_2	17.9	17.3	17.8
	Air	19.1	17.9	18.7

S.V.C., superior vena cava.
I.V.C., inferior vena cava.
[a] The three dogs from Group III were used for this experiment.

Increase of superior vena cava to inferior vena cava flow ratio was found in total bypasses under the influence of decreased flow and was related to changes in blood pressure. In cases of lowered blood pressure, flow in the superior vena cava increased mainly at the expense of the hepatic flow. What was expected but not found was a decrease of cardiac output by increase of oxygen tension. Frequently, cardiac output dropped under the influence of increased oxygen tension at the start of the study. On the other hand, when the cardiac output was low under certain conditions, increase of oxygen tension produced increase of cardiac output. There appeared to be a range of cardiac output where high oxygen tensions did not alter the cardiac output. This was confirmed during electromagnetic flow measurements in the aortic arch. Under con-

ditions of our experiments, most of the dogs were in these ranges.

The biochemical values clearly indicate the greater oxygen availability at 3 ata. The CO_2 elimination was somewhat more difficult when the oxygen tension was high. The highest CO_2 values were usually found in the hepatic veins. During oxygen ventilation, the lactic acid levels dropped. The differences were rather small because the periods of ventilation with a specific gas mixture lasted for only 15 min.

SUMMARY

1. Except in cases of hypoxia, changes in pO_2 do not change the flow distribution from splanchnic, renal, and infrarenal areas significantly.

2. High CO_2 tensions increase the flow from the splanchnic area.

3. The ratio of superior vena cava to inferior vena cava flow is not changed by changes in pO_2 (except in cases of hypoxia). Under the conditions of these experiments, high oxygen tensions do not produce a change in cardiac output.

4. The high pO_2 values demonstrate an increased oxygen availability; that this oxygen can be used is shown by the lower lactic acid levels during oxygen ventilation compared to the levels during air ventilation.

DISCUSSION

DR. E. LANPHIER, *Session Chairman (Buffalo, N. Y.)*: Would you repeat the type of anesthesia you used?

DR. MEIJNE: It was Nembutal, a barbiturate.

DR. LANPHIER: Have you tried similar determinations using halothane or chloralose?

DR. MEIJNE: No, we used only barbiturates.

DR. LANPHIER: In work done in our laboratory, the anesthesia seemed to make a considerable difference in whether the often-reported flow reductions occurred. They tended not to with barbiturate anesthesia.

Hyperbaric Oxygen Therapy of Acute Cerebral Vascular Insufficiency

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The concentration of substrates essential to neural cells falls during acute cerebral ischemia, resulting in disturbed function and neurologic deficit. Since hypoxia appears to be most important among the many factors influencing survival of ischemic tissues, increased oxygen availability should be of therapeutic value.[1] Unfortunately, inhalation of oxygen at normal environmental pressures does not enhance its transport significantly; respiration of pure oxygen raises content only 1.5 vol % in arterial blood if hemoglobin is already normally saturated.

The quantity of oxygen physically dissolved in blood will increase as a linear function of partial pressure; experimental observations in animals and normal man confirm this anticipated response during hyperbaric oxygenation.[2,3] This demonstration of increased oxygen transport to tissues led to the present study of hyperoxygenation therapy for patients with ischemia of the central nervous system.

Materials and Methods

Twenty-five patients with acute neurologic deficits caused by various forms of cerebral ischemia were treated with hyperbaric oxygenation. In 18, the time interval between onset of acute neurologic symptoms and hyperbaric treatment was less than 7 hours; in the remaining seven patients, the duration of the illness was considerably longer, their symptoms having been present for 7–30 days prior to treatment. The clinical manifestations of cerebral ischemia appeared suddenly and usually included hemiplegia, hemisensory loss, aphasia, and loss of consciousness.

After thorough neurologic evaluation, each patient entered the hyperbaric chamber and breathed oxygen (from a mask or head tent of special design) at normal and increased atmospheric pressures (Figure 1).[4] In most cases, myringotomies were performed before pressurization to reduce the incidence of barotrauma in these ill stuporous patients. With brief exposures to selected compression levels of 2–3.04 ata, high oxygen tensions could be attained without undue risk from toxicity. In the initial studies, the usual treatment regimen required exposure to 3.04 ata of oxygen for less than 1 hour. In later treatments, this schedule was modified so that patients breathed oxygen at pressures below 2.5 ata, thereby permitting pro-

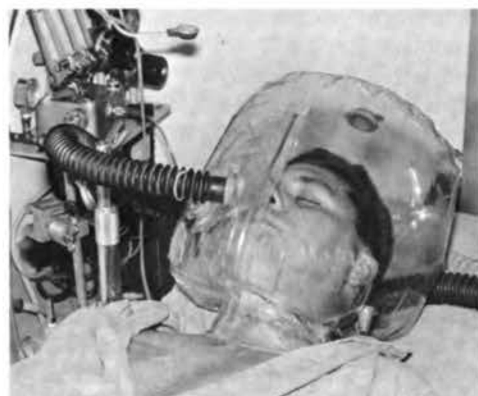

FIGURE 1. The subject breathes pure oxygen spontaneously from a head tent which allows him to communicate easily with attendants. This method of oxygen delivery is tolerated exceptionally well by dyspneic patients.[4]

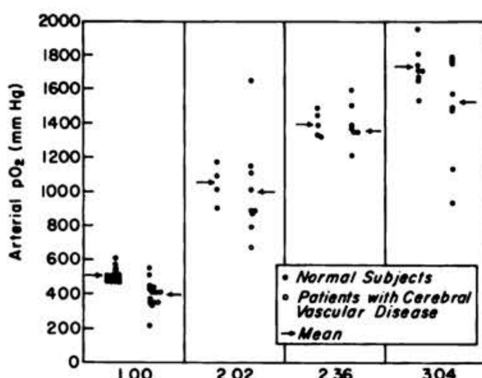

FIGURE 2. Changes in arterial oxygen tension during oxygen inhalation at various atmospheric pressures (indicated on horizontal axis). (Reproduced with permission from A. Heyman: Circulation Supplement. In press.)

longed exposures. In each patient, samples of arterial blood were analyzed periodically for pO_2, pCO_2, and pH by methods described previously.[3] In seven patients, blood was also sampled periodically from the internal jugular vein for similar analyses.

RESULTS

For a given hyperoxic exposure, the oxygen tension of arterial blood averaged less in patients than in normal young men, the mean pO_2 being 991 and 1520 mm Hg at 2.02 and 3.04 ata in patients compared to average values of 1056 and 1726 mm Hg in normal young men (Figure 2). The pCO_2 and pH in arterial blood of patients did not change significantly during hyperbaric oxygenation. In jugular venous blood, the oxygen tension rose from a mean control value of 34 to 105 mm Hg during hyperbaric oxygenation, while the average pCO_2 increased 6 mm Hg above the base-line measurement and the pH fell (Figure 3).

During hyperoxygenation an electroencephalogram was recorded but failed to reveal significant changes in the four patient studies. None of these four patients, however, demonstrated significant improvement during hyperbaric oxygenation.

The patients were closely observed for clinical and neurologic changes throughout the hyperbaric procedure and at frequent intervals thereafter. Of the 18 patients whose symptoms began within the preceding 7 hours, five improved dramatically during OHP with complete or almost

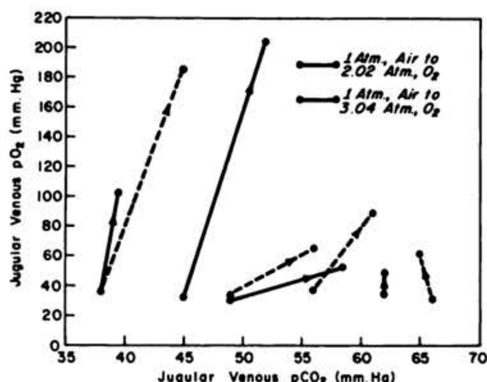

FIGURE 3. Oxygen tension (pO_2) plotted against carbon dioxide tension (pCO_2) in jugular venous blood before and during OHP therapy of patients with acute cerebral vascular insufficiency. The increases in oxygen tension were variable, but values exceeding 150 mm Hg were obtained in two subjects. The pCO_2 rose as well, reflecting in part reduction of cerebral blood flow. (Reproduced with permission from A. Heyman: Circulation Supplement. In press.)

complete restoration of neurologic and mental function. These impressive responses included immediate reversal of paralysis, with return of both strength and the ability to perform fine controlled movements. In addition, mentation improved, with increased awareness and responsiveness. Aphasic patients regained speech and comprehension. Each of these striking responses occurred within 5 to 10 min of beginning hyperoxygenation. Three of the five patients were thought to have had cerebral embolism; two developed hemiplegia after carotid arteriography.

Improvement persisted permanently in only two of these patients, however. One of these (J.K.) was a 61-year-old hypertensive Negro male who, during the preceding 8 months, had manifested atrial fibrillation and intermittent congestive heart failure. He suddenly developed hemiparesis and aphasia which progressed during a 5-hour period to hemiplegia and stupor. Hyperbaric treatment began with an exposure to 2.02 ata of oxygen. Within 5 min after OHP was begun, the patient awoke, moved his extremities, spoke, and responded appropriately to instruction. This dramatic improvement persisted after decompression, and the patient was discharged 10 days later with only a mild residual neurologic deficit. The other patient (C.H.) who maintained improvement was a 58-year-old Negro male who suddenly developed a severe neurologic deficit after carotid arteriography. This patient had been admitted to the hospital in mild confusion, complaining of persistent headache. Focal neurologic deficits were absent at this time, but pulsations could not be obtained over the left common carotid artery; this vessel could not be visualized arteriographically and occlusion was suspected. Arteriography of the carotid system revealed an occlusion of the right middle cerebral artery. Immediately after this procedure, the patient developed a right hemiplegia and became unresponsive to commands and painful stimuli. During the next 2.5 hours, neurologic abnormalities persisted. Ten minutes after OHP was begun, however, the patient responded to simple instructions and answered questions appropriately. He raised his right arm on command, opened and closed his hand, and performed rapid alternating movements with fingers and thumb. Although he could move the right leg, partial paralysis of this limb persisted. Further improvement in muscle strength, speech, and awareness occurred during a 5-hour period of OHP therapy at 2.36 ata. A significant increase in oxygenation of blood was demonstrated as well, with the tension in arterial blood reaching a level of 1364 mm Hg. After decompression the patient maintained the observed neurologic improvement.

Dramatic but temporary improvement occurred in three patients during hyperbaric oxygenation. One of them (D.G.) developed complete aphasia, right hemiplegia, and stupor, presumably due to cerebral embolism during transseptal catheterization of the right heart. The neurologic deficit increased in severity during the 1-hour lapse between the onset of neurologic symptoms and hyperbaric therapy. At this time the patient was exposed to 2.36 ata of oxygen pressure. Within 6 min she became completely normal, without residual paralysis or other neurologic abnormalities. Aphasia and partial paralysis returned, however, 2 hours after she left the hyperbaric chamber. A second course of OHP again produced prompt dramatic recovery, but 2 hours after the second decompression the patient again relapsed. No lasting significant improvement was observed during the third exposure to OHP. Her convalescence was characterized by gradual but incomplete recovery from hemiparesis and language defects. Another patient (B.H.) showed essentially the same improvement, followed by relapse with each of two exposures to OHP. Cerebral embolism was also suspected in this patient because the

attack occurred 6 days after insertion of a Starr aortic prosthesis.

In a fifth patient (O.W.), with a right hemiplegia and aphasia after carotid arteriography, reversal of paralysis in the right leg and improvement of speech occurred within a few minutes after OHP therapy was begun. This improvement was maintained until 6 hours after decompression, when the neurologic deficit recurred. At this time carotid endarterectomy was performed and, except for moderate residual paresis of the right arm, normal neurologic function returned.

Less dramatic but favorable responses to hyperbaric oxygenation were observed in eight other patients, all but one of whom had developed a neurologic deficit during the preceding several hours. The evidence of improvement during oxygenation included cleared mentation and partial restoration of motor activity. In this group definite neurologic dysfunction persisted, however, and in most instances the clinical picture reverted to the pretreatment level immediately after decompression. None of these patients received further hyperbaric therapy, and the subsequent clinical course conformed to the natural history of the illness.

None of the remaining 12 patients improved during OHP therapy. Symptoms developed during the preceding several hours in six of these, in whom neurologic deficit occurred much earlier than in the others. The subsequent clinical course in these patients did not differ from that usually observed for this type of illness, except in one patient (L.M.) who developed hemolysis similar to that observed in tocopherol-deficient mice exposed to toxic levels of oxygen pressure.[5] In this patient, jaundice and anemia occurred shortly after hyperbaric treatment. In addition, blood pressure rose strikingly during the procedure. Ultimately, the patient recovered from the anemia but died 3 months later from complications of the cerebral vascular disease.

Discussion

Physiologic observations in these patients confirmed the predicted relationship between environmental pressure and transport of oxygen by blood, and were similar to observations made previously in normal subjects[3] except that the rise in arterial pCO_2 was less than that observed in normal controls. This can probably be accounted for, however, by differences in age, concomitant pulmonary disease in some patients, and dilution of inspired oxygen due to leakage of environmental air into respiratory assemblies in patients.

Available experimental evidence indicates that the observed enhanced oxygenation of blood provides a more abundant supply of oxygen to neural tissue despite concomitant vasoconstriction. After temporary interruption of retinal blood flow, vision persists in the eye of normal man for approximately 1 min at 4 ata of oxygen pressure, whereas visual blackout occurs within 10 sec at 1 ata of air pressure.[6] Similarly, the normal electroencephalogram persists for a significantly longer interval after total interruption of oxygen transport to the brain in hyperoxygenated animals.[7]

In the total absence of blood flow, however, protection from hypoxia has been brief at all levels of oxygen pressure tested thus far; animals subjected to absolute circulatory arrest for more than 10 min have shown distinct neuropathologic lesions despite OHP.[8] Furthermore, in patients with acute focal retinal arterial occlusions, brief therapeutic trials of hyperbaric oxygenation have not altered the clinical course or visual deficit significantly despite the delivery of hyperoxygenated blood in choroidal capillaries to within 0.15 mm of the ischemic area.[9]

In other studies, however, using less reliable techniques for total interruption of cerebral blood flow, hyperoxygenation seemed to provide protection from neuronal hypoxia for periods as long as 1 hour.[10] In such instances, a small amount

of blood may have reached the central nervous system, suggesting that the hyperoxygenated perfusate in very small volumes can preserve neuronal viability.

In this group of patients, the arterial pCO_2 and pH did not change significantly during OHP and presumably did not contribute in a major way to changes in cerebral perfusion or function. Significant changes of pCO_2 and pH did occur in cerebral venous blood, however, in part reflecting improved oxygenation of hemoglobin in cerebral venous blood with a resultant impairment of isohydric transport of acid. The rise of pCO_2 may also have been due to a decrease in cerebral blood flow during OHP.

In most of these patients, brief exposures to OHP did not restore neural function. The two major factors underlying treatment failures in patients suffering from extensive vascular insufficiency and neurologic damage probably were: (1) irreversible damage to neural tissue from prolonged hypoxia, and (2) severe reduction in perfusion so that viable neural cells remained hypoxic despite augmentation of oxygen transport in the remaining nutrient capillaries. The avascularity associated with extensive cerebral vascular disease may render certain areas of brain inaccessible to any attainable gradient of oxygen across the capillary wall; the maximum distance that oxygen can traverse in the brain after leaving the capillary has been computed to be about 0.1 mm.[11] Furthermore, Brown and colleagues have calculated that even exposure to 3 ata of oxygen pressure provides an increase in the theoretical limiting distance from capillary to neural cells of only 0.073 mm.[12]

Aside from hypoxia, other factors may account for the poor results observed in some patients. These additional limitations to successful therapy include deficiencies of substrates (such as glucose), too brief periods of treatment in some early studies, and possibly the limiting effect of vasoconstriction upon oxygen transport. Whether hyperoxygenation of blood causes vasoconstriction in ischemic tissues has not been determined in a hyperbaric setting.

The clinical improvement has been most impressive in patients with evidence of cerebral ischemia due to embolism or arteriographic complications, although assessment of these results is hampered by the fact that these patients often showed sudden and dramatic improvement even without treatment. In this series, however, the remarkable improvement observed was thought to be caused by OHP, both because of the extremely rapid improvement once OHP had begun and because the extent of recovery was so great within a short period of time. Furthermore, a repeated reversal during hyperbaric oxygenation of major neurologic deficits in the same patient can be explained by factors other than OHP only with great difficulty. We have not observed the natural course of this illness to produce as complete or rapid a clinical recovery with traditional modes of therapy. Other observers have noted a transient restoration of neurologic function during OHP in cerebral ischemia.[13]

These results indicate that prolonged impairment of neurologic function may occur without loss of neuronal viability. Similar observations have been reported in patients with cerebral ischemia due to atherosclerosis and following carotid endarterectomy for arterial occlusive disease.[14] Therefore, a more optimistic attitude seems justified in attempting therapy of acute cerebral ischemia. OHP may have value as supportive therapy for acute cerebral ischemia, although the low incidence of sustained improvement suggests a somewhat limited application. Perhaps additional therapeutic adjuncts, such as vasodilators (*e.g.*, carbon dioxide), hypothermia, and hypertensive agents, which could increase cerebral perfusion, may increase the therapeutic usefulness of OHP. Means for inhibiting oxygen toxicity which would allow more prolonged exposures to hyperoxia would also enhance practical

use. Ultimately, hyperbaric oxygenation may prove most useful in preserving viability and function while the physician attempts to remove the occlusive lesion.

SUMMARY

The usefulness of OHP in increasing oxygen transport and in restoring neural function was evaluated in 25 patients with recent neurologic deficit caused by cerebral ischemia. OHP produced a significant elevation in the content and tension of oxygen in arterial and cerebral venous blood in this group of patients. Furthermore, dramatic improvement in neurologic function occurred in five patients, persisting in two of these. In two other cases, the neurologic deficits recurred a few hours after the patients were removed from the hyperbaric chamber, and repeated exposures to high oxygen pressures restored neural function only temporarily. In the fifth patient who improved dramatically during treatment, the neurologic deficit recurred some hours later. In this instance, carotid endarterectomy was performed with subsequent restoration of neurologic function. In eight other patients, there was some evidence of clinical recovery immediately after the onset of hyperoxygenation, but the neurologic deficit usually returned during decompression. The other 12 patients did not improve during treatment.

These observations indicate that neural tissue remains viable for some hours after loss of function in acute cerebral ischemia. In such instances, an increase in oxygen transport may reverse cerebral hypoxia, restore neural function, and possibly prevent death of cerebral tissue. The ultimate role of OHP in the treatment of acute cerebral ischemia is unclear, but the encouraging results suggest that it may restore neurologic function and maintain neuronal viability in some patients, thereby allowing time for removal of the occlusive lesion by surgery or other means.

ACKNOWLEDGMENTS

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DISCUSSION

DR. J. JACOBSON (*New York, N. Y.*): We all know that the average stroke patient, if given enough time, will have a large degree of recovery. This means that there are many cells which, though they are not functioning at the time, are still alive and will eventually recover. I think the evaluation of this technique, or abandoning its use in a great number of patients, on the basis of immediate improvement is not a fair one. There may be areas of brain which can be kept alive by hyperbaric oxygenation, although return of function is not immediately apparent. Therefore, I would think that when definitive studies are done, it will be necessary to treat the acutely ill patient for sustained periods (probably over 4 or 5 days) which are long enough for collaterals to open, and then to have a long-term follow-up to gauge the ultimate return of function.

DR. SALTZMAN: I must comment further on that point. Brief exposure to high oxygen pressures will only delineate part of the story, but long-term follow-up of complex illnesses is very difficult to evaluate. The problem of oxygen toxicity must be solved, at least in part, before we can indulge in the higher risk of substantially prolonged therapy.

DR. R. PENNEYS (*Philadelphia, Pa.*): Dr. Saltzman, were there any distinctive clinical features in the patients who were helped and those who were not that could help us select the proper ones for hyperbaric oxygen?

DR. SALTZMAN: I think recent onset was the most obvious difference. A second feature was a clinical setting in which the cerebral ischemia was expected to have been caused by cerebral embolism due to a transseptal catheterization or atrial fibrillation. In these instances, we have seen dramatic changes. Dramatic changes have also been observed in cerebral hemorrhage. We have seen one such impressive example, and Drs. Ingvar and Lassen (Nord. Med. 72:1352, 1964) have reported a dramatic response in a similar patient. In both instances, however, the patients expired because of continual intracerebral bleeding.

Effects of Hypobaric and Hyperbaric Oxygen on Experimental Brain Injury

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The therapeutic application of hyperbaric oxygenation in diseases of the central nervous system has received much attention recently. Jacobson *et al.* used hyperbaric oxygenation during carotid endarterectomy and found that, although cerebral oxygenation increased, it was insufficient to obviate the need for intraluminal bypass during the procedure.[1] Saltzman *et al.* reported improvement from OHP therapy in four of 12 cases treated for acute cerebral vascular accident.[2] Beneficial results were obtained recently in four cases of cerebral ischemia treated with oxygen inhalation at 2.0–2.5 ata.[3] In two animal studies, conflicting results were reported for the OHP treatment of occlusion of cerebral vasculature in dogs.[4,5] Illingworth reported prolongation of "safe" time of bilateral occlusion of carotid and vertebral arteries, while Jacobson noted larger infarcts of the cerebrum with occlusion of the middle cerebral artery when hyperbaric oxygen was used. There has been some uniformity of results in these and other reports, however; electroencephalographic changes from cerebral ischemia have been delayed with high pressure oxygen or hypothermia, and they have had a complementary effect when used together.[1,4,6,7]

Applying Rosomoff's modification of Clasen's technique to produce experimental brain injury, we were able to evaluate brain damage incurred from a standardized exposure to liquid nitrogen and to compare the mortality and morbidity in normobaric, hypobaric, and hyperbaric oxygen atmospheres.[8–10] In addition, the effects of barbiturate and halothane anesthetics were evaluated.

Materials and Methods

Healthy adult mongrel dogs weighing 11–22 kg were randomly selected. All animals were fasted 12 hours before surgery and received intramuscularly 0.4 mg atropine sulfate, 100 mg diphenylhydantoin sodium (Dilantin), and 600,000 units benzanthine penicillin G (Bicillin) preoperatively. Anesthesia was induced and maintained with either halothane or thiopental (Pentothal) and pentobarbital combination as indicated below. The animals were intubated with an endotracheal tube with saline-filled cuff, and respiration was assisted manually or with a Bird Mark IV–VII respirator to maintain a positive pressure of 5–15 mm Hg and a negative pressure of 1–5 mm Hg.

The dura mater was exposed through a 1.56-cm burr hole made in the left cranium just posterior to the frontal boss. A brass-tipped cannister (Figure 1), placed in the burr hole to fit snugly against the dura, was then filled with liquid nitrogen (−196° C) and kept full for 3 min (pilot experiments had determined that this duration of exposure produced an LD_{50} lesion averaging 5.5 cm^3 in volume). After the cannister was removed, the defect was filled with bone wax, the area was covered with tantalum wire mesh, and the wound was closed in three layers. All animals were allowed to recover spontaneously from anesthesia, and postoperatively each animal received 100 mg Dilantin twice daily.

On the seventh postoperative day, the animals were sacrificed with an overdose of barbiturate, and the brains were removed and placed in 10% buffered formalin. The brains of those animals that died before the seventh day were treated in the same manner. Seven days after death or sacrifice, the brains were cut coronally into sections 0.5 cm thick. Tracings of the perimeter of the lesions in each section were made and planimetrically measured independently by two observers. From these measurements, the total volume of each lesion was calculated.

The animals were divided into six groups, described in Table 1.

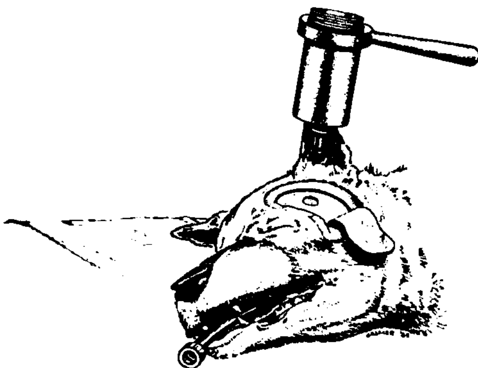

FIGURE 1. Exposure of dura mater in anesthetized dog and application of liquid nitrogen by cannister.

Group A (*Normobaric Normoxic*). Ten normal animals, used as controls, were anesthetized with thiopental and pentobarbital and exposed to liquid nitrogen at 1 atm while inspiring room air. Postoperatively they remained at 1 atm and breathed room air.

Group B (*Hypobaric Normoxic*). Nine animals lived at 27,000 ft simulated altitude (4.99 psia, 0.33 ata) breathing 97% oxygen for 7 days before surgery. They were anesthetized with thiopental and pentobarbital and exposed to liquid nitrogen at ground level while inspiring room air. Immediately after surgery, they were returned to the same simulated altitude and 97% oxygen for 7 days. Their alveolar oxygen tension at this altitude was calculated to be 165 mm Hg; thus, normal arterial oxygen saturation was adequately maintained in this environment.

Group C (*Hypobaric Hypoxic*). Eight animals breathing ambient air lived at 10,000 ft simulated altitude for 6 days and then progressed 2000 ft every 2 days, so that they were at 18,000 ft simulated altitude (7.34 psia, 0.5 ata) for 2 days before surgery. They were anesthetized with thiopental and pentobarbital and exposed to liquid nitrogen at 1 atm while inspiring room air. Immediately after surgery, they were returned to 18,000 ft simulated altitude, breathing room air for 7 days. Their estimated alveolar oxygen tension at 18,000 ft was 40–43 mm Hg, with an arterial oxygen saturation of 73–78%.

Group D (*Hyperbaric Hyperoxic*). Nine animals were anesthetized with thiopental and pentobarbital, breathing room air, and exposed to liquid nitrogen at 1 atm. They were immediately taken to 66 ft simulated seawater depth (44.1 psia, 3 ata), breathing 97% oxygen within 2 min, and they remained at this pressure for 2 hours before they were decompressed to 1 atm pressure at a rate of 60 ft/min.

TABLE 1. Mortality and Brain Lesion Sizes in Control and Experimental Dogs

Group	Pressure (psia)	Inspired gas	Anesthetic	No. dogs	Mortality		Brain lesion size (cm^3)		
					Rate	Probability[a]	Mean	S^2	Probability
A	14.7 (ground level)	Room air	Thiopental-pentobarbital	10	50%	—	5.81	4.7290	—
B	4.99 (27,000-ft alt.)	97% O_2	Thiopental-pentobarbital	9	11%	0.040	6.28	4.8556	NS
C	7.34 (18,000-ft alt.)	Room air	Thiopental-pentobarbital	8	63%	NS	8.90	20.3115	<0.05
D	44.1 (66-ft seawater depth)	97% O_2	Thiopental-pentobarbital	9	0%	0.004	5.33	1.6758	NS
E	14.7 (ground level)	97% O_2	Halothane	12	17%	0.038	5.00	1.4094	NS
F	44.1 (66-ft seawater depth)	97% O_2	Halothane	11	18%	0.066	4.59	3.7792	NS

S^2, variance.
NS, not significant.
[a] Probabilities obtained by comparison of values with those of Group A controls.

Group E (Normobaric Hyperoxic). Twelve animals were anesthetized with halothane, using 97% oxygen, and their brains were exposed to liquid nitrogen at 1 atm. They remained on 97% oxygen during the closure of the wound and until fully recovered from anesthesia at 1 atm, which was usually for a period of 30–45 min after exposure.

Group F (Hyperbaric Hyperoxic). Eleven animals were anesthetized with halothane, using 97% oxygen, and exposed to liquid nitrogen at 1 atm. They were immediately taken to 66 ft simulated seawater depth in 2 min and remained there breathing 97% oxygen for 2 hours, after which they were decompressed at a rate of 2 ft/min.

Eleven animals were anesthetized with thiopental and pentobarbital and exposed to OHP without surgery or liquid-nitrogen exposure. Six of these breathed 97% oxygen at 66 ft simulated seawater depth for 2 hours, and five animals breathed room air at 66 ft for 2 hours. One other dog received the same anesthetic and had a sham operation in which room-temperature water was placed in the cannister instead of liquid nitrogen; this animal was then taken to 66 ft simulated seawater depth breathing 97% oxygen for 2 hours. All of these animals were decompressed at a rate of 60 ft/min and had a normal recovery and postoperative course.

In both the hypobaric and hyperbaric exposures, a continuous ventilating flow of oxygen or room air was maintained throughout the experiment. Oxygen levels were monitored with a Neville oxygen sensor, and the oxygen and carbon dioxide levels were checked by micro-Scholander technique on samples of chamber gas.[11] There was never more than 0.30% carbon dioxide in the inspired air.

RESULTS

Mortality

Table 1 shows the mortality and mean brain lesion sizes for each group. The mortality for all the groups receiving 97% oxygen was less than that of the control animals of Group A. There was, however, a significant difference ($P<0.05$) only for Groups B, D, and E. The mortality in Group F, although not within the probability of statistical significance, was much lower than the expected rate of 50%. There was little difference in death rates between the hyperbaric groups (D and F), which were comparable except for the anesthetic agents used and the rates of decompression.

Tests for statistical significance showed no significant mortality differences between Groups B, D, E, and F, despite the wide range of atmospheric pressures to which they were exposed. Inconsistent with this result was the difference in mortality between Groups A and B, where both groups were relatively normoxic but exposed to different atmospheric pressures (14.7 and 4.99 psia). Group C, comprising hypoxic animals, had more deaths than the control group (A), but the difference was not statistically significant.

There was a total of 15 deaths, 13 of which occurred in the first 3 postoperative days. The days of death in the groups showed no pattern related to group differences.

Morbidity

The volume of the lesions was measured by two observers independently, and the mean of their measurements was used in the final data. The difference between the two observations averaged 0.388 cm^3. Comparison of the mean lesion sizes and analysis of variance revealed that only Group C (hypobaric hypoxic) had significantly larger lesions than the controls in Group A. This fact is in agreement with the increased mortality in this group.

Groups D, E, and F all had smaller lesions and less variances than Group A, but this finding was not statistically significant. There were no differences between these three groups when they were compared to each other, indicating that

there was little benefit from the use of halothane instead of barbiturate anesthesia and that no improvement resulted from the use of hyperbaric oxygen instead of 97% oxygen at 1 atm.

Group B dogs (hypobaric normoxic) had lesions similar in volume to those of the control animals; this similarity in volume was more consistent than the differences in mortality.

When the lesion size was related to the death rate in those groups with two or more deaths (Groups A, C, E, and F), the animals that died were noted to have larger lesions than the survivors except in Group E. In this group, one animal that died had a 5.50-cm^3 lesion and the other had a 4.35-cm^3 lesion.

Other Findings

Nine animals had 21 convulsions in the immediate postoperative course, and all of these were in Group F, which received halothane anesthesia and treatment with OHP. The time of onset of the first convulsion varied between 35 and 50 min after reaching full compression, except in one case where it was 90 min. When an animal convulsed, it was taken off of oxygen, given room air to inspire for 15 min and then placed back on 97% oxygen. The total time of oxygen inhalation at full compression in these animals averaged 1 hour 30 min. One animal convulsed five times while receiving 1 hour 6 min of OHP and died in the second postoperative day with a lesion of 8.57 cm^3 in volume. Another animal had seven convulsions during 1 hour 5 min of OHP. It survived the 7-day postoperative period in apparent good health and had a 5.90-cm^3 lesion.

One animal in Group E convulsed on the fifth postoperative day. It continued to convulse despite all anticonvulsant therapy and died 12 hours after the first seizure with a 5.55-cm^3 lesion.

Most of the animals showed some evidence of neurologic deficit during the early recovery phase, but this was usually transient, exceptions being the animals which died and the survivors with larger lesions.

DISCUSSION

Exposure of the brain to liquid nitrogen, with the dura intact, simulates the type of injury seen with contusion of the brain. Because of the low thermal conductivity of cerebral tissue, the damage remains focal, and the exposure time can be used to control the volume of the lesions and the mortality.[8–10,12] The volume of the lesion is easily measured because of the well-defined borders in both grey and white matter. The progress of events shown to occur in both freezing and acceleration or impact brain injury is: hemorrhage and vasodilation followed by vasospasm, with resultant ischemia, edema of the surrounding tissue, and glial reaction at both the lesion site and surrounding tissue.[8,12,13]

The reaction in the adjoining tissue has been shown to be reversible despite the liquefaction of the immediately damaged tissue.[12] Clasen and his co-workers reported the development of edema in the adjacent areas 4 hours after freezing. This fact correlates with the findings of White *et al.*, who demonstrated onset of swelling in 1 hour, reaching a peak in 4 hours, after trauma inflicted by striking the freely movable head of anesthetized cats with a pendulum.[14] Despite these similarities, differences in metabolic responses cannot be excluded on the basis of information available.

If this pathogenesis of brain injury is accepted, however, hypoxia should inflict larger lesions associated with a higher mortality. Our results confirmed this, although the change in mortality was not significant. In animals and humans, acid–base accommodation to hypoxic altitudes is stabilized by the end of the first week, but erythrocyte changes and complete acclimatization are not achieved until approximately 6 weeks. The animals were still hypoxic, therefore, but not alkalotic. The results in this group demonstrated the

ability of the experiment to reflect the deleterious effects of decreased cerebral oxygenation upon acute brain injury.

Increased atmospheric pressure alone made little difference in the outcome of the exposure to liquid nitrogen—yet reduced pressure with normal arterial oxygen saturation, as in Group B, reduced the mortality and produced lesions comparable to those in the control animals of Group A. Little is known of the effects of reduced atmospheric pressure with normal tissue oxygenation on the pathogenesis of tissue injury. Experiments on incisional wound healing in rats exposed to 27,000 ft simulated altitude, breathing 97% oxygen, have shown that wound healing is impaired.[15] This finding is incompatible with our results and may be due either to unknown hemodynamic changes in the cranium of animals exposed to reduced atmospheric pressure, or to the slightly increased oxygen tensions which this atmosphere provides (arterial pO_2 165 mm Hg). Neither seems very plausible, however, and the results certainly warrant further investigation to determine the mechanism of protection in reduced atmospheric pressure with normal arterial oxygen saturation.

A more substantial increase in inspired oxygen tensions in Groups D, E, and F also reduced mortality and lesion size, although the changes in lesion size were not statistically significant. The interesting aspect in comparison of these groups was our inability to demonstrate a relationship between increasing oxygen tensions and either mortality or morbidity. We originally hypothesized that OHP should exert a beneficial effect by one of two mechanisms. The tissues surrounding the damaged area should be provided with highly oxygenated capillary blood and thereby reduce the neuronal damage in this area due to ischemic changes. It should also reduce diapedesis secondary to vasodilation and lessened extracellular edema formation. Both of these effects may have been provided by simply changing the inspired gas to 97% oxygen at normal atmospheric pressure. Additional benefit from OHP was not manifested in our results, and this may have been due to the cerebral vasoconstrictive effect of OHP, noted by others.[16,17]

We are aware of no other studies in which OHP has been used to treat experimental brain injury. Several studies have been done on experimental vascular occlusions, however, which may be helpful in understanding the application of OHP to brain injury. In two studies, bilateral occlusion of both common carotid and vertebral arteries in dogs was performed,[4,18] although this type of experiment usually provides incomplete cerebral ischemia because of available collateral circulation in most dogs. In both of these studies, the vessels were occluded at 1 atm before and after treatment with 100% oxygen at 2 ata. Obliteration of electroencephalographic waves occurred within 1 min at ambient pressure during air-breathing, before exposure to OHP. During and after OHP occlusion, no (or only transient) electroencephalographic changes occurred in 10–30 min. It was evident that the hyperoxygenation provided additional time for the development of adequate collateral circulation.

Other studies have used techniques of complete occlusion of blood supply to the entire central nervous system or parts thereof.[4,5,19] Illingworth occluded the entire cardiac output and return in hypothermic dogs (27° C) and noted that the time of "safe" occlusion (no electroencephalographic changes) was extended from 20 to 30 min. Jacobson's study, on middle cerebral artery occlusion, did not demonstrate any reduction in cerebral damage when OHP was employed. Fuson *et al.* have recently reported experiments in which the cerebrospinal fluid pressure was elevated above the systemic arterial pressure until cessation of electroencephalographic output. They noted little benefit from OHP unless carbon dioxide content of the inspired gas was increased. Both of these studies and those on partial occlusion imply some benefit from OHP to the

central nervous system when cerebral vascular occlusion is incomplete. During complete ischemia, either generalized or focal, however, OHP is of little use unless used with carbon dioxide. Our experiments confirm these findings; the lesion which had total ischemia secondary to tissue destruction was unaffected by OHP. The reduction in mortality in the groups inspiring high oxygen concentration was probably due to adequate oxygenation of the tissue adjacent to the lesion and the remainder of the brain and subsequent reduction of edema, ischemia, or both. Our results might have been more dramatic if carbon dioxide had been added to the inspired gas mixture, but this mechanism, used to reduce or neutralize the vasoconstrictive effects of high oxygen tensions, is complicated by the added risk of cerebral oxygen toxicity. Again, we should point out that our animals benefited as much from increased oxygen at ambient pressure as under high pressure.

The only manifestation of central nervous system oxygen toxicity was the seizures in the animals in Group F (halothane + OHP). None of these animals had a seizure during anesthesia but rather in the immediate postoperative period, after recovery from halothane anesthesia (which was typically rapid). They were breathing oxygen at high pressure and were fully recovered when the seizures started. Flushing the chamber with air for 15 min stopped the seizures in all cases, but return to oxygen-breathing on some occasions initiated another or repeated seizures. The fact that Groups D and E had no seizures led us to conclude that the combination of brain trauma, OHP, and rapid recovery from halothane anesthesia were the contributing causes. Halothane itself should not be incriminated; rather, the characteristic rapid recovery from this anesthetic agent and the cortical damage from the liquid nitrogen must have been predisposing factors. The barbiturate-anesthetized dogs had no convulsions, but neither did they recover from anesthesia until well after completion of the hyperbaric oxygen therapy.

SUMMARY

To evaluate the usefulness of high pressure oxygen in treatment of central nervous system injury, we performed experiments on anesthetized dogs using Rosomoff's modification of Clasen's method of inducing experimental brain injury. Categories of treatment conditions were:

A. Ambient pressure, room air, barbiturate anesthesia.
B. Reduced atmospheric pressure (equivalent of 27,000-ft altitude), 97% oxygen, barbiturate anesthesia.
C. Reduced atmospheric pressure (equivalent of 18,000-ft altitude), room air, barbiturate anesthesia.
D. Increased atmospheric pressure (3 ata), 97% oxygen, barbiturate anesthesia.
E. Ambient pressure, 97% oxygen, halothane anesthesia.
F. Increased atmospheric pressure (3 ata), 97% oxygen, halothane anesthesia.

Group A animals had a 50% mortality and the lesions averaged 5.81 cm^3 in volume. Group C had a 62.5% mortality, but the lesions averaged 8.90 cm^3. The other groups had mortalities ranging from 11% to 18%, and the mean lesion volumes ranged from 4.59 to 6.26 cm^3. Lesions did not differ significantly in volume except for the increased size in those animals exposed to the hypoxic environment (*i.e.*, Group C). The mortality was significantly less in all of the groups receiving 97% oxygen, but there was no significant difference between the high pressure oxygen group and the other two groups which received 97% oxygen at 1 atm.

Although inhalation of gas with increased oxygen tensions reduced mortality in these experiments, there was no evi-

dence that breathing oxygen under increased pressure was any more beneficial. Inhalation of reduced oxygen tension increased both the mortality and the size of the lesions. Both halothane and pentobarbital–thiopental anesthetics were used, and there was no significant difference attributable to the use of either.

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Four Cases of Postabortal Neurologic Accident Treated with Hyperbaric Oxygenation

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The dramatic neurologic accidents which sometimes occur after illegal procedures to induce abortion can, we believe, be effectively treated in many cases with oxygen at high pressure. There appears to be general concurrence that these accidents are caused by cerebral air embolism,[1] and hence hyperbaric oxygenation should effect reduction in volume of the circulating air or even complete dissolution. In addition, since postmortem findings have indicated that the patients who died very rapidly of postabortal air embolism had cerebral lesions typical of anoxia,[2] hyperbaric oxygenation should also serve to reduce the hypoxia of the cerebral tissue.

Since July 1964, we have used OHP on four patients, each of whom, following intrauterine abortal procedures, manifested an acute neurologic accident.[3] The treatment consisted of pure oxygen inhalation in a small Vickers oxygen chamber. Exposures usually lasted for 1 hour at a constant pressure of 2 ata, plus about 15 min of compression and decompression.

Case Reports

Case 1. A 26-year-old woman, discovered in a state of coma with intermittent convulsive seizures immediately after having introduced an intrauterine catheter in the 10th week of pregnancy, was admitted to the hospital and found to have hyperactive deep tendon reflexes, bilateral Babinski responses, a symmetrical mydriasis, and no signs of neurologic localization. About 36 hours after the onset of symptoms, her condition suddenly worsened as she developed generalized convulsive seizures beginning in the right upper extremity. Because of this change, she was transferred to the Neurorespiratory Revival Center of the Raymond Poincaré Hospital. She was admitted in a state of coma with a right hemiparesis and bilateral Babinski responses. Generalized convulsive seizures, beginning in the right arm and lasting 2–5 min, were observed. Between seizures, spontaneous hypertonic spasms occurred, bringing the trunk into extreme opisthotonos; they were prolonged by a movement of axial rotation towards the left, while the limbs were held in hyperextension. The first session of OHP was performed under continuous electroencephalographic monitoring, about 48 hours after the clinical onset of the seizures and about 12 hours after the patient's condition had deteriorated. At the 40th min and 50th min of compression at 2 ata, two convulsive seizures occurred, tracings of which were recorded (Figure 1). Decompression was decided upon during this second seizure. At the end of this first session, the neurologic examination was unchanged, but during the

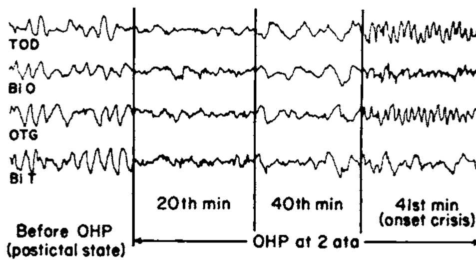

FIGURE 1. Electroencephalographic tracings of 26-year-old woman (Case 1) before and during first session of hyperbaric oxygen therapy. OHP at 2 ata was instituted about 48 hours after clinical onset of seizures and 12 hours after deterioration of patient's condition. Decompression was begun after occurrence of a second seizure at the 50th min.

following 2 hours there were no convulsions nor hypertonic spasms, and the motor activity of the right side of the body improved.

The second treatment with OHP was performed 2 hours later. Treatment had to be stopped within 45 min, however, because of a new seizure. In the following hours (beginning at 54 hours after onset of symptoms) the patient showed signs of real awakening, responding to simple orders. The right-sided hemiparesis had completely disappeared and plantar reflexes were normal.

On the third day after onset of neurologic symptoms, a third and last session of OHP was administered, during which no convulsive phenomenon nor any electroencephalographic disturbance was observed. Following this, consciousness was normal and there was no neurologic deficit. The electroencephalogram improved daily and by the fifth day was practically normal (Figure 2). The patient was discharged on the seventh day without any neurologic, psychic, or electroencephalographic sequelae. Complete recovery was verified 1 month later.

Case 2. A 36-year-old woman, having introduced an intrauterine catheter in the 12th week of pregnancy, immediately lost consciousness with no convulsive phenomena. She was referred to our department from another hospital 30 hours after the onset, and was admitted in a state of coma with conjugate deviation of the eyeballs to the left and right hemiparesis. The eye grounds were normal. Six sessions of OHP

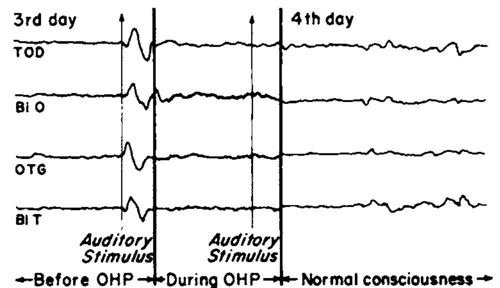

FIGURE 2. Electroencephalographic tracings from Case 1 during third session of OHP at 2 ata, performed on the third day after clinical onset of seizures.

at 2 ata, lasting 1 hour each, were performed over 4 days, the first three sessions occurring within the first 36 hours after admission. At the 45th min after compression during the first session, we noticed clonic movements of cheek and eyelids on the right side diffusing afterwards to the left without any abnormal movements of the rest of the body. These clonic movements disappeared on decompression. During the subsequent sessions, no convulsive manifestations were noted. The sessions of OHP seemed to have no immediate effect on the neurologic picture, but the electroencephalogram did improve with OHP therapy (Figure 3). The state of consciousness improved progressively from the fourth day and the hemiparesis regressed. Recovery continued and 4 months later, when she was discharged, the persisting

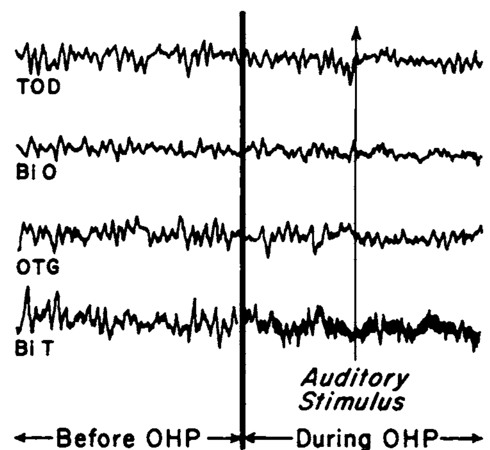

FIGURE 3. Electroencephalographic improvement in Case 2 with OHP therapy at 2 ata.

sequelae were discrete pyramidal signs in the right upper extremity and considerable loss of visual acuity of both eyes with tunnel vision.

Case 3. A 26-year-old woman introduced an intrauterine catheter during the 15th week of pregnancy. This catheter was left in place during the night and was lost when the patient suddenly lost consciousness during an effort of micturition. She was admitted to our department 5 hours later in a state of deep coma, showing no reaction to nociceptive stimuli, absent cutaneous and deep tendon reflexes, fixedly open eyelids, bilateral tight myosis, and abolished corneal reflexes. A few moments after admission, she had a convulsive episode which subsided only after intravenous infusion of Hemineurine. Circulatory collapse occurred without any blood loss and the systolic blood pressure could be maintained above 80 mm Hg only by means of Metaraminol infusion. Spontaneous breathing was weak and a tracheostomy was performed with ventilation accomplished by an Engstrom respirator. The convulsive seizures subsided by about 7 hours after onset, and the patient was then given 1 hour of treatment with OHP at 2 ata. As soon as the pressure was constant, clonic twitching of the lower limbs was noted for a few minutes. When the patient was removed from the chamber, the neurologic picture was not improved, and the tendency to vascular collapse became worse, necessitating an increase in doses of vasopressor drug. Examination of the arterial blood showed a metabolic acidosis (pH 7.33, CO_2 content 11 mEq, arterial pCO_2 20 mm Hg, and O_2 saturation 94%). One hour later, a second session of OHP was performed at 2.5 ata. The patient's condition continued to worsen, and we noted suppression of thermic, respiratory, and circulatory regulation, and disappearance of the cerebral electrical activity. The patient expired, and autopsy permission was not granted.

Case 4. A 26-year-old patient had introduced an intrauterine catheter during the 10th week of pregnancy, with immediate onset of neurologic disturbances. She was transferred to our department from another hospital 6 days after the onset of these disturbances. On admission, she was in coma with conjugate deviation of the head and eyeballs toward the left, left central facial paralysis, bilateral Babinski responses, and absence of spontaneous movement of all the extremities. Six sessions of OHP at 2 ata were performed from the sixth to the 10th days, during which time no complications were noted. There was no immediate change in the clinical state of the patient, but interesting electroencephalographic activity was noted (Figure 4). Recordings after the first OHP session showed response to pinching. On the next day, an electroencephalographic response to noise while under OHP was observed, and during the sixth session an acceleration of the basic rhythm and a normal voltage were apparent.

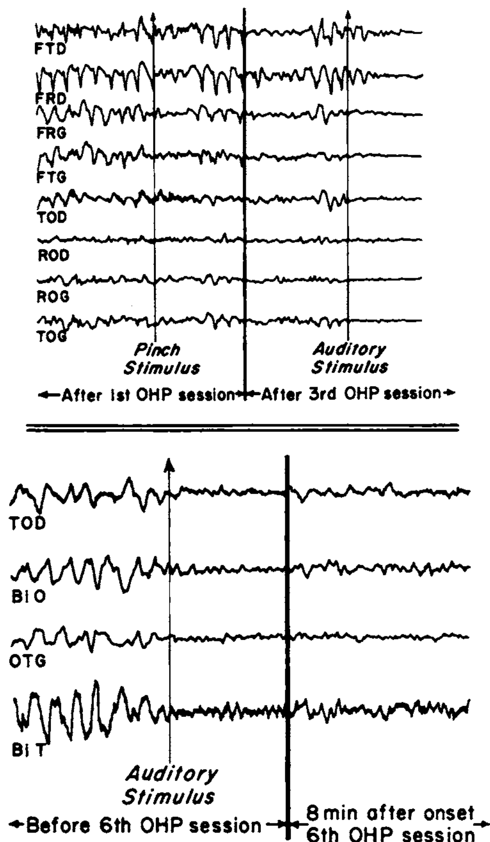

FIGURE 4. Electroencephalographic tracings made in Case 4. Top tracing shows response to pinching after first OHP session and response to being called by name after third session. Bottom tracing shows response to call before sixth session and the acceleration in basic rhythm, along with normal voltage, after compression to 2 ata.

DISCUSSION

The likelihood that multiple air emboli are responsible for the neurologic accidents which sometimes occur immediately after illegal abortion procedures is strong indication for the use of OHP to achieve rapid dissolution of the gaseous emboli. The diffusion of nitrogen from an air bubble in the blood is directly proportional to the difference between the partial pressures of nitrogen in the bubble and in the blood. Prolonged inhalation of pure oxygen at 1 ata causes a progressive denitrogenation of the body which, after a few hours, may be almost complete, so that the gradient of partial pressures of nitrogen in the bubble and in the blood may then be very nearly 1 atm, which is an eminently favorable condition for the dissolution of the bubble. Clearly, under these conditions the same result can be obtained much more quickly by OHP. There is the additional mechanical action of OHP, wherein every increase of 1 atm occurring in the environmental pressure reduces the volume of the bubble by half.[4]

Whatever the circumstances of the occurrence of cerebral air embolism, OHP is effective. This was confirmed in a case where penetration of air into the venous system occurred due to a technical error during a phlebotomy performed with a blood transfusion apparatus. The patient was a 59-year-old male with chronic bronchopneumonia and emphysema being treated during an episode of acute respiratory insufficiency with severe polycythemia. He lost consciousness rapidly after the accident and was transferred to our department 4 hours after the first neurologic symptom. On admission, he was comatose, had bilateral Babinski responses, and showed no localizing neurologic signs. About 10 min after start of the OHP session at 2 ata, the state of consciousness improved suddenly, and the patient asked questions about his condition. During his stay in the chamber, he showed no sign of confusion and no disorientation. The neurologic recovery persisted after the return to normal atmospheric pressure. However, 35 min of OHP had induced a respiratory acidosis, and he had to be tracheotomized for artificial ventilation. Ultimate recovery was complete.

The use of increased pressure in cerebral air embolism of uterine origin was previously reported by Vigouroux and Nivelleau.[5] Their case involved a woman who had been injected with 200 ml of ozone in the uterus for a chronic metritis associated with an unrecognized pregnancy. Immediate coma ensued, with generalized hypertonia alternating with muscular paroxysms. An hour and a half later she was moved into a frogman's recompression chamber where she was treated for 90 min at 6 ata, using environmental air. Decompression time was about 30 hours. Consciousness was regained about 36 hours after treatment was instituted. A left upper extremity paresis was noted at that time which subsided slowly during the following months along with the psychic and memory disturbances.

Since the treatment of air embolism with environmental gas at high pressure depends primarily upon elimination of nitrogen, it is preferable to use pure oxygen as the ventilating gas. Even at lower pressures, the dissolution of the bubbles can be achieved in less time because of the rapid denitrogenation when pure oxygen is used. This was demonstrated in the case reports of two caisson workers who, following rapid decompression, developed sudden acute bone pain. In one case, the upper end of the humerus was involved and, in the other, the leg; these pains were probably due to air embolism in the bone marrow. After recompression with environmental air, the pain disappeared, but it recurred in both cases as severely as before when decompression was achieved (decompression was performed in strict observation of the usual stages). The patients were then referred to us. The painful symptoms cleared up after one ses-

sion of OHP at 2 ata. These observations warrant the attitude adopted by Anderson *et al.*,[6] who systematically give 100% oxygen to subjects working in the hyperbaric chamber as soon as decompression reaches a level of 26.8 psig.

It is important to take into consideration the role of OHP in the sequence of convulsive phenomena. Two of our patients (Cases 1 and 3) were in a neurologic state which necessitated the administration of heavy doses of sedatives before we could move them into the chamber. In these two cases, the seizures recurred in the chamber. In Case 1, the beginning of the electroencephalographic recording corresponded to a postictal tracing. Under OHP we observed a progressive slow wave activity in the tracing, then the onset of a spiking activity, and, at the 40th min and 50th min, two electroclinical seizures which interrupted the session (Figure 1). The same sequence of electroencephalographic abnormalities was observed during the second OHP session given 2 hours after the first session. This also had to be interrupted at the 45th min because of an electroclinical seizure. One should take into consideration, however, that in this patient the convulsive phenomena did not prevent a rapid improvement in the neurologic state. These convulsive phenomena did not recur during a third session of OHP performed 24 hours later. In Case 3, continuous generalized clonic phenomena were observed during the second session where pressure was given at 2.5 ata. In Case 2, where there were no spontaneous epileptic phenomena, the first OHP session caused a small amount of right facial twitching in the paralyzed area. Therefore, OHP seems likely to increase the possibility of epileptic seizures at the initial stage of postabortal neurologic accident, as hyperoxia lowers the epileptogenic threshold of the brain. This is why the concomitant use of anticonvulsive drugs is necessary. It is certainly preferable not to exceed a pressure of 2 ata of pure oxygen and to use a short session of a maximum of 1 hour, to be repeated if necessary. Continuous electroencephalographic monitoring is indispensable in order to detect changes which indicate time to begin decompression.

The effectiveness of OHP in this type of neurologic accident depends on the seriousness of the neurologic lesion and on how soon the treatment is begun. Cases 2 and 4 were referred to us 32 hours and 6 days, respectively, after the onset of symptoms. The clinical picture progressed toward recovery, although it was slow in one case and very slight in the other, as usually observed in this type of accident. When applied very late, OHP seems to have no appreciable influence on the progress of the illness. Immediately after admission, Case 3 showed respiratory and circulatory disturbances, deep coma, and a convulsive state which was extremely difficult to control, all signs indicating a diffuse encephalic anoxia with serious damage to the brain stem. Although OHP was introduced early, it did not prevent the dramatic outcome and death occurred in a few hours.

Case 1, on the other hand, is an example of surprisingly favorable progress. It is certainly impossible to predict the clinical course of the patient had she not been treated with OHP; and, since deep coma and vegetative disturbance were absent, we can presume that she probably would not have died. However, the convulsive state, the hypertonic seizure, the right hemiparesis, and the degree of electroencephalographic disturbances seen at the onset were serious enough to make very slow regression and persistent sequelae likely in the absence of OHP. In this patient, the unusual rapidity of improvement and the complete lack of any sequelae lead us to believe that OHP was a predominant feature in the favorable outcome. The treatment was given 48 hours after the onset of the accident, but the secondary deterioration during which she relapsed into convulsive seizures occurred

only about 10 hours before treatment was begun. Furthermore, treatment consisted only of sedatives and intravenous infusion of low molecular weight dextran (Rheomacrodex), theoretically given to improve the cerebral circulation and to counteract the vasoconstrictive effect of hyperoxia. It seems to us that the amazingly favorable course, free from any sequelae, can be attributed to OHP.

We believe, therefore, that in the absence of any other effective therapeutic method OHP warrants use as soon as possible in any case of air embolism, whether it be from postabortal accident or from any other cause.

ACKNOWLEDGMENTS

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DISCUSSION

Discussion of papers by Dunn and Connolly (pp. 447–454) and Rapin et al. *(pp. 455–460).*

DR. H. A. SALTZMAN (*Durham, N. C.*): We did not observe significant EEG changes in six monitored patients with acute cerebral ischemia.

DR. E. LANPHIER, *Session Chairman* (*Buffalo, N. Y.*): I think some of the cases that Dr. Rapin mentioned were just too far advanced to be salvaged. If I got hold of an early air embolism, I would want to compress the bubble and get prompt relief of the symptoms. And if it's *my* brain, and somebody puts *me* on oxygen at 2 atmospheres and then sits down and watches *my* EEG waiting for something to happen, if I pull through, *he* won't! The basis, in part, for this point of view is that if there is a massive circulatory obstruction due to a bubble, and the blood is not moving in the affected area, it matters little how much oxygen we supply to the lungs. I do not deny that oxygen is valuable in resolving bubbles, and Dr. Rapin brought that point out very nicely. For example, if you simply compress a person to the point of relief, the symptoms may indeed return if you decompress immediately thereafter. A prolonged period of oxygen-breathing may be very desirable to ensure not only the compression, but the total disappearance of the bubble.

DR. C. LUNDGREN (*Lund, Sweden*): Such cases, I believe, are rather uncommon. It would probably interest you to hear a case with a somewhat different etiology, but which was treated with high pressure according to your prescription and which made us wonder what the trouble really was. A woman who had open-heart surgery performed developed bubbles in the circulation. The surgeons recalled that this had happened when she did not wake up properly from the anesthesia. Twenty-four hours after the operation she was still comatose and had a spontaneous Babinski in the left foot. Lacking facilities in our laboratory to treat her with more than 2 atmospheres of oxygen (although we tried this for a brief period with no positive results), we moved her to a Navy chamber in another town. There we exposed her to 6 atmospheres absolute with no beneficial effect. In fact, she developed convulsions during treatment. Consequently, after an hour or so at that pressure, we decided to return to normal pressure. During that time the spontaneous Babinski had disappeared, but it returned when we lowered the pressure to the equivalent of 6 or 9 feet of water. By slightly raising the pressure again, we could dispose of the plantar response entirely, and with still more pressure elicit a normal plantar response. When we returned to slightly lower pressure we could see her go backwards through the different types of reactions again. So, having seen a definite sign that the bubble was growing and decreasing under pressure, we maintained enough pressure to make the symptoms disappear. Then she was brought to normal pressure, still unconscious, but with less frequent seizures. She recovered completely. We also had a similar experience with a case of altitude sickness with coma. The point is that nothing was done after this compression (which lasted only a couple of hours), and still the patient recovered completely during the following 48 hours. I wonder whether someone could enlighten me on the point that this is a question of brain edema when you have disposed of the bubbles.

DR. P. G. LINAWEAVER (*Groton, Conn.*): In reference to Dr. Lanphier's comment concerning treatment at 2 atmospheres, we have seen a number of cases of acute air embolism in our submarine escape training and have found the need for deep pressure (4 to 6 ata). Therefore, I am concerned that hyperbaric facilities are being established around the country with only 2 or 3 atmospheres maximum working pressure. Mark my words, some day these facilities will wish for another 5 atmospheres of capability. Concerning the question Dr. Lundgren posed, I have treated several cases

of air embolism where cerebral edema was a problem and, using the neurosurgical technique of massive doses of Decadron, have been able to influence the outcome favorably, without altering the pressure.

DR. LANPHIER: I think the danger is that we sometimes assume that the problem is edema, rather than a bubble, when this is not the case, and we can rarely tell the difference at the time.

DR. SALTZMAN: Having been through one experience of treating a very ill patient at 74 psig, and not being anxious to repeat that experience or recommend it to my friends, I would strongly urge that one try the 30-minute stop at 60 feet with oxygen before electing the option of going to deeper pressure.

DR. LANPHIER: All right, if it's your brain we'll wait 30 minutes; if it's mine, we'll go right to 165 feet!

UNIDENTIFIED SPEAKER: We have created some artificial air embolisms in dogs and have taken them very quickly to a depth of 7 atmospheres with complete resolution of the bubbles in a few seconds. At this pressure, the bubble size reaches such a small diameter that the surface tension will completely resolve. I cannot emphasize enough that prompt deep pressurization is perhaps the most important factor in the treatment of air embolism.

DR. LANPHIER: Well, now that I have heard exactly what I wanted to hear we'll terminate the discussion.

Hyperbaric Oxygenation as an Aid to the Surgery of Abdominal Aortic Aneurysm

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Resection of abdominal aortic aneurysm still presents a formidable challenge to the surgeon. While the procedure can be done with an acceptable risk, associated cardiovascular disease generally places the patient in a group particularly sensitive to the consequences of hypoxia and hypotension. Hyperbaric oxygenation seems to offer a means for providing added safety during surgical operations by keeping the body better oxygenated during aortic cross-clamping and possibly by compensating for blood loss.

Following is a report of our small series of abdominal aortic aneurysm resections performed in patients in a hyperbaric chamber under OHP. As we gained clinical impressions, their validity was tested in animal experimentation, which will also be reviewed herein.

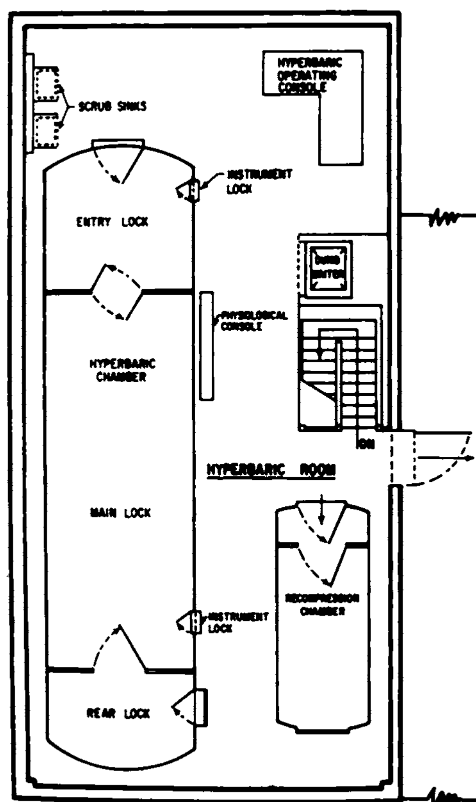

FIGURE 1. General floor plan of Mount Sinai Hospital hyperbaric facility.

HYPERBARIC FACILITY

The hyperbaric chamber used for major surgery in our hospital (Figure 1) contains an operating-room lock (Figure 2) 12 ft in diameter and 21 ft long. The actual floor width in the chamber is 10.5

FIGURE 2. Operating-room lock of Mount Sinai Hospital hyperbaric facility.

ft. The front lock is left in continuity with the operating-room lock during surgery, providing a place for additional storage of supplies and for blood gas determinations, while the rear lock remains free for exit and entry. While the unit has functioned effectively, space is quite limited, particularly on either side of the operating table. To conserve space, a special Simmons Contourflex operating table is used which has a contoured top and a small base. Only essential supplies are kept in the chamber during pressurization, ancillary supplies being passed in through equipment locks as needed.

Clothing, drapes, and linens are pretreated to render them nonflammable (presenting a minor problem of reduced absorbency due to the flame-proofing process). Aqueous disinfectants are used for skin preparation to prevent explosive vapors (*e.g.*, alcohol) from entering the chamber atmosphere. Thiopental (Sodium Pentothal) and halothane (Fluothane), the only two anesthetic agents used in the chamber, have had effects on patients no different than at normal atmospheric pressure. Endotracheal balloon cuffs are inflated with saline rather than air, and intravenous fluids are administered from plastic bags to avoid problems of entrapped-air volume changes.

SURGICAL PROCEDURE UNDER OHP

Nine patients, ages 53–77, underwent aortic aneurysm resections and prosthetic graft replacements. Anesthesia was induced at normal atmospheric pressure and bilateral myringotomies were performed. Surgical operation was begun at normal atmospheric pressure. Approximately 15 min before aortic cross-clamping, the chamber was pressurized at 2–3 psig/min until 30 psig was reached. The aneurysm was then resected and replaced with a prosthetic graft in conventional fashion. After removal of the vascular clamps, decompression was begun according to U. S. Navy standard decompression schedules.[1] Personnel breathed 100% oxygen during the last 10 min of decompression. The times required for wound closure and decompression were usually about equal, so that time spent by personnel in the operating room was not prolonged.

Blood samples were drawn from the aorta and iliac vein before aortic cross-clamping. Just before release of the clamps, blood samples were again taken from the abdominal aorta central to the proximal occluding clamp and from the iliac or femoral arteries peripheral to the distal occluding clamps. Iliac vein samples were again taken.

Blood pressure was monitored with a Statham P-23 Db transducer connected to an EFM recorder. Blood pO_2, pCO_2, and pH were measured with the Astrup apparatus and a Clark oxygen electrode.

CLINICAL RESULTS

Performance of surgery under OHP did not impose any unusual strain on personnel, although the staff required to carry out the operative procedure included three additional members not required during surgery in a conventional operating room; these were the two chamber operators and a circulating nurse outside the chamber.

TABLE 1. Changes in Systolic Blood Pressure (mm Hg) During Surgery for Abdominal Aortic Aneurysm

Patient	Control value	Aortic occlusion: Immediate	Aortic occlusion: Before release	After clamp release: 5 min	10 min	15 min	20 min
DG	100	100	100	80	110	110	110
WC	120	120	120	80	120	130	130
AK	130	130	130	100	80	100	120
MG	130	130	120	60	70	110	130
RS	140	140	140	100	80	90	100
AM	130	130	110	80	80	90	100
JR	120	160	140	120	130	130	140
SG	150	180	140	120	120	130	160
RR	120	140	150	110	120	120	120

The most significant finding in this series of cases was absence or reduction of hypotension following clamp removal (Table 1). At normal atmospheric pressures, one must release occluding clamps gradually over 5–10 min in order to minimize hypotension. Under hyperbaric oxygenation, clamps could be removed at once.

Blood gas and pH values appear in Tables 2 and 3. It is noteworthy that postocclusion (33–108 min) venous oxygen tensions during hyperbaric oxygenation were higher than preocclusion tensions under normobaric conditions. The possible significance of lower oxygen tensions in arterial blood samples peripheral to occluding clamps will be commented on in the discussion section.

Eight of the patients operated upon in the chamber had an uncomplicated recovery, and one patient died of a myocardial infarction on the second postoperative day. This series is too small, however, to permit inferences regarding the effect of OHP on postoperative mortality and morbidity.

EXPERIMENTAL EVALUATION OF CLINICAL FINDINGS

Since no control patients were operated upon by the same team, we felt it desirable

TABLE 2. Oxygen Tension (mm Hg) During Surgery for Abdominal Aortic Aneurysm: Patient Breathing 98.5% Oxygen + 1.5% Halothane

Patient	1 ata: Arterial pO_2 Aorta	1 ata: Venous pO_2 IVC	3 ata: Arterial pO_2 Preocclusion Aorta	3 ata: Arterial pO_2 Postocclusion Proximal aorta	3 ata: Arterial pO_2 Postocclusion Iliac artery	3 ata: Venous pO_2 Preocclusion IVC	3 ata: Venous pO_2 Postocclusion IVC
DG	320	35	1220	1580	1020	60	40
WC	510	53	2000	—	1780	100	60
AK	330	47	1400	1580	1340	100	280
MG	500	52	1840	1800	1580	80	60
RS	350	60	1740	2100	1640	160	54
AM	460	50	1940	1260	1000	460	24
JR	380	51	1800	2200	2100	750	45
SG	370	36	—	—	—	—	790
RR	—	—	1350	1250	1250	510	36

IVC, inferior vena cava.

TABLE 3. pH Values During Surgery for Abdominal Aortic Aneurysm: Patient Breathing 98.5% Oxygen + 1.5% Halothane

	1 ata		3 ata				
			Arterial pH			Venous pH	
				Postocclusion			
Patient	Arterial pH Aorta	Venous pH IVC	Pre-occlusion Aorta	Proximal aorta	Iliac artery	Pre-occlusion IVC	Post-occlusion IVC
DG	7.59	7.50	7.58	7.53	7.55	7.51	7.44
WC	7.48	7.42	7.50	—	7.49	7.46	7.43
AK	7.52	7.49	7.49	7.58	7.58	7.44	7.54
MG	7.48	7.43	7.50	7.48	7.51	7.41	7.31
RS	7.46	7.45	7.52	7.55	7.57	7.46	7.47
AM	7.39	7.33	7.43	7.43	7.38	7.36	7.31
JR	7.56	7.48	7.55	7.61	7.61	7.50	7.48
SG	7.51	7.46	—	—	—	—	7.50
RR	—	—	7.49	7.48	7.48	7.44	7.40

IVC, inferior vena cava.

to evaluate our clinical findings with experimental observations. This work was reported in detail elsewhere,[3] but a summary appears below.

In brief, dogs underwent occlusion of the infrarenal abdominal aorta for 1 hour. Animals were anesthetized with intravenous pentobarbital (Nembutal) and ventilated with 100% oxygen, using a Takaoka respirator. The effect of aortic occlusion on blood gases and on excess lactate was determined under both normobaric and hyperbaric (3 ata) conditions. Figure 3 shows comparative blood gas values, and Table 4 shows the relative

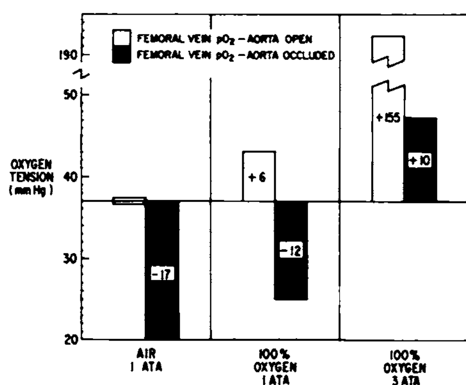

FIGURE 3. Femoral venous pO_2 before and after aortic occlusion. Bars represent changes from control (air at 1 ata before occlusion).

difference in excess lactate values between the two groups. Protection against hypotension following clamp release is illustrated in Figure 4 and the blood oxygen gradient proximal to the distal artery is shown in Figure 5.

DISCUSSION

We have found hyperbaric oxygenation to be a useful adjunct during resection of abdominal aortic aneurysms. Our initial goal was to protect patients against blood loss and hypotension, the rationale being based upon observations that OHP reduces mortality from hemorrhagic shock and decreases hypoxia in tissues temporarily subjected to diminished arterial inflow. The essential elimination of hypotension after clamp release was unexpected.

Controversy exists in the surgical literature whether hypotension after clamp release is due to sudden opening of a previously occluded large vascular bed and/or whether it is caused by the release into the circulation of accumulated metabolites from hypoxic limbs. Our findings support the latter hypothesis. The fact that the venous oxygen tension remains elevated after prolonged arterial

TABLE 4. Excess Lactate After 1 Hour of Infrarenal Aortic Occlusion in Dogs

Air at 1 ata		100% O_2 at 3 ata	
Dog no.	Excess lactate (mg%)	Dog no.	Excess lactate (mg%)
1	1.49	7	0.0
2	4.53	8	0.0
3	14.0	9	0.92
4	3.24	10	1.76
5	5.27	11	0.72
6	3.51	12	1.37
Mean	5.34	Mean	0.63

occlusion suggests that oxygen requirements have been met. The absence of excess lactate production indicates aerobic metabolism, although normal lactate values may be misleading under OHP because certain enzyme alterations may prevent a rise in excess lactate. In any case, absence of hypotension under hyperbaric oxygenation in a group of patients notoriously prone to complications from this condition is a clinically worthwhile adjunct.

Our impression that oxygenation was improved in the distal ischemic bed lacks proof, of course, because of the absence of blood flow and regional oxygen consumption determinations. These studies were not undertaken initially because of the problems associated with electromagnetic flowmeters in a hyperbaric chamber. We hope that these problems will be resolved with the newer ultrasonic flowmeters currently being developed. Nevertheless, the decrease in hypotension after clamp release and the decrease in excess lactate production support our interpretation that higher partial pressures of oxygen in the venous blood indicate less hypoxia at the tissue level.

One might think that since resection of aortic aneurysm is ordinarily associated with a low morbidity and mortality, the inconvenience of operating in a hyperbaric chamber is unjustified. The frequent discussions concerning use of vasopressors, mannitol, and compression devices for the legs to prevent or treat the consequences of hypotension after clamp release attest the seriousness of the problem, however. Breslau and Schwartz [2] have demonstrated protection against spinal cord injury after prolonged thoracic

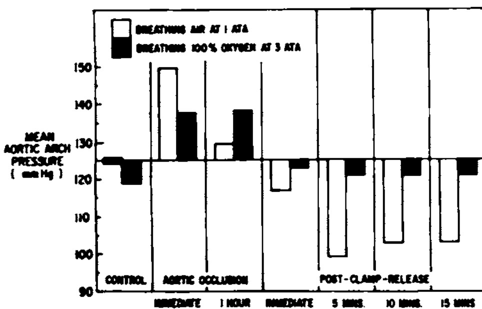

FIGURE 4. Infrarenal aortic occlusion and mean aortic arch blood pressure. Bars represent changes from control (air at 1 ata before occlusion).

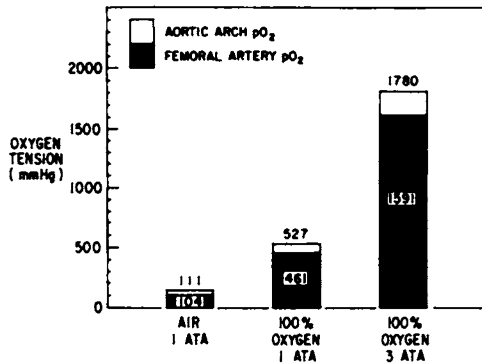

FIGURE 5. Arterial pO_2 proximal and distal to aortic occlusion.

aortic cross-clamping in the dog at 4 ata, with some protection evident at 3 ata. We selected 3 ata rather than 4 ata in our clinical study to avoid excessively long decompression times and exposure of the patient to the hazards of oxygen toxicity.

Our clinical and experimental observations that the partial pressure of oxygen is greater proximal to than distal to an arterial block are interesting. Clinically, we have seen this in other patients operated upon in the hyperbaric chamber where a block was present in the abdominal aorta or superficial femoral artery. Further experimental work is needed to evaluate the mechanism. It is possible that thin-walled collaterals called into service during arterial obstruction can act like capillaries, *i.e.*, oxygen can pass directly through the vessel walls into the surrounding tissues. An alternative explanation is that the blood flow is so sluggish that oxygen is metabolized by the blood cells and vascular endothelium. This seems less likely, however, in view of the rapid transit of blood through collateral vessels as evidenced by arteriographic observation and the maintenance of relatively high distal blood pressures.

Summary

A group of patients with abdominal aortic aneurysm had resection of the aneurysm carried out under hyperbaric oxygenation. The oxygen tension in venous blood from the legs during aortic cross-clamping was higher under OHP than at normal atmospheric pressure before cross-clamping of the aorta. Hypotension after clamp removal was essentially eliminated.

Experimental studies supported the clinical impression. Excess lactate production in ischemic limbs was eliminated, suggesting that anaerobic metabolism was prevented.

The interesting finding of a drop in arterial oxygen tension distal to the site of an arterial obstruction is described, with the possible interpretation that collateral vessels can take on the role of capillaries in releasing oxygen during main arterial occlusion under hyperbaric oxygenation.

ACKNOWLEDGMENTS

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DISCUSSION

DR. C. HITCHCOCK (*Minneapolis, Minn.*): Where did you take your venous blood samples? Was it in the inferior vena cava at the bifurcation, or in the common iliac? Or did you get them from the femoral vein, or possibly even as low as the popliteal vein? The reason that I am asking is that the superior mesenteric artery in the type of

surgery which Dr. Jacobson has discussed (*i.e.*, lower abdominal aortic aneurysm) is well above the area where we cross-clamp. In fact, these were obviously infrarenal, were they not?

DR. JACOBSON: They were infrarenal and at the bifurcation of the aorta, in our experimental work.

DR. HITCHCOCK: The point is that the superior mesenteric, supplying blood through the midcolic artery, can vascularize the pelvis nicely through the so-called arcade of Drummond, which is the peripheral arcade in the colon, and which then carries blood down into the pelvis. Through the rich plexus in and around the rectum, one gets a plentiful blood supply on a collateral basis through the colon down into the pelvis. This blood, of course, will drain back into the venous drainage from the pelvis into the inferior vena cava, as well as into the portal veins. The question is, how far down into the extremities were you getting what you thought was an adequate oxygenation of the tissues? Unless you had an increased amount of oxygenation in the venous blood from well down in the limbs, you were really just stopping at the pelvis.

DR. JACOBSON: We were aware of this. In the 50 procedures in the dog, this was corrected experimentally by occluding the inferior mesenteric. In the many patients (some of whom had Leriche's syndrome), we were well down in the superficial femoral artery and femoral vein. Some had superficial femoral artery obstruction, and, in this case, sampling was from the popliteal vein.

DR. HITCHCOCK: You got the samples well down peripherally?

DR. JACOBSON: Yes, we did. In this group of nine aortic aneurysms, the samples were taken as far down the iliac vein as was practical surgically, so that your argument has some validity there. However, the same observation was made both in the experimental animals and in the more peripheral arterial surgery. Therefore, I believe our conclusion is valid. The strongest evidence for adequate oxygenation we have is this lack of hypotensive episodes when the clamps are removed. You, as a surgeon, know that this is quite a change from what we are used to.

DR. HITCHCOCK: I was going to bring that point up. I believe in very porous grafts. This is quite a technical surgical point, Dr. Lanphier, but I believe in using only the knitted graft rather than the woven (*e.g.*, Dacron or Teflon). They are very porous, so they leak worse than the proverbial sieve when blood begins to flow through them. Neither preclotting nor gelatinizing influence has helped before operation; therefore, we have learned to open the clamp approximately 1/10 second, allowing a gush of blood to flow, and then to clamp back down and leave it for 4 or 5 minutes. In other words, serially and sequentially open the clamp and allow a calculated loss of about 400 or 500 cc. Eventually, clots form in the interstices, and the graft is completely open after about 12 minutes. With this method, it is possible to reconstitute flow without having more than a 15 or 20 mm Hg drop in the blood pressure.

DR. JACOBSON: If you took the clamp off immediately and left it off, however, there would be a profound hypotension. I should think you would be more interested in hyperbaric oxygenation than most. We use woven grafts, you use knitted grafts and you are used to a lot of hemorrhage. We have had cases of Leriche where suture lines tore and profound hypotension resulted. In such cases, when the patient has a blood pressure of 50 mm Hg for the 10 or 15 minutes that you need to get out of trouble, it is nice to know that he is fairly well oxygenated. We have had no problems with insufficient renal function postoperatively, and I believe this represents an improvement in surgery. We have not done enough cases to be able to say anything about the mortality rate, and, in fact, the clinical situation is so variable that we may never know, but I am convinced at this point that hyperbaria does add a measure of safety for the patient.

DR. H. URSCHEL (*Dallas, Texas*): In reference to hypotension following the removal of an aortic cross-clamp, Jesse Thompson has reported 450 cases of aortic

aneurysm and occlusive disease surgery with a mortality of 1.5%. By using 1.5 liters of Ringer's solution per hour of surgery, the average drop in blood pressure following prompt removal of the clamp is 10 mm Hg. We feel that Ringer's lactate is less expensive than the hyperbaric chamber.

DR. JACOBSON: I want to emphasize that I am not saying that this kind of surgery should not be done outside the chamber. Obviously, we have the chamber and want to use it, but there is no question in my mind, on the basis of our first 50 cases, that it does add a measure of safety. If this were as simple as using a bottle of Ringer's you would not hesitate to use it; yet you fight against this because it happens to be in a hyperbaric chamber. I must say that the time involved in the surgery is not increased. We just pressurize as we are ready to clamp the aorta, and depressurize during closure and complete the operation in the same total length of time.

DR. E. LANPHIER, *Session Chairman (Buffalo, N. Y.)*: It is valuable to consider this proposition: if this particular application were the only one that existed, we would not have many chambers. I have been impressed with the number of possible indications for the use of hyperbaric oxygen which may not be crucial, but if you have a chamber and can use it as readily and efficiently as Dr. Jacobson does, in the final analysis you would probably use it. I do not think we should lose sight of this type of application. The percentage of increased safety for the patient may not be very great, but if I happen to be the patient I think I would prefer to chance Dr. Jacobson's method. I am sure that not only this particular application, but also a number of others involving the same general principle, deserve much serious consideration.

An interesting recent development is the use of hydrogen peroxide, and there have been times when I have thought that perhaps a dripping bottle of peroxide could substitute for a chamber.

Effect of Increased Oxygen Resulting from Hydrogen Peroxide Degradation on Plasma and Tissue Lipids

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In the past few years, there has been a renewal of interest in the physiologic and medical applications of high pressure oxygen. A number of problems attend the systemic administration of oxygen at increased pressure, however. Because of this, we have attempted to evaluate an alternative method for increasing oxygen delivery to the tissues, using intra-arterially infused hydrogen peroxide (H_2O_2) in a regional system.[1-7] Our studies have made it apparent that the rapid decomposition of H_2O_2 in blood or other biological fluids results in supersaturation of the fluid with oxygen, bringing the oxygen concentration to a level comparable to that which would result from oxygen-breathing under several atmospheres of pressure.[8]

One of the sequelae to OHP administration noted by several investigators [9-11] is formation of lipid peroxides. We have observed a consistent reduction in the severity of atherosclerotic disease of the vessels in the infused area of patients receiving intra-arterial H_2O_2. Following is a preliminary report of a study undertaken to elucidate this finding. Three approaches were used: (1) gross, histologic, and chemical evaluation of aortas taken at autopsy from patients treated for long periods with intra-arterial H_2O_2, (2) *in vitro* studies of human aortas incubated with dilute H_2O_2 in saline or incubated in saline under oxygen at increased pressure, and (3) *in vivo* studies of changes in total serum lipids in animals and humans following the intra-arterial infusion of H_2O_2.

Materials and Methods

Study of Aortic Tissue from Patients Receiving H_2O_2 Infusions

Postmortem examination was performed on several patients who had, during life, received intra-arterial infusions with H_2O_2 as an adjunct to irradiation therapy for malignant disease. These patients had been treated with daily infusions of 250 ml H_2O_2 (0.36–0.48%) in an electrolyte carrier solution (Ionosol T) for periods ranging from 4 to 16 weeks. During autopsy, the H_2O_2 infusion catheter was left in place, the aorta was split longitudinally, and the location of the tip of

the catheter was marked. Sections were prepared from the aorta immediately above and below the catheter tip for comparative histologic evaluation by oil red O and hematoxylin–eosin stains. Weighed sections of the vessel were then prepared for total lipid analysis. This was accomplished by homogenizing the samples, extracting with chloroform-methanol (2:1 v/v), and separating the components (cholesterol, cholesterol esters, phospholipids, triglycerides, and free fatty acids) by thin-layer chromatography by the method of Mangold.[12] The material was taken from the thin-layer chromatogram and quantitated by gas chromatography by the method of Stoffel *et al.*[18]

In vitro *Study of Aortas Incubated with* H_2O_2

Aortas were taken at autopsy from a number of adults who had died from various causes and had received no H_2O_2 infusions during their hospital course. The tissue from several aortas was cut into 5-mm squares, pooled, washed 15 times in cold saline, and divided into a number of 15-gm samples. These samples were studied according to three protocols: (1) incubation in 100 ml of 0.36% H_2O_2 in saline, (2) incubation in 100 ml of saline under oxygen at 2, 4, and 6 ata, and (3) incubation in 100 ml of saline in room air at normal pressure (as a control). At the end of 2 hours of incubation, the tissue was removed by two filtrations, and the lipid was extracted from the remaining fluid and analyzed as described earlier.

In vivo *Studies of Total Serum Lipids After* H_2O_2 *Infusion*

Animal Studies. Rabbits were made atherosclerotic by being fed high-fat, high-cholesterol diets over 4–8 months. At the end of this period, experiments were begun. Into each animal were inserted two catheters: one in the femoral artery, passing retrograde to the diaphragm for H_2O_2 infusion, and the second in the vena cava for withdrawing blood samples. Venous blood samples (10 ml) were drawn before H_2O_2 infusion to serve as controls. The animals were then infused with 10 ml of 0.12% H_2O_2 in saline at a rate of 1 ml/min. Immediately after infusion was concluded, a second blood sample was drawn for total serum lipid determination (carried out as previously described).

Patient Studies. Four patients, receiving intra-arterial infusions of H_2O_2 for various reasons, were infused with 250 ml of H_2O_2 into the abdominal aorta; in three cases a 0.48% solution was used and in the fourth a 0.24% solution was given. Venous blood samples were collected from the cubital vein immediately before and after the infusions, the total time lapse between withdrawal of the two samples being 20–30 min. Serum lipid determinations were performed as described earlier.

RESULTS

Study of Aortic Tissue from Patients Receiving H_2O_2 *Infusions*

The aortas taken at autopsy from patients treated with intra-arterial H_2O_2 showed distinct variations in the sections exposed to the H_2O_2. On gross examination, the distal segment of the aorta receiving the infusion was found to be different from the proximal aorta (not receiving the infusion). There were fewer and less severe atheromatous plaques and an increase in flexibility and elasticity of the vessel.

Figure 1 shows a section of aorta taken from a 70-year-old man who had completed therapy 6 months before autopsy examination. The thoracic aorta contained numerous fibrofatty plaques, some of which eroded into the lumen. Or-

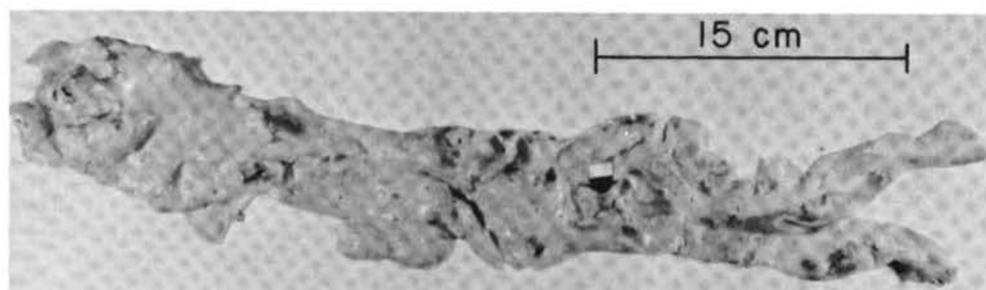

FIGURE 1. Aorta of a 70-year-old man taken at autopsy examination 6 months after the intra-arterial infusion of H_2O_2. The point where the H_2O_2 infusion catheter was inserted is marked with the arrow. Note the lack of raised atheromatous lesions from the catheter location to the bifurcation.

dinarily one expects to find an increase in the number and severity of atheromatous lesions from the thoracic to the abdominal aorta, but this patient had no raised lesions from the point of the catheter to the bifurcation, and the iliac arteries themselves were relatively free of gross disease except for some intimal and subintimal fibrosis. Histologic evaluation of sections stained with oil red O showed a decrease in total subintimal lipid deposits. When weighed samples of the vessels were extracted and total lipids determined, it was found that a reduction in total lipids of approximately 50% had occurred in the area infused with H_2O_2.

In vitro *Study of Aortas Incubated with H_2O_2*

Table 1 compares the relative quantities of lipids in the fluid after incubation of human aortas in 0.36% H_2O_2 or in saline under 6 ata of oxygen. A slightly higher total lipid value was achieved with incubation in H_2O_2. Table 2 shows the average figures from a number of individual tests in which human aortas were incubated in saline under oxygen at 2, 4, and 6 ata of pressure (relative lipid concentration in the samples expressed in mg lipid/100 gm of tissue). The reason for the slight decrease in total lipids at 4 ata of pressure has not yet been determined.

In vivo *Studies of Total Serum Lipids After H_2O_2 Infusion*

Table 3 shows the values obtained when total serum lipids were determined immediately before and after the intra-arterial infusion of 0.12% H_2O_2 in rab-

TABLE 1. *In vitro* Lipid Elution from Human Aortic Tissue After Incubation in H_2O_2 or Under OHP for 2 Hours

Sample	Cholesterol (mg%)	Chol. esters (mg%)	Phospholipids (mg%)	Triglycerides (mg%)	Free fatty acids (mg%)	Total lipids (mg%)
Incubated in saline at 6 ata of O_2	2.42	11.56	4.40	1.79	0.91	21.08
Incubated in 0.36% H_2O_2	3.32	13.44	2.93	1.94	1.97	23.60

TABLE 2. *In vitro* Lipid Elution from Human Aortic Tissue After 2 Hours of OHP

O_2 pressure	Cholesterol (mg%)	Chol. esters (mg%)	Phospholipids (mg%)	Triglycerides (mg%)	Free fatty acids (mg%)	Total lipids (mg%)
2 ata	3.38	11.10	2.84	1.27	1.46	20.05
4 ata	2.84	10.38	2.80	1.38	1.41	18.81
6 ata	2.42	11.56	4.40	1.74	0.91	21.08

bits rendered atherosclerotic by high-fat high-cholesterol diets. The first animal in this series had been on the diet approximately 8 months, the second animal for only 4 months, and the third animal was a control rabbit (not given the special diet). The total lipid increase after the infusion of H_2O_2 varied directly with the degree of atherosclerotic disease.

Table 4 shows the results of the study in which venous samples were collected from patients just before and after the intra-arterial infusion of H_2O_2. The patients are listed from 1 through 5, in descending order of degree of their atherosclerotic disease. Patient 5 was, for all practical purposes, free of atherosclerotic problems, being an 18-year-old male. Patients 1 and 2 were both 65-year-old men. Patient 1 received a 0.24% solution of H_2O_2 infused into the abdominal aorta, and the remaining four patients were infused with a 0.48% solution. (The total lipids are expressed as mg lipid/100 gm of tissue.) The total increase in lipids after infusion seemed to be related to both severity of disease and concentration of H_2O_2 infused.

SUMMARY

Gross and microscopic examination of aortas taken at autopsy from patients who had been treated with intra-arterial H_2O_2 for extended periods showed an increase in elasticity and a decrease in subintimal lipid deposits, *i.e.*, a 10–40% decrease in cholesterol and a 20–50% decrease in cholesterol esters in the infused area, when compared to the noninfused area of the same vessel.

In vitro studies were conducted with human aortas incubated with H_2O_2 in saline or in saline under high oxygen tension. Results showed the elution of cholesterol, cholesterol esters, phospholipids, triglycerides, and free fatty acids. Only a trace of lipid was detected in samples incubated in saline at normal pressures.

In vivo studies were performed in experimental animals and in humans, wherein total serum lipids were determined before and immediately after the intra-arterial infusion of H_2O_2. The results showed a rise in total serum lipids, with the greatest increase noted in patients with advanced clinical atherosclerotic disease and in patients receiving the higher concentrations of H_2O_2.

ACKNOWLEDGMENTS

This work was supported in part by the Syntex Laboratories, the Putman Estate, Fort Worth, Texas, the Moody Foundation, ACS Grant T-284A, the National Institutes of Health Grant CA-07127-03, and USPHS Grant DE-21.

The authors gratefully acknowledge the assistance of Mr. W. H. Meyer of Procter and Gamble Co., the advice and technical assistance of Dr. J. W. Miller, Director of the Department of Radiology, Dr. H. L. Dorman of the Department of Physiology, and Dr. M. J. Wagner of the Department of Biochemistry, Baylor University College of Dentistry, Mr. E. Lambert, Mr. H. Pingree, Mr. D. Clark, and Miss G. Davis, and the help of Mrs. M. Alexander in the preparation of this manuscript.

TABLE 3. Rabbit Serum Lipids Before and After Intra-Arterial Infusion of 0.12% H_2O_2

Animals	Sampling	Cholesterol (mg%)	Chol. esters (mg%)	Phospholipids (mg%)	Triglycerides (mg%)	Free fatty acids (mg%)	Total lipids (mg%)
1. High fat & cholesterol diet 8 months	Preinfusion	172.7	1246.9	309.0	309.9	44.8	2130.8
	Postinfusion	245.5	1467.2	392.0	391.1	39.2	2534.9
	(% change)	42.2	17.7	26.5	26.2	−12.5	19.0
2. High fat & cholesterol diet 4 months	Preinfusion	105.5	1012.0	234.5	64.5	35.0	1451.5
	Postinfusion	138.5	1170.0	253.0	49.0	29.5	1640.0
	(% change)	31.3	15.6	7.9	−24.0	−15.7	13.0
3. Normal diet (control)	Preinfusion	6.1	39.5	146.9	46.5	17.9	256.9
	Postinfusion	6.1	42.5	140.8	71.7	21.2	281.9
	(% change)	0.0	6.6	−4.2	54.2	18.4	9.7

TABLE 4. Human Serum Lipids Before and After Intra-Arterial Infusion of 0.48% H_2O_2

Animal	Sampling	Cholesterol (mg%)	Chol. esters (mg%)	Phospholipids (mg%)	Triglycerides (mg%)	Free fatty acids (mg%)	Total lipids (mg%)
1	Preinfusion	28.7	156.2	99.8	120.0	13.5	418.9
	Postinfusion	34.6	214.3	114.1	120.0	16.1	499.4
	(% change)	20.6	37.2	14.3	0.0	19.1	19.2
2	Preinfusion	9.9	135.6	39.4	22.2	38.0	245.2
	Postinfusion	14.4	177.2	64.2	34.2	30.4	320.4
	(% change)	44.6	30.7	62.9	54.1	−20.8	30.7
3	Preinfusion	18.6	175.4	75.8	46.8	46.0	362.6
	Postinfusion	20.0	198.8	117.8	55.2	43.6	435.4
	(% change)	7.5	13.3	55.4	17.9	−5.2	20.1
4	Preinfusion	15.8	142.2	49.2	20.8	52.6	280.6
	Postinfusion	19.6	137.4	58.4	46.8	47.8	310.0
	(% change)	24.4	−3.4	18.7	125.0	−9.1	10.5
5	Preinfusion	44.4	149.6	117.8	74.3	11.0	397.1
	Postinfusion	35.2	185.2	111.3	72.2	12.6	416.7
	(% change)	−20.2	23.8	−5.5	−2.9	14.7	4.9

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DISCUSSION

DR. J. HAMILTON (*Tonawanda, N. Y.*): Has anyone used, or considered using, this method for removing fat from the lungs in pulmonary fat embolism?

DR. FINNEY: The lung is an area which does not have this circulatory ability. It may work under oxygen at high pressure, but with our system we could not do any lung work.

Proceedings of the Third International Conference on Hyperbaric Medicine
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SESSION V

Hyperbaric Oxygenation in Anaerobic Infections

Chairman: DAVID C. SABISTON, JR.
Department of Surgery
Duke University Medical Center
Durham, North Carolina

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Diagnosis, Classification, and General Management of Gas-Producing Infections, Particularly Those Produced by *Clostridium perfringens*

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Throughout the ages, gas gangrene and other clostridial infections have been among the most dreaded complications of wounds of violence because of their rapid and fulminating course, their profound associated toxemia, their mutilating effects, and their high mortality.

Certain problems contribute to the difficulties in diagnosing these infections and evaluating the results of various types of treatment, the rarity of gas gangrene in civilian surgical practice being chief among them. Yet, bacteriologic studies of the flora of accidental and war wounds have shown percentages of clostridial contamination varying from 3.8% to 88%.[1,2] A review of the literature by Altemeier and Furste[1] revealed that clostridial contamination in 3027 reported wounds ranged from 3.8% to 39% (Table 1). Lindberg[2] has stated that, in the Korean war, careful studies of the anaerobic bacterial contamination of war wounds indicated that clostridia were present in approximately 88% of cases.

In view of the widespread distribution of *Clostridium perfringens* (*Cl. welchii*), particularly in soil and woolen clothing, this high incidence of contamination in wounds of violence is not surprising. What seems paradoxical is the infrequent development of clostridial infection. In a total series of 187,936 major open wounds of violence reported in the literature and reviewed by Altemeier and Furste,[1] the overall incidence of gas gangrene was only 1.76%, varying between 0.3% and 5.2% among the individual series. This is especially remarkable since there was often no appreciable difference in bacterial flora between the wounds that developed gas gangrene and those that did not.

Surgeons are confronted with this lesion so infrequently that they do not anticipate its development in accidental wounds or wounds of violence, and often are unfamiliar with its early signs and symptoms. Table 2 outlines a classification of clostridial infections, for purposes of discussion.

Clostridial Myositis

At our institution, we have made an intensive study of the problems encountered

TABLE 1. Incidence of *Cl. perfringens* Contamination in Wounds of Violence

	No. cases	No. wounds contaminated	Percent wounds contaminated
Altemeier and Gibbs	99	39	39
DeWaal	708	27	3.8
Dimtza and Gutscher	424	57	13.4
Meleney			
Soft-part wounds	926	138	14.9
Compound fractures	674	147	21.8
Levaditi *et al.*	61	10	16
Miles (military wounds)	105	24	23
Spooner	30	3	10
Total	3027	445	14.7

in the diagnosis and management of clostridial myositis. Forty-two patients with proven clostridial myositis of the spreading type (gas gangrene) have been studied.[3] Our experience and that of others [1,3–8] shows that, although clostridial myositis may occasionally follow relatively trivial injuries, it is most likely to develop in wounds in which there has been: (1) extensive laceration or devitalization of muscles, particularly in the extremities, (2) impairment of the main blood supply to a limb or muscle group, (3) contamination by dirt, clothing, or other foreign bodies, and/or (4) delay in surgical treatment.

Unfortunately, clostridial myositis is usually recognized by the clinical appearance of the patient and his lesion in the more obvious, far-advanced, and often irreversible stages of disease. The casts, splints, or large dressings necessary for the treatment of the major injury obscure the area of the wound and make the observation and interpretation of local signs difficult and indirect. For these reasons, dressings should be removed promptly and the wound inspected directly if there is the slightest suspicion of early symptoms of this infection. A high index of suspicion should always be held for patients with severe wounds associated with laceration or crushing of muscle.

TABLE 2. Classification of Clostridial Infections

- I. Clostridial myositis
  - A. Spreading or diffuse (gas gangrene)
    - 1. Crepitant
    - 2. Noncrepitant or edematous
    - 3. Anaerobic toxemia
  - B. Localized myositis
    - 1. Crepitant
    - 2. Noncrepitant
- II. Clostridial cellulitis (anaerobic or crepitant)
- III. Tetanus

Difficulties in recognizing gas gangrene seem to stem largely from: (1) confusion about the nature and types of gas gangrene, (2) the belief that this infection is characterized by the usual signs of pyogenic inflammation, with extending redness of the skin, cellulitis, lymphangiitis, purulent exudation, high fever, high leukocytosis, and frequent septicemia, and (3) the inability to correlate the presence of clostridia found in a wound with the etiology of the infection. The surgeon must realize that clostridial myositis may be caused by one or more of a variety of these anaerobes, and that the clinical picture may vary with the bacterial species present. The most important causative microorganism is *Cl. perfringens.* It has been present in all but three of the 42 cases in our series reported in 1957, either alone or in combination with other clostridia (*e.g., Cl. novyi, Cl. sporogenes,* and *Cl. sordellii*).

Clostridial myositis of the *spreading* or *diffuse* type represents true gas gangrene, and it may be manifested as the crepitant, the noncrepitant or edematous, the mixed, or the profound toxemic type. Essentially, it is an infection of the muscles, and the connective tissues may be affected comparatively little at first. As gas and edema accumulate in tissue spaces confined by fascial compartments or aponeurosis, pressure increases; this may be sufficient to produce further necrosis of muscle and compression of the regional lymphatics, veins, and even arteries. This opens the malignant phase of the infection, and the successive stages of muscle disintegration and bacterial invasion follow one another, often with great rapidity, to involve groups of muscles, an entire limb, or the torso. The involved muscles, at first hemorrhagic and friable, become discolored and noncontractile and exude a watery brownish-red foul discharge with or without bubbles of gas.

Localized clostridial myositis, on the other hand, presents a mild and self-limited picture and should not be considered as gas gangrene. It, too, may be either crepitant or noncrepitant.

Clinical Manifestations

A review of our cases has emphasized the importance of the following points in the early recognition of clostridial myositis of the spreading or diffuse type:

1. A variable interval exists between injury and the development of the lesion, sometimes as short as 6 hours, particularly in wounds associated with gross devitalization and contamination of muscle. Average incubation, however, is 53 hours in our experience.

2. Pain is the earliest and most important symptom, being secondary to the rapid infiltration of the infected muscle by edema and gas. It generally persists after primary treatment of the wound and progressively increases thereafter.

3. Rapidity and feebleness of the pulse usually follow the onset of pain and are characteristically out of proportion to the elevation of the temperature. In advanced or progressive lesions, the changes in the pulse may be increasingly apparent, heralding an abrupt, progressive, or severe circulatory collapse. The average pulse rate at the time of diagnosis in our series of 42 cases was 118/min, and the extremes were 100 and 160.

4. Early in the course of infection, the blood pressure is normal or slightly elevated. Later it may decrease significantly, falling precipitously in some instances to 80 mm Hg or less.

5. Temperature elevation in the early stages of this infection may vary considerably, the average being 38.3°C. The lowest temperature in our experience has been 36.1°C and the highest 41.7°C. Fever is not, therefore, a reliable index of the severity and extent of the infectious process. A low or subnormal temperature associated with a markedly rapid pulse may indicate a grave prognosis. A state of severe septic shock with anuria may also develop, as noted in five of our 42 patients.

6. The general appearance of the patient usually includes a peculiar grey pallor (in contrast to the usual malar flush associated with pyogenic infections), weakness, and profuse sweating.

7. The mental state is often one of apathy and indifference, the patient being conscious but usually unaware of the seriousness of his condition. Stupor, delirium, prostration, and coma are late symptoms indicative of an overwhelming infection.

8. Gastrointestinal symptoms are generally not marked, anorexia being a fairly constant finding but vomiting being uncommon.

9. As noted above, the appearance of the local lesion is not that of pyogenic inflammation. Early, the overlying skin is either white, shiny, and tense or essentially normal in appearance. An irritating dirty-brown watery discharge with a peculiar foul odor usually escapes from the

wound. As the swelling increases, the overlying skin becomes dusky, bronzed, or khaki-color in appearance. In far advanced cases, further discoloration occurs, and vesicles filled with dark-red fluid characteristically appear on the cutaneous surface (Figure 1). (Crepitation was palpable in 38 of our 42 cases, particularly as a relatively late sign.) The underlying muscle is usually discolored, edematous, and nonviable. It is frequently seen to herniate through a previously made fasciotomy incision. In advanced lesions, the temperature of the extremity distal to the infected wound is reduced, and the skin feels cold. In patients with myositis caused by *Cl. novyi,* marked swelling without crepitation may be present (Figure 2). Microscopic examination of the watery discharge usually reveals numerous red blood cells and many large gram-positive bacteria with squared ends, but without spores. In contrast to pyogenic infections, few pus cells are usually seen.

FIGURE 1. Far-advanced clostridial myositis caused by *Cl. perfringens* which complicated a compound comminuted fracture of tibia and fibula in a 53-year-old Negro woman. Note the herniation of the infected discolored edematous muscle protruding through the fasciotomy incision. The drainage of dark fetid fluid, the vesicles, the marked swelling, the crepitation, and the gangrenous patches of skin are characteristic of far-advanced gas gangrene. (Reproduced from *Surgery: Principles and Practice,* J. B. Lippincott Co., 1957, p. 63.)

Laboratory and X-Ray Aids to Diagnosis

Laboratory examinations often indicate marked reduction in red blood cells, hematocrit, or hemoglobin values. The leukocyte count is seldom elevated above 12,000–15,000. In our series, the lowest count was 8000, the highest was 57,000, and the average was 21,000. The average CO_2 combining power was 30 vol%.

In general, however, no satisfactory laboratory tests exist for the early diagnosis of gas gangrene, and one must remember that valuable time may be lost awaiting results of various diagnostic tests. Rapid spread of the infection may require only 2–4 hours, and irreversible changes may rapidly develop in the tissues. For this reason, immediate surgical exploration of any wound suspected of harboring clostridial myositis may be advisable. This permits early radical incision with decompression of the fascial compartments or excision of infected devi-

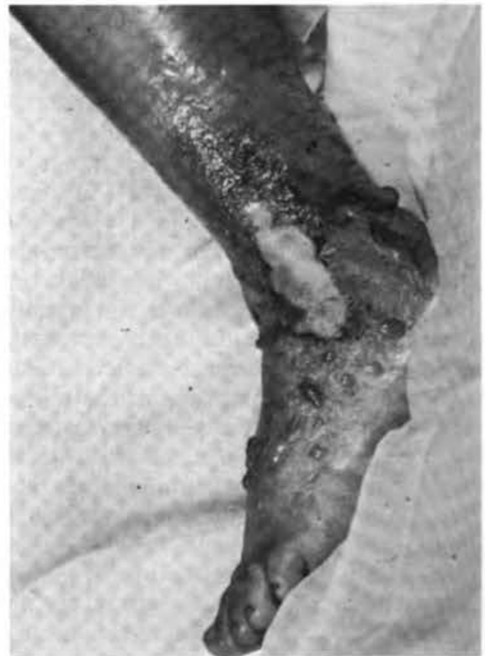

FIGURE 2. Clostridial myositis in a patient with a compound fracture; infection was caused by *Cl. novyi* and characterized by marked edema, multiple vesicles, and profound toxemia. There was no crepitation.

talized bundles of muscle before amputation of the part becomes necessary.

Valuable information can be obtained from the microscopic examination of exudate prepared with Gram's stain, however. *Cl. perfringens* and other clostridia usually appear as large gram-positive bacilli with squared ends, without evidence of sporulation. *Cl. perfringens,* the most important and commonest cause of gas gangrene, can be rapidly identified by inoculating infectious exudate into special milk media which are then incubated anaerobically for 4–5 hours. Typical stormy fermentation in the media and the presence of proliferating bacteria which are nonmotile indicate the presence of *Cl. perfringens.* (This method, developed in our laboratory,[9] has frequently helped us to reach diagnosis in our cases.)

While demonstration of this organism in wounds by bacteriologic methods suggests that it is the cause of the infection present, it does not prove it. In fact, a primary problem in diagnosing early gas gangrene at this time is our inability to determine whether the clostridia found in an infected area are contributing to the development of that infection and its associated toxemia, or whether they are present as nontoxigenic agents in mixed infections. Many strains of *Cl. perfringens* isolated from wounds are so low in virulence and toxigenicity that they are incapable of producing infections in experimental animals except in very large doses.

X-ray films taken at intervals of 2–4 hours may aid in the diagnosis by differentiating gas in the soft tissues produced by clostridial invasion from that due to mechanical or chemical causes. In our experience, such films have helped detect early or incipient gas gangrene, permitting a positive diagnosis 24 hours or more earlier than that possible by the clinical findings alone. If the visible gas increases in amount or presents a linear spread along the muscle and fascial planes, an early diagnosis of gas gangrene can be made (Figure 3). In far-advanced cases, large quantities of gas can be visualized in the muscular tissues by x-ray examination (Figure 4).

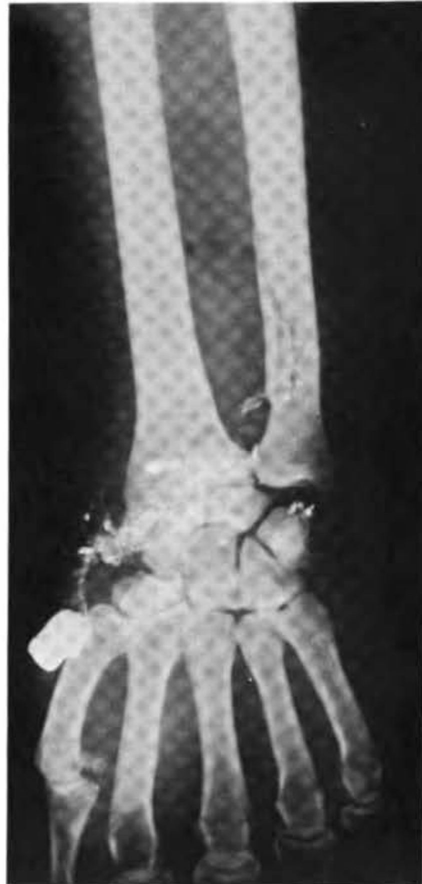

FIGURE 3. Roentgenogram of gunshot wound of wrist and forearm with early clostridial myositis. Early recognition of gas in tissues and its irregular linear spread made prompt diagnosis of the infection possible.

Clostridial Cellulitis

Clostridial cellulitis, also known as anaerobic or crepitant cellulitis, may easily be confused with clostridial myositis by the clinician. Indeed, cellulitis and myositis may coexist in the same patient, but cellulitis can exist in the absence of clostridial myositis and usually does. Clostridial cellulitis is a septic crepitant process involving the epifascial, retroperitoneal, or

FIGURE 4. Roentgenogram showing extensive dissection of muscles and fascial planes in lower leg produced by far-advanced *Cl. perfringens* gas gangrene.

other connective tissues (Figure 5). Its incubation period is usually 3–4 days, and the onset is ordinarily more gradual than in gas gangrene. Systemic effects may be slight unless the wound is also highly septic, and this relative mildness of the general reaction helps distinguish it from true gas gangrene. Anaerobic or clostridial cellulitis, however, is not a condition to be regarded lightly. The spread of infection in the tissue spaces may be rapid and extensive, necessitating radical surgical drainage even within a few hours.

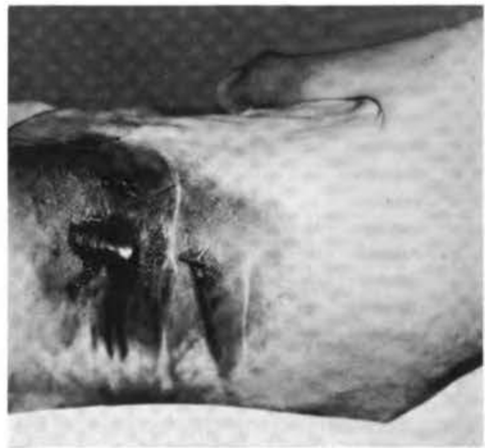

FIGURE 5. Postoperative *Cl. perfringens* retroperitoneal and subcutaneous cellulitis with crepitation, vesicle formation, and gangrene of skin. There was no evidence of clostridial myositis.

Nonclostridial Lesions Simulating Gas Gangrene

Many other lesions simulate clostridial infections, and diagnostic skill depends largely upon familiarity with the characteristics of these nonclostridial lesions for differentiation. The most important of these are listed in Table 3.

Aerobic Aerogenic Infections

Aerobic aerogenic infections are being seen more frequently in clinical practice,

TABLE 3. Classification of Crepitant Nonclostridial Lesions

I. Aerobic aerogenic infections
A. Coliform
B. Mixed
II. Anaerobic streptococcal infections
III. Hemolytic streptococcal gangrene
IV. Bacteroides infections
V. Nonbacterial lesions

and we believe that these far outnumber clostridial infections. Those caused by the coliform organisms such as *Escherichia coli* or *Aerobacter aerogenes* have been associated with the production of variable amounts of gas in the tissues. These aerobic aerogenic infections are much less severe than clostridial myositis and usually involve the subcutaneous or epifascial tissues. They may also involve the deep fascial compartments of an extremity, however, producing clinical pictures very similar to those noted in true gas gangrene (Figure 6). In many instances, these lesions are polymicrobic in etiology, and one or more aerogenic bacterial species may be active.

Streptococcal Myositis

Streptococcal myositis is a massive infection of muscle characterized by discoloration, edema, serous exudate, gas formation, local pain, and generalized toxemia. Neglected cases may progress to a true gangrene of muscle. It differs from clostridial myositis in that: (1) cutaneous erythema is more pronounced and extensive, (2) the muscle involved, although edematous and discolored, is still alive and reactive to stimuli, (3) the odor is different, and (4) microscopically, a muscle smear stained by Gram's method reveals no gram-positive bacilli but vast numbers of streptococci among masses of pus cells.

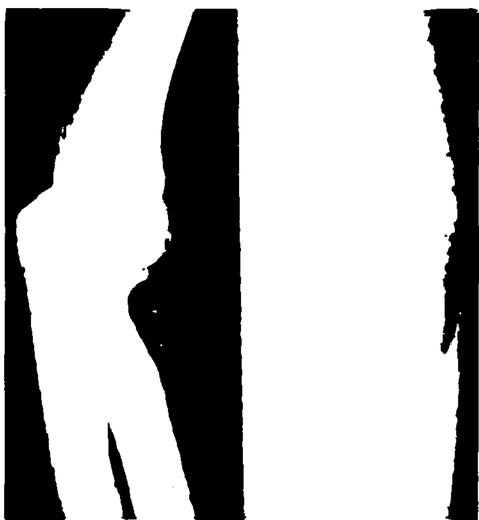

FIGURE 6. Roentgenograms of elbow and upper arm with extensive myositis and cellulitis. Note gas in soft tissues. Infection caused by *A. aerogenes*. No clostridia were present.

Hemolytic Streptococcal Gangrene

Hemolytic streptococcal gangrene occasionally follows some relatively minor operative procedure or injury. It is essentially an epifascial spreading subcutaneous gangrene with thrombosis of the nutrient vessels and resultant slough of the overlying skin. It usually occurs in the extremities, although the perineum, face, and other parts of the body may be involved. It is characterized by increasing pain and marked swelling at the wound site, chills, temperature elevation to 38–40°C, rapid pulse, toxemia, prostration, and a spreading painful cellulitis which undergoes bullous formation and a peculiar patchy and extending necrosis. Hemolytic streptococci are found in the subcutaneous gangrene and bullae, often in pure culture.

Staphylococcal Gangrene

Acute infectious staphylococcal gangrene may rarely simulate gas gangrene. Morrison [10] reported such a case in a 5½-year-old boy who developed a rapidly spreading cellulitis with pain, swelling, brawny induration, patchy discoloration of the skin, and elevation of temperature to 40.6°C 3 days after injury. No organism other than *Staphylococcus pyogenes* var. *aureus* was demonstrated on aerobic and anaerobic culture of the pus obtained by multiple incisions in the involved tissues. Similar cases have been observed by the author, particularly in association with acute fulminating osteomyelitis (Figure 7).

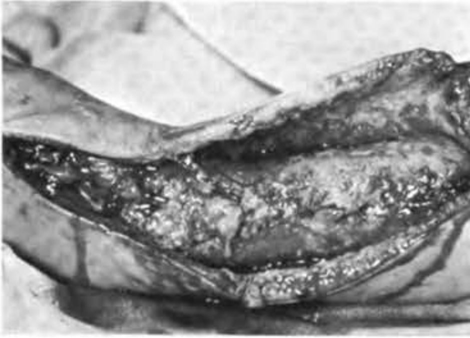

FIGURE 7. Extensive cellulitis of leg complicating acute hematogenous osteomyelitis of lower tibia caused by *S. aureus*. Marked swelling, large vesicles, patches of gangrenous skin, and profound toxemia made the clinical picture similar to clostridial myositis. (Reproduced from *Surgery: Principles and Practice*, J. B. Lippincott Co., 1957, p. 55.)

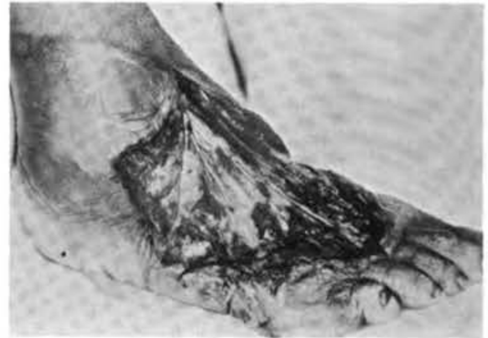

FIGURE 8. Gangrenous infection of foot with grey-black discoloration and crepitation of subcutaneous tissues and tendons. Cultures revealed a mixed bacterial flora including bacteroides and anaerobic streptococci. No clostridia were present. (Reproduced from Surg. Gynec. Obstet. 87:206, 1948.)

Bacteroides Infections

The bacteroides also produce lesions simulating those caused by the clostridia. The bacteroides are gram-negative anaerobic bacilli which may produce crepitant infections involving either the subcutaneous tissues, the retroperitoneal tissues, or the muscles. The appearance of these infections may simulate that of gas gangrene. The rate of local spread and the overall progression of bacteroides infections are considerably slower, however, than those seen in true clostridial myositis, and the tissues may show brown or black discoloration (Figure 8).

Nonbacterial Lesions

Many nonbacterial factors may cause the appearance of gas in wounds of violence, detectable either by palpation or by roentgenographic means. Examples are the irrigation of wounds with hydrogen peroxide, the sucking of air into wounds either as a direct result of or at the time of trauma, and the hypodermic injection of gas-producing chemicals. For example, the accidental injection of benzine into areas has been described as causing the volatilization of the benzine and marked crepitation of the tissues.

CURRENT PROBLEMS IN PREVENTING CLOSTRIDIAL MYOSITIS (GAS GANGRENE)

Early and adequate surgery is still the most effective means of preventing gas gangrene, and recent experimental and clinical evidence has indicated that antibiotic therapy alone cannot be relied upon to prevent the occurrence of clostridial myositis.[3]

Although there is considerable evidence that the prophylactic administration of gas gangrene antitoxin at the time of injury or shortly thereafter is of little or no practical value in the prevention of clinical gas gangrene, most surgeons continue to give it at the time of injury. The prophylactic use of gas gangrene antitoxin was discontinued at our hospital in 1943. Since then, the Subcommittee on Trauma of the National Research Council and the Department of Defense have recommended that gas gangrene antitoxin not be given prophylactically at the time of injury because of its ineffectiveness in preventing the disease.

The Departments of Biochemistry and Surgery of the University of Cincinnati

have, in cooperation, developed effective toxoids for *Cl. perfringens* and *Cl. novyi* which have proven very effective in preventing experimental gas gangrene in animals,[11,12] but they are not yet available for clinical use. Volunteers (medical students) inoculated with two or three injections of these toxoids have responded satisfactorily, with the development of adequate circulating antitoxin levels, thus suggesting the readiness of the preparations for clinical trial.

Problems in Treating Established Clostridial Myositis (Gas Gangrene)

The principal problem in the treatment of established gas gangrene arises from the necessity for early diagnosis. Because of the rapid spread of this infection, an irreversible process may develop within a few hours, and a 24-hour delay may be fatal to some patients. Delays in operative treatment may result from the surgeon's indifference to the rapidly progressive nature of this infection or from his inability to obtain operating-room facilities which are occupied at the time.

Most surgeons agree that early and adequate surgery is the most effective and primary means of treating clostridial myositis.[1,3,13–21] If the diagnosis is made early, while the gangrene is relatively localized and incipient, radical decompression of the involved fascial compartments by free longitudinal incisions and excision of the infected muscle usually arrest the process and eliminate the need for amputation. Local excision of a single muscle or group of muscles is preferred to amputation, whenever possible, to conserve a functional extremity. If diagnosis is reached when the process is extensive and has caused irreversible gangrenous changes implying permanent loss of function of the limb, open amputation of the guillotine type or some modification becomes necessary. At the moment, it is inconceivable that any type of serotherapy or chemotherapy could replace good surgery in the treatment of established gas gangrene.

Serotherapy

There is some evidence that the use of antitoxin in conjunction with adequate surgery aids in the control of the associated toxemia. Many surgeons, however, doubt the efficacy of gas gangrene antitoxin.

The main purpose of using antitoxin has been to prevent death from toxemia, by therapeutic doses given every 4–6 hours until surgery and antibiotic therapy can control the infection. The recommended intravenous injection of a therapeutic dose consists of 27,000 units of *Cl. perfringens* antitoxin, 13,500 units of *Cl. septicum,* and 27,000 units of *Cl. novyi* every 4–6 hours. Of our 42 patients, 13 received gas gangrene antitoxin in varying doses for severe toxemia, and the mortality in this group was 30%, as compared with 50% mortality in a similar group of patients receiving no serotherapy for severe generalized toxemia from gas gangrene.[8]

Antibiotic Therapy

Experimental and clinical evidence has indicated that penicillin is of value only when given in massive doses of 1 million units every 3 hours, intramuscularly or intravenously.[3,22] Other experimental evidence indicates that the tetracyclines are the drugs of choice in this condition, chlortetracycline (Aureomycin) and oxytetracycline (Terramycin) being preferable. Chloramphenicol (Chloromycetin) is also useful, but most other antibiotic agents are of dubious help.[13,23]

Supportive Treatment

The need for adequate supportive measures in the management of patients with spreading clostridial myositis may be great and may be overlooked. These measures include daily blood transfusions,

maintenance of fluid and electrolyte balance, adequate immobilization of the infected injured parts, oxygen therapy, and relief of pain. Frequent blood transfusions help to treat associated shock and to correct the profound hemolytic anemia which may be present in this condition. Care must be taken, however, not to produce pulmonary edema in the presence of impaired circulation. In cases of severe toxemia with septic shock, the intravenous administration of steroids should be considered.

The surgical management of patients with clostridial myositis, which at our hospital has not included hyperbaric oxygen therapy, may be summarized as follows:

1. Radical operative treatment as soon after diagnosis as possible, consisting of multiple incisions and fasciotomy for decompression and drainage of the fascial compartments, excision of the involved muscles, or open amputation when necessary.

2. Adequate immobilization of the affected part.

3. Injection of penicillin intravenously in large doses of 1 million to 3 million units or more every 3 hours before and after operative treatment.

4. Administration of a tetracycline compound, preferably chlortetracycline or oxytetracycline, given intravenously in 500-mg doses every 4–6 hours.

5. The administration of polyvalent gas gangrene antitoxin before and after operation, 50,000 units being given every 4–6 hours for 24–48 hours in patients with profound toxemia and hypotension.

6. Adequate additional supportive therapy, including administration of steroids in cases with overwhelming toxemia and septic shock.

7. Postponement of secondary operative procedures designed to facilitate healing or restore function of the wounded extremity until the infection has been brought under complete control.

Under this general therapeutic regimen, the mortality in the 42 cases treated by or under the direction of the author on the surgical services of our institution has been five cases (12%).

SUMMARY

A variety of clostridial infections are seen in clinical practice, but they occur only rarely. They are most likely to develop in wounds with extensive damage to muscle masses, with impairment of regional blood supply, with gross contamination by dirt or other foreign bodies, and with significant delay in adequate surgical treatment. Since they may be caused by one or more species of clostridia, the individual cases may vary greatly in appearance and in associated pathophysiologic changes, both local and systemic. Clostridial myositis is the most dreaded type, and it may be localized or diffuse (gas gangrene) and crepitant or noncrepitant. In comparison, clostridial cellulitis may occur without involving muscle, with considerably less toxemia but with similar clinical appearance to true gas gangrene.

Many factors contribute to the difficulty in diagnosing clostridial myositis, including confusion as to the nature and types of myositis, the belief that it is associated with the usual signs of pyogenic infection, inadequate bacteriologic studies, and the inability to correlate the presence of clostridia in wounds with the etiology of the infection in a given case. Perhaps one of the most important factors, however, is its clinical resemblance to the commoner types of crepitant infections caused by aerogenic bacteria such as *E. coli*, *A. aerogenes*, the anaerobic streptococci, and the bacteroides. Since the introduction of penicillin therapy in 1942, the incidence of gram-negative infections has been progressively increasing, making crepitant infections caused by this aerogenic group of bacteria more frequent in clinical practice.

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Treatment of Anaerobic Infections with Hyperbaric Oxygen

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Since 1960, 55 patients have been treated with hyperbaric oxygen in our clinic for active clostridial infections.[1-10] The favorable effects of OHP in halting the progressive process have been confirmed by other authors[11-15] who have reported similar success in their own cases. These findings contrast to those of animal experiments, in which OHP had little therapeutic effect upon iatrogenic *Clostridium perfringens* (*welchii*) infections; it served only as a preventive measure.[16-20] Perhaps the greater susceptibility of the small laboratory animal to pulmonary damage from OHP treatment explains this discrepancy, as suggested by the work of Stavrakas.[21]

Clinical Materials and Methods

Fifty-five patients with active clostridial infection were treated in the hyperbaric chamber. The cases included traumatic clostridial infection, endogenous infection, infection after surgery for diabetic or senile gangrene, postabortal *Cl. perfringens* septicemia, and iatrogenic infection. Most patients had a clostridial myositis. Since it has been our policy not to operate on these patients before submitting them to hyperbaric oxygen therapy, initially we could not tell which patients had clostridial myositis and which had clostridial cellulitis only.

Each patient was placed in a hyperbaric chamber, and the atmospheric air was compressed to 3 ata over a 10-min period. At 3 ata, the patient was given pure oxygen to breathe through a close-fitting B.L.B. mask at a flow rate of 10–12 liters/min. (The efficiency of oxygen administration was confirmed by the finding of arterial oxygen tensions between 1600 and 1700 mm Hg.) Oxygen inhalation continued for 1 hour 30 min, followed by decompression according to our standard schedule, published previously.[2] Total treatment consisted of seven sessions over a 3-day period: three 2-hour sessions on the first day, two 2-hour sessions on the second day, and two 2-hour sessions on the third day. In some cases (*e.g.*, Case 26) where the peripheral circulation was severely compromised by the trauma, the treatment was continued for 1–2 weeks (2 hours twice daily at 3 ata).[10]

Although 10 of these patients had received surgical treatment elsewhere, these attempts were unsuccessful and all 10 patients exhibited active progressive infection when admitted to our clinic. Seven patients were operated upon before or during hyperbaric oxygen therapy, and in

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those cases only local incisions were performed. In some cases, corrective surgery was later necessary to remove tissue which was already necrotic by the time OHP was administered. Operation was deferred when possible for three reasons: (1) surgery would not have to be performed on a patient in a toxic condition; (2) surgical excision could be confined to the removal of necrotic tissue, and radical amputative surgery could be avoided (limbs apparently doomed to be lost were repeatedly saved in this way); and (3) necrotic tissue could be removed with much less trauma because of sharper demarcation following hyperbaric oxygen therapy. Corrective surgery, apart from simple procedures such as skin grafting and/or removal of necrotic sloughs, was necessary in 17 patients. On 23 patients, no operations were undertaken before, during, or after OHP treatment.

Antibiotic drugs were administered for the clostridial infection in all but 12 cases. Antibiotic therapy consisted of penicillin or penicillin and streptomycin except in four cases (Cases 5, 20, 42, and 49). In five cases (Cases 7, 8, 26, 45, and 55), antibiotics were given only to combat secondary nonclostridial infections (beginning from 4 days to 2 weeks after OHP therapy). A few patients in the series had received small doses of anti-gas gangrene serum elsewhere before admission to our clinic. We used this form of therapy only once (Case 1).

The clinical problem of eardrum rupture was circumvented in our series in the following manner. During our initial work with patients in the chamber, we performed myringotomy (1) if the eardrum showed red discoloration when retracted or bulging; (2) if the patient was under general anesthesia; (3) if the patient was too ill, too old, or too young to swallow adequately; and (4) if the patient suffered an intractable earache during compression. More recently, however, myringotomies have been performed as a routine measure. In no case has it been necessary to repeat the myringotomy in the course of the 3-day therapy for anaerobic infections.

Results

The results of treatment appear in Table 1. Of the 55 patients, 10 died: in two cases, death was clearly due to the gas gangrene (Cases 6 and 51) and in two others death was probably indirectly attributable to the gangrene (Cases 5 and 32). We lack adequate explanation for Cases 5 and 32 because postmortem anaerobic cultures were negative; furthermore, in Case 32 our time for observation was too short to differentiate between death from gas gangrene and death from the patient's basic illness, from which he was already *in extremis*. In the remaining six patients who died, causes of death were: uremia due to aortic thrombosis 2 months after clinical cure (Case 1), *Escherichia coli* septicemia 1 week after cure of anaerobic infection (Case 10), pulmonary embolism 14 days after cure (Case 4), heart failure (Case 30), toxic shock from pseudomonas septicemia (Case 36), and electrolyte imbalance 1 month after cure (Case 43).

During our brief exposure times of 90 min at 3 ata, oxygen toxicity occurred only in patients with high fever (Cases 1, 2, and 20), probably because the increased metabolism of the central nervous system makes it more susceptible to this hazard. The initial symptoms of oxygen toxicity were pallor, hyperhidrosis, hyperpnea, and salivation. The patient would toss his head restlessly from side to side and pluck the blankets. Subsequently a staring gaze developed, with dilated pupils, and the head usually remained turned to one side. These manifestations disappeared within a few minutes after discontinuing oxygen administration. Surprisingly, subsequent administration of oxygen was often tolerated without further difficulty. When the initial symptoms were not recognized in time, convulsions occurred (Cases 2 and

TABLE 1. Results of Hyperbaric Oxygenation in 55 Patients with Active Clostridial Infection

Case	Age and sex	Lesion	Cause and causative microorganism	Surgical intervention previous to hyperpressure	Surgical intervention after hyperpressure	Results regarding anaerobic infection	Results regarding life and death	Cause and time of death
1	67 M	Gas gangrene in M.psoas and diffuse in abdominal and thoracic wall	Infection after upper leg amputation elsewhere for arteriosclerotic gangrene, *Cl. welchii*	Disarticulation at the hip joint elsewhere, 24 hours before	None	Cure	Dead	Uremia due to arteriosclerotic aortic occlusion, 2 months after cure
2	13 M	Gas gangrene, upper arm stump, shoulder girdle	Compound fracture forearm, *Cl. welchii*	Upper arm amputation elsewhere	None	Cure	Alive	
3	68 M	Gas gangrene, scrotal, inguinal, and lumbar region, and abdominal wall	Decubital ulcer of scrotum, *Cl. welchii*	8-cm inguinal incision elsewhere	None	Cure	Alive	
4	72 M	Deep-seated pelvic gas gangrene	Infected hemorrhoids, *Cl. welchii*	Two times excision elsewhere	None	Cure	Dead	Pulmonary embolism 14 days after cure
5	46 M	Gas gangrene, arm, up to middle upper arm	Injection of barbiturate, *Cl. welchii*	None	None	Cure	Dead	Unknown, hyperthermia 4 days after admission
6	80 M	Deep-seated pelvic gas gangrene	Prostatectomy, *Cl. welchii*	None	None	No cure	Dead	Gas gangrene 6 hrs. after diagnosis
7	45 M	Gas gangrene, from foot up to groin	Comminutive fractures lower leg, *Cl. welchii* + *Cl. tertium*	Local incision	Lower leg amputation	Cure	Alive	
8	52 M	Gas gangrene, lower leg up to middle thigh	Compound fracture ankle, *Cl. welchii*	None	None	Cure	Alive	
9	62 M	Gas gangrene, lower leg stump up to groin	Lower leg amputation for chronic ulcer elsewhere, *Cl. welchii*	None	None	Cure	Alive	
10	56 F	Gas gangrene, one leg + intra-abdominal	Cholecystectomy elsewhere, *Cl. fallax*	Incision upper	None	Cure	Dead	Anuria and *E. coli* sepsis 6 days after cure of infection
11	8 M	Gas gangrene, up to middle thigh	Compound fracture lower leg, *Cl. welchii*	6-cm incision upper leg	None	Cure	Alive	
12	59 F	Wound infection + sepsis (?)	Radical mastectomy for ulcerating carcinoma, *Cl. welchii*	None	None	Cure	Alive	

13	67 M	Sepsis (?)	Total gastrectomy for carcinoma, *Cl. welchii*	None	None	Cure	Alive
14	58 M	Gas gangrene, upper leg stump	Amputation for arteriosclerotic gangrene elsewhere, *Cl. welchii*	None	None	Cure	Alive
15	36 F	Endometritis + sepsis	Criminal abortion, *Cl. welchii*	Curettage elsewhere	None	Cure	Alive
16	36 M	Gas gangrene, up to groin and buttock	Lower leg amputation elsewhere for necrosis and comminutive fracture, *Cl. welchii*	None	None	Cure	Alive
17	24 M	Gas gangrene, leg up to groin	Compound lower leg fracture, *Cl. welchii*	Local incision	Lower leg amputation	Cure	Alive
18	35 M	Gas gangrene, arm up to shoulder	Arterial injury, elbow, *Cl. welchii* + *Cl. fallax*	Local incision	Upper arm amputation	Cure	Alive
19	18 F	Right leg gas gangrene, up to buttock, left leg up to middle thigh, comminutive fractures	Comminutive fractures, *Cl. welchii*	None	Upper leg amputation right side	Cure	Alive
20	32 M	Gas gangrene, lower leg stump and upper leg. Stop in femoral artery	Lower leg amputation, vascular graft, intramedullary nail upper leg elsewhere, *Cl. welchii*	None	Upper leg amputation through fracture for non-specific osteomyelitis	Cure	Alive
21	54 M	Unilateral gas gangrene, abdominal and thoracic wall	Lumbar sympathectomy elsewhere, *Cl. fallax*	None	None	Cure	Alive
22	73 M	Gas gangrene, abdominal wall (intraperitoneal?)	Cholecystectomy, *Cl. welchii*	None	None	Cure	Alive
23	17 M	Gas gangrene, upper leg and abdominal wall	Intramedullary nail femoral neck, *Cl. welchii*	Partial excision at the thigh elsewhere	None	Cure	Alive
24	28 M	Gas gangrene both lower leg stumps and one upper leg	Traumatic amputation both lower legs, *Cl. welchii*	None	Stump correction	Cure	Alive
25	17 M	Gas gangrene one thigh	Compound fracture upper leg, *Cl. welchii*	None	None	Cure	Alive
26	28 M	Gas gangrene one leg from toes up to middle thigh	Lacerated wounds calf, multiple vascular and nerve lesions, *Cl. welchii*	None	None	Cure	Alive

TABLE 1.—Continued.

Case	Age and sex	Lesion	Cause and causative microorganism	Surgical intervention previous to hyperpressure	Surgical intervention after hyperpressure	Results regarding anaerobic infection	Results regarding life and death	Cause and time of death
27	51 M	Gas gangrene, upper leg	Lacerated wound, *Cl. welchii*	Partial excision necrotic muscles elsewhere	None	Cure	Alive	
28	45 M	Gas gangrene, leg	Compound comminutive fractures, *Cl. welchii*	None	Lower leg amputation	Cure	Alive	
29	27 M	Gas gangrene, leg to inguinal region	Compound fractures lower leg, *Cl. fallax*	None	None	Cure	Alive	
30	52 M	Gas gangrene, abdominal and thoracic wall, upper leg	Traumatic colon perforation, *Cl. welchii*	None	None	Cure	Dead	Heart failure 4 days after admission
31	20 M	Gas gangrene, lower leg	Compound knee luxation, lacerated wound lower leg, *Cl. welchii*	None	Upper leg amputation	Cure	Alive	
32	59 M	Gas gangrene, upper leg	Infection or metastasis in leukemic patient, *Cl. welchii*	Local incision elsewhere	None	Cure(?)	Dead	Monoblastic leukemia, heart failure, hepatorenal syndrome, 1 day after admission
33	20 M	Gas gangrene, leg up to groin	Compound fracture lower leg, fracture femur, *Cl. welchii*	None	None	Cure	Alive	
34	20 M	Gas gangrene, leg up to groin	Compound comminutive fracture lower leg, fracture femur, *Cl. fallax*	None	Exarticulation knee joint	Cure	Alive	
35	24 M	Gas gangrene, lower leg	Compound fracture femur, compound comminutive fracture lower leg, *Cl. welchii*	None	Lower leg amputation	Cure	Alive	
36	33 F	Gas gangrene, upper leg, lateral side trunk	Compound comminutive fracture lower leg, *Cl. welchii*	Upper leg amputation elsewhere	None	Cure	Dead	Toxic shock, pseudomonas septicemia 2 days after admission

37	42 F	Gas gangrene, lower leg	Compound fracture lower leg, *Cl. welchii*	None	Lower leg amputation	Cure	Alive	
38	67 F	Gas gangrene, lower leg stump and upper leg	Lower leg amputation for diabetes gangrene elsewhere, *Cl. welchii*	None	None	Cure	Alive	
39	29 M	Gas gangrene, lower leg up to knee joint	Compound fracture lower leg, *Cl. welchii*	None	Lower leg amputation	Cure	Alive	
40	37 F	Gas gangrene, leg, inguinal region	Osteotomy femur elsewhere, *Cl. welchii*	None	None	Cure	Alive	
41	38 F	Sepsis	Mitral valvulotomy, *Cl. welchii*	None	None	Cure	Alive	
42	56 M	Gas gangrene, leg up to groin	Compound fracture lower leg, *Cl. welchii*	None	Lower leg amputation	Cure	Alive	
43	35 M	Gas gangrene, upper leg up to groin	Intramedullary nail upper leg, *Cl. welchii*	None	None	Cure	Dead	1 month after cure anaerobic infection, electrolyte imbalance caused by fulminant diarrhea after dialysis for anuria
44	20 M	Gas gangrene, lower leg	Compound fracture lower leg, *Cl. welchii*	None	None	Cure	Alive	
45	45 M	Gas gangrene, abdominal wall	Cholecystectomy, *Cl. welchii*	None	None	Cure	Alive	
46	45 M	Gas gangrene, lower arm	Compound fracture lower arm, *Cl. welchii*	None	Lower arm amputation	Cure	Alive	
47	50 M	Gas gangrene, upper leg stump	Compound fractures upper and lower leg, *Cl. welchii*	Upper leg amputation elsewhere	None	Cure	Alive	
48	66 M	Gas gangrene, upper leg stump	Upper leg amputation for arteriosclerotic gangrene, *Cl. welchii*	None	None	Cure	Alive	
49	26 M	Gas gangrene, leg up to groin	Compound fracture lower leg, *Cl. welchii*	None	Exarticulation knee joint	Cure	Alive	

TABLE 1.—Continued.

Case	Age and sex	Lesion	Cause and causative microorganism	Surgical intervention previous to hyperpressure	Surgical intervention after hyperpressure	Results regarding anaerobic infection	Results regarding life and death	Cause and time of death
50	19 M	Gas gangrene, lower leg	Wound lower leg, *Cl. welchii*	Local incision during OHP therapy	None	Cure	Alive	
51	59 M	Gas gangrene, upper leg stump	Upper leg amputation for arteriosclerosis, *Cl. welchii*	None	None	?	Dead	6 hours after admission, gas gangrene septic shock
52	58 M	Gas gangrene, lower leg up to knee	Compound fracture lower leg, *Cl. welchii*	None	Lower leg amputation	Cure	Alive	
53	19 M	Gas gangrene, upper leg up to groin	Compound fracture upper leg, *Cl. welchii*	Local incision during OHP therapy	Upper leg amputation	Cure	Alive	
54	32 M	Gas gangrene, upper leg	Wound upper leg, *Cl. welchii*	None	None	Cure	Alive	
55	19 M	Gas gangrene, lower leg	Compound fractures upper and lower leg, treated by intramedullary nailing, *Cl. welchii*	None	Lower leg amputation	Cure	Alive	

20). Euphoria was observed in one patient (Case 1). Our clinical work with highly febrile gas gangrene patients has taught us that the overt central nervous system signs of oxygen toxicity can be prevented by premedication with 1–2 gm chloral hydrate per rectum, 45 min before exposure to oxygen at 3 ata.

Discussion

The most impressive feature of hyperbaric oxygen treatment of clostridial infection is the immediate response of the patient. Several comatose patients, for instance, awakened within 24 hours and started to eat spontaneously. This seems paradoxical in view of the finding by others that oxygen under pressure exerts only a bacteriostatic effect on *Cl. perfringens* [22] and our finding of live *Cl. perfringens* bacilli in the wounds of patients clinically cured. Also, van Unnik,[18] in his recent *in vitro* experiments on the influence of OHP at 3 ata, found that (1) *Cl. perfringens* strains isolated from gas gangrene lesions in patients who had been successfully treated with OHP retained their potential ability to produce alpha toxin (under suitable conditions), and (2) 3 ata of oxygen for 90 min did not inhibit growth but did selectively (temporarily) inhibit alpha toxin production in broth culture if the oxygen tension was 250 mm Hg or greater. (Several times, Schoemaker and the author measured clinically with a Beckman polarographic oxygen electrode inserted into an area of clostridial infection an oxygen tension of approximately 250–330 mm Hg while the patient inhaled oxygen at 3 ata.) Since it is the alpha toxin which determines the clinical fate of the patient, and hyperbaric oxygen does nothing to affect the quantity of this toxin already present before institution of OHP, how can the dramatic improvement of these patients be explained?

Several investigators [23–25] have maintained that the alpha toxin is rapidly "fixed" *in vivo*, and one pair of workers [23] reported that free toxin injected into muscle tissue of rabbits could no longer be demonstrated after 30 min. This suggests that within a short time after inhibition of alpha toxin production by OHP, free toxin has virtually disappeared from the tissues and the hemolytic and necrotizing activity has ended. It would certainly explain the rapid improvement in the general condition of these patients.

For the time being, we must rely on hypothesis to explain the absence of lethal toxin activity (or production) after completion of hyperbaric oxygenation, even though this cannot be readily reconciled to van Unnik's finding that *in vitro* the clostridia remain capable of producing alpha toxin when returned to suitable anaerobic conditions. Our preliminary working hypothesis is that the temporary arrest of alpha toxin production during hyperbaric oxygenation at 3 ata and the simultaneous elimination of that toxin which is already present create a situation in which the clostridia cannot surround themselves with alpha toxin sufficiently concentrated to overcome local tissue defenses. When a second session of OHP is instituted several hours after the first, the same suppressive influence on the required minimal alpha toxin concentration becomes greater. This repeated suppression of toxin production leads, in turn, to a change in the environment of the clostridia, so that the requirements for optimal clostridial activity are no longer met. The transiently increased oxidation–reduction potential, the arrest of the activity of proteolytic enzymes in the tissue and the consequent arrest of the release of amino acids in the lesion during the 3 days of oxygen treatment may create a condition of the surrounding tissues unsuitable for the functioning and multiplication of *Cl. perfringens*. This change in the environment may persist even after discontinuation of the hyperbaric oxygen treatment.

This hypothesis would explain the rapid subjective improvement in the general

condition of patients with clostridial infection treated with hyperbaric oxygenation. Perhaps the strongest argument for this hypothesis is the fact that no clostridial infection was ever seen to progress at all once hyperbaric oxygen therapy at 3 ata had been started. Also, one must bear in mind that the environmental change in human infections is of an entirely different character from the readily reversible artificial change in broth media *in vitro*.

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Hyperbaric Oxygen Therapy in Recurrent Progressive Cutaneous Gangrene in Female Patients

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Over the past several years, four fertile female patients have been treated with hyperbaric oxygenation in our clinic for progressive cutaneous gangrene resembling the hemolytic streptococcal gangrene first described by Meleney in 1924.[1] The one exceptional finding in our patients was the absence of streptococcal or other apparent infection on bacteriologic study. In one patient, the cutaneous gangrene occurred after sterile operation in the postpartum period, and it recurred 4 years later after sterile adnexal surgery. In the second patient, the gangrene occurred after two caesarean operations performed 3 years apart. The third patient had one episode of the gangrenous process following sterile adnexal surgery, and the last patient had cutaneous gangrene involving the lower leg.

Case Reports

Case 1. A 22-year-old woman, hospitalized in 1956 for spontaneous childbirth, developed acute abdominal pain on the third day post partum with tenderness of the right lower quadrant and an abrupt rise in temperature to above 39°C. The patient was transferred to the surgical unit where a normal-appearing appendix was removed. After operation, a progressive cutaneous gangrene developed from the surgical wound (Figure 1A) and the patient was critically ill with severe toxemia and a continuous temperature of 40.5°C. She was treated by wide surgical excision of the diseased region, well into the unaffected tissues (Figure 1B), and no further complications arose (Figure 1C).

In September 1960, the patient was readmitted, in the second trimester of pregnancy, for extirpation of the left adnexa because of 180° adnexal torsion in which two dermoid cysts existed. Paramedian laparotomy was performed on September 6, and postoperatively the patient's temperature gradually rose to 39°C. The wound area was abnormally painful and an area of reddish discoloration became visible around it. On September 10, several greyish-blue bullae were observed in the wound margin, from which a bacterial culture was obtained. This proved negative. A day later, when it was clear that a colliquative necrotic process involved the wound edges, the wound was opened to expose the fascia and cleansed with hydrogen peroxide. The next day the process appeared to have stabilized. On September 13, the patient spontaneously delivered a 400-gm fetus. The cutaneous necrosis spread still further, the patient became icteric, and the temperature rose to

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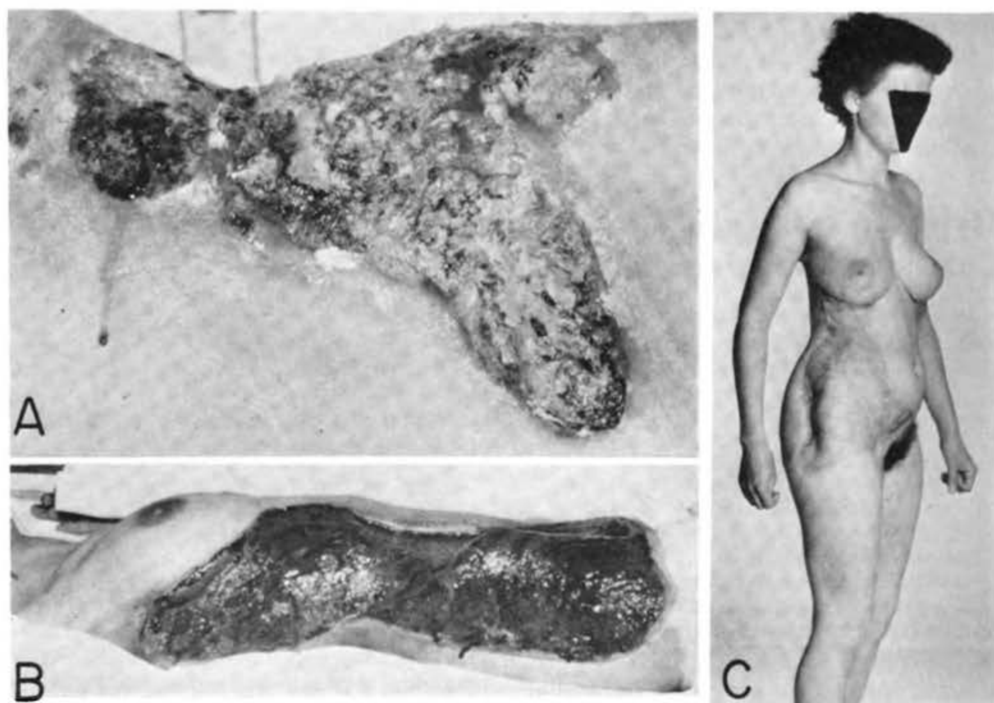

FIGURE 1. A, progressive cutaneous gangrene (Case 1) developing after appendectomy (December 16, 1956). B, wide surgical excision of diseased region (January 9, 1957). C, patient after healing complete, in 1958.

40°C. Repeated aerobic and anaerobic cultures recovered from the wound were again negative. On September 14, the patient was removed to the surgical ward, and the affected pathological area was excised to a margin of 2–3 cm beyond the diseased area (necrotic tissue being excised as far as the rectus abdominus and the aponeurosis of the external oblique muscles). This created a defect of 20 × 25 cm.

The bottom of the defect appeared to be well supplied with blood. Histologic examination of the excised material revealed a nonspecific necrotic process in the skin and subcutaneous tissues with inflammatory reaction, and bacterial culture again failed to demonstrate the presence of either aerobes or anaerobes in the excised skin section. The patient received 2 gm of tetracycline HCl (Achromycin) daily. The temperature declined temporarily but thereafter rose again to 40°C, and necrosis of the wound edges was again apparent. The wound area was further excised, without effect. Administration of antibiotics was discontinued. The phlegmonous periphery of the necrotic skin advanced to include the upper abdomen, reaching along the thoracic wall as far as the left axilla. The size of the affected area rendered further excision of tissue impossible, and the process failed to respond either to antibiotics or to local application of oxidizing agents.

In the absence of any further rational therapy at this point, the prognosis appeared extremely grave. Finally, on September 22, it was decided to make a last attempt to halt the progressive necrosis by increasing the available oxygen to the tissues by means of OHP. Consequently, the patient was placed in the hyperbaric chamber of the surgical department and given oxygen by mask at 3 ata. On 5 consecutive days, the patient was treated in this way for 2 hours daily. On the second day, she resumed taking some food and the fiery red phlegmonous spread toward the axilla decreased in intensity. She felt much better, although her temperature remained elevated. By the fifth day of high pressure therapy, the process had become

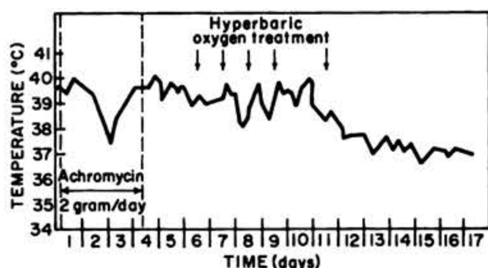

FIGURE 2. Temperature chart (Case 1), showing period of high fever and subsequent decline after the 5-day hyperbaric oxygen treatment.

localized and granulation was satisfactory. Subsequently, the disease gave no more cause for anxiety, the fever declined (Figure 2), and the wound surface was repaired with Thiersch's skin grafts (Figure 3).

Case 2. A 22-year-old woman was admitted to the Amsterdam University Women's Clinic at term of her third pregnancy on December 27, 1961, after labor pains of several hours. Considering her obstetrical history of two previous caesarean sections due to pelvic contraction, it was decided to again use this method. A few hours after admission, a suprasymphysial cervical section was performed without incident, and a 4160-gm male infant was delivered. Two days after delivery, the patient's temperature began to rise but the only physical abnormality which could be found was an "infection" in the operative wound just above the symphyses. After material had been collected for a bacterial culture, antibiotic therapy was instituted (1 million units of penicillin and 1 gm of streptomycin every 24 hours). Despite this treatment, the temperature rose rapidly during the next few days and was attended with chills. It reached its peak (40.4°C) on January 1, 1962.

At the same time, a progressive lesion, emanating from the operative site, developed with bluish-red discoloration of the adjacent skin. It was very painful to the touch, and a wide erythematous areola had formed around the lesion. In the affected skin area, which had extended to cover an area of 20 × 17 cm, bullae filled with turbid fluid developed; epidermolysis was apparent in the area (Figure 4). Repeated aerobic and anaerobic cultures from blood specimens and material collected from the wound area proved to be sterile. Since antibiotic therapy failed to affect the rapid spread of the lesion, it was suspended on January 1.

Because of our previous encouraging experience in similar cases, we decided to treat this patient also with oxygen inhalation at high pressure. Consequently, the patient was given oxygen by mask in the hyperbaric chamber at 3 ata during five 2-hour sessions (on January 2, 3, and 4). Thereafter, the process appeared to be arrested, and the temperature gradually dropped. By January 10, the temperature was normal, and the wound had cleared to the extent that a fine granulating surface had formed. On January 21, 1962, the patient was discharged completely healed.

Three years later, the patient was delivered of her fourth child, again by caesarean sec-

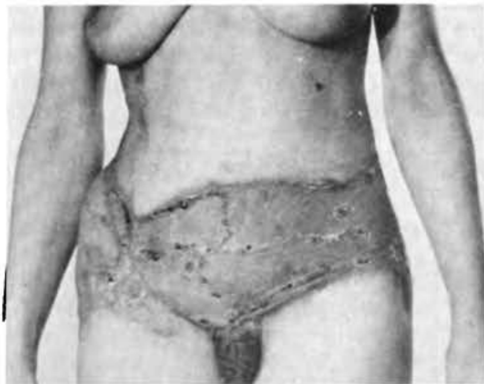

FIGURE 3. Case 1, extent of lower abdominal involvement from second bout of progressive cutaneous gangrene (see also Figure 1). Photograph was taken after grafting and healing.

FIGURE 4. Progressive cutaneous gangrene (Case 2) of abdominal wall after caesarean section (December 1961). The lesion healed completely after a course of hyperbaric oxygen therapy.

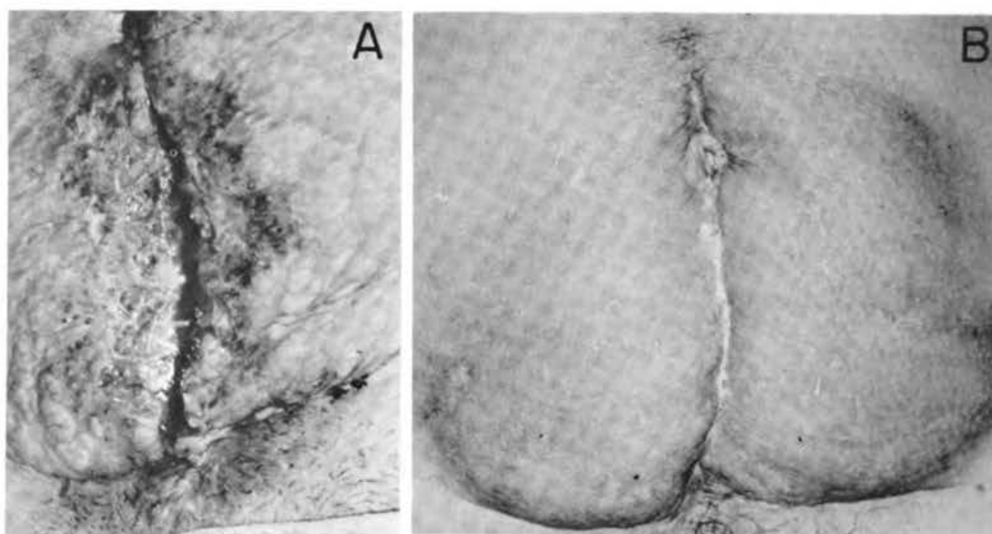

FIGURE 5. A, second bout of abdominal wall skin gangrene in Case 2, following another caesarean section in 1965. B, complete healing a few weeks after termination of OHP therapy.

tion. Five days after delivery, the abdominal wall around the midline incision showed the same rapidly progressive lesion as 3 years before (Figure 5A). This area was extremely painful and the patient exhibited severe toxemia; she had chills and the temperature rose to 40°C. Biochemical investigation of liver function, electrolyte balance, and blood proteins showed no abnormalities. Aerobic and anaerobic cultures from wound fluid, blood, urine, and vaginal smears were repeatedly negative. No antibodies against striated muscle or nuclear antigens were demonstrable, nor were there complete or incomplete cytotoxic antileukocyte antibodies. No antibiotics were administered.

Because of our previous experience with this patient in 1961, we decided to again attempt hyperbaric oxygenation. Oxygen was delivered by mask in the chamber at 3 ata during 14 2-hour treatment sessions (given twice daily, for 7 days). On the third day, the patient volunteered that she felt much better and she started to eat. From then on, the temperature decreased (Figure 6), and the whole course of the disease became favorable. The wound, which had already spontaneously cleared, started to granulate and epithelize. The patient was discharged well 25 days after the caesarean section had been performed (Figure 5B).

Case 3. A 39-year-old married woman underwent curettage for dysmenorrhea at another hospital in May 1963. Two days after curettage, a Pfannenstiehl laparotomy was performed and the right cystic adnexa extirpated. Four days later the temperature rose to 39°C, and a reddish-blue infiltrate with epidermolysis developed (Figure 7A). At this time, she was transferred to our institution. Blood and urine cultures were negative. The wound fluid yielded an occasional staphylococcus which the bacteriologist presumed not to play any causative role. Nevertheless, penicillin and streptomycin therapy was instituted. The patient was submitted to hyperbaric oxygen therapy (2 hours, twice daily for 4 days). Subsequently, she felt much better and the fever decreased, but the local subcutaneous defect seemed to progress. On the fifth day, the entire pathological area including the anterior rectus

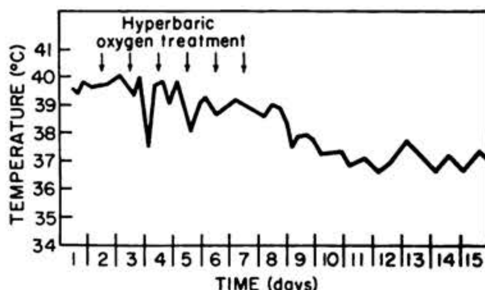

FIGURE 6. Temperature chart (Case 2), showing decline in fever after hyperbaric oxygen treatment.

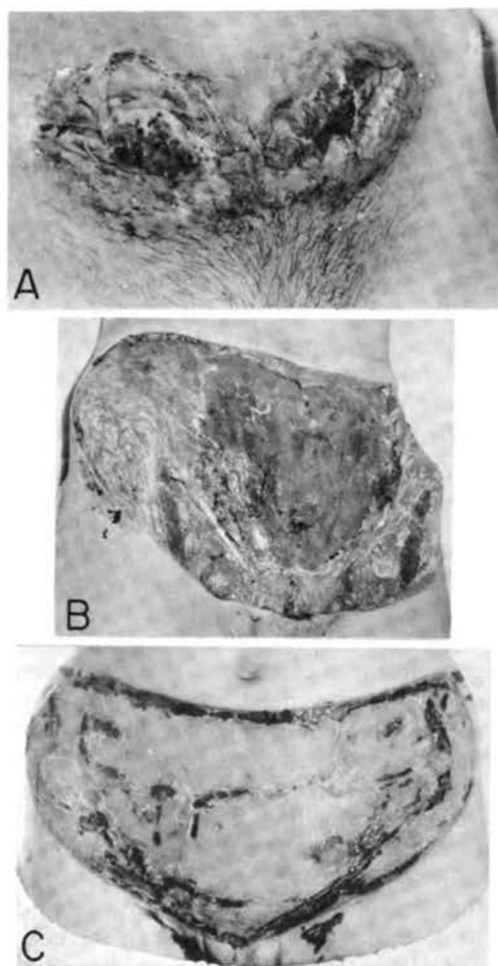

FIGURE 7. A, progressive cutaneous gangrene (Case 3) of abdominal wall just before hyperbaric oxygen therapy. B, 11 days later after wide excision of involved gangrenous area subsequent to OHP treatment. C, healing of grafted defect 5 weeks later.

sheath was excised (Figure 7B). The further course with plastic repair (Figure 7C) was uneventful.

Case 4. A 48-year-old woman, mother of 12 children, developed a progressive reddish-blue infiltrate with extensive epidermolysis on the anteromedial side of the lower leg which, within a short time, resulted in a large ulcerous defect with undermined edges. (History failed to uncover any traumatic origin of which the patient was aware.) A reddish infiltrate extended upward to within 10 cm of the knee joint (Figure 8A). Cultures from wound fluid and blood were repeatedly negative. Biochemical tests for blood proteins, liver function, serum electrolytes, *etc.*, were normal. Immunological investigation for antileukocyte nuclear antibodies was negative. The patient was treated at another hospital with massive doses of penicillin, streptomycin, tetracycline HCl (Achromycin), and erythromycin, in that order, but she failed to respond favorably. The patient was transferred to our clinic, with a temperature of 40.2°C. We stopped all antibiotic therapy and decided, on the basis of this patient's clinical resemblance to Cases 1–3, to give hyperbaric oxygenation a trial. Oxygen was given by mask at a chamber pressure of 3 ata during seven 2-hour sessions over a period of 3 days. The local lesion responded favorably, and the patient's general condition improved within 48 hours; in 7–10 days the wound cleared spontaneously and showed fresh granulations (Figure 8B). The patient was discharged in good condition after 4 weeks.

DISCUSSION

The clinical course of these patients closely resembled the picture described

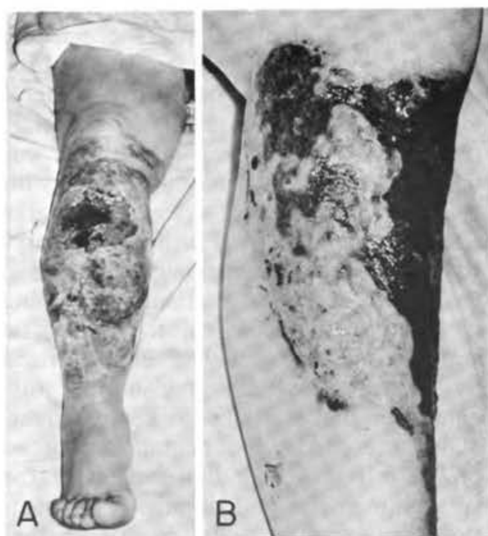

FIGURE 8. A, large ulcer surrounded by undermined area of progressive cutaneous gangrene of anterior lower leg (Case 4). B, clearing of lesion with fresh granulations and ingrowth of new epithelium 10 days after a course of hyperbaric oxygenation.

by Meleney as characteristic of hemolytic streptococcal gangrene.[1-3] They all exhibited a rapidly progressive cutaneous gangrene preceded by necrosis of subcutaneous tissues, and toxic manifestations were severe and generalized. They deviated from his description only in that *Streptococcus hemolyticus* could not be recovered from the necrotic foci (an important variation, to be sure). Later reports[4] have appeared in the literature, however, describing patients with similar lesions in whom all bacterial cultures proved negative for both aerobes and anaerobes.

Typically, hemolytic streptococcal gangrene occurs at the site of a deep wound (either accidentally or surgically inflicted), but occasionally the causative trauma is so minor as to suggest spontaneous origin of the condition. Careful history, however, almost always reveals that a cut, scratch, or even small injection puncture was present at the site where gangrenous infection started.

This infection usually follows a rapid course with severe toxic manifestations. Frequently, as early as 24 hours following onset, the temperature may reach 39°C or higher. Hemolytic streptococci can usually be recovered from the zone of infection, particularly around its periphery. Unlike the clinical course of erysipelas, the onset of hemolytic streptococcal cutaneous gangrene is seldom attended with chills, and the infected area is not markedly erythematous. Furthermore, erysipelas is not accompanied by necrosis, the center of the infected area retaining its normal inflamed color. Hemolytic streptococcal gangrene is characterized by skin necrosis with grayish-black discoloration that starts in the central areas. This is preceded by a subcutaneous spread of the infection with liquefaction of the subcutaneous fat and dermis. The infection may spread rapidly, covering rather wide areas of a part, with increasing toxic and febrile reactions in the patient.

Another form of cutaneous gangrene is the more chronic variety first described by Cullen in 1924[5] and later, in 1926, shown by Brewer and Meleney[6] to be a synergistic infection caused by a microaerophilic nonhemolytic streptococcus and a hemolytic staphylococcus (var. *aureus*). This latter infection, although progressive and frequently resistant to therapy, is not usually accompanied by serious toxic or systemic symptoms as in the cases cited above.

Ordinarily, hemolytic streptococcal gangrene is responsive to antibiotic treatment, particularly if started early in the course of the infection. However, our cases, which did not reveal the hemolytic streptococcus, were resistant and progressed despite what was considered adequate antibiotic therapy. While hyperbaric oxygen treatment appeared to bring about striking improvement in our four patients with acute cutaneous gangrene,[7-10] we are uncertain as to the mechanism by which this improvement occurred, particularly since no offending organisms were cultured. Perhaps the efficiency of increased delivery of oxygen to the periphery of the spreading infection acted in our cases to deter the process. Although the effect of OHP in these cases seemed impressive, it should be noted that there was a lapse of 2–3 days between the beginning of therapy and the onset of significant improvement. This was in contrast to our observations in the treatment of clostridial gas gangrene by hyperbaric oxygenation, where improvement was usually noted shortly after the first exposure to OHP.

Whether the obstetrical or gynecological histories of our patients had any bearing on the etiology of this pathological condition is not clear, but the circumstances suggest a possible relationship.

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Hyperbaric Oxygen Therapy in *Clostridium perfringens* and *Peptococcus* Infections

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Since the report of Brummelkamp, Hoogendijk, and Boerema in 1961,[1] on the treatment of anaerobic infections with oxygen under increased pressure, gas gangrene has been viewed with renewed interest by several investigators using this form of therapy.[1-3] From April 1964 to date, we have used hyperbaric oxygenation to treat 16 patients with clostridial infections (Table 1).

Our 16 patients have ranged in age from 3 to 70 years, and almost all have been referred to us for hyperbaric treatment by physicians from Minnesota and six surrounding states. In most cases, gas gangrene occurred in contaminated fracture wounds, although a variety of other types of trauma were also present in these cases.

TABLE 1. Types of Infection and Debridement Before OHP

	No. patients	Debrided before OHP
Cellulitis	2	2
Localized myositis	4	2
Cellulitis + localized myositis	1	1
Diffuse myositis	8	3
Uterine infection	1	1
Total	16	9

TREATMENT PROCEDURE

Our procedure has been to evaluate the patient's general physical status and the condition of his wounds on admission, the latter being done in the operating room under anesthesia when necessary. When a large amount of obviously devitalized tissue is present, debridement is performed at this time. Also, specimens for aerobic and anaerobic culture are taken. Blood cultures are obtained if the patient is febrile. When thorough evaluation of the patient has excluded any contraindications to hyperbaric therapy, the patient is pressurized to 3 ata and given oxygen by a tight-fitting oronasal mask equipped with a demand valve. After 2 hours at 3 ata, decompression is started. (At first, we used U. S. Navy standard decompression tables, but more recently we have been using oxygen decompression tables modified for hyperbaric chamber use by R. D. Workman and E. H. Lanphier.[4,5]) When trache-

otomy is performed, a respirator is used to administer oxygen.

The basic schedule for our gas gangrene therapy is: three treatments in the first 24 hours, two treatments in the second 24 hours, and one treatment in the third 24 hours. More seriously ill patients are treated eight or nine times, however.

Since we consider hyperbaric oxygen to be only a powerful adjunct in the management of this disease, vigorous supportive measures are also used as indicated. Unless allergy is present, all patients receive large doses of intravenous aqueous penicillin in addition to a broad-spectrum antibiotic, usually tetracycline hydrochloride or chloramphenicol. Other antibiotics are given as indicated. In five patients, surgical debridement had been done prior to admission; four patients were debrided by us before institution of hyperbaric therapy (column 2 of Table 1 shows which cases were debrided before hyperbaric treatment).

Gas gangrene antitoxin was used in only two patients early in the series. Six patients had received varying amounts of antitoxin before referral for hyperbaric oxygen therapy; all were becoming progressively more ill at the time of transfer.

Illustrative Cases

Case 1. Our first patient, an 18-year-old boy who had sustained multiple fractures (one of which was a compound fracture of the left femur), was the only one to die before infection was brought under control. Thirty-six hours after treatment of the fractures, the patient developed profound shock, which was vigorously treated by his surgeons. Gas gangrene was diagnosed. Extensive debridement of the anterolateral thigh muscles was performed and the patient was referred for hyperbaric oxygen therapy. On admission, he was in shock, oliguric, and moribund. Two treatments were given. The patient's condition remained precarious, and cardiac arrest developed 6 hours after the second treatment and 20 hours after admission. He could not be resuscitated. This is the only death due to gas gangrene in our series.

Case 2. The second patient was a 70-year-old man, referred to us after developing a fecal fistula and gas gangrene involving the abdominal wall, flank, and chest wall up into the left axilla, following a left hemicolectomy for cancer. The patient was in shock, had renal failure, was unresponsive, and was in severe respiratory distress on admission. He was resuscitated with intravenous fluids, aspiration of the tracheobronchial tree, and respiratory assistance. Approximately 1 hour after admission, the patient had a cardiac arrest. He was successfully resuscitated, a tracheostomy was done, and respiratory assistance was continued. An electrocardiogram showed a large myocardial infarction. Blood pressure was maintained with vasopressors, and, after 12 hours, hyperbaric oxygen therapy was started. After a total of six treatments in the chamber, the infection seemed to be under control, so hyperbaric therapy was discontinued. During exposure to hyperbaric oxygen, smaller doses of vasopressors were needed to maintain blood pressure. After the fourth treatment, vasopressors were no longer necessary. Six hours after the last treatment, the patient developed cardiac arrest and could not be resuscitated. Autopsy permission was refused.

Case 3. Our third patient who had localized clostridial cellulitis and myositis around the elbow, secondary to a compound fracture, died 3 weeks after his infection had been brought under control by three hyperbaric oxygen treatments. Death was due to peritonitis secondary to perforated duodenal ulcer which had been patched. He had multiple injuries on admission. This death was not related to the clostridial infection.

The following selected case histories will demonstrate some of the problems in the management of these patients:

Case 4. An 11-year-old boy had sustained a compound fracture of the right lower leg when a tractor ran over him 2 days before admission to our facility. He was treated in his home town by debridement, primary closure, reduction of the fracture, and ap-

plication of a cast. Two days later, the cast was removed and foul-smelling discharge and bubbles were escaping from the wound. The wound was opened widely but not debrided. One dose of polyvalent gas gangrene antitoxin was given and one dose of erythromycin, and the patient was transferred to the Hennepin County General Hospital.

On admission, the youngster was acutely ill, with a temperature of 40.2°C and a large foul-smelling wound of the right lower leg. Bubbles were exuding from the surface of the wound, and bone was exposed. There was edema and discoloration of the thigh up to the groin. No pedal pulses were palpable. X-ray photographs showed extensive dissection of gas along the tissue planes. The patient was allergic to penicillin and was given Chloromycetin and tetracycline. *Clostridium perfringens* was cultured from the wound.

After three treatments in the chamber, the patient was taken to the operating room for debridement. When the muscles of the calf were cut, there was no bleeding nor reaction in the muscles. It was obvious at this point that a crush injury of the lower leg had taken place initially. A guillotine amputation was performed through the open wound 10 cm below the knee. Despite five more chamber treatments, the patient did not respond to therapy until he was started on sodium nafcillin in high doses. The infection was then brought under control. A total of 4000 ml of blood and 1250 ml of plasma was given to this patient as supportive therapy.

Case 5. A 13-year-old boy had been injured 4 days before transfer to our hospital, when a tractor on which he was riding was hit by an automobile. He sustained bilateral compound fractures of his lower legs. Two days after injury, evidence of gas gangrene was present, and a fasciotomy was done on the dorsum of the left foot, with some improvement. Twenty milliliters of gas gangrene antiserum, lincomycin, and tetracycline hydrochloride were administered.

On admission, the left lower leg seemed to be nonviable almost to the knee. There was induration and edema of the entire leg up to the iliac crest and also patches of induration of the right leg. Very definite crepitation was felt in the necrotic skin area of the left lower leg. The patient was taken to the operating room, where debridement of the left lower leg was carried out, along with amputation of the distal third of the tibia and fibula. Tetracycline (500 mg)was given intravenously every 6 hours. He was reported to be allergic to penicillin. Cultures of the excised tissue revealed *Cl. perfringens.*

The patient subsequently had a total of nine hyperbaric oxygen treatments, with good response. He received a total of 4000 ml of blood and a total of 1750 ml of plasma. He needed no further treatment except revision of the amputation stump and was discharged 24 days after admission.

Case 6. A 20-year-old woman had a classical caesarean section in our hospital because of a transverse lie. Postoperatively she developed evidence of a pelvic inflammatory disease believed to be an infected broad ligament hematoma. The abdominal wound disrupted at 9 days, and the patient was taken to the operating room where the hysterotomy wound was found to be disrupted and the uterine muscle necrotic. A subtotal hysterectomy, appendectomy, debridement, and closure of the abdominal wound was done. Cultures revealed *Cl. perfringens*, peptococci, alpha streptococci, and pseudomonas organisms. Postoperatively, her course was characterized by ileus and toxemia for 4 days. Multiple antibiotics were given. A total of nine hyperbaric oxygen treatments were given, with dramatic response. No additional surgical treatment was necessary. She was discharged 9 days after completion of hyperbaric treatment. A total of 4500 ml of blood and 5250 ml of plasma was given to this patient during 4 weeks of hospitalization.

Case 7. A 13-year-old boy fell from a tree and was impaled on a steel post. The post penetrated the left upper quadrant of the abdomen, entered the left chest, injured the left lung, and fractured the posterior sixth and seventh ribs. Immediate surgical treatment consisted of splenectomy, repair of the rent in the left diaphragm, repair of perforations in the stomach, placement of tubes in the left pleural space, debridement of the abdominal wound, and tracheostomy. Two days after operation, he became febrile and toxic. Brownish discoloration of the skin

developed over the left abdomen, flank, and back and the left hemithorax. There was subcutaneous emphysema. Cultures from the left chest revealed a gram-positive rod thought to be *Cl. perfringens*, and the patient was referred for possible hyperbaric oxygen treatment.

On admission to our hospital, the boy was cyanotic and respirations were labored at a rate of 50/min. The blood pressure was 110/60 mm Hg and the pulse rate was 160/min. Chest x-ray films showed a hydropneumothorax on the left side, an infiltrate and segmental atelectasis in the right lung, and subcutaneous and intramuscular emphysema in the soft tissues along the left lateral hemithorax and upper abdomen. There was a foul-smelling thin purulent drainage coming from around the chest tubes. A smear of this fluid showed gram-positive rods, and cultures demonstrated *Cl. perfringens*.

New chest tubes were placed in the left pleural space, assisted ventilation with a positive-pressure respirator was accomplished through the tracheostomy tube, and intravenous fluids and large doses of intravenous penicillin and Chloromycetin were given. The systemic arterial pO_2 was 43.3 mm Hg, the pCO_2 was 38.5 mm Hg, and the pH was 7.3.

We felt that hyperbaric oxygenation was indicated despite the damaged left lung and the probable pneumonia in the right lung. Table 2 summarizes the blood gas and pH studies. At 1 ata, the boy was cyanotic on the respirator, breathing 100% oxygen. The systemic arterial pO_2 was 28.5 mm Hg, the pCO_2 was 16.7 mm Hg, and the pH was 7.41. Because of the bilateral pulmonary damage, we decided to pressurize to 2 ata and determine systemic arterial oxygen tensions. At 2 ata, the systemic arterial pO_2 was 129 and 165 mm Hg on two determinations. The pressure was increased to 3 ata, and systemic arterial readings of 574, 702, and 2121 mm Hg were obtained. The discrepancy in these three readings, with the one high value, cannot be explained.

After 33 min at 3 ata, the boy developed profuse sweating and restlessness. Both lungs were wet and difficult to ventilate, indicating a decrease in compliance. The respirator was detached from the endotracheal tube and cyanosis developed. The blood pressure rose to 160 mm Hg systolic, and the heart developed an irregular rhythm. Because of the cyanosis and cardiac irregularity, oxygen was restarted and the patient's condition improved. After approximately 1 hour at 3 ata, decompression was started and was well tolerated. At 1 ata, while on the respirator using 100% oxygen, the patient's color was good and the arterial pO_2 was 212 mm Hg. The bilateral pulmonary edema was much improved.

During the second and third treatments at 3 ata, the lungs again became wet, with watery blood-tinged secretions, and there was decreased compliance of the lungs. The boy had a total of eight treatments. The last five were tolerated well, and arterial pO_2 values of 1362, 1336, and 1526 mm Hg were measured. With each treatment the patient's general condition improved, and, at the end of the eighth treatment,

TABLE 2. Blood Gas and pH Values of 13-Year-Old Boy with *Cl. perfringens* Infection During OHP Therapy

Treatment session	Pressure (ata)	pO_2* (mm Hg)	pCO_2 (mm Hg)	pH
1	1	28.5	16.7	7.41
	2	129	17.7	7.43
	2	165	18.3	7.42
	3 (15 min)	702	42.6	7.33
	3 (35 min)	2121	56.3	7.40
	3 (45 min)	574	52.1	7.40
	1	212	35.7	7.48
3	3	1362	39.4	7.44
5	3	1336	30.4	7.51
7	3	1526	17.3	7.39

* On respirator using 100% oxygen.

the patient was interested in food and his surroundings. After the third treatment, there was an improvement in the pulmonary status.

The patient's hospitalization was complicated by an episode of a bloody enterocolitis, with *Candida albicans* overgrowth, and the development of infectious mononucleosis with mild hepatitis. A left empyema was converted to open drainage. The boy was discharged well approximately 7 weeks after admission.

Discussion

The evaluation of one form of therapy compared with another is difficult when various therapeutic procedures are combined.[6] Initially we thought, as did others,[7] that surgical debridement would not be needed until the systemic aspects of the infection were brought under control. Our experience has shown that debridement of obviously devitalized tissue should not be delayed until after hyperbaric treatment is concluded. In this regard, we concur with Smith and associates.[7] The second point of importance is demonstrated by Case 1—that in case of penicillin allergy a suitable bactericidal drug should be substituted for control of the infection. However, this is not invariably true, because in Case 2 only tetracycline was administered.

The value of antitoxin is as yet unsettled. Until more conclusive evidence on its effectiveness is available, we will refrain from its use.

Although a distinct improvement has been noted in most patients after the first day of treatment, we have not encountered the dramatic response reported by the Amsterdam group so early in the course of treatment.[1,2] The need is clear for defined indications for hyperbaric oxygen therapy in this disease. We believe that simple cellulitis of the extremities is curable by appropriate surgical and antibiotic management alone. Localized myositis of the extremities could possibly be treated with surgical debridement and antibiotics alone, but sometimes local myositis becomes diffuse and spreading and the disease is much harder to control. On the other hand, acute spreading clostridial myositis is a clear-cut indication for hyperbaric oxygen treatment. Case 7 demonstrates clearly the difficulties which may be encountered in accomplishing hyperoxygenation in patients with lung damage. It is well known that the two essentials for hyperoxygenation are healthy lung parenchyma and absence of shunting of blood outside the lungs (right-to-left shunts). This case also emphasizes the need for monitoring the pO_2 of every patient receiving oxygen therapy under increased pressure.

ACKNOWLEDGMENTS

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DISCUSSION

Discussion of papers by Altemeier (pp. 481–491), Brummelkamp, (pp. 492–500 and 501–507), and Arnar et al. *(pp. 508–513).*

DR. D. C. SABISTON, JR., *Session Chairman (Durham, N. C.)*: This response of anaerobic infections to hyperbaric oxygenation is one of the most important and controversial issues in this field. For this reason, it is particularly important to have an accurate and concise definition and classification of the various gas-forming types of infection. Dr. Altemeier is a surgical scholar who has had wide experience in surgical bacteriology, and who is a recognized authority in the field of antibiotics for surgical infections. More important, he has carefully evaluated a large group of patients with gas-forming infections due both to clostridial and non-clostridial organisms. This has indeed been an unusually objective presentation, beautifully supported by objective data.

DR. V. FREDETTE *(Montreal, Canada)*: I am inclined to believe that the mechanism of action of hyperbaric oxygen in *Clostridium perfringens* infections is not as simple as Dr. Brummelkamp would have us believe. First, we must contend with the rapidly invasive power of the *perfringens* bacillus; second, the alpha toxin is not the only cause for the toxicity of the *perfringens* bacillus. Beta-hemolysin and kappa-toxin (collagenase) are equally lethal. In our own experiments concerning the genetics of the virulence in the *perfringens* bacillus, we have been able to permanently suppress the capacity of our strains to produce alpha toxin, but we are still left with cultures virulent enough to kill guinea pigs.

DR. C. HITCHCOCK *(Minneapolis, Minn.)*: Dr. Altemeier, what would be the mortality rate in critical patients who have true clostridial myositis of the disseminated type, such as those studied by our group and by Dr. Brummelkamp? Also, what would be the mortality of patients treated without hyperbaric oxygen? We are in complete agreement with you that the cellulitis cases would probably do well without hyperbaric oxygen, but of our 10 patients who were critically ill we lost only two, which would be a fairly respectable survival for a serious infection of this kind.

DR. ALTEMEIER: It is difficult, of course, to arrive at a realistic mortality figure for true cases of clostridial myositis. The usual death rate reported in large series of the true, rapidly fulminating, spreading clostridial myositis is approximately 40%. In the 42 cases which we studied, the mortality rate was 12%. This was with the methods of treatment which I described, using active diagnostic methods, early introduction of surgical decompression or amputation as indicated, vigorous supportive therapy, and intensive antibiotic therapy. Massive doses of penicillin and a broad-spectrum agent, usually tetracycline, were administered intravenously in most instances in recent years.

UNIDENTIFIED SPEAKER: Dr. Altemeier, what are your feelings about the use of antiserum?

DR. ALTEMEIER: This has been debated many times. The general experience and the thinking in this country are that the polyvalent clostridial antitoxin is of limited or no value. Those who believe it to be of limited value agree with the British who have indicated the polyvalent antitoxin may have some usefulness in the overwhelming toxic case by providing or sustaining life for a period of 2, 3, or more days until other treatment is effective. As a result of our own experience, we have not used gas gangrene antitoxin prophylactically since 1943. We still use polyvalent antitoxin in the overwhelmingly toxic lesions, however.

Gas Gangrene Treated in a One-Man Hyperbaric Chamber

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In late 1962, we began using hyperbaric oxygen in the treatment of gas gangrene at the Karl Bremer Hospital.[1-3] Several patients had come to us during that year for treatment, and having no compression chamber at our institution we were offered the use of a collapsible one-man Dräger unit by the South African Naval Base at Simonstown. This chamber was used for all our patients until April 1965, when our Vickers one-man unit was delivered.

Following is a brief overall review of our clinical experience with the treatment of clostridial gas gangrene, including the criteria for selection of patients to be exposed to hyperbaric oxygen, the timing of debridement, and the place of the administration of antiserum.

Patient Selection

In our 3 years of experience with OHP in the treatment of gas gangrene, 44 patients have been referred to us for therapy. Of these, however, 15 have been deemed unsuitable for treatment in the chamber, on the grounds of either clinical evaluation or bacteriologic findings. In none of these 15 patients could clostridial organisms be isolated. Thus, 29 patients were accepted for hyperbaric oxygen therapy. Although no clostridia could be cultured from 13 of these, they were selected for treatment in the chamber because the clinical picture was so suggestive of gas gangrene. Of the 16 patients with proven clostridial infection, 12 had gas gangrene resulting from surgical or accidental trauma, and four were diabetics in whom the gas gangrene arose spontaneously. In this latter group, two had infection involving the perineum, one the abdominal wall, and one the foot and leg.

Generally, the patients referred to us for hyperbaric oxygen therapy fall into four categories: (1) those in whom clostridial infection can be confidently ruled out and consequently OHP treatment also, (2) those in whom a diagnosis of clostridial gas gangrene is considered unlikely, with treatment deferred pending bacteriologic investigation, (3) those in whom the diagnosis is fairly certain, and for whom OHP treatment is begun with the decision to give further exposures depending upon the bacteriologic report and reaction of the patient, and (4) those

in whom the diagnosis is clinically certain and bacteriologic study is merely confirmatory. In the third group, even if the culture is negative, therapy may be continued if the patient responds encouragingly.

Treatment Methods

When these patients are admitted to our hospital, they are usually critically ill and in great pain. Thus, if a trial of hyperbaric oxygen is decided upon, time is taken only to: (1) administer an intravenous infusion of dextrose solution, with an appropriate covering dose of insulin for diabetics, (2) perform myringotomy, (3) draw blood for biochemical examination and blood compatibility tests (should transfusion be necessary at termination of the first OHP treatment), and (4) incise any tensely swollen areas, without anesthesia if overlying skin is dead and with local anesthesia if it is alive. Material for bacteriologic examination is obtained from such incisions, as well as from any open wounds or necrotic areas.

Hyperbaric oxygen therapy, previously administered in the Dräger unit, has, since April 1965, been given in the one-man Vickers chamber. Over a 15-min period, the patient is compressed to 2 ata in 100% oxygen supplied from cylinders. The pressure is maintained for 2 hours, after which staged decompression is carried out over a 30-min period.

Debridement is postponed until progression of the lesion has been halted by the hyperbaric oxygen (whether the infection involves muscles or only subcutaneous tissue). We have followed this course because debridement merely occasions delay by dealing with tissues already dead which play no further active role in the disease. It must be remembered that this delay may amount to several hours in our situation, where the patient cannot be directly attended during pressurization in the chamber. We therefore direct our treatment resources first to the actively progressing lesion in the tense phlegmon by administering increased oxygen to the tissues, on the rationale that there will be ample time for wide incision, debridement, or amputation after the first or subsequent treatments. Furthermore, when the progress of the lesion has been halted, the demarcation between dead and live tissue is more clearly defined, the patient's resources are less strained and anemia has been corrected, and, in the case of diabetics, insulin balance has usually been fully restored.

We have not used gas gangrene antiserum in the treatment of our patients, although two did receive it before they were referred to us. Any minor beneficial effect obtainable from the use of this preparation has seemed to us negligible in comparison with the benefits of hyperbaric oxygen. Antibiotic administration is a routine part of the therapeutic regimen, and usually consists of penicillin given in dosages of 10 million units/day at first, the subsequent choice and dosage of antibiotics depending upon bacteriologic findings.

Results

Twenty-nine patients with gas gangrene were treated with hyperbaric oxygenation at our hospital, 16 of whom had bacteriologically proven clostridial gas gangrene. Of the 13 patients with nonclostridial infections, eight showed no significant improvement from OHP therapy and died, two lived but showed no real change and recovered only after amputation, and three responded dramatically to hyperbaric oxygen therapy.

The results in the 16 patients with proven clostridial gas gangrene were much more impressive (Table 1). The effects of OHP in two cases are illustrated in Figure 1 (Case 11) and Figure 2 (Case 12). Fourteen responded dramatically to the OHP therapy, with rapid elimination of the clostridial infection. The two pa-

TABLE 1. Résumé of 16 Cases of Proven Clostridial Gas Gangrene Treated with OHP

Case no.	Age	Sex	Lesion	Extent of gangrene	Tissue loss	Outcome
1 [a]	56	F	Multiple infected areas at injection sites on anterior abdominal wall	Extensive areas of skin, subcutaneous tissue	Skin, subcutaneous tissue, with muscle aponeurosis	Died 2 months later from staphylococcal infection
2 [a]	48	M	Perineal abscess	Perineal tissues	Skin, subcutaneous tissue	Recovery with colostomy
3	47	M	Traumatic amputation at thigh	Skin, subcutaneous tissue, muscle	Slightly more than pre-OHP treatment excision	Recovery
4	35	M	Stab wound at elbow	All tissues of forearm, gas to middle of upper arm	Amputation at stab-wound level	Recovery
5	6	M	Compound fracture of forearm	All tissues of forearm	Skin, subcutaneous tissue, some muscle, three fingers	Recovery (with sound bone union)
6	37	M	Electric burn of upper arm	All tissues of upper arm, gas into neck	Amputation at level of injury	Recovery
7	20	M	Gunshot wound, buttock and loin	Buttock, loin, abdominal cavity		Died shortly after first treatment
8 [a]	52	M	Perineal abscess	All perineal soft tissues	Perineal skin and subcutaneous tissue	Died after OHP therapy, from diabetic nephropathy
9	68	M	Amputation of leg (arteriosclerotic gangrene)	All tissues of stump	Minor, local	Died 1 week later from stroke
10	23	F	Caesarean section	Abdominal cavity, uterus, abdominal wall (determined at autopsy)		Died at end of first treatment
11	40	M	Stab wounds of upper arm	Anteromedial aspect of arm, gas into neck	Amputation of little finger, flexor muscles of upper arm, injury to ulnar and medial nerves	Recovery
12 [b]	25	M	Traumatic amputation below knee, compound derangement of knee joint, compound fracture of femur, penetrating wound in groin	Fulminating gas gangrene of stump, phlegmon over posterior aspect of entire thigh, gas in thigh and groin wound	Post-treatment guillotine amputation through femoral fracture	Recovery
13 [b]	32	M	Compound fracture of tibia and fibula, with gross lacerations	Fulminating infection of leg, phlegmon into lower thigh, gas above knee	Debridement, leg fixed on splint, amputation below knee later	Recovery
14	42	M	Compound subtrochanteric fracture of femur	Entire hip region	Minimal soft-tissue loss (amputation averted)	Recovery, with chronic osteitis
15	17	M	Lacerated adductor muscles of thigh, compound fractures of femur and tibia	Entire limb	Amputation at hip joint for useless limb	Recovery
16 [a]	62	F	Limb ischemia	Lateral side of calf	Midthigh amputation after treatment	Recovery

[a] Diabetic patients.
[b] Both of these young men were riding a motor scooter together when it was struck by a car.

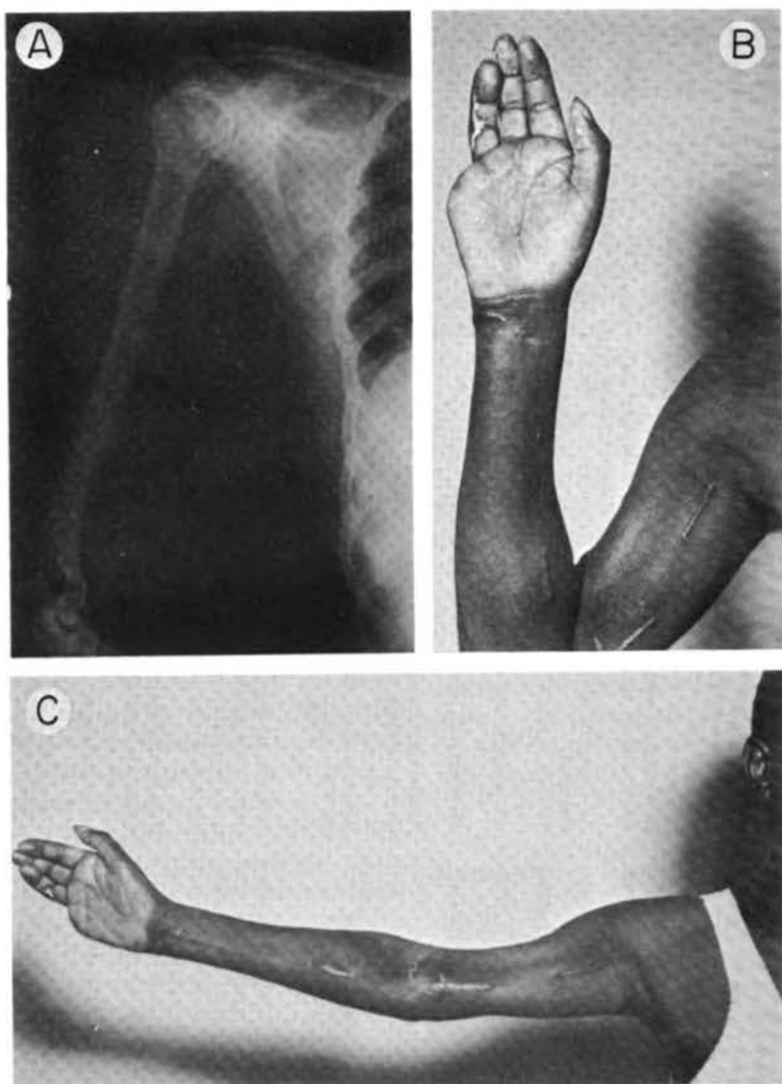

FIGURE 1. A, roentgenographic evidence of gas infiltration of shoulder region in a patient who, on his job as a watchman, was stabbed in the upper arm just above the elbow and subsequently developed clostridial infection in the wound site. B and C, healing of wound (ragged scar) after OHP therapy. (Other scars resulted from surgical incision.) The little finger had to be amputated because of necrosis, but the remainder of the hand and arm was saved. Recovery of function was delayed by concomitant nerve injury.

tients who failed to benefit both died at the end of their first treatment (Cases 7 and 10), but this was not surprising as both were moribund by the time they were referred to us. These were the only two deaths in the series occurring as a direct result of the clostridial infection.

Four diabetics with proven clostridial gas gangrene (Cases 1, 2, 8, and 16) all had lesions of the cellulitic type which were not the consequence of any gross trauma. Only two of these four patients have been long-term survivors (Cases 2 and 16).

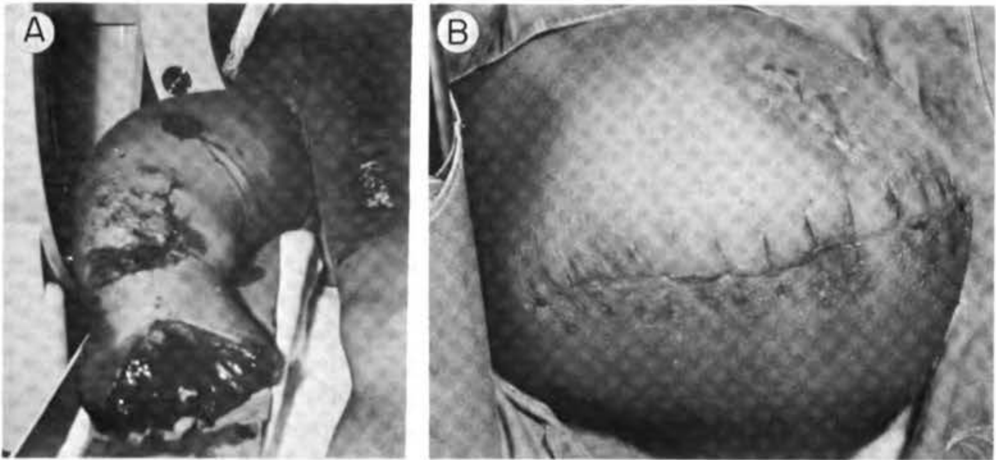

FIGURE 2. A, extensive gas gangrene of right leg, gluteal region, and flank of a young man whose leg had been mangled in a motor-scooter accident. Amputation and debridement had been carried out elsewhere, and, when the patient reached our hospital 2 days later, the characteristic coppery discoloration of the skin covered the entire infected area. His hemoglobin had dropped to 4.2 gm/100 ml. He was promptly treated at 3 ata in the chamber, and, 2 days after his first OHP treatment, a premeditated amputation was performed above the knee, through an area which at the time of admission was grossly infected. No signs of infection or gas were apparent in the open stump. B, uneventful healing followed secondary suture.

In only three of our 29 OHP-treated patients did difficulties arise which were associated with the compression procedure. One small boy became grossly distended during decompression, due to aerophagy. By extension of decompression to 90 min, he was able to release this gas through eructation. One of the severely ill patients, who died after the first compression, became so dyspneic that treatment had to be cut short. A third patient tolerated one full treatment without any problems, but on subsequent occasions he became so disoriented that treatment periods had to be reduced. He was anemic at the time, with a blood urea nitrogen of over 300 mg/100 ml, but he responded excellently and recovered spontaneously from his renal failure.

DISCUSSION

As we gathered experience in the treatment of gas gangrene at the Karl Bremer Hospital, we became more critical and accurate in our evaluation of referred patients. (The last five patients accepted for OHP therapy all proved to have clostridial infection.) In retrospect, some of the 13 patients with nonclostridial infection would not now have been accepted for treatment in the chamber. Yet, we were then, and still remain, impressed by the results in three of these cases. We would rather err in the direction of giving unnecessary OHP therapy than risk depriving a patient of its benefits who might later prove to have clostridial infection or who might show a favorable response despite our failure to culture clostridia.

In describing these patients, no distinction has been made between fasciitis and myositis. In all 16 patients with proven clostridial infection, local and systemic responses to OHP were equally excellent in patients with myositis and in those with fasciitis. This contrasts markedly to the lack of response in the majority of patients with nonclostridial infections, in whom myositis was, of course, not a feature. We are convinced that myositis and fasciitis respond identically to hyperbaric oxygen and should, therefore, be regarded

in exactly the same way for treatment purposes. It would be a pity if the impression was gained that while it is correct to treat clostridial myositis with hyperbaric oxygen, it is somehow wrong to apply the same treatment to fasciitis or cellulitis, particularly since the diagnostic distinction can often be made only in retrospect.

The high incidence of diabetes in our series is noteworthy. Of the total of 29 patients, 10 (34%) were diabetics. Among the 16 patients with proven clostridial infection, four (25%) had diabetes. This contrasts remarkably with the 10% incidence in 156 patients with proven clostridial infection reported by various other workers in this field.[4]

SUMMARY

In all, more than 100 hyperbaric treatments have been administered to 29 patients in one-man chambers at the Karl Bremer Hospital. Despite the reservations that have been expressed concerning compression chambers in which the patient is inaccessible to medical personnel during pressurization, we are satisfied as to the effectiveness and safety of these units in the treatment of clostridial gas gangrene. Close supervision and care are, of course, essential, but the risks seem small compared to the rewards, particularly when the choice is one of employing the one-man chamber or doing without the benefits of OHP. Indeed, if hyperbaric oxygen finds its main therapeutic application in the treatment of carbon monoxide poisoning and clostridial gas gangrene, the proliferation of small chambers such as ours may offer an economically feasible way to treat such patients on a broad scale.

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Hyperbaric Oxygen in Infection

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Oxygen under pressure is toxic to many bacteria, causing inhibition of their growth. *In vitro* studies [1,2] have shown this effect for both anaerobes and aerobes. Other workers [3] have demonstrated that hyperbaric oxygen prevents the formation of *Clostridium perfringens* alpha toxin. The successful treatment of gas gangrene has also been reported,[4,5] using large compression chambers, with the patient inhaling oxygen from a face-mask.

Below are described our clinical experiences in the treatment of anaerobic and aerobic infections with oxygen under pressure. In our series, treatment has been given in a one-man transparent high pressure oxygen chamber (first described by Emery *et al.*[6]).

ANAEROBIC INFECTIONS (*Clostridium perfringens* GAS GANGRENE)

The Amsterdam workers [3] have shown experimentally that clostridial alpha-toxin formation in the phlegmon is inhibited by oxygen at 3 ata, and they believe that a pressure of 3 ata is required for the treatment of gas gangrene. In our experience, however, 10 patients with gas gangrene admitted to the Whipps Cross Unit were satisfactorily treated at 2.5 ata (22 psig), and five patients successfully treated at Heatherwood Hospital received only 2 ata. One wonders if our good results reflect the greater efficiency of oxygen administration in a small chamber. Of the total of 15 cases of gas gangrene treated in both of our units, all were bacteriologically proven *Cl. perfringens* infections (Table 1). Nine patients had clostridial myositis of limbs, two after crush injury and two from complicating amputation for diabetic gangrene. Three patients had uterine gas gangrene, two after criminal abortion and one after caesarean section for prolonged labor. This last patient had originally responded to conventional treatment with antitoxin and antibiotics but relapsed after 3 days. The remaining three patients had spreading clostridial cellulitis with gas in the subcutaneous tissues; in one, this was periorbital.

In severely ill patients, we administer intermittent 2-hour exposures to 2 or 2.5 ata (depending upon the unit to which they are admitted) for the first 24 hours, the rest-periods between exposures lasting a maximum of 2 hours. Further shorter exposures are given in the next 2 days. As a supplement, large doses of penicillin are given. In cases of mixed infection, penicillin is usually combined with

TABLE 1. Classification and Origin of Clostridial Infection and Incidence of Renal Failure in 15 Patients

Classification	Total no. patients	Cause of infection	Renal failure (no. patients)
Anaerobic cellulitis	4[a]	Incision of abscess in upper lid, secondary orbital cellulitis (1 case)	0
		Wound debridement for compound tibial-fibial fracture (1 case)	
		Caesarean section, pelvic cellulitis, and thrombophlebitis (1 case)	
		Traumatic arm injury (1 case)	
Bacteremia	2	Septic abortion	2
Gas gangrene myonecrosis	9	Traumatic injury to:	
		Leg (1 case)	
		Leg and pelvis (1 case)	
		Leg and abdominal wall (1 case)	2
		Thigh and groin (1 case)	
		Amputation of leg for peripheral vascular disease (2 cases)[b]	0
		Surgery for neoplastic disease (2 cases)	0
		Infection of perianal abscess involving perianal region, thigh, and buttock (1 case)	0

[a] In three of these four patients, infection emanated from surgical wound site.
[b] One of these two patients was a diabetic.

ampicillin. We do not use streptomycin because it is contraindicated in renal failure, an apparently common complication of gas gangrene following severe trauma or postseptic abortion. Only one of the patients treated at Whipps Cross Hospital received anti-gas gangrene serum following commencement of hyperbaric oxygen therapy, but serum administration was continued in the patients treated at Heatherwood Hospital.

Because of the frequency of renal failure, it is imperative to record urinary output and to institute a renal-failure regime at the onset of oliguria. We now think that the best method for tiding these patients over until the gas gangrene infection is under control is to alternate peritoneal dialysis with hyperbaric oxygen therapy, provided there is no peritoneal sepsis or previous intraperitoneal operation within 12 days.

A typical case treated was a 24-year-old woman who had a septic abortion with *Cl. perfringens* septicemia complicated by acute tubular necrosis with oliguric renal failure. The patient had douched herself with carbolic soap when 22 weeks pregnant and was admitted to the hospital on the same day. The next day she aborted spontaneously and at that time went into shock. She was noted to be slightly jaundiced and her serum was dark red–brown. Spectroscopically, the serum was shown to contain methemalbumin and free oxyhemoglobin.

Cultures from high vaginal swab, the fetus, placenta, and blood confirmed the presence of *Cl. perfringens*. The patient was treated with penicillin and gas gangrene antitoxin and, on the same day, was transferred to Whipps Cross Hospital. On admission she was critically ill, hypotensive, jaundiced, and toxic, with a pulse rate of 150/min. She received six hyperbaric treatments for a total therapeutic period of 11.5 hours at 2 ata. After therapy, her pulse rate settled to 100/min, her blood pressure rose, and her general condition improved. It was noted, however, that over a 24-hour period she had passed only 28 ml of urine and that the

blood urea had risen from 78 to 170 mg/100 ml.

Two days after the abortion, a total hysterectomy and left salpingo-oophorectomy was performed, and the uterus was found to be honeycombed and yellow-green, with the left tube and ovary black and gangrenous.

The patient made an uneventful recovery from her operation and was treated with an acute renal-failure regime. Seven days after the abortion, she was transferred to the London Hospital where she received five hemodialyses over a period of 8 days. She had a total of 21 days of oliguria, and it was another 28 days before the blood urea had fallen from 460 mg/100 ml at the commencement of dialysis to 82 mg/100 ml. She was discharged in good condition on a low-protein diet.

A second case was a 39-year-old gamekeeper who sustained an accidental shotgun wound of the left knee. There was gross damage to the bones, but on exploration the neurovascular bundle was found to be intact. The wound was debrided, skin closure effected, and the limb placed in a plaster cast with the intention of carrying out an arthrodesis at a later date. He was given antibiotics, antitetanus serum, and anti-gas gangrene serum. Three days later he developed severe pain in the limb and became toxic and febrile. Edema of the thigh was noted above the level of the plaster, with bronze discoloration extending to the groin. A diagnosis of clostridial myositis was made, and he was transferred to Heatherwood Hospital for hyperbaric oxygen therapy.

On admission, the patient was toxic, jaundiced, apprehensive, and not fully oriented. There was extensive brawny edema of the thigh and discoloration. The knee wound was reopened, and gas and foul-smelling fluid discharged. (Cultures from this showed *Cl. perfringens.*) Gas was not palpable in the thigh but was apparent in the muscle planes on x-ray films. He was given anti-gas gangrene serum and penicillin, 2 megaunits every 6 hours. Hyperbaric oxygen therapy was started immediately; in all, he had 16 sessions of 2 hours each (three times daily for the first 4 days). There was no increase in the extent of the discoloration after the first session, and evidence of systemic toxicity disappeared after the second. He was discharged on the eighth day and has remained well since.

After apparent cure of gas gangrene, relapse may occur, and one of our cases reinforces the contention of Hitchcock,[7] at the last Conference, that adequate surgical excision of dead tissue is necessary. A 57-year-old male diabetic had amputation below the knee for gangrene of the left foot. Gas gangrene developed in the stump, with crepitus extending halfway up the thigh. Temperature was 39.4°C and x-ray examination confirmed the presence of soft-tissue gas. Four 2-hour cycles of high pressure oxygen were given, resulting in apparent cure. There was doubt, however, whether the original amputation had been high enough; after 3 days, gas and toxemia recurred although the toxemia was not as severe as before. A further 6 hours of treatment was given in the oxygen chamber, followed by amputation above the knee. Another 6 hours of hyperbaric oxygen therapy resulted in complete cure. We feel that the poor circulation prevented antibiotics and oxygen at high partial pressure from adequately dealing with the clostridia in the amputation stump; thus, when therapy was stopped, the organisms again began to multiply in the relatively ischemic tissues. Only after adequate surgical excision could cure be achieved.

We believe that hyperbaric oxygen may have additional application in the prophylaxis of gas gangrene, especially where there is pre-existing sensitivity to anti-gas gangrene serum. In illustration, a 16-year-old boy who sustained a severely lacerated and dirty soft-tissue gunshot wound around the left elbow was admitted for treatment. He was known to be sensitive to horse serum. Gas gangrene was thought to be a possible complication,

although there was no bacteriologic evidence. He was given daily 2-hour treatments for 1 week, and the wound healed cleanly.

Aerobic Infections

Experimental work by Ross and McAllister[8] has shown that hyperbaric oxygen can exert a protective effect *in vivo* during aerobic infection, and Schreiner[9] has shown that in *Staphylococcus pyogenes* var. *aureus* infection, OHP and penicillin have an additive effect on growth retardation of the organism. Stansell[10] has reported cure of systemic *Pseudomonas pyocyaneus* infection. Frequently severe burns become heavily (secondarily) infected with staphylococci and pyocyaneus, and the resulting toxic absorption can render the patients very ill indeed. We have had encouraging results in the treatment of three such cases.[11]

One of our patients, a 70-year-old woman, had approximately 30% truncal burns heavily infected with *S. aureus*, unresponsive to antibiotics. When first seen by us, she was so ill with toxic absorption that we doubted whether she could be moved to the oxygen chamber. However, treatment was commenced in 2-hour daily sessions, and on the second day there was some improvement in her general condition. After a week, she was much better, and discharging areas were drying up. Serial cultures showed reduction from a heavy to a moderate growth of organisms. After 2 weeks, treatment was stopped for 3 days. During this period, a heavy pyocyaneus infection and recrudescence of toxemia occurred. With further treatment, this infection slowly cleared, and 3 weeks later all areas were fully granulated and healed, with surprisingly little scarring.

While it would be wrong to draw conclusions concerning the treatment of aerobic infections from these three cases, the results do suggest that hyperbaric oxygen may have a role in the management of some surface infections.

Conclusions

Fifteen cases of gas gangrene have been successfully treated with hyperbaric oxygen in a one-man transparent oxygen chamber at pressures of 2 or 2.5 ata. We feel that the use of the one-man chamber is an efficient and economical method to treat gas gangrene.

The possible use of hyperbaric oxygen in the treatment of surface infections with aerobic organisms is discussed.

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High Pressure Oxygen Therapy for Patients with Gas Gangrene

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In February 1964, a research center for hyperbaric oxygen therapy was opened at our institution. Thus far, 22 patients with gas gangrene have been referred for treatment. The following is a brief account of our observations on these patients.

Treatment Method

The patient is placed in the pressure chamber and immediately given oxygen to breathe by mask. The chamber is pressurized to 28 psig (just below 3 ata) and left at this pressure for 1.5 or 2 hours, after which decompression is carried out according to the U.S. Navy decompression tables.[1] The patient breathes oxygen delivered in high flows through a clinical mask during the entire procedure, including the compression and decompression phases. An effort is always made to keep the mask applied tightly to the patient's face. During the first 24 hours, the patient usually receives three such treatments; on succeeding days, only two are given. The first exposure lasts 2 hours, subsequent exposures being limited to 1.5 hours. Initially, patients were treated for as long as 10 days, but recently the number of exposures has been reduced. The size of our chambers permits us to provide intensive nursing care within the unit while the patient is exposed to oxygen at the elevated pressures.

Most of our patients with gas gangrene were started on therapy with penicillin and chloromycetin before their arrival at our hospital. Administration of these antibiotics is continued without interruption, and if there is evidence of severe blood loss, blood transfusion is started as soon as possible, frequently during the first exposure to high pressure oxygen. (In some patients, however, transfusion was delayed for more than 3 hours.)

Patients

Our patients can be classified in six categories: (1) five patients with compound fractures and soft-tissue injuries, with gas gangrene of an upper extremity, (2) five patients with compound fractures and soft-tissue injuries, with gas gangrene of a lower extremity, (3) four patients with gas gangrene that developed following amputation because of ischemia due to

obstructive arterial disease, (4) four patients with gas gangrene of the abdominal wall and related structures, (5) two patients with questionable gas gangrene of the abdominal wall, and (6) two patients with gas gangrene following elective surgery.

Group 1: Compound Fractures and Soft-Tissue Injury of Upper Extremity

Table 1 summarizes data from the first group of five patients: four in whom gas gangrene developed following compound fractures of the forearm which had been treated with a constricting cast, and one in whom gas gangrene developed following soft-tissue injuries of the palm associated with metacarpal fractures.

Case 1. A 12-year-old boy with a compound fracture of the right wrist underwent debridement of the wound several hours after the accident, and a plaster cast was applied. When seen again 4 days later, the visible portion of the hand and fingers had become blue-grey and swollen, and all sensation in the hand was lost. Immediately after removal of the cast, he became acutely ill, developed a fever of 40.6°C, and suffered hallucinations. The cast had apparently acted as a tourniquet, predisposing the distal portion of the limb to necrosis, and permitting invasion with *Clostridium perfringens* (*Cl. welchii*).

Four hours after removal of the cast, the patient was seen at our hospital. He showed evidence of systemic toxicity. The phlegmon had spread to the middle third of the upper arm, and crepitation was apparent over the medial aspect just below the axilla. The hand and the fingers were swollen and darkly cyanotic, and there was tissue necrosis at the site of the original injury. *Cl. perfringens* and *Escherichia coli* were cultured from the wound. There was no evidence of hemolysis.

Because of the rapidly spreading anaerobic myositis, immediate disarticulation of the upper extremity seemed indicated, but in view of the results reported by Brummelkamp and co-workers,[2] a trial of hyperbaric oxygen therapy was decided upon. Since this patient was seen before the construction of our large pressure chambers, he was treated in a small steel experimental chamber (diameter 30 in., length 54 in., three 4-in. glass ports). He had to flex his knees and hips slightly, but was not uncomfortable in this "capsule." The chamber was flushed with oxygen and subsequently pressurized to 28 psig with 100% oxygen. Ventilation was maintained with a liberal flow of oxygen. In this pressurized environment, the color of the necrotic tissues of the hand and forearm changed from a dark grey-blue to an unnaturally vivid pink. Gas gangrene antitoxin was not given. The general condition improved rapidly. The temperature returned to normal within 36 hours, but the hand and forearm remained dark and lifeless. Amputation was carried out just below the elbow on the third day. The hand and the muscles of the forearm were necrotic.

Exposure to hyperbaric oxygen apparently arrested the toxic infection and made it possible to delay amputation until the third day. Moreover, it permitted preservation of the upper arm and the elbow joint. Live *Cl. perfringens* persisted in the wound, as demonstrated by cultures after several exposures to OHP.

Cases 14, 18, and 19 of this group (Table 1) had a similar history. All three patients entered our hospital quite ill, and all recovered rapidly, although amputation of the forearm was required in two later on, as the hand and the lower forearm proved to be necrotic. The last patient in this group, with hand injury, did not require amputation and was sent back to the referring hospital for skin grafting and further care.

Group 2: Compound Fractures and Soft-Tissue Injury of Lower Extremity

Table 2 shows data on the group of five patients with gas gangrene that developed after soft-tissue injury or compound fractures of a lower extremity. This group included our most severely ill patients, all but one of whom recovered (Case 2).

TABLE 1. Compound Fractures and Soft-Tissue Injury of Upper Extremity

Case no.	Age	Temp. (°C)	BUN (mg/100 ml)	Bilirubin (mg/100 ml)	Hgb (gm/100 ml)	Injury	No. OHP exposures	Result
1	12	39.4	20	0.8	7.6	Compound fracture of forearm (with constricting cast)	16	Recovered, with late amputation below elbow
14	18	39.3	16	1.18	13.8	Compound fracture of forearm (with constricting cast)	5	Recovered, with late disarticulation of elbow
18	9	38.9	10	0.73	11.5	Compound fracture of forearm (with constricting cast)	4	Recovered
19	12	38.6	9	0.89	12.7	Compound fracture	7	Recovered, with late amputation below elbow
21	27	38.7	13	1.18	11.7	Fractures and laceration of right hand	4	Recovered

TABLE 2. Compound Fractures and Soft-Tissue Injury of Lower Extremity

Case no.	Age	Temp. (°C)	BUN (mg/100 ml)	Bilirubin (mg/100 ml)	Hgb (gm/100 ml)	Injury	No. OHP exposures	Result
2	27	38.7	129	8	11.7	Compound fracture of right tibia	10	Death due to gastro-intestinal hemorrhage
3	36	38.9	7.7	—	7.3	Soft-tissue crushing injury of right gluteal area and pelvic fractures	16	Recovery
4	20	39.2	7.8	0.82	13.9	Soft-tissue crushing injury of left leg	10	Recovery without amputation
17	25	39.2	11	0.9	9.6	Multiple compound fractures of left leg with soft-tissue crushing injuries of thigh and calf	15	Recovery without amputation
20	46	38.4	17	0.69	13.8	Laceration of left thigh	3	Recovery without amputation

Case 2. A 27-year-old reserve army officer sustained a compound fracture of the right tibia, and the wound was debrided in a nearby hospital shortly after the injury. Twenty-six hours later, a diagnosis of gas gangrene of the right leg was made, and the patient was transferred to an army hospital where a midthigh amputation was carried out. He also received large doses of penicillin and gas gangrene antitoxin. Subsequently, crepitation in the stump was noted, and he was moved to our hospital. On admission, he appeared to be in a state of toxemia, and he was jaundiced, slightly cyanotic, and semicomatose. Blood pressure was 96/64 mm Hg. Temperature was 38.7°C and pulse was 148/min. His urinary output was 90 ml during the first 24 hours after admission and only 71 ml on the third day. Thereafter it increased slowly.

The stump of the right thigh was blue-grey, and a malodorous, nearly black drainage was present. Cultures were positive for *Cl. perfringens* and *Staphylococcus epidermidis.* Without delay, he was treated with OHP. The spread of the gas gangrene was readily arrested, and the general condition improved. The temperature returned to normal over a period of 4 days.

The patient was known to have had a peptic ulcer before the accident. On the sixth day, gastrointestinal hemorrhage developed, and on the seventh day he was transferred to a Veterans Administration Hospital for further care. Unfortunately, he died there shortly after arrival from massive gastrointestinal hemorrhage.

Case 3. A 36-year-old man with a large soft-tissue slough of the right gluteal area following an automobile accident underwent immediate debridement of the wound, but on the third day his temperature rose rapidly, and on the morning of the fourth day a diagnosis of anaerobic myositis and cellulitis was made. The wound was again debrided, after which he was transferred to our hospital.

The infectious process involved the right buttock and flank, extending over the anterior aspect of the abdomen to the left anterior superior spine and down the lateral aspect of the right thigh; the spread was manifested by bronzing of the skin and crepitation. The patient was semicomatose, the heart rate was 150/min, and the temperature was 38.9°C. The crushed area was again debrided and OHP therapy was started. Within 45 min he became more alert and showed an interest in his surroundings. No progression of the disease was noted thereafter, and he improved rapidly. Treatment was continued for 10 days. No gas gangrene antitoxin was given.

Cultures from the wound showed *Cl. perfringens,* as well as *S. epidermidis, Aerobacter aerogenes,* and *Bacillus subtilis.* Subsequent cultures continued to show *Cl. perfringens* despite rapid clinical improvement. The patient returned to his home on the tenth day in good general condition, with healthy granulation tissue covering the wound.

Case 4. A 20-year-old man with a soft-tissue crush injury of the left leg developed anaerobic myositis and cellulitis of the left calf within 48 hours. Thirty-six hours later he was transferred to our hospital. Inspection of the wound showed that the posterior aspect of the leg had been widely opened by previous debridement of the upper two-thirds of the calf, the popliteal fossa, and the lower third of the thigh. There still remained, however, patchy areas of black discolored muscle and skin, and a foul odor arose from the wound. Crepitation was found over the medial aspect of the upper portion of the thigh and over the left lower quadrant of the abdomen. Cultures of the infected area were positive for *Cl. perfringens, Staphylococcus pyogenes* var. *aureus, A. aerogenes,* and an unidentified gram-negative organism.

No progression of the gas gangrene was noted after the beginning of hyperbaric oxygen therapy. The temperature, which was 39.2°C, returned to normal over a 1-week period. The patient returned home in excellent condition for plastic repair of the wound, which at that time was covered with healthy granulation tissue.

Case 17. A 25-year-old woman was transferred to our center on the third day after an automobile accident which had resulted in a compound fracture of the left femur and tibia, in addition to causing crushing injuries of the soft tissues of the thigh and calf. On admission, her temperature was 38.3°C, pulse rate was 120/min, and blood pressure was 118/52 mm Hg. Toxemia was

evident, and a foul odor arose from the left leg. A large area of the anterior, medial, and lateral aspects of the thigh, including skin and muscle, was necrotic, as well as a large area of the calf. A long incision from the midthird of the thigh extending to an inch above the ankle had been made and subsequently closed (after drains had been inserted) prior to her transfer to our institution.

She was treated with OHP three times during the first 18 hours. Thereafter, she underwent surgery, and most, but not all, of the necrotic tissue was removed. Hyperbaric oxygen was continued for 6 days, with two exposures per day. The dressings of the left leg were left in place and further surgical procedures were postponed. On the twelfth day, the remainder of the necrotic tissue was removed and a program of reconstruction was started. The temperature returned to normal over a 5-day period. She was transferred to another hospital, in excellent condition, for reconstructive surgery and skin grafting.

Group 3: Amputation for Ischemia from Obstructive Vascular Disease

Table 3 summarizes data on the group of four patients with gas gangrene that developed after amputation of a lower extremity for ischemia due to obstructive vascular disease.

Case 8. In a 46-year-old man with rheumatic heart disease, a large embolus occluded the terminal aorta, and an attempt was made to remove this clot. This was not completely successful, however, and a left midthigh amputation was carried out. Two days after amputation, a diagnosis of gas gangrene of the stump of the left leg was made and disarticulation of the hip was carried out. Subsequently, the man was transferred to our hospital.

On admission, the patient showed signs of moderate systemic toxicity. The temperature was 37.6°C. Apical heart rate was 80/min and blood pressure was 130/90 mm Hg. Crepitation was present over the left lower quadrant of the abdomen and below the left costal arch. The right leg was cold, mottled, and without sensation up to the level of the knee, due to diminished blood supply. Treatment with oxygen was begun at a pressure of 28 psig. With two exposures per day, the gas gangrene did not progress further, and the patient's general appearance improved. In addition, the color of the right leg improved markedly and was considered normal after 18 exposures, although the right calf remained tender and "stiff" and never entirely regained normal function, despite an additional 11 exposures to OHP.

Case 10. A 60-year-old man developed gas gangrene following a midthigh amputation for dry gangrene due to chronic arterial disease and diabetes mellitus. He responded readily to OHP therapy, although final wound healing was delayed considerably due to the poor blood supply. Hyperbaric oxygen was continued, therefore, in an attempt to encourage the formation of granulation tissue. It is, of course, difficult to determine whether the increased oxygen tensions contributed to the final wound healing.

Case 15. This 72-year-old man was similar to the patient described above; he had diabetes mellitus and chronic kidney disease, as well as chronic peripheral vascular disease, and he developed dry gangrene of his right foot. Amputation had been recommended but was refused initially. After the patient finally consented to amputation, he developed gas gangrene of the stump. Although the gas gangrene was readily arrested with hyperbaric oxygen, the patient died 3 days later in uremia.

Case 16. Similarly, this 61-year-old man developed gas gangrene in the stump following amputation for chronic ischemic disease of the leg, associated with diabetes mellitus. He responded rapidly to OHP and recovered after reamputation, which was carried out 3 days later.

Groups 4–6: Abdominal Wall and Torso Involvement

Table 4 describes four patients with gas gangrene of the abdominal wall and related structures. One patient in this group (Case 7) arrived at our center in shock and died shortly after the first exposure

TABLE 3. Amputation for Ischemia from Obstructive Vascular Disease

Case no.	Age	Temp. (°C)	BUN (mg/100 ml)	Bilirubin (mg/100 ml)	Hgb (gm/100 ml)	Injury	No. OHP exposures	Result
8	46	37.6	16	—	13.2	Rheumatic heart disease, midthigh amputation following embolization of aorta and femoral artery	29	Recovery
10	60	38.7	51	1.6	7.0	Obstructive atheromatosis of terminal aorta and femoral arteries, with midthigh amputation	29	Recovery
15	72	37.6	106	19.14	10.0	Midthigh amputation following ischemic gangrene of foot	4	Gas gangrene arrested, death due to uremia 3 days later
16	61	38.2	16	0.98	11.3	Amputation below the knee because of advancing ischemic gangrene	7	Recovery

Table 4. Gas Gangrene of Abdominal Wall and Related Structures

Case no.	Age	Temp. (°C)	BUN (mg/100 ml)	Bilirubin (mg/100 ml)	Hgb (gm/100 ml)	Injury	No. OHP exposures	Result
13	80	38.6	14	0.29	10.3	Slowly advancing gangrene of abdominal wall following bowel surgery	6	Rapid improvement
6	57	38.3	8	—	8.3	Slowly advancing gangrene of abdominal wall following bowel surgery	9	Rapid improvement
7	48	36.9	—	—	14.2	Gas gangrene of perianal structures and liver cirrhosis	1	Death due to hepatic coma
9	70	37.0	37	—	8.7	Gas gangrene of abdominal wall following surgery for strangulated left femoral hernia	7	Rapid improvement, but death due to another cause a few days later

to OHP. Two patients (Cases 13 and 6) had slowly advancing necrotizing myositis, and both responded well to hyperbaric oxygen therapy. Similarly, the last patient (Case 9) had a form of slowly necrotizing myositis which responded well to OHP and advanced no further. However, when the necrotic material was removed, there was no abdominal wall remaining, and the patient died a few days later.

Table 5 lists data for two patients with abdominal wall infections which developed after surgery for perforated appendix with peritonitis. The diagnosis of gas gangrene in these two patients was subject to question. In the first patient (Case 22), we were inclined to retain this diagnosis, although we were not certain about the presence of any myonecrosis. Our own cultures failed to show any clostridia, although *Cl. perfringens* had been cultured in the referring hospital. In the second patient (Case 12) we felt we could not make a diagnosis of gas gangrene on clinical grounds, even though cultures showed a rich growth of *Cl. perfringens*. Both patients recovered rapidly after exposure to OHP.

Table 6 lists data for two patients with gas gangrene that developed after elective surgery. One was a 62-year-old man with a spiral fracture of the shaft of the left femur (Case 5). After a stabilizing rod had been placed in the marrow cavity, he developed gas gangrene with uremia and mild jaundice. He responded rapidly to hyperbaric oxygen therapy. The second patient is described below.

Case 11. A 53-year-old man operated on for a herniated lumbar disc underwent fusion of the spine, which was performed by using chips from the left fibula. The postoperative course was uneventful until the third day, when he started to complain of pain in the right side of the chest. An infectious process was found around the surgical site which had spread to the right lateral aspect of the chest. The skin of this area was red-brown, and a chest x-ray film showed collections of subcutaneous gas in this area.

The patient arrived at our hospital 15 hours after the onset of pain in the chest. He had generalized toxemia and was in shock. The blood pressure was 95/70 mm Hg, the heart rate was 140/min, and the respiratory rate was 56/min. The lips and nails were cyanotic, and the skin was cold and clammy. The sclerae were yellow. Urinary output was limited to a few milliliters per hour. The patient was exposed to hyperbaric oxygen, but no improvement was noted. After an interval of 3 hours 45 min, he was exposed a second time, and at the beginning of this exposure the blood pressure was too low to be recorded. It did not improve, nor did the patient respond to administration of digitalis, vasoconstrictors, and hydrocortisone. He died 30 min after completion of the second exposure.

Before transfer to our institution, the patient had undergone debridement of the wound, and long deep incisions had been made through the infected area. One of these incisions had perforated the chest wall. Autopsy examination showed the right lung to be collapsed. The right pleural cavity contained 50 ml of pus. Cultures of the infected surgical wound grew *Cl. perfringens*, *Staphylococcus albus*, and anaerobic streptococci.

Discussion

The case histories presented above suggest that oxygen at a pressure of 28 psig is an effective agent for the treatment of infection associated with gas gangrene. According to our treatment program, surgical procedures, including removal of necrotic tissue, are delayed and priority is given to hyperbaric oxygen. From our experience and that of others,[2] it would appear that removal of necrotic material can usually be delayed until the patient has improved. There are, however, exceptions. If, for example, one is faced with swelling of muscles within a tight fascial compartment, a decompressive fasciotomy is equally important, and in such patients surgery can be combined with hyperbaric oxygen therapy.

The diagnosis of "gas gangrene" is

TABLE 5. Questionable Gas Gangrene of Abdominal Wall

Case no.	Age	Temp. (°C)	BUN (mg/100 ml)	Bilirubin (mg/100 ml)	Hgb (gm/100 ml)	Injury	No. OHP exposures	Result
22	37	39.3	100	8.69	12.4	Appendicitis with perforation, peritonitis; cellulitis of right half of abdominal wall; cultures revealed no anaerobic organisms	9	Recovery
12	8	37.2	3	—	14.2	Appendicitis with perforation; cellulitis and dehiscence of abdominal wall; clinical picture not consistent with gas gangrene, but *Cl. perfringens* cultured from wound	6	Rapid recovery

TABLE 6. Gas Gangrene Following Elective Surgery

Case no.	Age	Temp. (°C)	BUN (mg/100 ml)	Bilirubin (mg/100 ml)	Hgb (gm/100 ml)	Injury	No. OHP exposures	Result
5	62	38.9	80	1.8	11.6	Spiral fracture of left femur with introduction of stabilizing intramedullary rod	11	Recovery
11	53	—	—	—	15.2	Herniated intervertebral disc	2	Death from irreversible shock

made on clinical grounds, and the continued presence of live clostridia in the afflicted part is not a guide to when hyperbaric oxygen therapy can be discontinued. Arbitrarily, we now discontinue OHP treatment within the chamber after four exposures. It is quite possible that one or two exposures would suffice.

A satisfactory explanation of mechanisms of the sudden improvement observed in these patients has not been found. Certainly, OHP resulted in rapid amelioration of systemic toxicity, which could be due either to inactivation of circulating toxin or to sudden interruption in the production of toxins. In addition to the systemic effect, in all these patients we saw a sudden halt of the progression of the local gas gangrene. Whether the reduction of gas bubbles in the tissues to about one-third of their original size is significant is doubtful. We treated one patient with a *Clostridium novyi* infection (not presented above) who had no gas in the tissues, and improvement was as rapid as that of most of the patients discussed above.

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In vitro and *in vivo* Experimental Effects of Hyperbaric Oxygen on *Clostridium perfringens*

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The encouraging reports of the clinical usefulness of hyperbaric oxygen in gas gangrene[1-4] have raised a need for parallel laboratory work to elucidate the effect of OHP on the causative organisms. The problem is twofold: (1) to elucidate the basis and manner of the action of OHP against the anaerobic organism, and (2) to evaluate OHP as a therapeutic measure in experimental gas gangrene. Therefore, both *in vitro* and *in vivo* experiments were performed using *Clostridium perfringens* as a model.

GENERAL METHODS

All experiments were performed using a strain of *Cl. perfringens* isolated from a patient with gas gangrene. The isolate was obtained pure on second subculture and a stock culture lyophilized so that a constant source of organisms would be available for each experiment. Quantitation of viable organisms was done in duplicate by the log-dilution pour-plate method using Brewer jars and illuminating gas to obtain an anaerobic environment. Hyperbaric oxygen was delivered with a variable temperature tank using 100% O_2 at 30 psig. White mice weighing over 23 gm were used for all *in vivo* experiments.

In vitro EXPERIMENTS

Method

The *in vitro* studies were performed using series of 10-ml log-dilution pour plates made from a 4.5- to 5-hour growth of the organism. An original control titer was made by immediate anaerobic incubation of these plates, while other dilution series of plates were exposed to hyperbaric oxygen for varying lengths of time at 37°C and afterwards incubated anaerobically to determine the number of viable organisms remaining.

Results

Data were obtained on the inactivation of *Cl. perfringens* in 100% O_2 at 30 psig, and it was found that when the organisms were suspended in plain trypticase soy agar, the number inactivated was time-dependent. During the course of these experiments, however, it was noted that 2% whole human blood included in the medium provided remarkable protection from the inactivation of the organism due

to OHP. An 18-hour OHP exposure time was chosen for further experiments regarding this observation.

Blood was studied according to its different constituents and derivatives to determine where this protective effect might reside. Table 1 illustrates how, when plain trypticase soy agar was used, the viable count dropped from an initial 4.9×10^8 bacteria/ml to less than 10^2 bacteria/ml after 18 hours of hyperbaric oxygenation. When 2% whole blood was added to the medium, however, very little drop in the titer was seen. Washed red blood cells were seen to be responsible for this protective action, as opposed to serum, which showed very little effect. Lysed red blood cells were found active as well as lysed cells in which the hemoglobin had been converted to methemoglobin by use of sodium nitrite. Crystalline human hemoglobin also proved to be effective.

At this point, titrations of lysed cells and crystalline hemoglobin in decreasing concentrations pointed to the hemoglobin as being active at concentrations typical of an enzyme. For this reason, catalase activity was suspected and subsequently found present in the crystalline hemoglobin. Thus, when bovine crystalline catalase was tested, it was found to be protective at the 500 μg/ml concentration level as well as in much lower concentrations. Figure 1 shows an end-point titration of crystalline catalase and hemoglobin for its protective effect during 18-hour OHP exposure of the clostridia. On a concentration basis, catalase used as an additive was seen to show a steady level of protective action beyond the point at which hemoglobin had lost this activity. Thus, all indications pointed to catalase or catalase activity as responsible for the protective action found in the various human blood derivatives (Table 1).

Evidence that the enzymatic action of catalase in decomposing hydrogen peroxide is responsible for the protective action was obtained by use of a specific catalase inhibitor, 3-amino-1,2,4-triazole, which

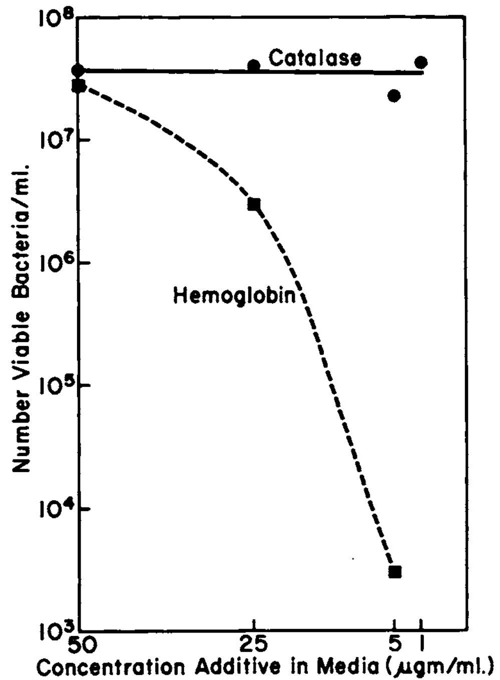

FIGURE 1. End-point titration of protective effect of catalase and hemoglobin on OHP inactivation of *Cl. perfringens*. OHP consisted of 100% O_2 at 30 psig for 18 hours (constant temperature 37°C).

causes irreversible inhibition of catalase when hydrogen peroxide is present.[5,6] The effect of catalase and aminotriazole on OHP inactivation of *Cl. perfringens* is shown in Figure 2, which illustrates the loss of the protective effect when the enzymatic inhibitor was added to the catalase. This resulted in a drop in titer of viable organisms like that observed with plain trypticase soy agar, in contrast to the curve obtained when catalase alone was used as an additive. Small amounts of catalase had to be used in these experiments wherein some protective effect was sacrificed due to the necessity of using aminotriazole in low concentrations and achieving a balance of the two substances. Higher concentrations of aminotriazole were found to be inhibitory to the organism. Aminotriazole alone in trypticase soy agar was included in these experiments as a further control, and it, like

TABLE 1. Protective Effect of Whole Blood and Certain Derivatives on Inactivation of *Cl. perfringens* by OHP[a]

Additive to medium (trypticase soy agar)	Concentration of additive in medium	Titer (viable bacteria/ml)	
		Initial titer (control)	After 18 hr OHP (test)
None	—	4.9×10^8	$< 10^2$
Whole blood	2%	4.2×10^8	2.6×10^8
Washed RBCs	1%	5.9×10^8	3.4×10^8
Serum	1%	6.7×10^8	2.0×10^8
Lysed RBCs	1%	7.3×10^8	3.6×10^8
Methemoglobin lysed cells	1%	7.6×10^8	3.6×10^8
Crystalline human hemoglobin	500 μg/ml	7.6×10^8	1.3×10^8
Crystalline catalase	500 μg/ml	6.2×10^8	9.1×10^7

[a] OHP consisted of 100% oxygen at 30 psig, constant temperature 37°C.

plain trypticase soy agar, provided no protective effect.

In vivo EXPERIMENTS

Method (Agar Disc)

Subcutaneously implanted agar discs impregnated with *Cl. perfringens* were used for *in vivo* experimental study because this method offered the advantage of easy quantitation of the organisms. Small trypticase soy agar discs seeded with a constant number of organisms for an initial inoculum were implanted in mice through an incision in the lower abdomen. The discs were placed subcutaneously well up into the chest wall and the incision was closed with sutures. After the desired time interval had passed, the mice were sacrificed, the discs were recovered intact, and quantitation was performed by grinding the discs and performing log-dilution titrations. Growth within the disc in mice closely paralleled that within similar discs placed anaerobically in Brewer jars.

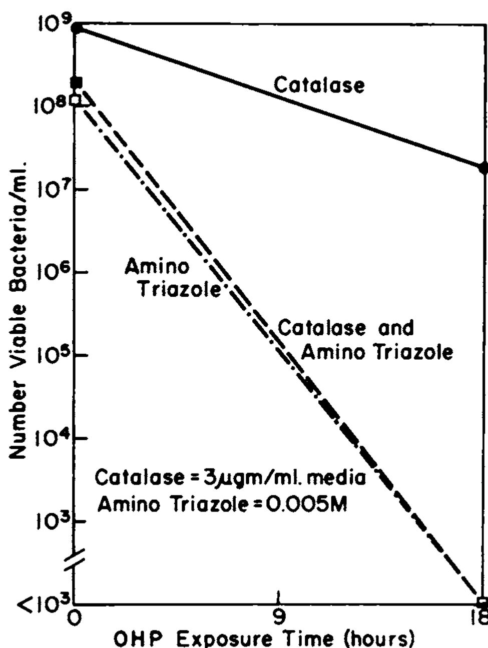

FIGURE 2. Effect of catalase and aminotriazole on OHP inactivation of *Cl. perfringens*. OHP consisted of 100% O_2 at 30 psig (constant temperature 37°C).

Results

With an initial inoculum of approximately 1000 organisms per disc, 48 hours' growth in control mice increased the titer to well over 1,000,000, whereas in an OHP-treated group of mice only an occasional organism would remain (Figure 3). The OHP-treated mice were given four treatments of 90 min each with 100% O_2 at 30 psig over this 48-hour period. Again, however, it was found that inclusion of 2% whole blood in the medium from which the discs were cut would protect the bacteria from the inactivating effect of OHP. Thus, the 48-hour titer of over 10,000,000 organisms was essentially the same for blood agar discs with

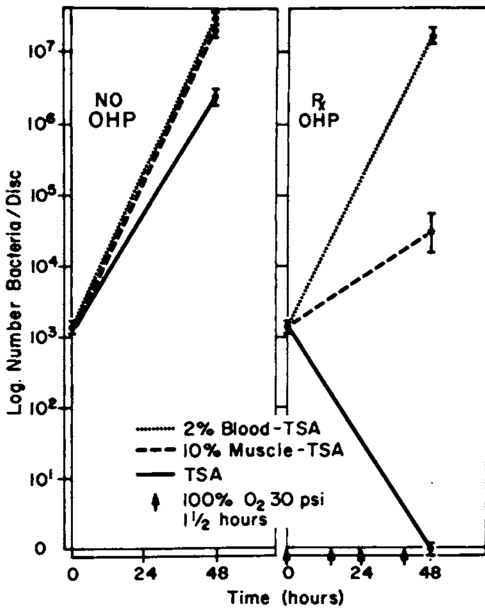

FIGURE 3. *In vivo* effect of OHP on *Cl. perfringens* by the agar-disc method.

or without OHP treatment. Muscle was also tested for its protective action by including a mince in the agar for the discs, and it was found to protect to a lesser, more variable extent.

Method (Experimental Gas Gangrene)

The effectiveness of hyperbaric oxygen therapy in experimental gas gangrene was evaluated by designing a model infection in mice which would simulate as closely as possible the pathology of gas gangrene in humans. The infection was initiated with washed organisms suspended in 10 μg of epinephrine and injected intramuscularly into the hind leg. The picture of infection resembled that of a human case with extension through the muscle and up the flank, accompanied by pain, edema, gas production, toxicity, prostration, and, later, hemoglobinuria. The hind limb assumed a distinctive slate-grey to black color as myonecrosis set in.

Results

When the OHP-treated mice (seven sessions, 90 min each, 100% O_2 at 30 psig) were compared with the untreated controls for cumulative mortality at time periods after injection, a significant difference was apparent (Figure 4). The control group had approximately 80% mortality by 48 hours, whereas the treated group had only about 20%. However, other experiments in which OHP sessions (still 90 min) were spaced farther apart in the early course of the infection showed only slight prolongation of the average survival time for treated mice, with an overall mortality similar to that of the controls. Further experiments were also performed in which the initiation of the successful scheme of therapy (Figure 4) was delayed for varying lengths of time after injection, and these likewise resulted in an overall mortality like that of the controls, except for some slight benefit shown at the 2-hour-delay level.

DISCUSSION

Presumably, catalase protects *Cl. perfringens* against OHP inactivation by decomposing the hydrogen peroxide produced by the organism during hyperbaric

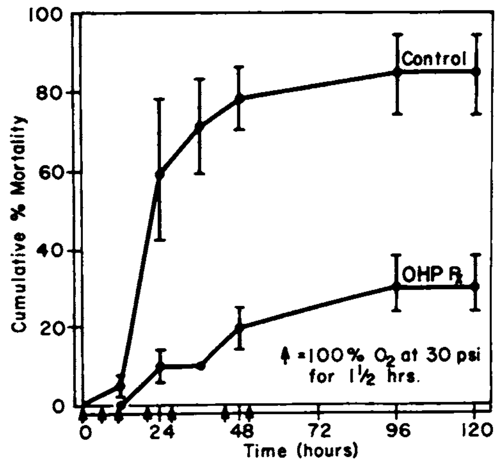

FIGURE 4. Effect of OHP therapy on *Cl. perfringens* infection in mice.

oxygenation. This anaerobic organism lacks catalase, and it has been debated for a number of years whether the inability of clostridia to grow aerobically is due to its production of H_2O_2 when oxygen is present.[7-9] The production of H_2O_2 by clostridia has been demonstrated,[7,10,11] but there has been no conclusive proof that it is this agent which inhibits aerobic growth.[9] Apparently, under OHP conditions, sufficient H_2O_2 is produced to inactivate the organism unless catalase is provided. In this case, catalase prevents the lethal effect of the H_2O_2, but the amount of undestroyed H_2O_2 and/or other factors continue to inhibit multiplication under OHP conditions, and growth is not resumed until the organisms are removed from the hyperbaric oxygen and placed in an anaerobic environment.

The term "inactivation" has been used to describe the effect of OHP on *Cl. perfringens,* but reference was made above to a "lethal" effect. As far as it has been possible to determine, OHP is bactericidal, the number of organisms killed being time-dependent as long as catalase or other decomposition of H_2O_2 does not take place. It has been reported that viable clostridia have been cultured from wounds of patients cured of gas gangrene with hyperbaric oxygen.[4] We suggest that this may be partly due to some protection afforded by catalase present in the patient's muscle tissues. In this case, although OHP has inactivated many organisms and made tissue conditions unsuitable for further proliferation of the clostridia, some organisms remain because of this protective action of catalase.

The contrast between our gas gangrene model experiments shown in Figure 4, in which significant benefit was derived from the hyperbaric oxygen treatments, and our other experiments, in which different regimens of OHP treatment afforded little or no benefit, emphasizes the need for prompt and sufficient hyperbaric oxygen treatment in order to control the rapidly progressive nature of the disease.

In conclusion, the *in vitro* observations on the protective effect of catalase on OHP inactivation of *Cl. perfringens* are offered as evidence for the previously proposed theory that the production of hydrogen peroxide is responsible for the toxic effect of oxygen in these anaerobic bacteria. In addition, the *in vivo* observations are offered as further support of the clinical usefulness of hyperbaric oxygen therapy in patients with gas gangrene.

Summary

In vitro and *in vivo* studies on the effect of OHP on the anaerobe *Cl. perfringens* indicated that inactivation of the organisms was time-dependent when the bacteria were exposed in ordinary trypticase soy agar pour plates, but inclusion of 2% whole human blood in the medium protected the clostridia from inactivation by the OHP. Experiments to determine the factor in whole blood affording the protection indicated that catalase or catalytic activity was responsible. Results of OHP treatment of mice implanted with small trypticase soy agar discs containing *Cl. perfringens* were similar to those found *in vitro.* Organisms were inactivated in plain trypticase soy agar discs, but were protected by the inclusion of whole blood or muscle in the medium. A model infection of *Cl. perfringens* in mice was significantly benefited by OHP treatments, as measured by survival. These results were briefly discussed in relation to the treatment of patients with gas gangrene.

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Mechanism of Action of High Pressure Oxygen in *Clostridium perfringens* Toxicity

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A precise understanding of the mechanism of action of high pressure oxygen (OHP) has been sought ever since its therapeutic value in clostridial infections became known. The concept of delivering increased oxygen into areas of anaerobic infection is not new, but the methods attempted before the reports of the Amsterdam investigators were unsatisfactory.[1] Although the initial use of OHP in experimental gas gangrene in guinea pigs was quite effective,[2] the dramatic results obtained by Boerema and his co-workers in human beings were the main stimulus for further extensive research.[3]

The persistence of live clostridial bacteria in the wounds of patients apparently cured of their disease by OHP therapy posed a problem in interpretation. The term "detoxification" was used by early investigators to describe the beneficial effects of OHP and may be responsible for some of the confusion. Although it was obvious to some medical personnel in this field that Boerema and his associates referred to clinical detoxification of the patient, others assumed that the term applied to the effect of OHP on the toxin itself.

Clarification of the mode of action of OHP on clostridial infections should result from research in four areas: (1) the action of OHP on bacteria (*in vitro* and *in vivo*), (2) the production of bacterial exotoxin, (3) the detoxification of the exotoxin, and (4) the systemic effects of the toxin (Figure 1). Although the first two areas have already been extensively reviewed, studies on the effect of OHP on toxin itself have been lacking, and the action of OHP on the systemic effects of the toxin has been studied only with respect to the effects of OHP on clostridial myositis. Studies on the effect of OHP in various types of shock other than exotoxin shock may be relevant.[4,5]

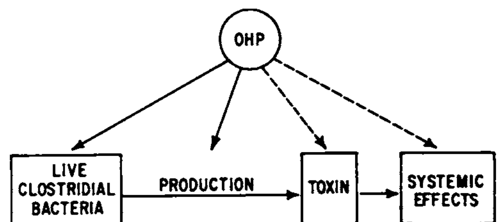

FIGURE 1. Possible inhibitory actions of hyperbaric oxygen.

Fredette has shown that 3 atm of 100% oxygen exerts a profound bacteriostatic effect on *Clostridium perfringens*.[6] This effect is strictly bacteriostatic, even when a pressure of 6.5 atm of 100% oxygen is used. Hopkinson and Towers[7] have demonstrated the bacteriostatic effect of OHP on the bacterial flora of rat feces. Nuckolls and Osterhout[8] found an inhibitory effect of OHP on the growth of *Cl. perfringens in vitro. In vivo* studies, both experimental and clinical, tend to confirm the finding that OHP has a bacteriostatic effect on the live bacterium.[2,9,10]

Van Unnik[11] has shown that 3 atm of 100% oxygen is required to inhibit alpha toxin production *in vitro*. The oxygen tension, as measured in culture tubes, was 240 mm Hg. Corresponding levels of tissue oxygen tension in clinical cases were also found by inserting probes into the phlegmon of patients with gas gangrene. On the basis of these findings, the action of OHP in clinical clostridial myositis was described as one of inhibition of alpha toxin production by the bacterial cells.

To our knowledge, no one has evaluated the effects of OHP on *Cl. perfringens* exotoxin *per se*. It has been demonstrated that certain enzymes containing sulfhydryl radicals are inhibited *in vitro* by OHP. Other enzymes whose activity appears to be inhibited by OHP are specifically those involved in reactions associated with high-energy phosphate bonds.[12]

Our study was designed to examine the effects of OHP *in vitro* and *in vivo* on the alpha exotoxin of *Cl. perfringens*, an enzyme identified as lecithinase C. The experiment was divided into two parts: (1) determination of the effects of OHP on the cell-free toxin (*in vitro* and *in vivo*), and (2) study of the physiologic responses of dogs injected with lethal doses of exotoxin during exposure to OHP.

In vitro STUDIES WITH *Cl. perfringens* TOXIN

Preparations of cell-free toxin of *Cl. perfringens* have been shown to cause a relatively specific reaction with egg-yolk medium, the so-called "lecitho-vitellin reaction."[13] The *Cl. perfringens* exotoxin used in this experiment was the lethal alpha toxin described as lecithinase C— a crude unpurified toxin prepared by anaerobic incubation of *Cl. perfringens* in a horsemeat infusion broth. When the peak toxin potency was reached, a filter aid was added to the crude culture, which was clarified by passage through a coarse Seitz filter and then sterilized through a fine Seitz filter. At this point, the toxin-containing filtrate was cell-free. Thimerosal was then added in a concentration of 1:10,000 and the toxin tested for potency. When 0.25 ml of the cell-free toxin (lecithinase C) was mixed with 5 ml of egg-yolk medium (Difco), opalescence resulted within 5 min and heavy clouding within 15 min.

We put 0.25 ml of cell-free toxin in two open-end test tubes and placed them in the experimental hyperbaric animal chamber (Medical and Biological Research Corp.). (This small amount makes possible a suitable condition so that maximum oxygen tension can be exerted at the gas–liquid interface.) The chamber was then pressurized to 3 ata for 2 hours. Next, by means of polyethylene tubing which had been placed into each tube before pressurization, 5 ml of egg-yolk medium was injected into the tubes containing the cell-free filtrate after 2 hours of maximum pressure. In one tube, egg-yolk medium alone was injected, while in the other tube 0.25 ml of *Cl. perfringens* antitoxin was also given. Simultaneously, a tube containing 0.25 ml of cell-free toxin was left outside the chamber and also mixed with 5 ml of egg-yolk medium. A second tube containing 0.25 ml of cell-free toxin was also kept outside of the chamber and mixed with 5 ml of egg-yolk medium plus 0.25

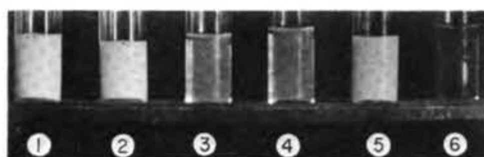

FIGURE 2. *In vitro* results of lecitho-vitellin reaction—positive or negative: (1) egg yolk plus toxin plus OHP, positive; (2) egg yolk plus toxin, positive; (3) egg yolk plus toxin plus antitoxin plus OHP, negative; (4) egg yolk plus toxin plus antitoxin, negative; (5) serum plus toxin plus OHP, positive; (6) serum plus toxin plus antitoxin plus OHP, negative.

ml of *Cl. perfringens* exotoxin. After pressurization at 3 ata for 30 min more, decompression was accomplished in 15 min.

Similar experiments were also performed to evaluate the effects of the cell-free filtrate on serum with and without antitoxin under the same conditions used with the egg-yolk medium.

Results

No significant alteration in the reaction of the cell-free filtrate and substrate was produced by OHP. No evidence of delay in onset of either opalescence or clouding of egg-yolk medium or serum was seen by subjecting the toxin alone to OHP before mixing. The egg-yolk medium, when mixed with antitoxin, prevented any reaction by the exotoxin whether inside or outside the chamber (Figure 2).

In vivo STUDIES WITH *Cl. perfringens* TOXIN

Thirty small white mice were used to determine the approximate LD_{50} of lecithinase C, the alpha exotoxin of *Cl. perfringens,* in a 24-hour period. This was done by injecting various dilutions of the exotoxins in quantities from 0.1 to 0.5 ml in the subscapular area. After determination of a dose close to LD_{50} (0.5 ml of exotoxin) for this type of mouse, 220 other mice were divided into three groups and subjected to: (1) toxin alone, (2) toxin plus OHP, and (3) OHP alone. Twenty additional mice (controls) had 0.5 ml of sterile saline solution injected into the subscapular area and were left outside the hyperbaric chamber.

All animals treated in the chamber were subjected to 3 ata of pressure with 100% oxygen. Compression and decompression were accomplished at the rate of 2 psig/min. The duration of maximum compression varied with each group (Table 1).

TABLE 1. *In vivo* Studies of the Effect of OHP on *Cl. perfringens* Toxin

No. mice	Duration of max. compression	Treatment	Post-treatment survivors		
			24 hours	48 hours	1 week
20	1 hour	Toxin + OHP	8(40%)	8(40%)	8(40%)
20	—	Toxin + room air	12(60%)	12(60%)	12(60%)
20	1.5 hours	Toxin + OHP	12(60%)	11(55%)	11(55%)
20	—	Toxin + room air	11(55%)	11(55%)	11(55%)
20	1.5 hours	Saline + OHP	20(100%)	20(100%)	20(100%)
20	2 hours	Toxin + OHP	10(50%)	9(45%)	9(45%)
20	—	Toxin + room air	11(55%)	10(50%)	10(50%)
20	2 hours	Saline + OHP	19(95%)	19(95%)	19(95%)
20	3 hours	Toxin + OHP	11(55%)	11(55%)	10(50%)
20	—	Toxin + room air	12(60%)	11(55%)	10(50%)
20	3 hours	Saline + OHP	19(95%)	19(95%)	19(95%)
20	(Control)	Saline + room air	20(100%)	20(100%)	20(100%)

Results

OHP did not prolong survival time in mice injected with cell-free clostridial toxin. Table 2 shows the higher survival rate in untreated mice, as compared to that of mice subjected to toxin plus OHP.

PHYSIOLOGIC RESPONSE OF DOGS SUBJECTED TO OHP AND INJECTED WITH LETHAL EXOTOXIN

Fifteen dogs were used for this part of the study. Initially, five dogs were employed to develop a satisfactory experimental model and also to determine an approximate dose of exotoxin which would be lethal in 3 hours after injection.

Blood pressure, pulse, and respiration were recorded during the experiment. Arterial and tissue oxygen tensions were determined with Beckman microelectrodes and a Beckman 160 gas analyzer. The oxygen tension studies were performed with the Liston modification of a Clark microelectrode. This electrode can be used for both arterial and tissue oxygen determinations. We found that the calibration procedures frequently had to be repeated to determine whether the electrodes were responding satisfactorily. Being aware that all determinations of oxygen tensions are subject to certain variables, we regarded all measurements in relative terms. Thus, absolute values were disregarded in favor of percent of increment or decrement from initial readings.

The animals were anesthetized with intravenously injected sodium pentobarbital (1 grain/11 kg), and endotracheal intubation was performed. One femoral vein was isolated, and a polyethylene catheter filled with dilute heparin solution was inserted and attached to a three-way stopcock. The dog was then given an injection of heparin, 2 mg/kg. Both femoral arteries and the other femoral vein were isolated. One femoral artery was clamped with bulldog clamps placed about 8 cm apart, and the vessel was transected. T-shaped glass cannulas were inserted into both ends of the cut vessel and tied in place. A #18 Riley needle was inserted into the sidearm of the cannula. The distal and proximal bulldog clamps were then released, allowing for continuous flow of blood.

TABLE 2. Survival Rate of Mice Given Injections of *Cl. perfringens* Toxin With and Without OHP

Group	Total no. mice	Survivors at 1 wk
Toxin + OHP	80	38(48%)
Toxin alone	80	43(54%)
OHP alone	60	58(97%)

Next, a polyethylene catheter filled with dilute heparin solution and attached to a blood pressure manometer was inserted into the opposite femoral artery. After function of the electrodes within the cuvette was checked, one of the electrodes was inserted into a sidearm of the cannula. Another electrode was placed inside a second Riley needle inserted into the adductor muscle in the same thigh. A temperature gauge was placed in the rectum of the animal. Base-line determinations of blood pressure, pulse, and oxygen tensions in both the femoral artery and thigh muscles were then recorded.

The endotracheal tube was attached to the inflow line of 100% oxygen at 1 ata. During this period, the response of the electrode was again noted. If, for any reason, the electrodes appeared to be functioning improperly, they were removed and the appropriate membranes evaluated within the cuvette. If necessary, membranes were replaced and the entire calibration procedure repeated until the proper conditions were obtained (Figure 3).

The dog was placed within the chamber, and pressure was raised to 3 ata. Although the chamber itself was compressed with air, the dog received 100% oxygen

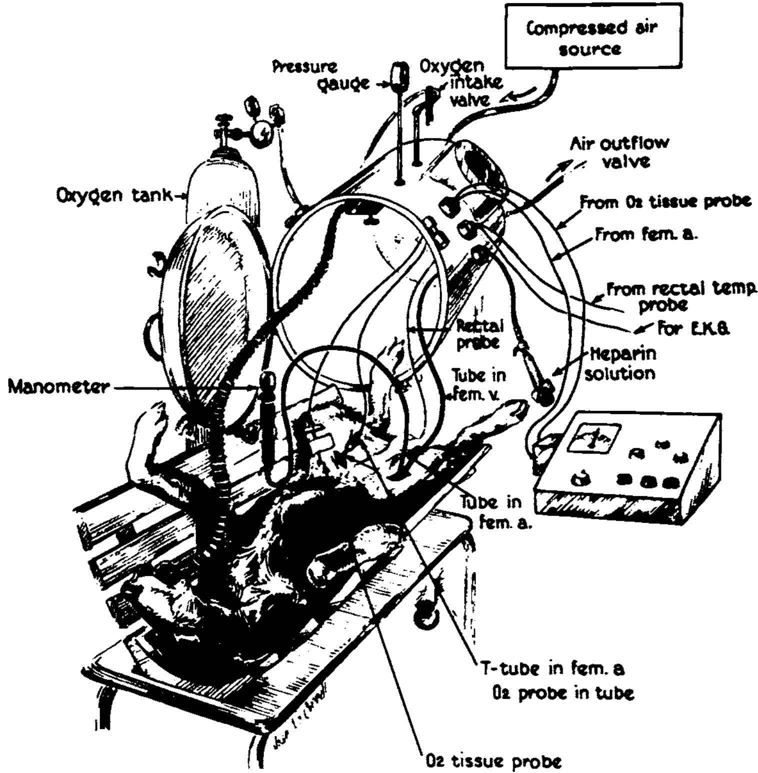

FIGURE 3. Method employed to obtain simultaneous readings of arterial and tissue oxygen tensions both inside and outside the chamber.

through the oxygen inflow line, maintained at a pressure of 50 psig. After the dog was pressurized to 3 ata and the electrodes were responding satisfactorily, a lethal dose of *Cl. perfringens* exotoxin (20–40 pigeon MLD/kg body weight) was given through the intravenous catheter which had been attached to an outlet in the chamber before compression. Pulse, blood pressure, respiration, and arterial and tissue oxygen tensions (pO_2) were noted.

Chamber pressure was maintained at 30 psig for 1 hour, after which decompression was begun. When 1 atm was reached, the dog was removed from the chamber and its responses noted. In order to shorten the recording time of the dog's physiologic reactions until death, it was frequently necessary to administer an additional dose of exotoxin.

Results

Immediately after compression, a slowing of respiration was noted. When the animals were then injected with exotoxin, a short period of bradycardia and hyperpnea followed. Thereafter, it appeared that the animals' usual responses to intravenous injection of exotoxin under normobaric conditions were modified by OHP in that there was a relatively slower respiration rate. Changes in pulse rate were minimal, with occasional irregularities. Whereas arterial pO_2 gradually increased with compression, remaining elevated until decompression, tissue pO_2 showed a much different pattern. The tissue pO_2 slowly increased with compression, remaining relatively stable until the toxin injection. Within 10 min of toxin injection, it began to decrease, although the blood pressure (after its initial

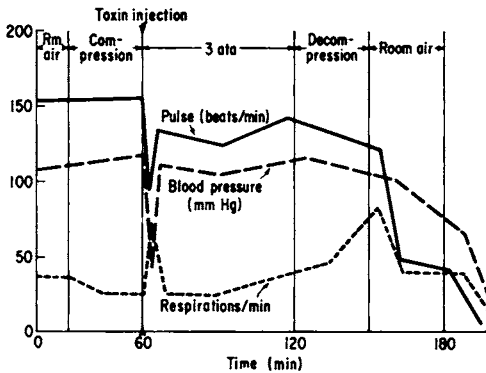

FIGURE 4. Typical response pattern of pulse, blood pressure, and respiration in dogs exposed to OHP and injected with *Cl. perfringens* toxin.

biphasic response) and arterial pO_2 remained steady (Figure 4). The tissue pO_2 values varied greatly from dog to dog, but the pattern of change was relatively consistent (Figure 5).

The arterial pO_2 was maintained until there was a profound decline in the arterial blood pressure. The temperature of the animals usually declined steadily after exotoxin injection. The amount of decrease was about 11°C, the initial temperature of the dogs being about 34–35°C.

DISCUSSION

Our *in vitro* studies demonstrated no inhibitory effect of OHP on the toxic effects of the clostridial exotoxin. *In vivo* studies tended to substantiate the conclusions reached by other workers, that OHP has no influence on exotoxin which has already formed.[11] Intravenous injections of lethal doses of lecithinase alone produce a typical response in dogs,[14] consisting of an initial biphasic blood pressure response with the return of blood pressure to non-shock levels and maintenance of blood pressure until shortly before death. The respiratory rate gradually increases as the dog's condition deteriorates. We found, as many investigators have demonstrated previously, a typical pattern of usual physiologic responses due to OHP alone. However, as soon as these dogs were subjected to a lethal toxin while under the influence of OHP, a temporary bradycardia and hyperpnea resulted, from which they recovered only enough to exhibit once again a pattern rather characteristic of the effects of OHP alone.

Because it is widely accepted that inadequate perfusion of tissues and organs with oxygenated blood is a basic feature of shock regardless of etiology,[15] it was felt that any change of oxygen concentration at the tissue level might give insight into disturbance of cellular physiology. Also, the higher the tissue oxygen tension rises, the more meaningful are its measurements. Therefore, the animals in our experiment were placed under hyperbaric conditions and then injected with exotoxin. The precipitous fall in tissue pO_2 was taken to indicate signs of irreversibility and occurred long before the more gross physiologic phenomena (such as pulse, blood pressure, and respiration) showed any appreciable change.

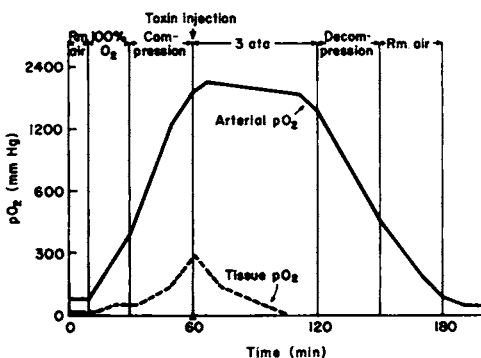

FIGURE 5. Pattern of arterial and tissue pO_2 in dogs exposed to OHP and injected with *Cl. perfringens* exotoxin.

Other investigators[4] have shown that tissue pO_2 decreases in hemorrhagic shock, while arterial pO_2 rises even in the absence of OHP. This effect is apparently due to compensatory hyperpnea. When animals are subsequently treated with OHP, an even greater rise occurs. Arterial oxygen tension in septic shock also rises, and shows a further rise when the

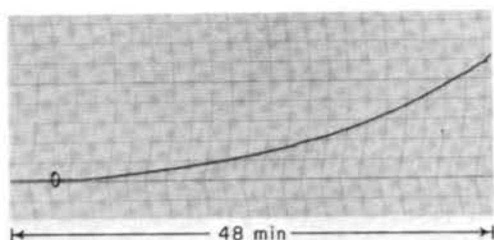

FIGURE 6. Photograph of recording on graph paper of tissue pO_2 measurements in a dog subjected to OHP and injected with *Cl. perfringens* exotoxin. Graph reads from right to left. Sloping curve represents decline in tissue pO_2 within 4 min after exotoxin injection. Within 50 min, tissue pO_2 was usually undetectable.

animal is treated with OHP. We were unable to find comparable references of simultaneous readings of arterial and tissue pO_2 in clostridial toxicity (shock) under hyperbaric conditions (Figure 6).

In these experiments, tissue pO_2 measurements showed evidence of the irreversibility of the toxic state much earlier than simultaneous arterial pO_2 findings. Our studies tend to strengthen the concept that the mechanism of OHP in clinical cases of gas gangrene is not one of action against exotoxin which has already been produced as much as it is an inhibitory action on bacteria, preventing them from producing exotoxin.

SUMMARY

Our experiments indicate that OHP does not neutralize the cell-free exotoxin either *in vitro* or *in vivo*. Tissue oxygen tension measurements indicated that the irreversible pattern of lethal exotoxin toxicity can be demonstrated by this means long before the usual physiologic assessments (such as pulse and blood pressure) reflect irreversibility. We believe these early changes are evidence of early but irreversible effects of the exotoxin on cellular metabolism.

ACKNOWLEDGMENT

This work was supported by the Oliver C. Schmidt, Sr., and Oliver C. Schmidt, Jr., Research Foundation.

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DISCUSSION

Discussion of papers by van Zyl et al. (*pp. 515–520*),
Slack et al. (*pp. 515–525*),
van Elk et al. (*526–537*),
Hill and Osterhout (*pp. 538–543*),
and Nora et al. (*pp. 544–551*).

DR. D. C. SABISTON, JR., *Session Chairman* (*Durham, N. C.*): Dr. van Zyl has a cumulative study of 204 patients with gas gangrene, 168 of which have been proven to be due to clostridia. I think it might be interesting if he would give a brief comment on this collected study.

DR. J. J. W. VAN ZYL: In an effort to determine the extent to which OHP is being used in the treatment of clostridial gas gangrene, questionnaires were sent from this medical center to 100 workers known or thought to be active in this field. Seventy replies have been received and series of cases have been reported in 23. These series were generally small; in 12, the patients numbered five or fewer. The largest series is, of course, that of the Amsterdam group under Professor Boerema and described by Dr. Brummelkamp earlier in this session. In these replies, a total of 170 proven clostridial infections were reported, and I would like to review some of the important findings briefly at this time.

First, there was a preponderance of males, only 53 of the 170 patients being females. This 2:1 ratio of males to females would be expected, however, because of greater exposure of males to severe trauma. Table 1 shows the age distribution of the patients.

Trauma preceded the onset of gas gangrene in 156 patients, while in only 14 did it develop with no (or minimal) injury. In 23 of the cases (13%), the clostridial infection developed in the surgical wound site after premeditated surgery (Table 2). The presence of clostridia in the gallbladder is well recognized. The development of this infection in amputation stumps, where the operation is done for peripheral ischemia, is probably at least partly due to deficient blood supply in the remaining tissues.

TABLE 1. Age Distribution of Patients

	Age				
	<17	17–30	31–50	51–70	>70
No. pts.	16	60	40	41	13

TABLE 2. Gas Gangrene in 23 Cases after Premeditated Surgery

Operation	No. pts.
Cholecystectomy	5
Other abdominal surgery	5
Amputation for ischemic disease of leg	4
Elective osteotomy	2
Miscellaneous	7

Hyperbaric treatment facilities are still few and far between, and yet nearly 80% of the patients reported in the questionnaires were transported less than 50 kilometers. Vast geographical areas and huge population masses, therefore, fall outside the effective transportation range. One can only speculate on the true incidence of this infection as a cause of postoperative complications and mortality, where it is found to make up 13% of this collected series.

Diabetic patients are particularly liable to all forms of sepsis, including that due to *Cl. perfringens.* Still, there is a noteworthy contrast between the 15% incidence in the total series (15 of 98 patients)* and the 25% in my own series (4 of 16 patients) from the Karl Bremer Hospital, Cape Town.

Distinction between purely cellulitic lesions and those combined with myositis was frequently difficult. In 98 cases where this

* Between the time of the conference in November, 1965, and June, 1966, the incidence of diabetes in the Duke series increased to 54%.

TABLE 3. Tissue Involvement [a]

	No. pts.
Myositis only	11
Cellulitis only	10
Myositis + cellulitis	74
Positive blood culture only	3
Total	98

[a] Clinical distinction between myositis and cellulitis was possible in 29.

assessment was attempted, the distinction could be made with reasonable confidence in only 29 cases. Table 3 shows the distribution of myositis, cellulitis, and the two in combination.

The use of antitoxin was associated with a remarkably high death rate, as shown in Table 4. While I am not prepared to unreservedly condemn it as a causative factor in these deaths, I cannot help but harbor suspicions. Its employment was not necessarily dictated by the severity of illness in the fatal cases. Other contributors, who achieved better results and avoided the use of antiserum, also had their share of desperate cases.

Antibiotics were used routinely, except in 15 of the cases of Professor Boerema and his associates, where no antibiotics were given. Two of these patients died, but in neither case could withholding antibiotics be considered to have played a part in causing the deaths.

Causes of death are listed in Table 5. The mortality in cases of gas gangrene treated with OHP remains high, the overall death rate approximating that quoted by Dr. Altemeier in this discussion for cases receiving conventional treatment. Deaths directly due to gas gangrene do, however, include a large proportion of moribund patients who, by all standards, are beyond saving. Eighteen of the 43 patients who died in the series of 170 collected by questionnaire fell into this category and did not survive beyond the first day of OHP therapy.

TABLE 4. Use of Antitoxin

	No. pts.	Deaths
Antitoxins used	52	19 (37%)
No antitoxin	55	10 (18%)

TABLE 5. Causes of Death in 43 Patients [a]

Cause	No. pts.
Primary disease [b]	23 (13%)
Complications	13 (8%)
Unrelated conditions	7 (4%)
Total	43 (25%)

[a] Percentages based on total number of 170 patients in the series.
[b] Five of these patients (3% of series) died after the first day of OHP treatment.

If these 18 are subtracted from the 23 who died as a direct result of the gas gangrene, the amended death rate due to the primary condition is reduced to 3% (5/170 cases) among those living long enough to begin to derive benefit from the OHP treatment.

Excision of necrotic tissue and amputation is still required, but the extent of loss is much less than that resulting from obligatory tissue ablation associated with the conventional management of gas gangrene (Table 6). For 50 survivors reported in the questionnaires, the actual level of amputation after OHP was diagramatically recorded, along with the contributor's estimate of what radical amputation would have been necessary had the patient not received OHP therapy. From these diagrams, the percent of tissue preservation was assessed. Thus, where below-knee amputation was performed instead of above-knee amputation, or a mid-thigh amputation instead of a

TABLE 6. Amputations in Survivors with Gas Gangrene Involving Extremities, after OHP Treatment [a]

Involved extremity	No. pts.	Amputations	Radical [b]	Conservative [c]
Arm	12	6	0	6
Leg	46	16	5	11

[a] The survival rate just within the group of patients with arm involvement was 92%; in the group with leg involvement it was 81%.
[b] Radical amputation refers to the type of tissue ablation considered necessary in the conventional form of treatment.
[c] Conservative amputation is that done so as to retain all viable tissue compatible with a good functional result.

TABLE 7. Estimated Percent of Additional Tissue Preservation Due to OHP in 50 Survivors [a]

Added tissue saved	No. pts.
<10%	1
10–20%	3
20–40%	16
40–60%	3
60–90%	4
90–100%	23

[a] Based upon expected tissue ablation by conventional treatment methods.

disarticulation at the hip, a saving of 40% was recorded. Where a whole limb was saved (although scarred, or with some muscle loss) 90-100% preservation was recorded. Table 7 shows the contributors' assessments of tissue preservation in their patients on this basis. Such an assessment is, of course, subject to considerable error, but this table is presented as an indication of tendencies rather than of absolute values. Seen against the background of the case reports to which we have listened today, however, these estimates are probably fairly accurate, and tissue preservation in these patients certainly contrasts with the tissue loss necessitated by the conventional treatment plan described by Dr. Altemeier.

Finally, I wish to express my sincere appreciation to those who have taken such great pains to supply the information on which this survey has been based.

DR. J. MENDELSON (*Edgewood Arsenal, Md.*): We have been working for many years on the subject of *Cl. perfringens* infection and its various forms of therapy, and I think it necessary that we consider all factors involved. For example, what is it that we are treating when the infection is associated with lowered oxygen tension in the tissues and there is some evidence of devascularized tissue of some type present? It may well be that, in obtaining good results with hyperbaric oxygen, one is not so much directly affecting the organisms which may be inaccessible, but rather raising the oxygen tension in the adjacent tissue, thereby providing a defense against the effects of the organism. It is known that one can put a great number of *Cl. perfringens* organisms into healthy tissue without adverse effects. Possibly one can use much lower doses of oxygen to achieve the beneficial effects that might occur. I noticed that one of the speakers had to terminate treatment because 3 atmospheres was too hard on the patient. We have found in our animal studies that 3 atmospheres is, indeed, the borderline of toxicity (J. Trauma 4:642, 1964). It was also mentioned that antibiotics did not appear to be beneficial. Indeed, if you have an area of devascularized tissue, the systemic antibiotics are not going to get there. We have found that where we have areas of devascularized tissue in experimental animals that otherwise always died of *Cl. perfringens* infection, we can prevent death in those animals without any debridement or other treatment if we apply a certain antibacterial substance topically (J. Trauma 2:239, 1962).

DR. S. ATTAR (*Baltimore, Md.*): I would like to comment on Dr. Nora's paper in which he showed that the oxygen tissue tension after exotoxin injection was nearly zero, compared to arterial oxygen tensions of about 2000 mm Hg. I am sure this discrepancy is so apparent that no serious conclusions should be attached to it. It only reflects the inadequacies of the method of measuring tissue oxygen tension.

DR. R. ASHFIELD (*London, England*): As there are so many variables in hyperbaric treatment, I would like to make a plea that workers adhere to either unit atmospheres, half-atmospheres, or, at the most, quarter-atmospheres for comparative purposes. I would, therefore, like to ask Dr. van Elk why he selected 28 psi, which is just below 3 atmospheres. Someone beside me cruelly suggested that that was all his compressors could give, but I am sure that's not true.

DR. J. VAN ELK: Our compressors do go beyond 30 psi. Twenty-eight pounds per square inch was chosen because it seemed to us adequate for our purposes, and I think the results have shown that a pressure of 28 pounds is indeed adequate.

Effect of Hyperbaric Oxygen on *Clostridium botulinum* and Its Toxin

V. FREDETTE

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Experiments from this laboratory to determine the effect of hyperbaric oxygenation upon *Clostridium perfringens, Clostridium septicum,*[1] *Clostridium tetani,*[2] *Streptococcus micros, Streptococcus parvulus,*[3] *Staphylococcus pyogenes* var. *aureus,*[4] and their toxins have suggested that OHP acts not so much upon the toxins,[5] when they are present, as upon the organisms themselves. It appeared that studies on *Clostridium botulinum* might help to elucidate the intimate mechanism by which OHP alters the course of diseases caused by the anaerobic bacteria, at least. In the case of botulism, it is widely accepted that the causative organism cannot multiply in the human body, the disease usually resulting from absorption of preformed toxin in contaminated, insufficiently heated food. We decided to utilize the marked biological activity of this powerful toxin as an experimental tool, to evaluate the effects of hyperbaric oxygen on toxin and on the metabolism of the organism itself.

Materials and Methods

Types A and C botulinum toxins were prepared by culturing the organisms in glucose VF broth (a peptic digest of beef muscle and liver) in a cellophane tube apparatus described previously.[6,7] Material containing an MLD (minimum lethal dose, mouse) of 10^6–10^7/ml was obtained upon harvest, and 1- and 5-ml volumes of these fresh toxins were exposed in Wassermann tubes or in petri dishes to 45 or 90 psig of 100% oxygen for 2 hours (Figure 1A). Identical samples were left under atmospheric pressure for the same period of time. Titration of these two lots was done by intraperitoneal injection into mice of decimal dilutions of the toxins in cold gelatin–phosphate buffer; gross alterations in the activity of the toxin could thus be detected. To date, eight such experiments have been performed.

In another study, similar dilutions of botulinum toxin were injected into guinea pigs, the animals were exposed for 2 hours to 100% oxygen at 45 psig (Figure 1A), and observations were made of symptomatic sequelae and time of death. Control guinea pigs, receiving identical doses of toxin, were left at atmospheric pressure in order to compare any differences in appearance of symptoms and time of

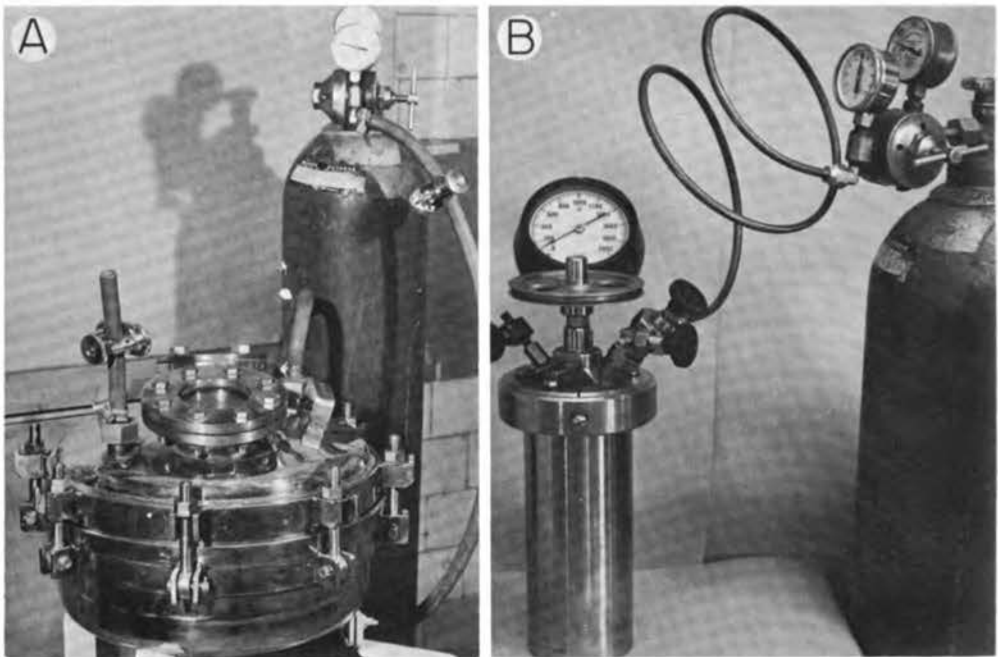

FIGURE 1. A, Pfaudler reagent vessel used to expose botulinum toxin or inoculated guinea pigs to oxygen at 45 psig. These vessels are guaranteed safe to only 120 psig of pressure; we found ours unsatisfactory even for the 90 psig of pressure, however, as the numerous closures and gaskets leaked abundantly. B, Parr hydrogenation bomb used to expose *Cl. botulinum* cultures to 90 psig of oxygen. This apparatus can be operated safely at very high pressures. Its disadvantage is that only plastic petri dishes may be used, and the apparatus will accommodate no more than 24 tubes at a time, even when the inside accessories are removed.

death. Seven such experiments have been completed.

Finally, the effect of OHP upon *Cl. botulinum* itself was studied. Decimal dilutions of fresh young cultures of types A, B, and C *Cl. botulinum* were prepared in two series of tubes containing freshly regenerated glucose VF broth. One series of tubes was placed in a Parr hydrogenation bomb (Figure 1B) which was pressurized to 45 or 90 psig with 100% oxygen. The entire apparatus was placed in an incubator, where it stayed overnight along with the second series of tubes which remained at atmospheric pressure for the same length of time. Comparison between the OHP-exposed and control dilutions which showed growth indicated the number of organisms which resisted OHP and multiplied under these conditions, and, conversely, it gave an indication of the ratio of organisms which were killed or inhibited by hyperbaric oxygen. Eighteen experiments form the basis of our results in this last study.

RESULTS

Table 1 summarizes the results obtained when type C and type A botulinum toxins were exposed to 45 and 90 psig of 100% oxygen, respectively. These titrations in mice showed no marked alterations of the toxin *in vitro*. Experiments 1, 3, 5, and 6 showed mortality differences of one dilution, that is, of the order of about 10 MLD/ml. Experiments 4, 7, and 8 would indicate the contrary, although in these three experiments differences occurred only in the numbers of mice dying for a given dilution, or in the survival time.

TABLE 1. Mouse Titers of Botulinum Toxins Exposed to Atmospheric Pressure and OHP

Expt. no.	Toxin type	Dilution	Mouse mortality ratio (deaths/no. inoculated)			
			1 atm		OHP	
			1 ml	5 ml	1 ml	5 ml
		10^{-5}	2/2 (24 hr)	2/2 (24 hr)	2/2 (24 hr)	2/2 (24 hr)
1	C	10^{-6}	2/2 (48 hr)	2/2 (48 hr)	2/2 (48 hr)	0/2
		10^{-7}	0/2	1/2 (48 hr)	0/2	0/2
		10^{-5}	2/2 (24 hr)	2/2 (24 hr)	2/2 (24 hr)	2/2 (24 hr)
2	C	10^{-6}	0/2	0/2	0/2	0/2
		10^{-7}	0/2	0/2	0/2	0/2
		10^{-5}	2/2 (24 hr)	2/2 (24 hr)	2/2 (24 hr)	2/2 (24 hr)
3	C	10^{-6}	0/2	1/2 (24 hr)	0/2	0/2
		10^{-7}	0/2	0/2	0/2	0/2
		10^{-5}	—	4/4 (24 hr)	—	4/4 (24 hr)
4	C	10^{-6}	—	4/4 (48 hr)	—	4/4 (24 hr)
		10^{-7}	—	1/4 (48 hr)	—	4/4 (120 hr)
		10^{-5}	—	4/4 (24 hr)	—	4/4 (24 hr)
5	C	10^{-6}	—	4/4 (48 hr)	—	4/4 (96 hr)
		10^{-7}	—	4/4 (96 hr)	—	0/4
		10^{-6}	—	4/4 (48 hr)	—	0/4
6	A	10^{-7}	—	0/4	—	0/4
		10^{-8}	—	0/4	—	0/4
		10^{-5}	—	4/4 (72 hr)	—	4/4 (72 hr)
7	A	10^{-6}	—	2/4 (72 hr)	—	2/4 (72 hr)
		10^{-7}	—	1/4 (72 hr)	—	2/4 (72 hr)
8	A	10^{-3}	—	4/4 (24 hr)	—	4/4 (24 hr)
		10^{-4}	—	2/4 (24 hr)	—	4/4 (48 hr)

When 1-ml volumes of toxin were exposed to OHP, no difference whatsoever could be detected (experiments 1, 2, and 3).

Table 2 shows the results obtained when guinea pigs inoculated with botulinum toxin were exposed to 2 hours of 100% oxygen-breathing at 45 psig. If one disregards accidental deaths, which were rare in healthy animals (experiments 3, 4, and 5), it appears here also that OHP had no pronounced effect on botulinum toxin *in vivo*. Three experiments (2, 6, and 7) showed mortality differences of one to two dilutions, but two other experiments (1 and 4) showed exactly the reverse, and the remaining two tests gave perfectly identical results.

Concerning the effect of hyperbaric oxygen upon *Cl. botulinum* itself, results with type C (Table 3) were far from convincing for the simple reason that this type of culture autoagglutinates spontaneously, thereby giving growth only in the first two or three tubes, even in the control series. In seven experiments with type A and nine others with type B, oxygen under pressure noticeably inhibited the growth of these anaerobic bacteria, as could have been expected. Growth was not completely suppressed, however, and the difficulty lies in determining the degree of inhibition.

Figure 2A illustrates this phenomenon; growth and abundant gas formation occurred readily underneath the stainless-steel ball bearing (which acts as a loose seal in the Hall tube), especially when the tubes were left at normal atmospheric pressure. When the tubes were pressurized to 45 psig in 100% oxygen (Fig-

TABLE 2. Effect of Atmospheric Pressure and OHP on Mortality of Guinea Pigs Injected with Botulinum Toxin [a]

Expt. no.	Dilution	Guinea-pig mortality	
		1 atm	OHP
1	10^{-4}	D (24 hr)	D (24 hr)
	10^{-5}	S	D (24 hr)
	10^{-6}	S	S
2	10^{-4}	D (24 hr)	D (24 hr)
	10^{-5}	D (24 hr)	S
	10^{-6}	D (48 hr)	S
3	10^{-4}	D (24 hr)	D[b]
	10^{-5}	D (24 hr)	D (24 hr)
	10^{-6}	S	S
4	10^{-4}	S	D (144 hr)[c]
	10^{-5}	S	D[b]
	10^{-6}	S	S[c]
5	10^{-4}	D (24 hr)	D (24 hr)
	10^{-5}	D (24 hr)	D[b]
	10^{-6}	S	S[c]
6	10^{-4}	D (24 hr)	D (24 hr)
	10^{-5}	D (24 hr)	D (24 hr)
	10^{-6}	D (72 hr)	S[c]
7	10^{-4}	D (24 hr)	D (24 hr)
	10^{-5}	D (48 hr)	S[c]
	10^{-6}	S	S[c]

D, died.
S, survived.
[a] Type C toxin was used in all cases. One guinea pig was used for each dilution within an experiment, except where indicated by footnote *c*.
[b] Died during decompression.
[c] Three guinea pigs were tested with this dilution.

ure 2B), growth was only partly inhibited underneath the loose seal. A difference in two tubes was easily seen (tubes 7 and 8), but the degree of inhibition elsewhere could not easily be measured, especially in the presence of a seeming prozone phenomenon, as in the first two tubes. The absence of gas in this latter case was due to loss during decompression and should be discounted.

The same difficulty in interpretation arose when these anaerobic bacteria were grown in straight-bore tubes, as illustrated in Figure 3. Here, it was evident that growth had been inhibited considerably by OHP, but the degree of inhibition was impossible to state without accurate measurements. An effort was made to record in gross form the results obtained. Table 4 presents the results when type B *Cl. botulinum* was exposed to 45 psig of 100% oxygen, and Table 5 presents the results obtained with type A under 100 psig of 100% oxygen.

CONCLUSIONS

These results, while not impressive in themselves, reinforce the conclusions drawn from previously recorded experiments using other anaerobic bacteria and toxins, particularly *Cl. perfringens* and *Cl. tetani, i.e.*, that oxygen under pressure acts not so much upon the toxins as upon the metabolism of the microorganisms themselves.

TABLE 3. Growth of *Cl. botulinum* C Under Atmospheric Pressure and OHP

Expt. no.	Dilution	Growth	
		1 atm	OHP
1	10^{-1}	4 +, gas	4 +, gas
	10^{-2}	Sediment, gas	Sediment, gas
	10^{-3}	—	Sediment, gas
	10^{-4}	—	—
	10^{-5}	—	—
	10^{-6}	—	—
2	10^{-1}	4 +, gas	3 +, gas
	10^{-2}	—	—
	10^{-3}	—	—
	10^{-4}	—	—
	10^{-5}	—	—
	10^{-6}	—	—

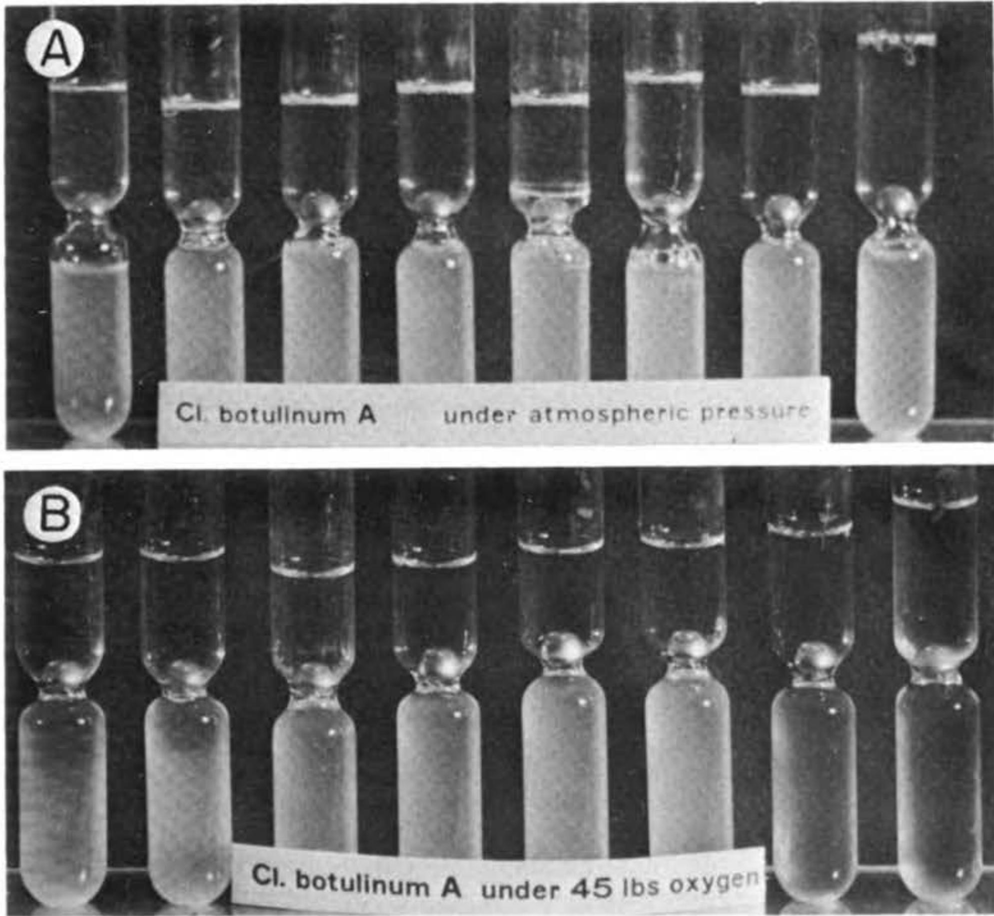

FIGURE 2. A, *Cl. botulinum* type A cultures kept at normal atmospheric pressure in Hall tubes. Decimal dilutions are from left to right. B, similar cultures pressurized to 45 psig in oxygen.

TABLE 4. Growth of *Cl. botulinum* B Under Atmospheric Pressure and OHP

Expt. no.*	Dilution	1 atm		OHP	
		Growth	Gas	Growth	Gas
1	10^{-1}	2+	+	1+	+
	10^{-2}	3+	+	2+	+
	10^{-3}	4+	+	3+	+
	10^{-4}	4+	+	3+	+
	10^{-5}	4+	+	3+	+
	10^{-6}	3+	+	2+	+
	10^{-7}	1+	—	—	—
	10^{-8}	—	—	—	—
	10^{-9}	—	—	—	—
	10^{-10}	—	—	—	—
2	10^{-1}	2+	+	2+	slight
	10^{-2}	2+	+	2+	slight
	10^{-3}	4+	+	4+	slight
	10^{-4}	4+	+	4+	slight
	10^{-5}	4+	+	4+	slight
	10^{-6}	4+	+	3+	slight
	10^{-7}	4+	+	3+	slight
	10^{-8}	4+	+	3+	slight
	10^{-9}	4+	slight	2+	slight
	10^{-10}	3+	—	1+	slight

* Seven other experiments gave similar results.

TABLE 5. Growth of *Cl. botulinum* A Under Atmospheric Pressure and OHP

Expt. no.		1 atm		OHP	
		Growth	Gas	Growth	Gas
1	10^{-1}	2+	+	2+	slight
	10^{-2}	3+	+	3+	slight
	10^{-3}	4+	+	4+	slight
	10^{-4}	4+	+	4+	slight
	10^{-5}	4+	+	4+	slight
	10^{-6}	3+	+	4+	slight
	10^{-7}	2+	+	1+	—
	10^{-8}	—	—	—	—
2	10^{-1}	4+	+	3+	—
	10^{-2}	4+	+	3+	—
	10^{-3}	4+	+	2+	—
	10^{-4}	4+	+	2+	—
	10^{-5}	—	—	2+	—
	10^{-6}	—	—	—	—
	10^{-7}	—	—	—	—
	10^{-8}	—	—	—	—

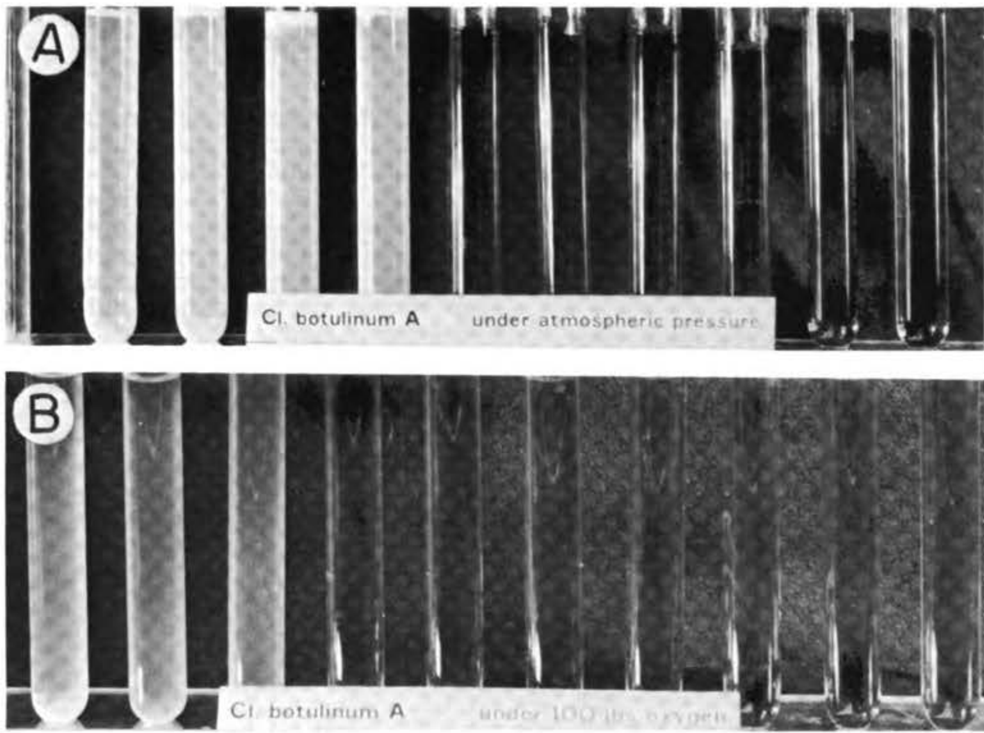

FIGURE 3. A, *Cl. botulinum* type A cultures kept at normal atmospheric pressure in straight-bore tubes. Decimal dilutions are from left to right. B, similar cultures pressurized to 100 psig in oxygen.

ACKNOWLEDGMENT

I wish to thank my colleague, Dr. G. Vinet, for preparing the types A and C toxin used in these studies.

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DISCUSSION

DR. A. R. BEHNKE (*San Francisco, Calif.*): One of the most powerful tools in biological analysis is the use of bacterial enzyme-substrate systems. Are reports of the effects of hyperbaric oxygen on the bacteria-induced reactions as extensively investigated in connection with irradiation phenomena?

DR. FREDETTE: Dr. Behnke's remark is well-taken. Unfortunately, we do not know exactly which enzymes are involved with anaerobic bacteria. However, he is right in thinking that we should now examine some of these basic mechanisms. We can always crush our bacteria to see what happens under hyperbaric oxygen, but this creates an immediate problem. One of my colleagues, working at the Pasteur Institute a few years ago, found that *Clostridium sporogenes*, a supposedly strict anaerobe, contains an enzyme which will actually use up oxygen in the presence of some amino acids. So the question of what is an anaerobe and what is an ordinary bacterium remains.

DR. I. MCA. LEDINGHAM, *Session Chairman* (*Glasgow, Scotland*): There is quite a gap between test tubes and mice and clinical cases of botulism. Your comments indicate that hyperbaric oxygen will have little, if any, effect on botulism, assuming that it is primarily a toxemia. Also, is it fair to draw conclusions from these tubes as to the mechanism of growth reduction in organisms exposed to a very high tension? As they are in a hyperbaric chamber at 45 and 90 psig, can one compare these with the tissues of small animals, in which lower oxygen tensions are present? What are the pO_2 tensions at the bottom of these test tubes as opposed to those at the top?

DR. FREDETTE: We used methylene blue as an indicator, and oxygen reaches down to the very bottom in small volumes, such as 1 ml. We also placed the toxin in petri dishes to be doubly sure that it was well exposed to oxygen. You are quite right, however, that artificial laboratory experiments should not be related to what will happen in the human body. I would like to repeat these experiments and attempt to simulate in the laboratory what actually goes on in the animal's body, *i.e.*, combining toxin with nervous tissue, red blood cells, *etc.* I purposely exaggerated the influence of oxygen by using 90 psig, in order to make doubly sure that there was no effect on the toxin itself.

SESSION VI
Hyperbaric Oxygenation in Infections, Traumatic Ischemia, and Wound Healing

Chairman: I. MCA. LEDINGHAM
Department of Surgery
Western Infirmary
Glasgow, Scotland

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Effects of Hyperbaric Oxygenation and Antibiotics on Aerobic Microorganisms

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High pressure oxygen (OHP) has received considerable attention in the medical literature recently because of its theoretical potential in the treatment of diseases involving anoxia. The underlying rationale is that large quantities of oxygen can be forced into physical solution in the fluids of the blood, thus supplementing the oxygen-carrying capacity of the blood cells. The dissolved oxygen may cross from the intravascular to the extravascular and intracellular spaces by diffusion, and thereby become available to the hypoxic cells.

Evidence is accumulating, however, which indicates that certain limitations imposed by many of these disease states cannot be overcome solely by presenting the cells with more oxygen in physical solution. In hemorrhagic shock, for example (where ultimate deficit would theoretically be oxygen lack at the cellular level), OHP is distinctly effective only when used early in the experimental shock preparation;[1] when applied later, it is of little value.[1,2] In conditions where long-standing hypoxia is the problem, the ability of the cells to utilize oxygen may be impaired. As the disease progresses, the simple presentation of oxygen in physical solution may be insufficient to reverse the disease.[3] In septic conditions, similar limitations seem apparent.[4-6]

Studies performed on common coliform organisms as well as on staphylococci have indicated that the response of bacteria to OHP both *in vitro* and *in vivo* is biphasic as oxygen tensions increase.[6] When oxygen tensions are increased to a critical point, bacterial growth is stimulated to proceed at an above-normal rate. Thus, with *Escherichia coli,* increasing oxygen tension *in vitro* from that found under normal atmospheric conditions (160 mm Hg) to that possible in pure oxygen (760 mm Hg) steadily increases the growth rate, as measured over a defined period of time. Above 2 atm of pure oxygen, however, inhibition of growth occurs.

Using survival time and bacteremia as parameters of intraperitoneal bacterial growth rate in the dog with fecal peritonitis, a similar phenomenon seems to occur *in vivo.*[6] Thus, the oxygen tensions actually reaching the bacteria are critical in deciding whether bacterial growth will be stimulated or depressed. Oxygen tensions reaching the locus of infection, especially in shock conditions where the

perfusion is decreased, do not approach the tensions breathed by the shocked animal.[6] We have demonstrated that breathing 3 atm of oxygen may result in intraperitoneal oxygen tensions of less than 100 mm Hg in septic shock, unless the peritoneal cavity is vented for equilibration with chamber tension. At these tensions, the intraperitoneal bacteria can proliferate more rapidly than if OHP were not used at all. It appears that OHP can be a double-edged sword in septic problems. Practical limitations determined by the actual oxygen tensions reaching the bacteria may limit its clinical application—a problem which warrants further study.

The following study is an *in vitro* analysis of the effects of varying combinations of high pressure oxygen and antibiotics upon bacterial growth rate. It was designed to determine whether the addition of OHP to standard antibacterial therapy would supplement or inhibit the effect of the antibiotic alone. Such knowledge would help define the restrictions that might be placed on the use of OHP in septic conditions and might also better delineate those situations where OHP might have a beneficial effect in the treatment of sepsis.

Materials and Methods

Thin-layer culture preparations (described previously [6]) were used for the measurement of growth rate as determined by optical density over a 3-hour period. To 8 ml of trypticase soy broth, placed in a sputum jar, was added 0.3 ml of bacterial suspension containing 10^9 bacteria/ml. An appropriate dilution of antibiotic was then added in a volume of 0.3 ml to make a total volume of 8.6 ml of medium in the sputum jar, resulting in a depth of approximately 5 mm.

The experimental preparations were placed in the hyperbaric chamber and pressurized. Control preparations, identically prepared, were placed in an incubator under room atmospheric conditions. At the end of 3 hours, the preparations were removed, their volumes measured, and aliquots taken for measurement of optical density in the Beckman DU spectrophotometer at a wavelength of 650 mμ. In each case, optical density was corrected for any volume difference due to differential rate of evaporation in the chamber under pressure as compared to that in the incubator. Relative percentage of growth rate was then calculated using the control as the 100% base line.

Figure 1 illustrates why we found it necessary to use a standard thin-layer broth preparation for these studies. The 5-mm thin-layer preparation placed in the chamber under 3 atm of oxygen resulted in a pO_2 which rose rapidly and reached inhibiting levels in 30 min or less. In a standard test-tube preparation, the pO_2, measured at a depth of 5 cm at 3 atm of oxygen pressure, failed to reach 760 mm Hg over a 3-hour period.

Oxytetracycline was used in combination with OHP in studies involving *E. coli* serotype 0127B8. The *E. coli* cultures were kept in skim milk in the freezer for indefinite periods. Periodically, they were subcultured on glycerinated trypticase soy agar slants which could be stored in the refrigerator for 3 weeks at 1–4°C. The afternoon before each experiment, the organisms were subcultured and placed in an incubator at 37°C, and the next morning the culture was har-

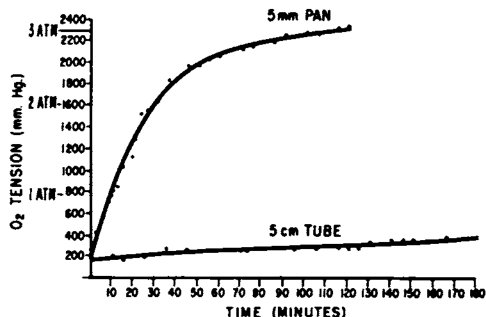

FIGURE 1. Rate of oxygen saturation in trypticase soy broth under 3 atm of oxygen pressure.

vested for use. The culture was periodically checked with the 0127B8 typing antiserum and was also scrutinized grossly and microscopically to be sure that it maintained its identity as pure *E. coli* 0127B8.

The same hyperbaric chamber was used for all experiments. A catheter was placed from the oxygen inlet in the top of the chamber to the bottom of the chamber to better ensure the uniformity of oxygen throughout and avoid problems that might exist concerning diffusion of oxygen through a column of compressed air trapped in the chamber. Before the experiment, the chamber was vigorously flushed with oxygen.

Oxygen tensions were measured with a Beckman physiologic gas analyzer (Model 160) and a platinum-tipped microelectrode. Measurements were standardized using a nitrogen gas zero and two known gas standards.

RESULTS

Figure 2 shows the relative growth rates of *E. coli* with varied concentrations of oxytetracycline under different pressures of oxygen. The percentages of growth were plotted relative to the growth in the incubator under ordinary atmospheric conditions. The curve showing the effects of OHP with no oxytetracycline is a reduplication of previously published curves.[6] Each curve representing a given dilution of oxytetracycline is essentially the same type of biphasic curve which has been shifted to a lower base line due to the inhibitory effects of oxytetracycline. A dilution of 0.35 mg of oxytetracycline for every 10^9 *E. coli* cells resulted in the identical curve, starting at a lower base line, so that at atmospheric conditions (0.2 atm oxygen) the base line began at 80%. This merely indicates that this concentration of oxytetracycline gave a 20% inhibition, resulting in only 80% of the growth that would occur if no antibiotic were added. At a dilution of 1.5 mg/10^9 organisms, the same curve was reproduced, beginning at a base line of only 35% of the growth that occurred normally.

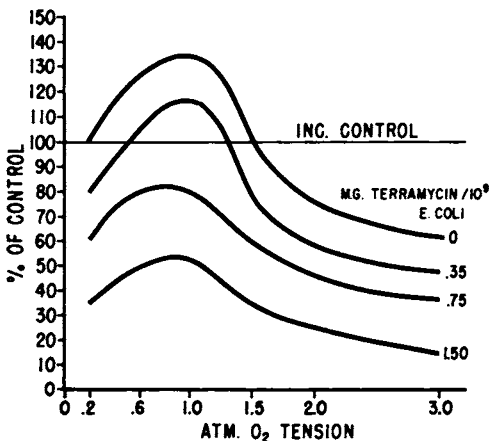

FIGURE 2. The combined effect of oxytetracycline and OHP on growth of *E. coli.*

With lower oxygen tensions, OHP actually reversed the effects of the antibiotic. Indeed, with a low dilution of oxytetracycline (0.35 mg/10^9 organisms), at 0.6 atm and at 1 atm the growth with oxytetracycline plus OHP was greater than that occurring in an untreated incubated control specimen. At this dilution, the OHP succeeded in more than reversing the effect of the antibiotic. At the other dilutions, the effects of the antibiotic relative to the incubator controls were partially reversed. When the oxygen tension was above 1.5 atm, there was a cumulative effect upon the bacterial growth which seemed to represent the sum of the inhibition caused by oxytetracycline plus that caused by OHP. Under these conditions, bacterial growth rate was less with a combination of oxytetracycline and OHP than with either of the agents alone.

Figure 3 shows essentially the same information plotted in a different manner, to emphasize certain information. Here, the percentage of inhibition relative to untreated controls was plotted as a function of the concentration of oxytetracycline with different atmospheric tensions of oxygen, represented by separate curves.

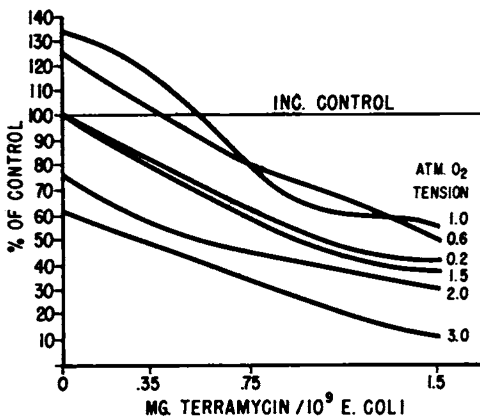

FIGURE 3. The effect of OHP and oxytetracycline on growth rate of *E. coli*.

This figure emphasizes the fact that at 0.6 and 1 atm of oxygen tensions, the effect of oxytetracycline was completely reversed, so that growth occurred more rapidly in the bacterial culture medium than it would have if no oxygen were applied at all. On the other hand, at 2 and 3 atm, growth occurred at a slower rate than with oxytetracycline alone. The effect of oxytetracycline alone is represented by the curve of growth rate at 0.2 atm, or normal atmospheric conditions. It is in a center position among the six curves.

The question arises whether the two variables, high pressure oxygen and oxytetracycline, exerted an effect on one another. Did increasing the concentration of oxytetracycline also increase either the stimulation phase or inhibition phase seen with OHP, and/or did increasing the high pressure oxygen change the sensitivity of the organism to oxytetracycline? In an attempt to answer this, data obtained from experimental preparations exposed to oxytetracycline and OHP were compared, not to the untreated incubator controls, but to the controls containing the same amount of antibiotic at normal atmospheric conditions (Figure 4). In this way, the effects of oxytetracycline cancelled each other out; it follows that if the antibiotic were affecting the sensitivity to OHP, the resulting curve should

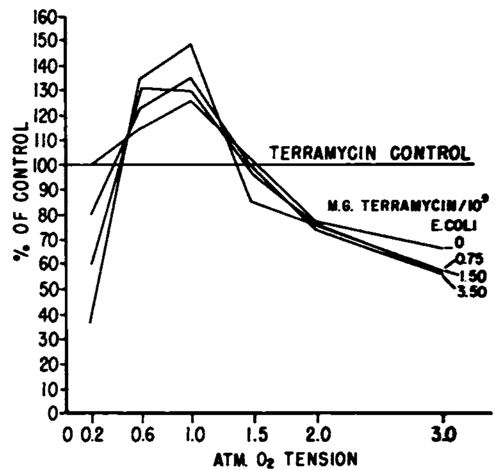

FIGURE 4. The effect of OHP on *E. coli* grown in varying dilutions of oxytetracycline.

be different than the curve obtained from the use of OHP alone. This did not appear to be the case; rather, the three curves produced by the various oxytetracycline dilutions seemed to be essentially the same as the curve produced with oxygen alone. The differences noted in the curves were not statistically significant.

In the same manner, the experimental preparations treated with OHP and oxytetracycline were compared with controls treated with OHP alone. The effects of high pressure oxygen were theoretically ruled out, and if the OHP had altered the sensitivity to oxytetracycline, the curve should have been different than that occurring with oxytetracycline alone under normal atmospheric conditions. Figure 5

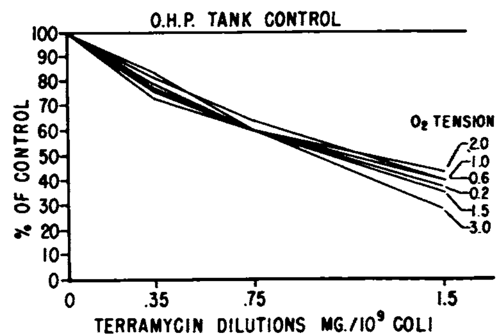

FIGURE 5. The effect of oxytetracycline on *E. coli* grown under varying conditions of OHP.

shows that the curves resulting from the various oxygen tensions approximated the curve of oxytetracycline alone at atmospheric conditions. They all seem to make essentially the same curve, implying that the two parameters affect the bacteria independently, the total effect representing their sum.

Discussion

For many years, bacteria of various types have been recognized as sensitive to changing oxygen tensions. For groups of bacteria classified as obligate anaerobes, such as the clostridia, the critical oxygen tensions were below those normally found under atmospheric conditions. Under such conditions, these bacteria were unable to multiply in the vegetative form and often assumed spore formation. When they were transferred to anaerobic conditions, with oxygen tensions much below those found in normal atmospheric situations, they reverted to the vegetative forms. Such conditions are present in anaerobic culture media or ischemic necrotic wounds where the blood supply is impeded and oxygen is not brought to the tissue in normal amounts.

Obligate aerobes are also susceptible to increasing oxygen tensions. Pneumococci respond with a bacteriostatic effect at first and then, with increase of either oxygen dose or exposure time, with a bactericidal effect.[7] In 1878, Bert demonstrated sensitivity of aerobic organisms to increased oxygen tensions.[8] In 1907, Berghaus demonstrated a bactericidal effect of 24-hour exposure to 2 atm of oxygen in five of 20 microorganisms studied.[9] In 1934, Thaysen noted a bactericidal effect on *E. coli* and staphylococci within 4 hours of OHP.[10] He also described the relationship between environmental temperature and oxygen effects, noting that lowering temperatures decreased susceptibility of the organisms to oxygen.

By the time interest in hyperbaric oxygen had been renewed for its therapeutic potential, considerable information had already been gathered concerning the sensitivity of both aerobic and anaerobic bacteria to increased oxygen tensions. The first clinical application was made by Brummelkamp and Boerema, when they used OHP to treat infections caused by the obligate anaerobe clostridia.[4] Gas gangrene was successfully treated, and this therapeutic use remains well established.

In 1963, two reports again appeared to reiterate the fact that a variety of microorganisms are sensitive to hyperbaric oxygen.[11,12] We published work in this regard showing that 3 hours of exposure to OHP under appropriate conditions resulted in inhibition of coliform bacteria as well as staphylococci. It was found that the response of bacteria to increasing oxygen tensions above atmospheric conditions was a biphasic one.[6] Initially, as the oxygen tension increased, the bacterial growth rate increased up to 1 atm. Once the 1-atm level was passed, the growth rate began to decrease.

It seems that clinicians applying hyperbaric oxygen in the treatment of aerobic infections will have to take cognizance of this biphasic phenomenon. If offending bacteria in a patient are exposed to oxygen tensions in the lower stimulating range, the increased oxygen tension should be reflected by increased bacterial growth rate and may do the patient harm. On the other hand, if the offending bacteria are exposed to inhibiting oxygen tensions above 1 atm, bacterial growth rate should decrease. While the growth rate of bacteria in an infection which is a struggle between host and parasite is not the sole determinant of the clinical course, it must certainly exert a considerable influence.

The question naturally arises regarding the effects of OHP upon some of the other treatment forms, such as antibiotic administration. In studies reported in 1963, disc sensitivities performed in solid-medium experiments done under hyperbaric conditions[12] showed no significant

increase resulting from exposure to OHP. Studies on the effects of hyperbaric oxygen on tubercle bacilli revealed that growth was inhibited after exposure to hyperbaric oxygen, and a cumulative effect seemed to occur when combinations of *p*-aminosalicylic acid, isoniazid, and streptomycin were used with OHP.[13]

The study reported in this paper consists of a quantitative determination of the combined effects of oxytetracycline and hyperbaric oxygen on *E. coli* over a 3-hour period. This short exposure was dictated by the 3- to 4-hour limitation of clinically applied hyperbaric oxygen. Our results have indicated that the same biphasic phenomena observed when hyperbaric oxygen is used alone on bacteria also occur when antibiotics are given concurrently.

The effects of combining antibiotics with hyperbaric oxygen depend upon the doses of both. If the antibiotic is combined with low doses of hyperbaric oxygen, the effects are antagonistic; if the dosage of antibiotic is low enough, its effect is completely reversed by low-dosage OHP, so that the bacteria will grow even faster under these conditions than they would with no treatment at all. On the other hand, if the pressure of hyperbaric oxygen is high (2-3 atm), the effects with the antibiotic are cumulative. Further studies have indicated that hyperbaric oxygen does not increase the sensitivity of *E. coli* to oxytetracycline, nor does oxytetracycline increase the sensitivity of *E. coli* to the effects of hyperbaric oxygen.

Clinical application of this information poses some problems, the first involving a knowledge of the actual oxygen tension reaching bacteria. In our previous publication, it was shown in a canine fecal peritonitis preparation that intraperitoneal oxygen tensions remained in the stimulating phase despite 3 hours of oxygen-breathing at 3 atm of pressure.[6] When the conditions of the experiment were altered so that the peritoneal cavity was brought into equilibrium with the chamber environment, the growth rate in the peritoneal cavity seemed to decrease, as indicated by two parameters: (1) the dogs lived longer, and (2) the bacteremia had a different pattern, with a depression for 2–3 hours under these circumstances, as opposed to the steady increase observed when oxygen was administered with a closed peritoneal cavity. Thus, if hyperbaric oxygen is to be applied in the treatment of aerobic infections, the oxygen tensions reaching the bacteria must be in the inhibiting ranges. If they are not, OHP may negate the efforts of other standard methods of treatment, such as administration of antimicrobial agents.

A second problem involves the possibility that each combination of each antibiotic with each particular organism and each particular dosage of hyperbaric oxygen may behave differently. If so, the myriad possible conditions would make clinical application virtually impossible. Further study, however, may demonstrate definite patterns which can be observed either for all bacteria and all antibiotics or for groups of bacteria and groups of antibiotics, according to their characteristics. Studies are presently being conducted on the behavior of a certain *Staphylococcus aureus hemolyticus*, coagulase-positive and penicillin-resistant by antibiotic disc tests, which indicate that this may be the case. A similar biphasic growth curve with and without penicillin and/or chloramphenicol has been observed. The same antagonistic effect is noted with oxygen tensions in the 1-atm range; the reported cumulative inhibition of bacterial growth with OHP and antibiotic is also seen at 2 and 3 atm.

It appears that some reasonable predictability might be arrived at for clinical infections with specific organisms or suspected organisms. Thus, in the case of a gram-negative infection in which the oxygen tensions reaching the source of the infection could be determined, one might be able to use a drug whose effect in combination with OHP was known, and

thereby predict the beneficial effects expected from the combination.

Only after extensive study will it be theoretically safe to use hyperbaric oxygen in the treatment of aerobic infections. Until such knowledge is available, there appears to be a very real danger of making the patient worse.

SUMMARY

Increasing the oxygen tension from that occurring under normal atmospheric conditions (pO_2 160 mm Hg) to 1 atm (pO_2 760 mm Hg) increases bacterial growth rate for *E. coli*. With increases of oxygen tension above 760 mm Hg, the bacterial growth rate is decreased; it is inhibited at 2 or 3 atm. When oxytetracycline is added during the experimental procedure, its inhibitory effect is reversed in the presence of oxygen tensions under 1 atm, whereas above 1 atm its inhibitory effect is supplemented by the inhibitory effect of the oxygen. There is no evidence for any change in sensitivity of *E. coli* to oxytetracycline as a result of hyperbaric oxygen or any change in sensitivity of *E. coli* to hyperbaric oxygen as a result of oxytetracycline. At present, it appears that the use of hyperbaric oxygen in aerobic infections has at least as much potential danger as potential good. Much more knowledge in this area is required before clinical applications are evolved.

ACKNOWLEDGMENTS

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Effect of Hyperbaric Oxygen on Experimental Infections in Rabbits

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High oxygen tensions inhibit or even prevent the growth of bacteria in culture media. There is little evidence, however, that such lethal tensions can be reached in tissue infections in the intact organism. It has been difficult to construct experimental conditions capable of yielding objective results pertinent to tissue infections as opposed to those of the bloodstream. Ross and McAllister [1] found that OHP prolonged survival during the septicemic phase of pneumococcal infections in rats. Nevertheless, most of their animals died, and the effect of oxygen on the tissue component of the infection is far from clear.

Some insight into this problem could be obtained if a model for the study of tissue infections were available. The ideal model for these purposes should provide an objective (preferably numerical) measure of bacterial as well as host survival. Furthermore, it should be possible to measure concentrations of the antibacterial agent, in this case oxygen, within the infection model. A method is described which can provide such information, and some results from its application to the study of clostridial infections will be presented.

Materials and Methods

The technique depends upon the creation of a stable dead space within tissue which can be infected and subsequently sampled at will. Stainless-steel wire-mesh cylinders, 1 cm in diameter and 4.5 cm long, were implanted under the dorsal skin of rabbits by the technique of Schilling and Milch.[2] The cylinders were made by cutting a rectangle of wire mesh (#40, 34-gauge) and removing 8–10 crosswires at each end. The wire was rolled into a cylinder and the loose longitudinal wires were folded over to form the ends. The cylinders were heat-sterilized before use and were implanted under sterile conditions (Figure 1). Six such chambers were placed deep to the panniculus carnosus muscle on the dorsal surface of each of 38 New Zealand white rabbits (2.0–3.5 kg) through three midline incisions. At operation, 3-mm cubes of muscle were

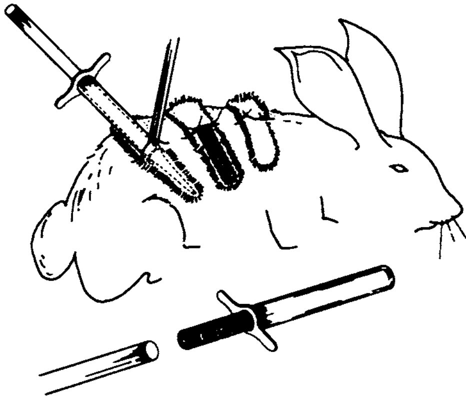

FIGURE 1. Technique for inserting wire-mesh cylinder.

taken from the paraspinal area and one was placed in each of the two most cephalad cylinders. A 3-mm cube of sterile polyvinyl chloride sponge was placed in each of the second two cylinders. The caudad cylinders were left empty. Wounds were allowed to heal for 7 days before the infection experiments were begun.

Group I: Bacterial Counts and Duration of Infection

On the seventh day after implantation, each cylinder in a series of 10 animals was infected with 0.2 ml of Robertson's cooked-meat medium containing *Clostridium perfringens* (*Cl. welchii*, type A) in numbers sufficient to produce a bacterial concentration of 3×10^5 to 7×10^6 organisms/ml, when diluted within the cylinder. This produced a nonfatal infection in which bacterial counts and the duration of infection could be determined.

At intervals from 1 to 3 days after inoculation, 0.2-ml samples of fluid were aspirated from each cylinder. The bacteria were then counted by the technique of Miles and Misra.[3]

All animals were infected in pairs. Both animals received identical volumes of the same culture. One of the pair was treated with hyperbaric oxygen at 2 atm according to the following schedule: a small-animal chamber was flushed with oxygen and the pressure raised to 2 atm over 10 min, pressure was maintained for 1 hour, and decompression was accomplished within 15 min. Three such treatments were given daily for 5 days.

Group II: Fatal Infections and Mortality Rate

In a series of 22 rabbits (10 pairs and two unpaired controls), a second type of inoculation was performed to produce a fatal infection. All three cylinders on one side of each animal were injected with *Cl. perfringens* (type A), this time in numbers sufficient to give a concentration of 7.5×10^4 to 9×10^5 bacteria/ml within the cylinder. The three cylinders on the other side were injected with *Cl. novyi* (*Cl. oedematiens*, type A) to give a concentration of 5×10^2 to 6×10^4 bacteria/ml of wound fluid. Again, animals were infected in pairs; both animals received identical volumes of the same culture material. One of the pair was treated with hyperbaric oxygen according to the basic schedule described above, and the control animal was untreated. Five animals received OHP at 2 atm and the other five at 3 atm.

Group III: Oxygen Tension Determination in Infected and Uninfected Chambers

Cylinders on one side of five additional animals were inoculated with *Cl. perfringens*, as in Group I. The next day, these animals were placed in the large hyperbaric oxygen chamber at 2 atm. Oxygen was given by mask at a flow rate of 12 liters/min. The mask consisted of a small polyethylene bag placed over the animal's head and fastened at the neck with a loose-fitting rubber band. The animals were lightly sedated with pentobarbital (Nembutal) so that they would tolerate the mask, but they were still awake. One milliliter of fluid was aspirated from each infected and uninfected cylinder and the pO_2 was immediately measured in a standard oxygen electrode within the hyperbaric chamber.

Group IV: Culture Filtrate Controls

Cultures of the same *Cl. perfringens* and *Cl. novyi* used previously were filtered through Millipore membranes. Two milliliters of the filtrate of each culture was injected intramuscularly into each of two rabbits. This corresponded to five times the volume of culture medium injected in any of the above experiments. The animals were then observed for signs of local or systemic toxicity. No oxygen therapy was given.

RESULTS

Bacterial Counts, Duration of Infection, and Oxygen Tensions (Groups I and III)

The fate of the injected organisms differed markedly in response to the contents of the wire-mesh cylinders. Figure 2 shows the number of cylinders that remained infected on any given day following inoculation. Any culture that was positive for the infectious organism, regardless of bacterial count, was considered infected. The bacteria showed a definite preference for the cylinders that contained devascularized autologous muscle. The presence of polyvinyl chloride sponge did not support prolonged infection.

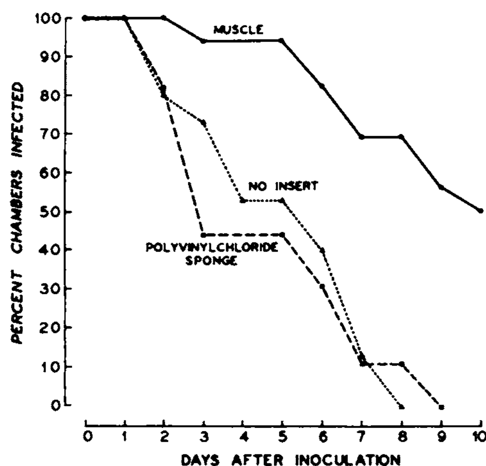

FIGURE 2. Duration of *Cl. perfringens* infection related to contents of chambers. Each curve represents the fate of 20 cylinders in 10 animals.

Oxygen tensions during air-breathing measured in uninfected chambers ranged from 5 to 28 mm Hg, with a mean of 15.5 mm Hg. (Most clostridia will multiply within this range of oxygen tensions.) At 2 atm of oxygen given by mask, oxygen tensions within the cylinders ranged from 36 to 152 mm Hg (mean 85 mm Hg) in 20 uninfected chambers in five animals. In 19 infected chambers measured during oxygen administration by mask at 2 atm pressure, the oxygen tensions ranged from 10 to 178 mm Hg, with a mean of 70. Ambient oxygen tensions delivered at 2 atm of oxygen by mask are probably lower, however, than those achieved in an oxygen-charged chamber. (Fluid oxygen tension has not yet been measured at 3 atm.)

Five of the 10 animals in Group I were exposed to OHP at 2 atm, with no difference between the treated and control groups in duration of infection (Figure 3). Furthermore, in the treated and control animals there was no difference between bacterial counts in chambers containing muscle inserts (Figure 4). Stained smears of the contents of the cylinder showed no sporulating forms in either the hyperbaric oxygen group or the control animals. Two animals in this group died. One animal from the oxygen-treated group died 9 days after infection, and its control died on the fifth day.

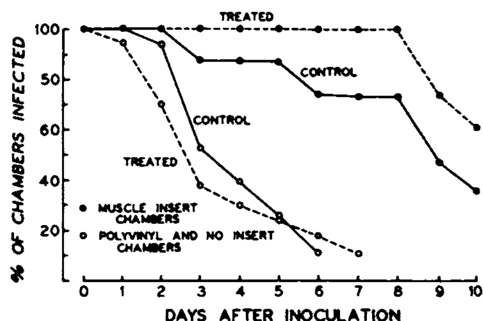

FIGURE 3. Duration of *Cl. perfringens* infection in hyperbaric-treated and control animals.

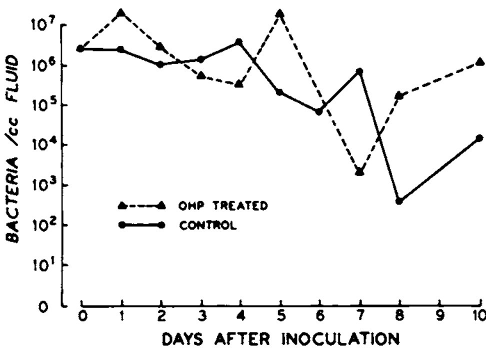

FIGURE 4. Bacterial counts in muscle-containing chambers of hyperbaric-treated and control animals.

There was no evidence of gas formation or edema in either animal, and the cause of death was unknown.

Mortality Rates and Toxicity (Groups II and IV)

All 12 control animals given the above-described mixture of *Cl. novyi* and *Cl. perfringens* died. Edema and crepitation of the thoracic wall appeared at 24 hours, and death occurred 32–76 hours after infection.

In the early experiments, hyperbaric oxygen was given at 2 atm, starting 24 hours after infection. Two treated animals and their paired controls died. The untreated animals survived longer (mean 44 hours for treated animals, mean 62 hours for controls). The next three animals were treated at 2 atm, but this time exposure was begun immediately after inoculation. All three animals and their paired controls died. In this case, however, all the treated animals survived longer than their controls (68 hours for treated animals, 42 hours for controls).

Five animals were given hyperbaric oxygen at 3 atm. In the first animal of this series, treatment was started 24 hours after inoculation, and the oxygen-treated animal died before the control animal. Thereafter, hyperbaric oxygen was given to four animals, starting immediately after inoculation. In this group, all four control animals died, but three of the treated animals survived, and the treated animal that died lived longer than its untreated control.

All 12 control animals died 32–76 hours after infection. All five animals treated at 2 atm of oxygen died. On the other hand, of the series of four animals treated with 3 atm immediately after inoculation, three survived. All animals showed signs of advancing edema and crepitation except the three survivors. The paired controls of the survivors received identical bacterial inocula and died an average of 60 hours after infection.

The two animals that were given a Millipore filtrate of the *Cl. perfringens* and *Cl. novyi* cultures equivalent to five times the volume used in the previous experiments survived without apparent systemic or local toxicity. Neither animal was treated with oxygen.

DISCUSSION

The primary aim of this study was to devise a precisely controlled infection which could be studied in terms of survival of both bacteria and host. The secondary goal was to apply this model to the study of the effect of hyperbaric oxygen on clostridial infections. It was possible to vary the dose of infecting agents so that chronic or rapidly fatal infections could be produced.

It was somewhat difficult to define the most effective conditions for study of hyperbaric oxygen therapy because of the difficulties in (1) determining the proper length of exposure, and (2) determining the most effective pressure and the best time to start treatment. These problems were attacked separately.

Brummelkamp,[4] in his clinical studies, has routinely used seven 2-hour exposures given over a 4-day period at 3 atm of oxygen by mask. We attempted to duplicate this schedule. Previous experiments in our laboratories [5] showed that uninfected animals of the same species and

size would withstand this schedule without apparent oxygen toxicity. However, in pilot studies, infected animals exposed three times daily for 2-hour periods soon began to lose weight and at autopsy were found to have pulmonary hemorrhages typical of oxygen toxicity. When exposure time was reduced to 1 hour, signs of toxicity ceased. Although no statistical studies have been done, it is our impression that infected animals are more susceptible to oxygen toxicity than uninfected animals. Recently, similar observations have been made by Grogan [6] during the treatment of staphylococcal infections with hyperbaric oxygen.

After the decision was made to use three 60-min exposures daily, it remained to determine the best time to start therapy and the most effective tensions to use. Treatment at 2 atm failed to change the duration of infection, bacterial count, or mortality rate. Oxygen given at 2 atm raised oxygen tensions within the cylinder only to a disappointing mean of 70 mm Hg. In some cylinders, oxygen tension rose not at all. Experiments were then begun using 3 atm. Since animals treated immediately after infection had done somewhat better than those treated 24 hours later, we decided to expose them to 3 atm immediately after infection. In the group of four animals so treated, three survived. All paired controls in this group died.

Relatively few animals were used in these experiments. If more had been treated at 2 atm, possibly some may have survived, but it is already apparent that the survivors would have been few. The more recent success with 3 atm indicates that this combination of infection and oxygen therapy will yield profitable information in future studies. It is apparent that the studies on bacterial counts and duration of infection must be repeated at 3 atm.

SUMMARY

The new model for inducing controlled clostridial infections, described above, depends upon the production of a stable dead space which can be inoculated with organisms of any type and in any number. Subsequently, the dead space can be sampled and bacteria counted.

In our experiments, the oxygen tension of infected fluid within the model was raised with oxygen given by mask at 2 atm pressure to a mean of 70 mm Hg and a high of 178 mm Hg. Hyperbaric oxygen at 2 atm failed to modify either the bacterial count or the duration of clostridial infections in rabbits.

A lethal infection was obtained by using a mixture of *Cl. perfringens* and *Cl. novyi* which invariably produced death within 32–76 hours in untreated animals. Hyperbaric oxygen at 2 atm failed to modify the mortality rate. However, recent experiments with 3 atm of oxygen started immediately after initiation of infection have produced survivors.

ACKNOWLEDGMENTS

This work was supported by a grant from the Medical Research Council of Great Britain. Dr. Hunt's participation was supported by USPHS Fellowship 6-F2-HE-29,219-01 from the National Heart Institute.

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OHP in the Management of Chronic Osteomyelitis

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Chronic osteomyelitis is an inflammatory process in bone, arising as a sequel to acute infection. The blood vessels supplying the bone are contained in rigid channels, and the inflammatory exudate produced by the acute inflammation compresses these vessels, which then thrombose, leading to necrosis and ultimately to the formation of sequestra (Figure 1). These are contained in cavities containing infected granulation tissue; sclerosis of bone ensues, and this is surrounded by dense fibrous tissue with a poor blood supply, which prevents drugs from reaching the infected bone. Sinuses may later form, and the course of the disease is often characterized by recurrent flares of acute inflammation that are unaffected by antibiotics.

Because of the dual aspect of local hypoxia and persistence of organisms, and because both these factors appeared likely to be influenced by administration of OHP, it seemed reasonable to treat patients who had discharging sinuses, since these might give an indication of response. In March 1964, we therefore decided to give hyperbaric oxygenation a trial in the management of patients with chronic osteomyelitis.[1]

Illustrative Cases

Case 11. A 48-year-old man had tuberculous bursitis of the left hip diagnosed in 1942, and numerous drainage operations were performed and antituberculous drugs administered between 1942 and 1956. Sequestra in the femoral head were demonstrated and sequestrectomy performed. In 1960, an osteotomy resulted in firm ankylosis. During 1942–1965, a sinus had been discharging intermittently.

When the patient was seen in January 1965, the discharge had persisted for 6 months, and *Staphylococcus pyogenes* was cultured from the exudate (Figure 2A, 2B). He was treated with 13 OHP sessions of 2 hours each at 2 ata over 9 days, during which time the sinus gradually dried up and healed (Figure 2C). During this time, the erythrocyte sedimentation rate (Westergren) fell from 123 mm in the first hour to 44 mm. Upon discharge from the hospital, this had fallen to 16 mm and it has remained at this level for 10 months.

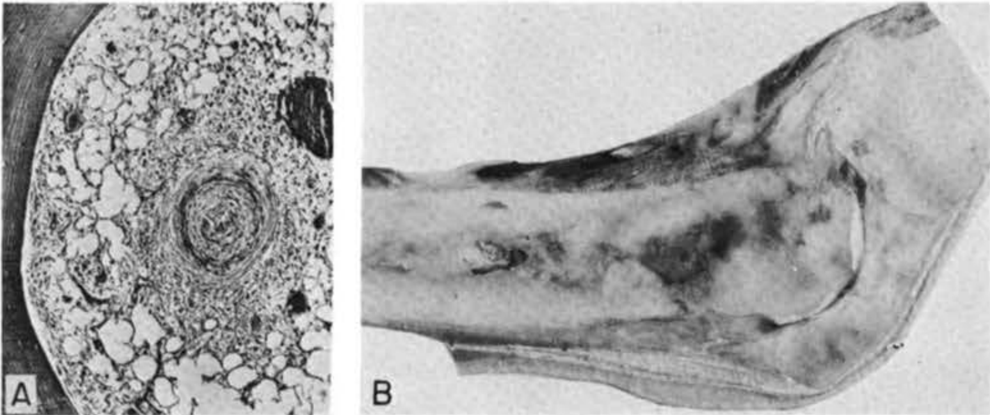

FIGURE 1. A, osteomyelitis seen in cross section through small cylindrical bone. A medullary vessel is completely thrombosed and is surrounded by inflammatory cells (×60). (Reproduced with permission from E. Aegerter: *Orthopedic Diseases,* W. B. Saunders, 1963, p. 246.) B, chronic osteomyelitis of femur. Museum specimen showing the characteristic cavities and sequestra in thickened sclerosed bone.

Case 14. A 70-year-old man had osteomyelitis of the left femur, unhealed for 40 years. In February 1965, the condition flared up, and when the patient was seen in April he had a foul-smelling discharge with edematous skin surrounding the sinus (Figure 3A). He was treated in the chamber at 2 ata for 49 hours over 32 days, with gradual improvement. By the end of treatment, the flare-up had settled completely, he was able to walk unassisted, and the offensive smell had disappeared (Figure 3B). Further treatment then had to be abandoned, because the patient developed a cerebral abscess for which a craniotomy was performed.

Case 13. A 66-year-old man had chronic osteomyelitis with a sinus at the lower end of the tibia for 49 years following a gunshot wound. When seen in June 1965, the patient had been suffering from a flare-up for a month previously. He was unable to move his ankle joint and was in some pain. A few days after hyperbaric treatment was begun, he reported less pain and had regained 30° of ankle movement (Figure 4A). After 16 days of treatment, the sinus had healed, but it became evident that a secondary sinus was developing (Figure 4B), so surgical exploration was performed, a large deep abscess cavity containing sequestra was exposed, and much debris was released

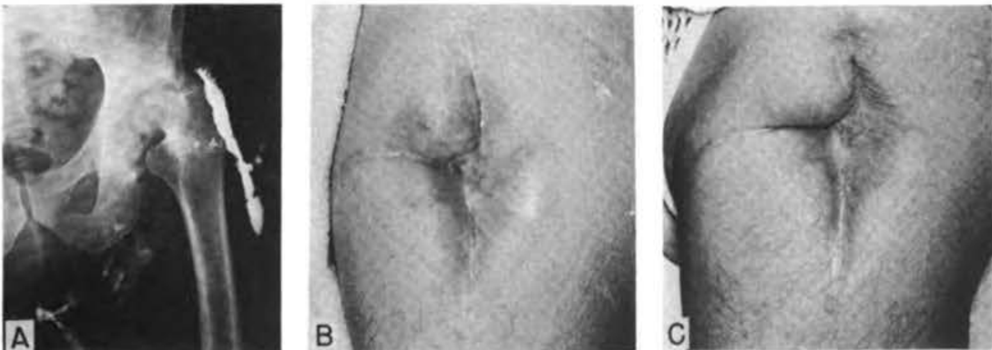

FIGURE 2. Case 11. A, sinogram of left hip joint. B, appearance of sinus before treatment. C, appearance 10 days later.

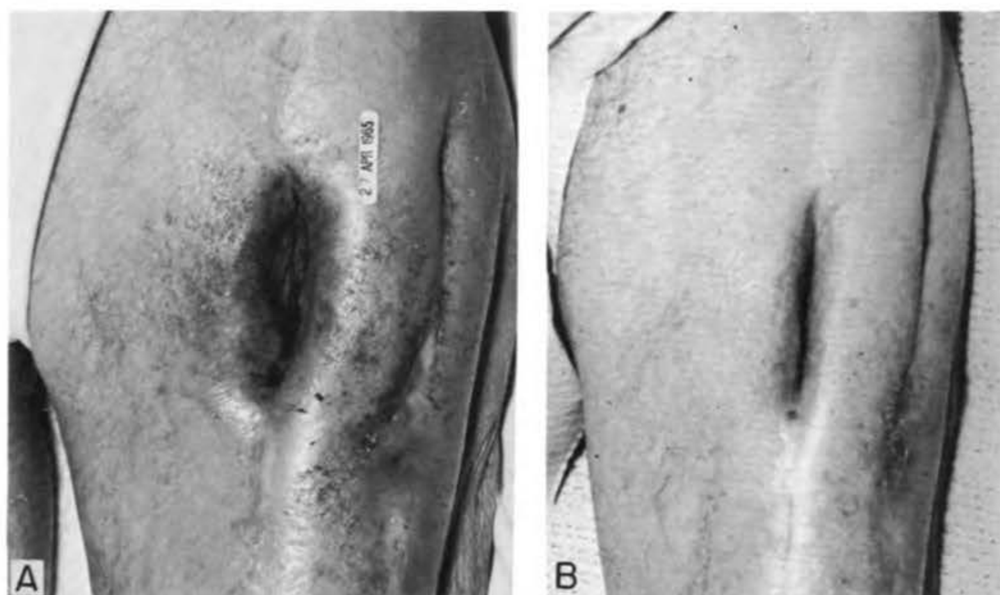

FIGURE 3. Case 14. A, sinus from chronic osteomyelitis of femur before treatment. B, appearance 1 month later after 2 hours OHP daily at 2 ata.

(Figure 5A). For the next 10 days the resulting cavity remained much the same (Figure 5B), but on recommencing hyperbaric treatment it closed so rapidly that the patient was able to walk and leave the hospital after 3 weeks with only a small residual sinus (Figure 5C).

Case 23. A 53-year-old man had osteomyelitis of the upper left tibia for 20 years, and in October 1965, a sinus had been discharging for 8 months (Figure 6A). After 14 hours of OHP therapy over 9 days at 2 ata, the sinus healed (Figure 6B) and it has remained so to date (November 1965).

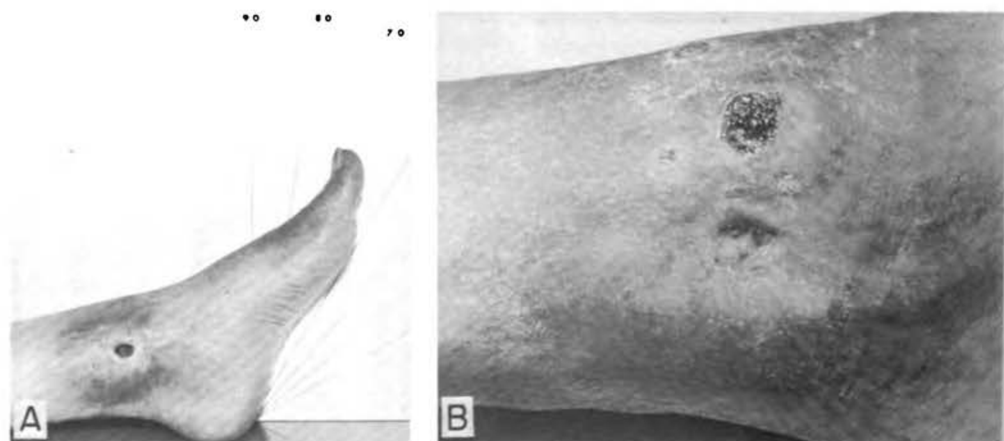

FIGURE 4. Case 13. A, movement of ankle joint achieved after 1 week of OHP treatment. B, sinus healed after 16 days of treatment. Note diminished surrounding pigmentation and a secondary sinus appearing.

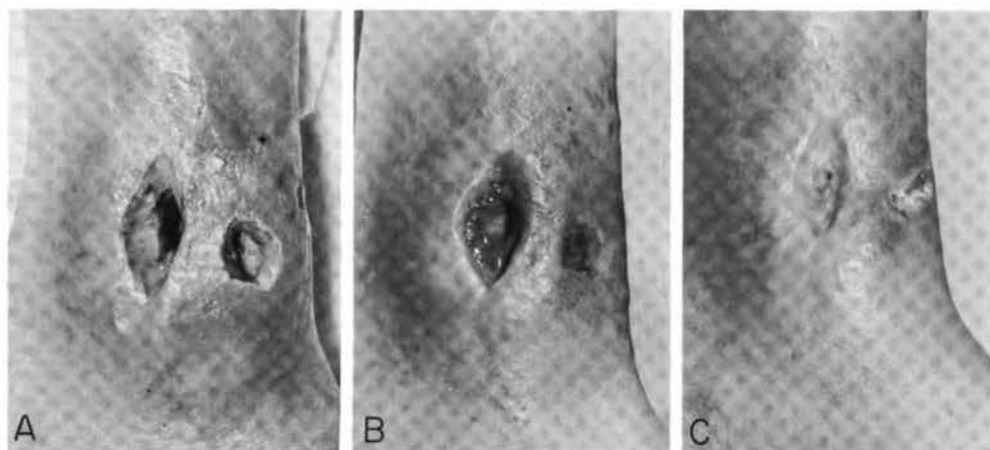

FIGURE 5. Case 13. A, sinus after sequestrectomy. B, appearance of sinus 10 days later. C, sinus after 3 weeks of OHP treatment.

Case 22. A 45-year-old man, who had undergone radical mastoidectomy at age 8, had a chronically discharging ear which had never healed. In July 1965, the mastoidectomy was revised and a temporal fascia myeloplasty performed. This procedure had no effect on his discharge, however, and 3 months later hyperbaric treatment was begun in the slight hope that it might help. He was given 1.5 hours of exposure to 2 ata daily for 4 days, when he elected to discontinue treatment. A week later he reported that the discharge had ceased for the first time in 37 years (Figure 7).

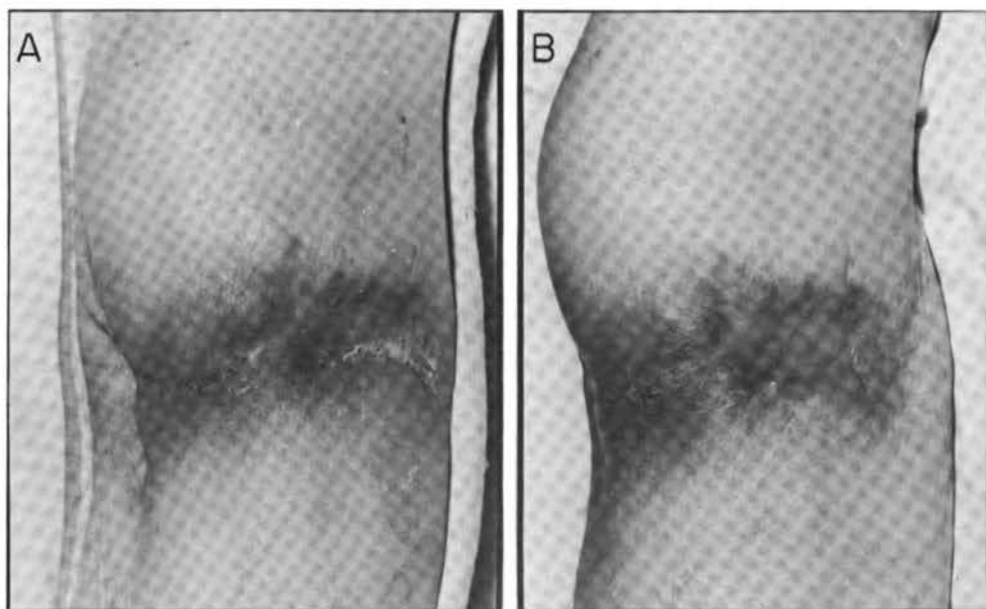

FIGURE 6. Case 23. A, appearance of sinus before treatment. B, after 9 days of OHP therapy, the sinus was dry.

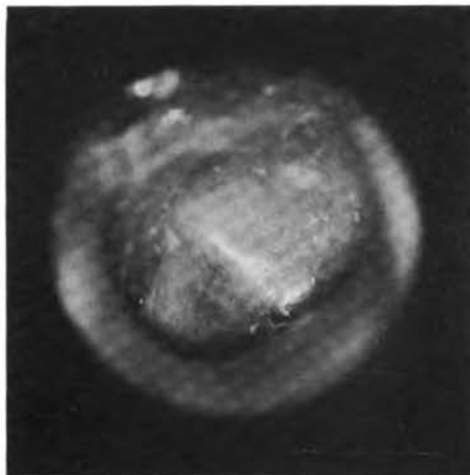

FIGURE 7. Case 22. View of tympanic membrane 1 week after commencement of hyperbaric treatment. Before OHP therapy, this membrane was completely obscured by thick discharge.

CONCLUSION

Among our three units, we have now treated 24 patients with chronic osteomyelitis presenting with sinuses of varying duration, all of which had failed to heal with conservative treatment. Throughout the management of these patients with OHP therapy, orthodox methods of treatment have also been maintained, including antibiotic administration and sequestrectomy. The clinical results are shown in Tables 1 and 2.

Based upon our limited experience to date, we have the impression that hyperbaric oxygenation can favorably influence the course of a persistent sinus in chronic osteomyelitis, and that given sufficient exposure most but not all cases will heal, at least temporarily. Present evidence does not justify an assumption, however, that the effects on the sinus necessarily represent an influence on the underlying disease process. We do not know whether the beneficial effects we have observed were achieved by (1) influencing oxygenation of the infected area, (2) inhibiting growth of the organisms, (3) potentiating the action of antibiotics, or (4) an interrelationship of these factors.

This report represents some initial observations on a condition whose natural history is unpredictable. Further study will be required to define the indications for OHP therapy.

TABLE 1. Response of Discharging Sinuses to OHP in Patients with Chronic Osteomyelitis

	No. cases
Total cases treated	24
Sinus healed [a]	17
Relapsed [b]	4
Discharge diminished	4
Sinus apparently uninfluenced	3

[a] Cases were recorded as healed only if complete epithelial closure was obtained.
[b] Cases were recorded as relapsed if the sinus recurred any time after cessation of OHP therapy.

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TABLE 2. Analysis of Cases Treated with OHP for Chronic Osteomyelitis with Discharging Sinus

Case	Age	Sex	Bone	Organism	Pressure (psig)	Exposure (hours in days)	Total history	Currently discharging	Result	Progress
1	55	M	Tibia	*Proteus* *Pseudom. pyocyaneus*	15	26/10	41 yr.	5 yr.	Healed	Remains healed for 6 mo.
2	59	M	Femur & tibia	*Staph. pyogenes*	15 15	14/7 18/9	8½ yr.	8 mo.	Healed	Relapsed in 2 mo. Relapsed in 9 mo.
3	21	M	Tibia	*Streptococcus* *Staph. pyogenes* *Pseudom. pyocyaneus*	15	18/9	2 yr.	13 mo.	Healed	Relapsed in 1 wk.
4	36	M	Tibia	*Staph. pyogenes*	15	56/24	1 yr.	6 wk.	Healed	Remains healed for 8½ mo.
5	46	M	Tibia	*Staph. pyogenes*	15	64/32	5 mo.	5 mo.	Healed	Remains healed for 2 mo.
6	15	M	Tarsus	*Staph. pyogenes*	22	24/30	6½ yr.	5½ yr.	Healed	Remains healed for 18 mo.
7	55	M	Hip joint	*Staph. pyogenes*	22	31/18	2 yr.	1 mo.	Healed	Remains healed for 4 mo.
8	60	F	Mandible	Oral commensals *Strep. viridans*	22	51/32	9 yr.	6 mo.	Healed	Remains healed for 3 mo.
9	19	M	Femur	*Staph. pyogenes*	15	30/15	7 mo.	3 mo.	Unhealed, improved	Healed after sequestrectomy
10	65	M	Tibia	*Staph. pyogenes* *Pseudom. pyocyaneus* β-Hemo. strep.	15	48/32	48 yr.	6 mo.	Unhealed, improved*	
11	48	M	Hip joint	*Staph. pyogenes*	15	26/10	23 yr.	6 mo.	Healed	Remains healed for 10 mo.

TABLE 2.—Continued

Case	Age	Sex	Bone	Organism	Pressure (psig)	Exposure (hours in days)	Total history	Currently discharging	Result	Progress
12	25	M	Tibia	*Proteus*	15	20/10	3½ yr.	3 yr.	Healed	Remains healed for 6 mo.
13	66	M	Tibia	*Proteus* *E. coli*	15	24/16	49 yr.	1 mo.	Unhealed, improved	Healed after sequestrectomy
14	70	M	Femur	*Proteus*	15	49/32	40 yr.	40 yr.	Unhealed, improved++	
15	43	F	Metatarsal	Coliforms	15	14/7	11 mo.	11 mo.	Healed	Relapsed in 2 mo.
16	64	F	Metatarsals	*Proteus*	15	12/6	?	?3 mo.	Healed	Remains healed for 8 mo.
17	25	M	Tibia	*Staph. pyogenes*	15	80/27	8 mo.	5 mo.	Healed	Remains healed for 8 mo.
18	52	M	Tibia	*Staph. pyogenes*	15	87/49	5 mo.	4 mo.	Healed	Relapsed in 6 wk.
19	25	M	Mandible (osteopetrosis)	Oral commensals	15	20/13	Congenital	3 yr.	Unhealed	
20	43	M	Mandible (radionecrosis)	Oral commensals	15	22/8	10 yr.	1 yr.	Healed	Remains healed for 4 mo.
21	46	M	Femur	*Proteus*	15	34/17	16 yr.	1 yr.	Unhealed	
22	45	M	Mastoid	*Pseudom. pyocyaneus*	15	6/4	37 yr.	37 yr.	Healed	Remains healed for 2 wk.
23	53	M	Tibia	*Staph. pyogenes*	15	14/9	20 yr.	8 mo.	Healed	Remains healed for 2 wk.
24	52	M	Tarsus	Micrococci	15	90/60	25 yr.	9 mo.	Unhealed, improved++	

Five Cases of Suppurated Pseudarthrosis (Osteomyelitis) Treated by Hyperbaric Oxygenation

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The results of conventional treatment of suppurative pseudarthrosis are often disappointing despite administration of antibiotics and repeated surgical procedures. Since chronic infection plays an important role in the absence or delay of bone union, and since oxygen at high pressure may exert a bacteriostatic effect, we believe that OHP might be of benefit in such cases. Consequently, we treated five cases of suppurative pseudarthrosis in a small Vickers chamber with a 1-hour exposure to pure oxygen at 2 ata, given daily for 18 to 23 days.

Case Reports

Case 1. I.M., a 37-year-old man, sustained a supracondylar fracture of the left femur after a fall on January 27, 1963, and was treated by intramedullary nailing. In March 1963, suppuration appeared at the fracture site along with disunion of the scar and fistula formation. The x-ray photographs showed no sign of callus formation. On April 24, 1963, the patient underwent curettage of the sinus which had formed. On August 16, 1963, because the suppuration and sinus persisted, the patient was taken to the operating room for removal of the nail, curettage of the walls of the sinus, and insertion of external fixation (Judet's method). During this procedure, an aspect of complete avascular pseudarthrosis was noted. The diaphysial femoral fragment had lost all of its neovascular attachments over 20 cm and had the whitish aspect of a sequestrum. The upper part of the epiphysial fragment was also whitish and devascularized. There was no trace whatsoever of reconstructive osteogenesis in the area. *Staphylococcus pyogenes* var. *aureus* was isolated from the cultures taken prior to the operation. In January 1964, another surgical procedure was performed because of the persistence of the sinus. Drainage was achieved by excision of a small fragment of external cortex, and it was noted that the entire devascularized part of the diaphysis was covered with nonadherent soft tissue (Figure 1A).

In June 1964, due to the persistence of suppuration and the absence of bone union, an amputation at the thigh was considered. We decided, however, to give OHP a trial first. Beginning on June 29, 1964, the patient received 1 hour OHP at 2 ata on each of 22 consecutive days. During this treatment, drainage ceased, and the sinus closed permanently.

When the patient was reoperated on 2 months after the last OHP session (September 17, 1964), only one avascular zone of 2–3 cm was seen to persist on the diaphysial fragment. Over a length of 12 cm, the diaphysis was revascularized and was adherent to the peripheral structures. The sequestrum

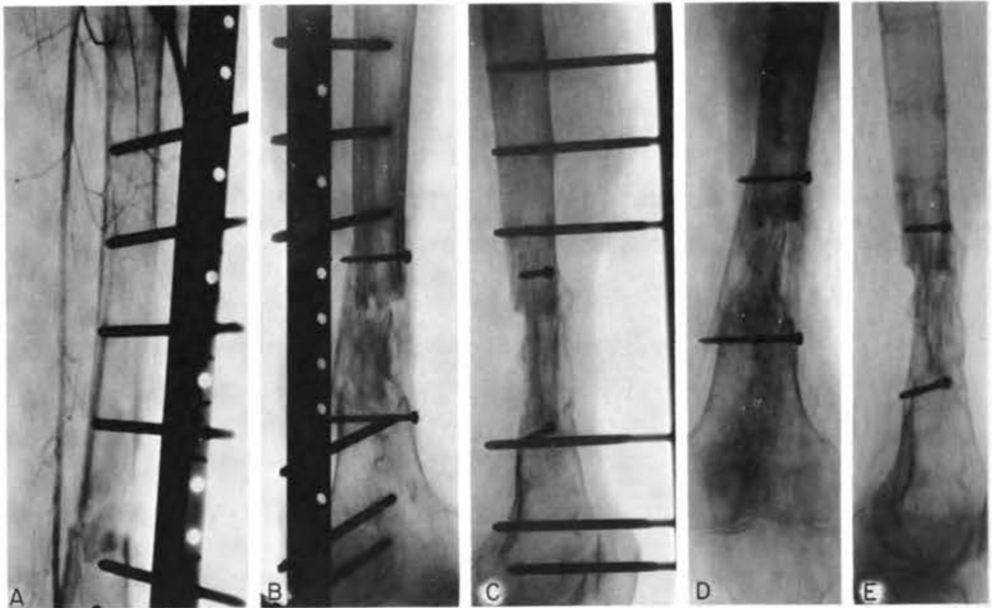

FIGURE 1. Case 1. A, pinned fracture of lower femur (June 16, 1964), showing areas of osteomyelitis at fracture site. Note the arteriographic demonstration of the avascular areas above and below the fracture. B and C, lateral and anteroposterior views of femur, February 22, 1965, showing firm union and healing after OHP therapy and grafting. D and E, lateral and anteroposterior views, May 1965, after removal of external fixation and after weight-bearing had begun.

was completely excised, and the femur bled when cut. This soon was filled by an autogenous graft and external fixation was applied. The excised fragment was cultured and a few colonies of *Staphylococcus pyogenes* var. *aureus* were isolated.

By February 1965, consolidation was complete (Figure 1B, 1C), and 2 months later the external fixation was removed. By May 1965, the patient could walk normally with an ischiatic support (Figure 1D, 1E). The patient was able to walk 8 km a day. In September 1965, a firm reconstitution of the femur was noted.

Case 2. R.H., a 33-year-old man, fractured the midthird of the right femur in a car accident on July 11, 1962. An operation that same day involved intramedullary nailing and circlings.

One month later, a purulent discharge occurred through a fistula in the upper thigh. Despite prolonged polyvalent antibiotic treatment and removal of the circling material, persistence of the discharge and complete absence of bone union were noted in March 1963. On March 14, 1963, another operation was performed with drainage of a 200-ml abscess around the site of the pseudarthrosis, removal of the centromedullary nail, resection of sphacelated osseous endings, and insertion of two external fixations. Purulent discharge persisted, bone union was attained in about 6 months but was very fragile, and when the external fixation was removed 1 year later, in April 1964, a refracture occurred immediately. Surgical reintervention was performed on April 16, 1964, with drainage of an infected hematoma on the line of the old scar, decortication, and insertion of fixation with compression. Six months later, in October 1964, the purulent discharge persisted. *Staphylococcus pyogenes* var. *aureus* was cultured from the wound, and there was complete absence of bone union (Figure 2A).

Beginning November 4, 1964, 1-hour sessions of OHP at 2 ata were given daily for 21 days. During this period of treatment, the discharge became serous and bloody instead of purulent, and in the following weeks the sinus dried up completely without any addi-

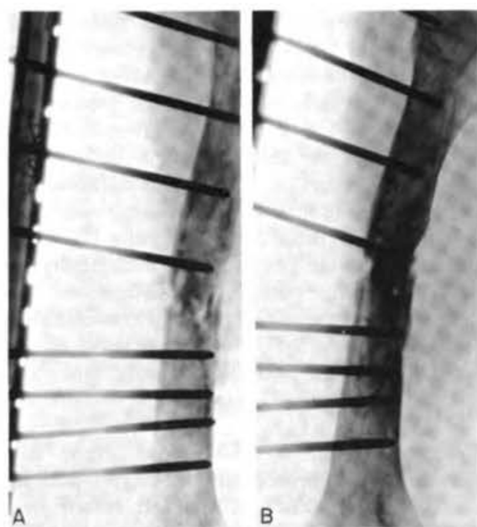

FIGURE 2. Case 2. A, fracture of femur after refixation in October 1964. B, appearance of femur in September 1965, 9 months after completion of OHP therapy and clearing of infection. By this time, bone union had occurred, permitting the patient to walk without support.

tional antibiotic treatment. From January 1964 on, successive x-ray photographs showed formation of an osseous callus and progressive consolidation. In June 1965, the fracture was considered as consolidated and walking was resumed without support except for the external fixation, which was maintained. In September 1965, the sinus was still closed, the bone union appeared radiologically satisfactory, and walking was authorized without support (Figure 2B).

Case 3. On November 16, 1963, K.J., a 13-year-old boy, underwent an osteotomy to correct a shortening of the left femur secondary to tuberculosis of the hip in infancy. In March 1964, during the fifth month after surgery, angulation of the bone fragment caused a cutaneous ulceration with suppuration. On March 24, 1964, the patient was taken to the operating room for curettage of the fracture site, removal of the metallic material, and application of external fixation. He was also started on polyvalent antibiotic treatment (penicillin, streptomycin, and colistin).

Because of the absence of bone union by July 18, 1964, the decision was made to perform a decortication of both bone extremities, an autogenous tibial graft, and an osteoplasty with a vitallium plate. When the plaster splint was removed in November 1964, bullous edema of the scar was found with a purulent fistula from which *Pseudomonas aeruginosa* was cultured. Bone union was poor, with interfragmentary discontinuity (Figure 3A).

Beginning November 19, 1964, 1-hour sessions of OHP at 2 ata were given daily for 23 days. During the treatment, the fistula dried up and finally closed. On January 12, 1965, the osteosynthesis material was removed. The bone had regained a better texture by March 1965; bone union was satisfactory by May 1965 (Figure 3B), and complete by July 1965, at which time walking with support was permitted.

Case 4. On October 29, 1964, G.O., a 16-year-old girl involved in a road accident, sustained a compound fracture of the midthird of the right femur along with a compound fracture of the tibia and fibula of the left leg with an intermediate tibial fragment.

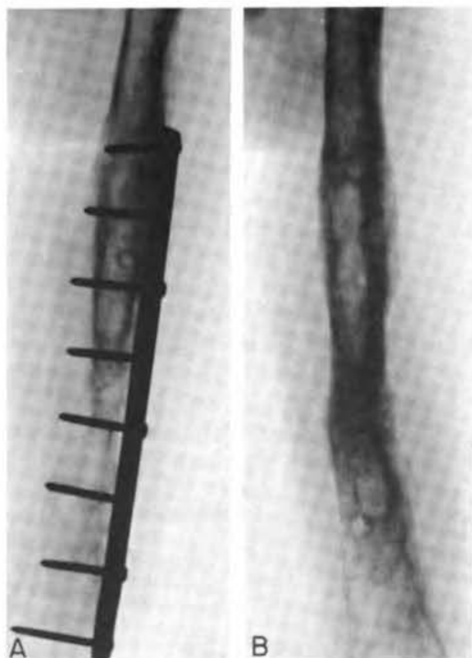

FIGURE 3. Case 3. A, femoral malunion and osteomyelitis at fracture site (November 1964). B, x-ray appearance of femur 4 months later (May 1965) after OHP treatment, demonstrating complete bone union and healing.

At operation the same day, the fractured femur was treated by intramedullary nailing and insertion of a vitallium plate.

The periosteum of the intermediate tibial fragment was lacking except at the upper part. This latter fracture was reduced and an osteosynthesis by external fixation was performed. On the 10th day after the accident, a cutaneous necrosis appeared, followed by suppuration and fistulization of the fracture. This was treated by prolonged polyvalent antibiotic therapy. Four surgical procedures were performed (November 10 and 24, 1964, January 30, 1965, and February 11, 1965) during which necrotic tissue was excised and the sinus drained. *Pseudomonas aeruginosa* and *Proteus vulgaris* were cultured from the wound. During the operation on February 11, 1965, it was noted that the intermediate fragment of the fractured tibia was completely avascular and sequestrated and that there was no bone union (Figure 4A, 4B). Callus formation was minimal at the level of the femoral fracture.

Beginning February 15, 1965, 1-hour sessions of OHP at 2 ata were given daily for 21 days. During this treatment, definite but incomplete decrease of the suppuration occurred. *Pseudomonas aeruginosa* was still cultured from the wound. On June 16, 1965, the intermediate fragment was surgically removed. It was completely sequestered and necrosed and the tibia appeared consolidated. By September 1965, the suppuration of the leg had cleared and the fracture was consolidated (Figure 4C, 4D).

Case 5. B.A., a 55-year-old man, sustained a comminuted supracondylar and intracondylar fracture of the left femur in a road accident. An osteosynthesis was performed with a MacLaughlin's plate. One

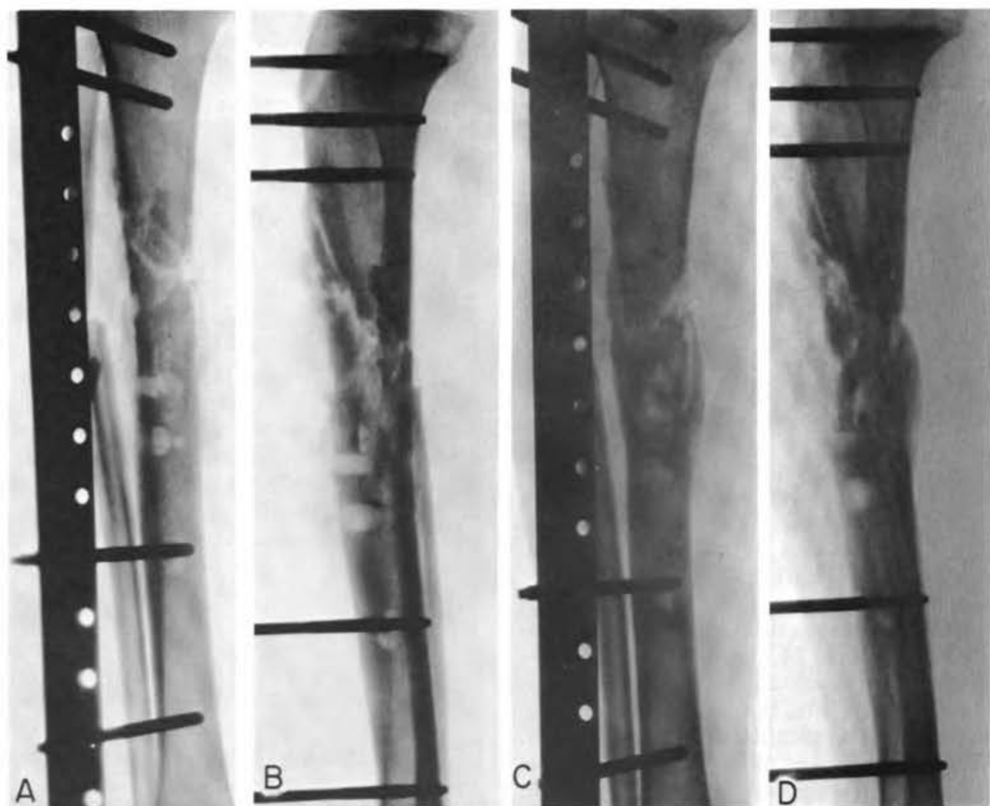

FIGURE 4. Case 4. A and B, x-ray appearance of infected left tibial and fibular fractures in February 1965, showing minimal callus formation and nonunion of bone. C and D, x-ray appearance 3 months after completion of OHP therapy and after infection had cleared (May 1965). Note callus formation and consolidation.

month later, an infected hematoma was noted over the posterior aspect of the thigh, and this was drained on August 1, 1964. In January 1965, despite prolonged polyvalent antibiotic treatment, the infection persisted. *Staphylococcus pyogenes* var. *aureus* was cultured from the wound (Figure 5A). On January 14, 1965, the fixation material was removed. A consolidation of the epiphysial fracture was noted along with a suppurative pseudarthrosis of the junction of the middle and lower thirds of the femur. The extremity of the diaphysial fragment was avascular. A fixation with compression was inserted. Despite this operation suppuration persisted. Beginning February 25, 1965, 1-hour sessions of OHP were administered at 2 ata for 18 days. During the treatment, the appearance of the thigh improved. The discharge subsided considerably but dried up completely only after removal of the sequestrum on March 8, 1965. The sequestrum was separated from the femur by a zone of elimination. The fracture was still mobile. By

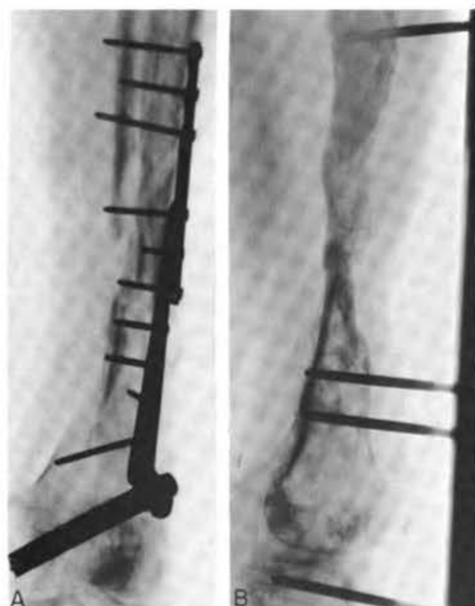

FIGURE 5. Case 5. A, infected supracondylar fracture of femur with nonunion in January 1965. B, appearance of femur 6 months after OHP therapy and after removal of sequestrum and clearing of infection. The original fixation had been removed and replaced with external fixation (shown). When this photograph was made, the patient was walking with support.

June 20, 1965, the fracture was on the way to consolidation. The fistula had closed, and walking was possible with ischiatic support and external fixation. By September 1965, the femur had improved (Figure 5B).

DISCUSSION

It seems probable to us that OHP played a part in the cure of these five patients with suppurative pseudarthrosis. In all of these cases, the failure of usual therapeutic methods was amply recorded (prolonged antibiotic therapy, curettage and drainage of the sinuses, removal of the metallic material of plasty, decortication, and insertion of an external fixation). In the first patient, OHP was tried as a last resort before deciding to perform an amputation through the upper thigh. Salvage of a limb in such a young patient truly emphasizes the value of OHP.

In these five patients (Table 1), infection at the fracture site was the only apparent cause of pseudarthrosis. In each case, there was inflammatory edema and a fistula which discharged purulent material (*Staphylococcus pyogenes* in three cases, *Pseudomonas aeruginosa* in two cases associated with *Proteus vulgaris* in one case). The effectiveness of OHP in halting the infectious process was uniform. In all cases, a decrease of the edema as well as the discharge was observed after the 10th session. In three cases (Cases 1, 2, and 3), the drying up and permanent closure of the fistula were obtained during treatment. In Cases 4 and 5, the drainage decreased under the influence of OHP, but it subsided completely only after sequestrectomy. When Cases 1, 4, and 5 were reoperated upon after OHP, a swab was taken and culture elicited only a few colonies of the causative organism. No active infection was observed, however.

The antibacterial action of OHP against certain microorganisms has been reported.[1,2] OHP has been reported to be capable of inhibiting *in vitro* the de-

TABLE 1. Clinical Course of Five Cases of Pseudarthrosis Treated with OHP

Case no.	Initial lesion	Secondary lesion	Type of suppuration	OHP at 2 ata	Clinical course
1 (I.M., 37-year-old male)	Supracondylar fracture of left femur Jan. 27, 1963	Avascular pseudarthrosis	*S. pyogenes* var. *aureus*	22 sessions June–July 1964	Bone consolidation Jan. 1965
2 (R.H., 33-year-old male)	Refracture of mid-third right femur July 11, 1962	Pseudarthrosis	*S. pyogenes* var. *aureus*	21 sessions Sept. 1964	Bone consolidation June 1965
3 (K.J., 13-year-old male)	Lengthening osteotomy of left femur Sept. 16, 1963	Pseudarthrosis	*P. aeruginosa*	23 sessions Nov.–Dec. 1964	Bone consolidation July 1965
4 (G.O., 16-year-old female)	Compound fractures of right femur, tibia, and fibula Oct. 1964	Avascular pseudarthrosis	*P. aeruginosa*	21 sessions Feb.–March 1965	Bone consolidation Sept. 1965
5 (B.A., 55-year-old male)	Condylar fracture of left femur June 23, 1964	Avascular pseudarthrosis	*S. pyogenes* var. *aureus*	18 sessions Feb.–March 1965	Bone consolidation Sept. 1965

velopment of such aerobic organisms as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Proteus vulgaris*. Experimentally, OHP has been shown to have a protective effect against pneumonococcal septicemia in mice.[3] Recently, Slack *et al.*[4] reported five cases of osteomyelitis treated successfully with OHP; three of these patients had a chronic bone infection secondary to a fracture. When there is a sequestrum, however, permanent healing at the site of active infection can be obtained only after sequestrectomy, as was the case with two of our patients (Cases 4 and 5) and one of Slack's patients. We have noted the secondary consolidation of the pseudarthrosis in all our patients. It is possible that the action of OHP on infection could have enhanced the formation of bone.

OHP may also have its own action on osteogenesis. The observations which we made in Case 1, where revascularization of a 12-cm-long diaphysial fragment was noticed after OHP, seems to suggest this possibility. Further investigation is necessary to elucidate the exact role of OHP in osteogenesis (increase of tissue pO_2, direct action on the phosphocalcic mechanism, and vasoconstrictive effect in the case of hypervascular pseudarthrosis [5]).

No pulmonary, osseous, or neurologic accidents occurred during or after the OHP therapy.

ACKNOWLEDGMENTS

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DISCUSSION

Discussion of papers by Perrins et al. *(pp. 578–584) and Goulon* et al. *(pp. 585–591).*

DR. W. H. BRUMMELKAMP (*Amsterdam, The Netherlands*): I appreciate the courage of Dr. Goulon to present his interesting case histories, for it is very difficult to explain the ultimate effect of hyperbaric oxygen in these cases. This has been the main reason why we have not mentioned our experience so far. We met, in our gas gangrene and mixed anaerobic series, several patients with infected fractures which had been treated by a plate or an intramedullary nail. We left these foreign materials intact for several weeks or months and, in some patients, complete conjugation and healing of these fractures occurred. In other patients, the plate or nail had to be removed after some time, and we had the impression that even in those patients the healing of the fractures was quicker and better than in many cases not treated with hyperbaric oxygenation.

DR. T. K. HUNT (*San Francisco, Calif.*): There are only two common classes of wounds which do not heal: those with insufficient circulation and those where dead tissue or an infected foreign body is present. Chronic osteomyelitic sinus is an example of both, *i.e.*, a piece of dead bone and poor circulation in the edge. Anyone who has excised one of these realizes that one can usually cut deeply into the tissue surrounding the sinus before reaching any appreciable circulation. It is not necessary, therefore, to postulate an effect on bacteria, because while hyperbaric oxygen cannot really accelerate normal healing, it can restore healing, made abnormal by the absence of good circulation, to a more normal state. I would not be surprised if this were the mechanism behind your observations. You have a tissue with very poor circulation, but once you dispose of the sequestrum, it is possible to restore healing toward normal with increased oxygenation.

DR. E. NEPTUNE, JR. (*U. S. Navy*): Would it not be possible to inject into the medullary cavities an experimental preparation such as krypton to measure the blood flow in the bone? Such a technique might be helpful in the clinical analysis of these cases.

DR. D. J. D. PERRINS: I am not in a position to discuss this academically. We are only presenting our observations. I agree with Dr. Hunt's comments. My own opinion is that we are simply allowing natural reparative processes to take place, rather than influencing the infective organism. We are, as Shore would say, simulating the phagocytes.

Effect of Hyperbaric Oxygen on Experimental Peritonitis

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Despite advances in the management of almost all other infections, diffuse peritonitis presents a formidable therapeutic problem in surgery. Even with proper fluid therapy, antibiotic administration, and appropriate well-timed surgery, the mortality remains high.

Because of the promise shown by hyperbaric oxygenation in recent years in the treatment of a variety of bacterial infections, both aerobic and anaerobic,[1-4] we initiated studies to evaluate the effect of OHP upon diffuse peritonitis. Previously, Ross and McAllister[5] demonstrated a protective effect in mice exposed to oxygen at 2 ata, after challenge with an otherwise lethal intraperitoneal inoculum of pneumococci. Ollodart and Blair[4] showed that survival time of dogs with fecal peritonitis was increased by exposing the animals to OHP at 3 ata, with the abdomen vented and the peritoneum directly exposed to oxygen. In addition, other investigators[6-9] have demonstrated an inhibitory effect of OHP upon some of the secondary physiologic responses to bacterial infection, such as endotoxin shock.

The peritonitis model selected for our studies was that used by Davis and Yull,[10,11] in which a pure culture of *Escherichia coli* and 4 gm% hemoglobin were injected intraperitioneally into animals. This model produced about 70% mortality in treated animals, while in contrast, injection of *E. coli* without hemoglobin was nonlethal. Although the potentiating action of hemoglobin is unclear, experimental evidence suggests that it either hinders host defense mechanisms or promotes *in vivo* bacterial growth. Following intraperitoneal injection of *E. coli*–hemoglobin mixtures, the animals become listless after 4–6 hours, develop diarrhea, refuse food and water, and gradually become less responsive. Shock supervenes and death follows approximately 20–24 hours after the initial challenge. Postmortem findings resemble those seen after lethal intravenous injection of endotoxin. There is massive congestion of all viscera, areas of necrosis are often seen in the bowel wall, and thrombi can be found in the microcirculation of all important organs. There is a purulent exudate in the peritoneal cavity.

In the present experiments, rats with peritonitis were treated by exposure to oxygen at 3 ata for two 2-hour periods,

and mortality and survival times were noted. In addition, the effect of OHP on the arterial blood gases of animals with peritonitis was measured. Since there is general agreement that the outcome in severe fulminating infections is determined relatively soon after the initial insult, OHP was started shortly after the induction of peritonitis.

Materials and Methods

Male white rats of the Walter Reed strain, weighing 225-275 gm, were used in all the experiments.* *E. coli* (0111:B4) were incubated with lysed red cells for 18 hours at 37°C as described by Davis and Yull.[10,11] The red cells were obtained from rats, lysed with distilled water, and adjusted to 4 gm% hemoglobin with nutrient broth. Cultures of *E. coli* in nutrient broth alone were used as control incoula. Before injection, bacterial counts were performed by serial dilution in saline and plating on blood agar; counts were 10^8–10^9 organisms/ml. A standard dose of 5 ml/kg body weight was injected intraperitoneally.

* The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

The stock *E. coli* cultures were stored on agar slants at 3–5°C. Organisms stored over 3 weeks lost their virulence in the hemoglobin system and had to be discarded. This fact was noted because one such nonlethal *E. coli*–hemoglobin mixture was used in one series of experiments.

Hyperbaric Conditions

The animals were pressurized in a standard Navy compression chamber consisting of two air locks which permitted the simultaneous treatment of animals with 3 atm of both oxygen and air. In the inner lock, animals treated with 3 atm of oxygen were placed in a sealed infant Isolette, within which a 95–99% oxygen environment was established by maintaining a high oxygen flow. The oxygen content within the Isolette was monitored by frequent sampling through special outlets and was analyzed by gas chromatography. In addition, carbon dioxide analyses of Isolette gas revealed no significant increase during the treatment period. Temperature and humidity were controlled by ventilation of the main chamber. Animals treated

Table 1. Experimental Conditions, Treatment, and Number of Rats in Each Group

	No. rats			
	No treatment	Treatment		
		O_2 (1 atm)	Air (3 atm)	O_2 (3 atm)
Lethal *E. coli*–hgb. peritonitis, treatment schedule A [a]	35	20	20	20
Lethal *E. coli*–hgb. peritonitis, treatment schedule B [b]	35	35	30	30
Nonlethal *E. coli*–hgb. peritonitis, treatment schedule B [b]	20	40	40	40
E. coli in nutrient broth [b]	12	12	12	12
Hemoglobin in nutrient broth [b]	12	12	12	12
Nutrient broth alone [b]	12	12	12	12
Total no. rats	126	131	126	126

[a] Treatment schedule A, 2 hours treatment given immediately after infection and again 6 hours later.
[b] Treatment schedule B, 2 hours treatment given 2 hours after infection and again 6 hours later (8 hours after infection).

with 3 atm of air were placed in the outer lock in open cages.

The animals challenged with intraperitoneal injection of *E. coli*–hemoglobin were divided into four treatment groups (Table 1). The experimental conditions, time of treatment, and methods of treatment were chosen to study the effect of hyperbaric oxygen in the early stages of peritonitis. All groups were kept at atmospheric pressure when not being treated. Staged decompression was not necessary after hyperbaric treatment.

Blood Gas Analyses

A model was devised whereby sufficient quantities of arterial blood could be obtained from the animals while awake and in the compression chamber. One or 2 days before the experiment, rats were anesthetized with pentobarbital (Nembutal), and the common iliac artery was approached extraperitoneally in the groin and cannulated with PE-50 polyethylene tubing. The tube was passed into the lower aorta below the renal vessels. The other end of the tube was tunneled beneath the subcutaneous tissues from the groin to the back of the neck, filled with heparinized saline, and capped. The arterial sample was obtained from the fully conscious rat by placing the animal in a snug Lucite cylinder perforated for access to the intra-arterial cannula. Since 3 ml of blood was needed for each series of blood gas determinations, each rat was bled only once and then sacrificed.

The animals which had been previously cannulated were divided into two groups: one group received the standard *E. coli*–hemoglobin intraperitoneal injection, and the other received an equal volume of saline. Two hours after intraperitoneal injection, the animals were placed in 3 atm of oxygen. Blood samples were drawn from one to three animals of each group at each of the following times: before intraperitoneal injection, just before compression to 3 atm, approximately 30, 60, and 120 min after compression, and, finally, 30 min after returning to normal atmospheric pressure. Samples were obtained from the rats in the sealed Isolette by means of rubber gauntlets fitted to the ports; into these the investigator could place his arms to perform manipulations and withdraw blood.

Arterial pO_2, pCO_2, and pH were measured to evaluate the effects of 3 atm of oxygen in normal rats (as noted above) as well as in those subjected to *E. coli*–hemoglobin peritonitis. The blood samples were analyzed within the compression chamber at ambient pressure using an Instrumentation Laboratories gas analyzer.

Postmortem Studies

Gross and microscopic examinations were carried out on each animal after death. Animals which did not die during the experiment were sacrificed and studied 30 hours after the peritoneal injection.

In a selected number of animals, bacterial counts of the peritoneum were performed at autopsy. Ten milliliters of 0.9% saline was instilled into the closed peritoneal cavity and, after thorough mixing with the peritoneal exudate, a 1-ml sample was withdrawn. Serial tenfold dilutions of this mixture were made, and 0.1 ml of each dilution was plated on a dry blood-agar plate. Bacterial colonies were counted 24 hours later, and the total number of organisms present in the peritoneal cavity was estimated.

Results

Gas Analysis

The results of blood gas analyses are shown in Table 2. The arterial pO_2 values of rats breathing oxygen at 3 atm were, as expected, much higher than base-line levels; values ranged between 1400 and 1600 mm Hg. The *E. coli*–hemoglobin peritonitis *per se* had no effect on arterial oxygen pressure, and values in the infected group were similar to those in the

TABLE 2. Effect of Hyperbaric Oxygen on Arterial Blood Values in Animals with and Without Peritonitis

	E. coli peritonitis				No peritonitis (control)		
	Time from onset infection (min)	pO_2 (mm Hg)	pCO_2 (mm Hg)	pH	pO_2 (mm Hg)	pCO_2 (mm Hg)	pH
Before compression	105	98	34	7.41	90	45	7.35
	105	94	36	7.32	92	49	7.34
During 3 ata O_2 therapy	160	1440	30	7.39	1400	36	7.41
	160	1400	34	7.38	1500	37	7.41
	210	1400	28	7.37	1500	31	7.35
	210	1540	32	7.34	1540	36	7.37
	250	1400	27	7.31	1600	32	7.31
	250	1460	24	7.42	1520	35	7.35
After decompression	290	97	28	7.45	92	41	7.36
	290	97	28	7.44	97	34	7.41

control group. Arterial pCO_2 and pH values, however, varied even among animals in the same group. This variation may have reflected the state of excitement of each animal and the degree of hypoventilation or hyperventilation at the time of sampling.

Mortality

The mortality and average time to death of rats infected with a lethal *E. coli*–hemoglobin mixture and treated immediately and 6 hours later are shown in Table 3. The purpose of this experiment was to study the effects of immediate oxygen therapy on the lethality of *E. coli*–hemoglobin peritonitis. There were no significant differences in overall mortality in any of the groups, whether they received no therapy, oxygen at 1 atm, air at 3 atm, or oxygen at 3 atm. The survival time in the group receiving oxygen at 3 atm, however, was significantly lower. The rats which received oxygen at 3 atm had an average time to death of 11.2 hours, while survival in the other groups was twice as long (21–22 hours).

Parallel results were found when therapy was begun 2 hours after the lethal *E. coli*–hemoglobin injection and repeated 6 hours later (Table 4). Here again, the mortality was not significantly different between groups, but the average time to death was highly significant. The average survival time in those receiving oxygen at 3 atm was 13.1 hours, compared to 21 hours in Groups 1, 2, and 3.

The use of a nonlethal *E. coli*–hemoglobin inoculum showed that increased

TABLE 3. Mortality in Rats with Lethal *E. coli*–Hemoglobin Peritonitis Treated Immediately and 6 Hours After Infection

	No treatment	Treatment		
		O_2 (1 atm)	Air (3 atm)	O_2 (3 atm)
No. rats	35	20	20	20
No. deaths	22	10	13	16
Mortality (%)	63	50[a]	65	80[a]
Av. time to death (hours)	21.1	21.3	22.0	11.2[b]

[a] Chi-square test, $P > 0.05$.
[b] t test, $P < 0.01$.

TABLE 4. Mortality in Rats with Lethal *E. coli*–Hemoglobin Peritonitis Treated 2 and 8 Hours After Infection

	No treatment	Treatment		
		O_2 (1 atm)	Air (3 atm)	O_2 (3 atm)
No. rats	35	35	30	30
No. deaths	22	21	19	23
Mortality (%)	63	60	63	77 [a]
Av. time to death (hours)	21.1	21.3	22.1	13.1 [b]

[a] Chi-square test, $P > 0.05$.
[b] t test, $P < 0.001$.

pressure alone had a detrimental effect on animals with peritonitis (Table 5). Hyperbaric oxygen treatment resulted in the death of 20% of the animals, and exposure to air at 3 atm resulted in the death of 13%, while no deaths from this inoculum were recorded in animals with no treatment or in those receiving oxygen at 1 atm. Unfortunately, the survival time was not noted in this series.

No deaths were recorded in the control animals injected with saline, hemoglobin in nutrient broth, *E. coli* in nutrient broth, or nutrient broth alone, and treated otherwise identically to animals in the other experiments.

Postmortem Findings

Regardless of the time of death or the method of treatment, all animals which died of *E. coli*–hemoglobin peritonitis had similar postmortem findings. A brownish exudate (1–2 ml) was present in the peritoneal cavity, the major organs were markedly congested on gross examination, and occasionally a pleural effusion was noted. Focal necrosis was invariably seen in the intestinal mucosa and occasionally in the liver. Microscopic examination also revealed severe congestion and some thrombi in the small vessels of the lung, liver, and intestinal tract. Demonstrable lesions appeared to have developed more rapidly in the oxygen-treated animals, since the survival times of the animals treated with 3 atm of oxygen were markedly decreased and the pathologic findings were identical to those in the other groups. There was no evidence of oxygen toxicity on microscopic examination of lung sections, and the pulmonary lesions were those usually produced in this peritonitis model. No significant pathologic findings were noted in the control animals at sacrifice.

Bacterial counts of viable organisms in the peritoneal exudates of animals infected with *E. coli*–hemoglobin showed no significant differences among the four treat-

TABLE 5. Mortality in Rats with Nonlethal *E. coli*–Hemoglobin Peritonitis Treated 2 and 8 Hours After Infection

	No treatment	Treatment		
		O_2 (1 atm)	Air (3 atm)	O_2 (3 atm)
No. rats	20	40	40	40
No. deaths	0	0	5	8
Mortality (%)	0	0	13 [a]	20 [a]

[a] Chi-square test, $P < 0.01$.

ment groups. In all cases, bacterial counts showed 10^8–10^9 organisms/ml. The fact that the bacterial counts were the same in all treatment groups, even those with more rapid deaths, may simply be indicative of the numbers of bacteria necessary for lethality in this model.

Discussion

Hyperbaric oxygenation failed to reduce mortality in our study of rats with *E. coli* peritonitis. In fact, if average survival time or increased mortality with normally nonlethal injections is used as a criterion, the OHP effects were detrimental to the animals. These findings seemingly contradict the results of other studies, which show a beneficial effect of oxygen therapy in aerobic infections. The apparent discrepancies, however, may be partly resolved by considering the studies of Blair and co-workers.[12] They demonstrated enhanced *in vitro* growth of aerobic organisms with an increase of oxygen tensions to about 1.5 atm (1000 mm Hg). Higher oxygen tensions, however, were bacteriostatic. In the present experiments, the oxygen tension in the peritoneum was not measured but can be estimated. Data from other studies in which arterial pO_2 was in the same range (1400–1500 mm Hg) showed that the venous oxygen tensions ranged between 100 and 200 mm Hg. Tissue oxygen tensions must be less than arterial and greater than venous tensions, but they are probably nearer venous values. It can be assumed, therefore, that the oxygen tension in the peritoneal cavity of these rats was below the value necessary for bacteriostatic activity, but was sufficiently high to enhance the growth rate of the *E. coli*. Further experimentation will be necessary to verify this assumption.

The beneficial effects of hyperbaric oxygen on aerobic infections reported by other investigators may result from differences in application. Slack *et al.*[13] studied a series of patients with osteomyelitis with open draining sinuses. The oxygen, in these cases, had direct access to the infected area through the sinuses. In mice with pneumococcal peritonitis, studied by Ross and McAllister,[5] the toxic effects of the infection were due to direct bacterial invasion of the blood. The beneficial effect of hyperbaric oxygen in this situation could result from direct contact between intravascular bacteria and the high arterial oxygen tension. Blair and co-workers[12] found that hyperbaric oxygen increased survival time in dogs with fecal peritonitis, but only when the abdominal cavity was vented. Here again, the organisms were in direct contact with the oxygen environment. These experiments would indicate that hyperbaric oxygen has definite beneficial effects if applied directly to the site of aerobic infection.

The postmortem findings in our experimental rats were similar to those found by other workers after injection of bacterial endotoxins. Evans *et al.*[6,7] demonstrated that hyperbaric oxygen prolonged survival time and delayed hemorrhagic necrosis in endotoxin shock. Since these investigators used only purified endotoxin and not living multiplying organisms, analogies between their studies and the present one would be presumptuous.

Oxygen toxicity *per se* was apparently not responsible for the observed decrease in survival time in our study. The animals had no convulsions and no pathologic changes in the lungs suggesting oxygen poisoning. In addition, no obvious signs of decompression sickness were observed in any of the animals. Other studies with man and animals have shown that the duration of oxygen administration and the applied oxygen pressures in our experiments were safe.

Hyperbaric oxygen could be beneficial in peritonitis if the tissue oxygen tensions are increased sufficiently to have a bacteriostatic effect. The most obvious solution is to administer oxygen at higher pressures, but the probability of oxygen toxicity increases rapidly with increasing pressures. The use of THAM[14] or gamma-

aminobutyric acid,[15] both of which protect against oxygen convulsions, may provide a method whereby higher oxygen pressures carry less risk of toxicity. Another method would be the direct exposure of the infected site to hyperbaric oxygen; this would be feasible for surface infections, such as burns, but technically impractical for treating infections not readily accessible. Hyperbaric oxygen would probably be useful in peritonitis if 3 atm of oxygen pressure could be maintained in the peritoneum. Hydrogen peroxide is currently under investigation as an intraperitoneal source of oxygen.

Naturally-occurring fecal peritonitis is generally a mixed bacterial infection, and in most instances anaerobes are present. It may be that hyperbaric oxygenation would be more beneficial in this clinical peritonitis than in the *E. coli*–hemoglobin model. The growth of the anaerobes could be inhibited by modest increases in oxygen tensions and hence one element of a lethal infection would be eliminated.

The effect of oxygen therapy in diminishing local blood flow[16–18] must also be considered when treating infections. Although the effects of hyperbaric oxygen on blood flow may be transient, repeated prolonged exposure may sufficiently decrease blood flow to the infected area so as to hinder the host's defense mechanism. Vasoconstriction is also potentially dangerous when septic shock complicates the course of the infection. The vasoconstrictive properties of high oxygen tensions, therefore, should be carefully considered before oxygen therapy can be recommended for infections associated with shock.

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DISCUSSION

DR. I. MCA. LEDINGHAM, *Session Chairman* (*Glasgow, Scotland*): This paper emphasizes the very important point that it may be dangerous to experiment with hyperbaric oxygen, especially in the infected patient, unless one is certain of the oxygen tension of the area that one is treating. The results may, in fact, be detrimental.

DR. R. M. OLLODART (*Baltimore, Md.*): Dr. Grogan presented work very similar to Dr. Sleeman's at the American College of Surgeons. He induced staphylococcal peritonitis in mice and showed that hyperbaric oxygen was detrimental in terms of survival. Both of these studies have suggested that if intraperitoneal tensions are low, OHP would be detrimental to the animal.

DR. J. B. GROGAN (*Jackson, Miss.*): Dr. Ollodart mentioned our work with staphylococci-infected mice. We showed that hyperbaric oxygen at 3 atmospheres for 3 hours was highly detrimental in the case of mice infected with staphylococci, but the same preparation with pseudomonas-infected mice showed no detrimental effects. However, there was certainly no beneficial effect noted with hyperbaric oxygen; so it may be, as far as aerobic microorganisms are concerned, that the response to hyperbaric oxygen therapy varies with the microorganism studied.

DR. A. R. BEHNKE (*San Francisco, Calif.*): Can these infections be controlled by antibiotics? If these animals are hit hard enough, neither hyperbaric nor any other type of therapy will help.

DR. GROGAN: With an LD_{50} preparation, 49% of our control animals and nearly 80% of the infected animals died. I did not use any type of antibiotic therapy.

DR. LEDINGHAM: This raises, of course, another problem—namely, that the interactions of hyperbaric oxygen and antibiotics may be complex.

DR. W. H. BRUMMELKAMP (*Amsterdam, The Netherlands*): We have had the impression that hyperbaric oxygen therapy had a much broader effect in some infections than could be expected from antibiotic therapy alone. Hyperbaric oxygen therapy, for example, results in better oxygenation of infected organs or organ systems. We observed this phenomenon in two patients with peritonitis who were treated in the hyperbaric chamber. One case of peritonitis was caused by bacteroides, and the other case was due to bacteroides, enterococci, and *E. coli*. In both cases, complete paralysis of the bowels existed. After 20 minutes of oxygen-breathing at 3 ata, peristalsis started, bowel movements could be heard, and the gastric tube started to produce intestinal fluids. The patients breathed oxygen at 3 ata for 90 minutes and were then decompressed. After the first session, the bowel movements stopped again 5 minutes after decompression. The same phenomenon was observed during the next hyperbaric session several hours later, but after decompression the bowel movements continued for at least 30 minutes. Our preliminary conclusion was that this rapid effect was directly related to better oxygenation of the bowel wall under hyperbaric conditions, rather than to an effect on the infection *per se*.

Effect of Hyperbaric Oxygen on Wound Healing in Rats After Acute Hemorrhage

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In a previous report,[1] we cited experimental results which indicated that high oxygen tension impaired the healing of incised-skin wounds in rats. It appeared that the more intense was the hyperbaric oxygen treatment, the more reduced was the tensile strength of the wounds. Although deleterious effects of OHP on the circulation may be one way to explain these findings, under certain other conditions of impaired circulation (such as irreversible shock), OHP may greatly improve circulation.[2] Since acute profuse hemorrhage is known to disturb wound healing by reduction of the peripheral blood flow,[3] we attempted to determine the effect of intensified oxygenation of the blood under such conditions.

Materials and Methods

Eighteen adult rats from a closed colony received, after being shaved, two symmetrically opposed wounds—one on each side of the back: a control incision at a first session, and an experimental incision at a later one. Thus, each animal acted as its own control. The control wound was allowed to heal for 5 days at normal atmospheric pressure, after which time sutures were removed and tensile strength in the incision site was measured. When the experimental incision was later made on the other side of the back (symmetrical to the first), amputation of the tip of the tail was also performed, so as to induce bleeding sufficient to cause anemia. Blood loss was adjusted to about 2.0 ml/100 gm body weight, resulting in a fall in hemoglobin concentration from an average initial value of about 13 gm% to about 8 gm% on the second day.

Six rats constituting one group were kept at 1 atm during the healing of their experimental wounds, while the remaining 12 rats in the second group were treated with hyperbaric oxygen for 2 hours twice daily during the 5-day healing period. (Simply raising environmental air pressure fails to affect wound tensile strength.[1]) Oxygen treatment was given by enclosing the animals in a special plexiglass container which was placed in the hyperbaric chamber. The chamber was then pressurized to 2 ata with air while the plexiglass container was flushed with oxygen; a continuous flow of oxygen was maintained which kept the CO_2 level below 0.5%. Tensile strength of the healed experimen-

tal incision was measured in both OHP-treated and untreated rats at the end of 5 days.

The tensile strength was measured *in situ* (during anesthesia) in the following manner. A specially designed tensiometer was equipped with a pair of levers having claw-shaped tips; these levers, which articulated to one another, were inserted carefully on each side of the healing incision after removal of the sutures. A steadily increasing separating force was applied perpendicularly through the wound by the levers, and the force required to rupture the wound was automatically recorded in gram-force. By this standardized technique,[4] the results were expressed by calculation of the percentage differences in tensile strength between symmetrical segments of the control wound and the experimental wound.

Results

The anemic rats without OHP therapy showed a statistically significant reduction in wound healing, as measured by tensile strength in the incision site. Mean decrease in the gram-force required to rupture the incision in the untreated group was 22%. In contrast, anemic rats treated with hyperbaric oxygenation showed normal healing of their experimental wounds (compared with their own control wounds).

Discussion

These results suggest that bleeding and OHP, which are known to impair the healing of incised skin wounds in rats when acting separately, can in combination result in normal healing capacity. These findings raise the question of whether the basis of this cooperative action lies in the production of an optimal oxygen tension, the restoration of blood flow and hence of supply of other agents important for healing, or both factors. This question cannot be answered now, as we do not yet know the necessary tissue oxygen tension measurements and local blood flow measurements.

An interesting additional finding has been that, if we give a drug known to dilate the skin vessels, the impairing effect of OHP on wound healing in rats is reduced.[5] Furthermore, it has been found in another study[6] that if rabbits are kept at an air pressure of 350 mm Hg during the healing of skin wounds, no reduction in healing occurs despite the lack of oxygen in the blood—provided perfusion of the skin is attended by denervation of it.

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DISCUSSION

Dr. C. Hitchcock (*Minneapolis, Minn.*): I believe that you are dealing in your studies with the skin only. This is a wound that involves the skin and vascular subcutaneous tissue. We tried to approach the problem more in terms of the wound that we commonly are dealing with in surgery by using the rectus abdominus muscle in the dog (*Hyperbaric Oxygenation*, Livingstone, 1965, pp. 397–408). We had a 50% increase in the tensile strength of the wounds in animals breathing 100% oxygen at 2.5 ata several times daily for 3- to 4-day periods postoperatively. There may be a reason why your animals, which had only a skin wound, showed no particular difference in the tensile strength (including the tensile strength of healing without the additional bleeding) as opposed to ours which involved a greater mass of tissue, *i.e.*, muscle, which has a better blood supply. I am intrigued with the combination which you present—that is, the bleeding and the apparent potentiation of the two factors.

Dr. J. Jacobson (*New York, N. Y.*): Dr. Lundgren, was the second wound made soon enough after the first to have affected the healing process of the second wound?

Dr. Lundgren: I made a point of the fact that if you allow ample time between the two wounds, and study their tensile strength, there is no difference. I am fully aware of the possibility that another type of wound might respond in a different way, but I also recall that there was another important explanation which Dr. Lowe suggested (*Hyperbaric Oxygenation*, Livingstone, 1965, p. 409), that is, that your dogs were anesthetized during the oxygen-breathing periods, whereas our rats were left awake. The point is that one must consider the possibility that with anesthesia the control dogs may have had a decreased tensile strength of the wounds.

Dr. I. McA. Ledingham, *Session Chairman* (*Glasgow, Scotland*): Are you proposing that there is an oxygen toxicity effect in unanesthetized animals?

Dr. Lundgren: No, but they have a better heal-line.

Dr. Ledingham: There is the possibility, of course, that we are dealing with a difference in actual flows between muscle and skin and their respective responses to hyperbaric oxygenation.

Dr. T. K. Hunt (*San Francisco, Calif.*): For the past year we have been doing much work on oxygen tensions in healing wounds. The oxygen tension in a dead space in the wound is quite low, averaging about 15 mm Hg, whereas carbon dioxide is rather high, averaging about 60 mm Hg. Pyruvate and lactate levels are both elevated. Fascia has a poor blood supply and is known to heal in a different kind of tensile-strength gain curve than skin. Skin gains strength along a sigmoid curve which is almost flat after about 14 days, whereas fascia continues to gain strength for 6 to 8 months. Thus, you may well be dealing with two tissues of different blood supplies. Since the oxygen tension seems to be fairly critical in granulation tissue, this may be part of the observed difference. It may be that skin withstands low oxygen pressures better and needs hyperbaric oxygen less than fascia, which is an entirely different structure.

Dr. S. Attar (*Baltimore, Md.*): It is questionable that if you add two negative effects you get a positive one. However, we have noticed similar findings in our experimental hemorrhagic shock and oxygen toxicity studies. We found that bleeding *per se* protects against the toxic effects of hyperbaric oxygen. There is no question that in a series of normal dogs exposed to 3 atmospheres of OHP for 4 hours, the incidence of oxygen toxicity is no less than 20 to 25%, whereas, in dogs in hemorrhagic shock treated with hyperbaric oxygen for 2 hours at 3 atmospheres it is less than 5%. If Dr. Lundgren would run experiments with lesser degrees of hyperbaricity and with varying degrees of exposure time, his results would be more acceptable. At least, they could be explained more easily than the present findings.

Hyperbaric Oxygen in Experimental Burn

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The effect of hyperbaric oxygen as an anti-inflammatory agent was first suggested in 1964 by Stansell,[1] whose study of the histopathology of ischemic injury in patients treated with hyperbaric oxygen revealed certain distinctive features which were interpreted as the consequences of an anti-inflammatory influence. The present study, reported below, was undertaken to evaluate the effect of hyperbaric oxygen in injury which is not essentially hypoxic in etiology and which is associated with a profound inflammatory response. We attempted to examine the effect of extensive deep thermal burn upon blood volume as influenced by hyperbaric oxygen.

Materials and Methods

Two paired groups of mongrel dogs were anesthetized with pentobarbital and, after control hematocrit determinations on jugular venous blood, were immersed to the posterior axillary fold in 70–72°C water for 30 sec. Thereafter, hematocrit was determined at 10-min intervals for 2 hours in both groups. The control group, consisting of 12 animals, was undisturbed after the burn and breathed room air through an endotracheal catheter. The hyperbaric group, consisting of 12 animals, was placed in the hyperbaric chamber immediately after the burn and pressurized to 30 psig breathing oxygen via a cuffed endotracheal catheter and non-rebreathing valve. High pressure oxygen was administered for 1 hour to the treated group, which was then decompressed to ambient air-breathing conditions for the rest of the 2-hour experimental period.

Results

The average hematocrit results (expressed as percent of initial hematocrit) in each group at each time interval are shown in Figure 1. A sharp rise in hematocrit occurred in both groups during the first 10 min after injury. However, at each time interval, hematocrit values in the hyperbaric group were consistently below those in the control group. Statistically significant differences were found between the average values in each group at the various time intervals. A strong tendency toward convergence of the average curves was suggested in the latter part of the second hour.

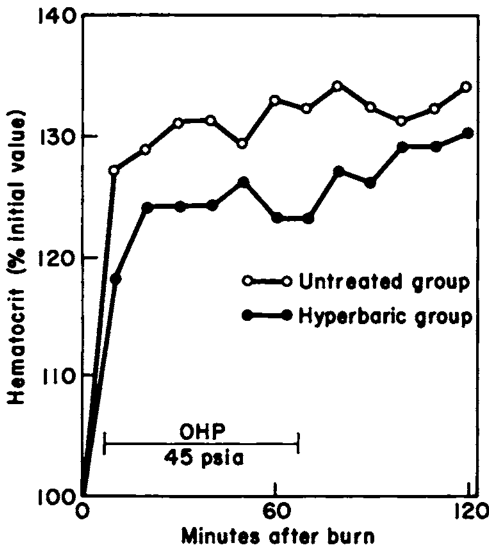

FIGURE 1. Average hematocrit results (expressed as percent of initial hematocrit) in OHP-treated and untreated animals given experimental burns.

DISCUSSION

Inflammation is the sum of a set of responses in living tissue to any injury. It is initiated by the release of humoral factors from damaged cells and is characterized by local vasodilation and increased capillary permeability. Edema and leukocyte infiltration are the inevitable consequences of these vascular changes. When the extent of the initial injury is small, the inflammatory response is compensatory, tending to limit injury and promote healing. When injury is massive, however, the inflammatory response tends to become decompensatory. Massive edema interposes diffusion barriers to tissue oxygenation, and hypoxic injury is superimposed upon initial injury.

Extensive, deep thermal burn produces this decompensatory variety of inflammation and rapidly provokes notable changes in circulating blood volume. The differences we observed between treated and untreated animals could be interpreted as reflecting the extent to which hyperbaric oxygen overcomes diffusion barriers and prevents superimposed hypoxic injury. In this event, further vascular changes and edema formation would be opposed and circulating volume would be spared in hyperbarically treated animals as against untreated animals. However, the vasoconstrictor effect of high partial pressures of oxygen is well known, and one might thus interpret the attenuation of fluid shift as a reflection of reduced blood flow in the injured area.

The present study provides no information helpful in identifying the means by which hyperbaric oxygen produces the observed result. If the vasoconstrictor activity of hyperbaric oxygen is insignificant in these circumstances, then the effect is compensatory and beneficial. If, on the other hand, vasoconstriction in itself accounts for the result, then the effect is probably decompensatory and deleterious.

Further studies of the influence of hyperbaric oxygen on the inflammatory response should include measurements which will determine whether tissue injury is enhanced or reduced by its application.

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Effects of OHP and THAM on Experimental Burns of Rats

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This study was undertaken to evaluate the effectiveness of OHP in treating experimental burns; it was prompted by evidence previously reported by Deleuze[1] that oxygen at normal atmospheric pressure has a beneficial effect in the management of burn cases. Also, Cowley *et al.*[2] have reported that OHP aids in the treatment of hemorrhagic and endotoxin shock. By overcoming the immediate effects of shock, one would expect to limit the area of necrosis, obtain earlier tissue granulations, and provide better protection against infection.

METHODS

Three hundred purebred rats of the hooded Long-Evans strain, weighing 170–350 gm, were selected for this study and were all maintained under the same conditions during the experimental procedures. Extensive and local burns were inflicted under complete ether anesthesia. Ten animals (Group I) were not burned and served as controls for OHP tolerance, 30 animals (Group II) received local burns, and the remaining 260 animals (Group III) received extensive burns. For extensive burns, unshaved rats were immersed up to the axilla in water at 70°C for 7 sec. The size of the burn was calculated to be 75% of the total body area. For local burns, the rats were shaved on the lateral and dorsal sides and a hot metallic plate was placed for 5 sec on a 40-cm^2 area.

Group I (the 10 control rats) were submitted during 30 days to 21 compressions of 60 min each in 100% oxygen at 3 ata. Gradual decompression was performed in the same way for all groups, with a period of 15 min at 1.6 ata and 30 min at 1.3 ata. Group II, animals with local burns, was divided into two subgroups: spontaneous cicatrization was allowed to develop in 14 control rats, and 16 rats were treated twice daily for 60 min with 3 ata of 100% oxygen, on 28 successive days.

The third and most important group, rats with extensive burns, was divided into seven series, each comprising at least 25 animals. After burning, the rats were not allowed to eat and drink before they were able to move about spontaneously in their cages. In Group III, Series 1 (control animals) had no treatment. Series 2 was treated every day for 60 min with OHP at 3 ata. In Series 3, after the burn a saline solution (distilled water with 0.9 gm% of NaCl) was injected intraperi-

toneally at a dose of 0.22 ml/10 gm of body weight. In Series 4, after intraperitoneal injection of saline, the rats were exposed for 60 min daily to OHP at 3 ata. In Series 5, the burned rats received a daily intraperitoneal injection of 0.3 M THAM titrated to a pH of 7.7 with HCl, at a dose of 0.22 ml/10 gm of body weight. Series 6 and 7 of burned rats were treated with both OHP at 3 ata and intraperitoneal THAM: Series 6 had a daily THAM injection (pH 7.7) and was exposed for 60 min at 3 ata, and Series 7 was treated only every other day (same exposure conditions, THAM pH 7.2).

Tolerance to OHP was determined from clinical observations (convulsions, during both compression and decompression) and from mortality rate. Criteria for efficacy of OHP were based on local tissue response and on total body condition. Locally, the speed and the quality of cicatrization were noted. The total body condition of these rats with 75% burns could be evaluated from the rate of survival. The survival rate was compared to survival of the control rats with extensive burns. During the whole experiment, the rats were weighed every day.

Results

Group I: Controls

It was first necessary to evaluate tolerance to OHP in control animals. The oxygen toxicity associated with hyperbaric oxygenation in humans[3,4] is known to be much less than that in many animal species, mice and rats being especially susceptible. In our study, of 10 rats receiving OHP for 30 days, one died on the fourteenth day for no apparent reason, and a second convulsed after 40–45 min. The eight remaining rats showed no symptoms of toxicity. They failed, however, to gain weight during this study as normal untreated rats did. It was evident that this breed of rats had a relative tolerance to OHP.

Group II: Local Burns

In the 30 animals given local burns, there was no modification of the burned areas during the first 12 days. In both subgroups (OHP-treated and untreated), third-degree burns were present and a crust had formed. After the crust peeled, the rats receiving OHP had quicker and better tissue granulation, producing spontaneous cicatrization around the periphery of the burn. Cicatrization was complete after 40–50 days. The formation of scar tissue in these rats was better and faster than in the control rats. In addition, the incidence of infection was lower in the OHP-treated rats. Infection in the control rats was primarily due to anaerobic microbes (*Clostridium perfringens*) or pathogenic staphylococci.

Group III: Extensive Burns

In the rats of the extensively burned group, the effects of shock were studied for 10 days in each series. In Series 1, six of 25 rats survived the 10-day observation period with a slight decline in mortality up to the fourth day, a rapid increase in mortality (40%) from the fourth to the sixth day, and a slowing of the mortality rate thereafter (Figure 1). It should be noted that during the first 4 days the rats neither ate nor drank. In Series 2, which received OHP every day, all rats died by the eighth day (Figure 1). This series was repeated, and the same results were obtained. OHP was therefore judged to be responsible for the deleterious effect, being accompanied by 50% mortality from the fourth to the sixth day.

When the burned rats of Series 3 were rehydrated with intraperitoneally injected saline solution (Figure 2) they did better than the control rats for the first 6 days. Since the rats were not able to eat or drink, it would seem that the administration of water and NaCl was beneficial. After the sixth day, however, the mortality rate increased markedly. In Series 4 (rehydration with saline solution and OHP) there

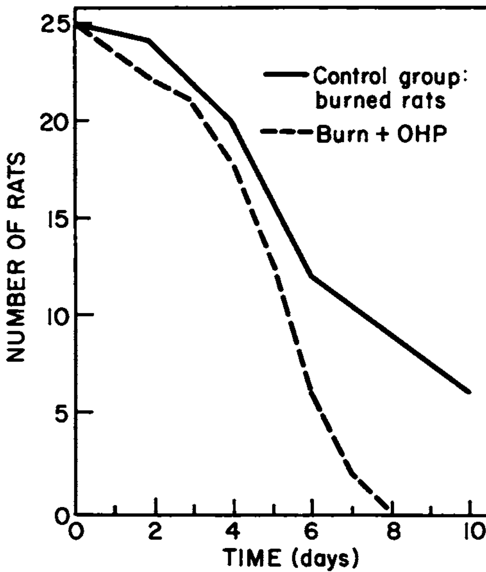

FIGURE 1. Survival curve of control rats in Series 1 (extensive burn, no treatment) is represented by solid line. Survival curve of rats in Series 2 (extensive burn treated with OHP) is shown by dashed line.

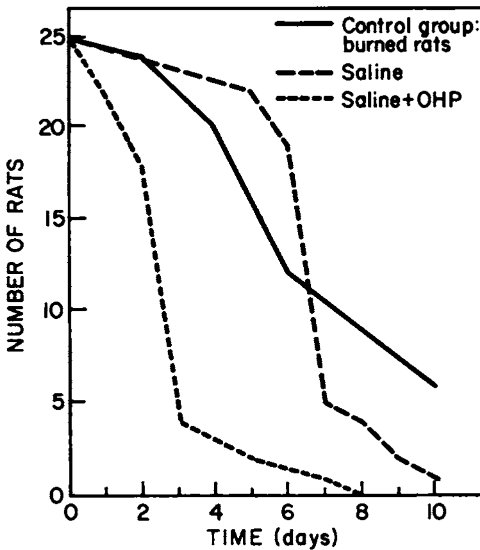

FIGURE 2. Survival curve of control rats in Series 1 (extensive burn, no treatment) is represented by solid line. Survival curve of rats in Series 3 (extensive burn treated only with intraperitoneal injection of saline solution) is shown by dashed line. Survival curve of rats in Series 4 (extensive burn treated with intraperitoneal injection of saline solution plus OHP) is shown by dotted line.

was also a high mortality, and again OHP appeared to have a deleterious effect.

Results in Series 5 (rats receiving THAM at pH 7.7) are shown in Figure 3. The curve in this case was similar to that obtained with the control rats of this group, with a small difference on the sixth day. Biopsies showed no injury due to the intraperitoneal administration of THAM. Figure 4 shows results obtained with Series 6 rats which received OHP and THAM titrated to a pH of 7.7. Results were identical to those obtained with rats treated with OHP alone. Rats in Series 7 received OHP and THAM (at pH 7.2) every other day. Survival rate was best in this series when compared to the reference curve, and especially when compared to the OHP curve.

DISCUSSION

In terms of the localized response, faster cicatrization was obtained with OHP treat-

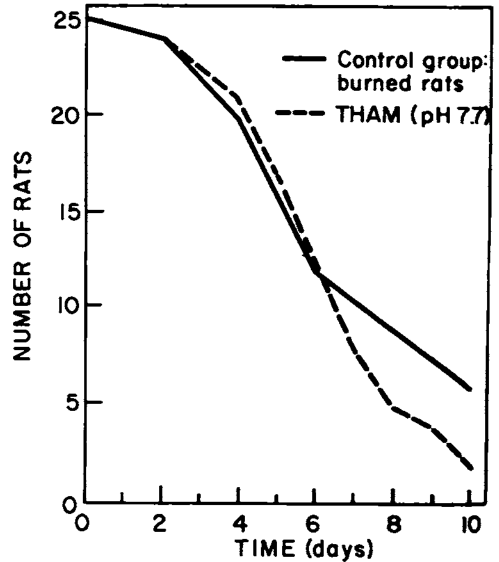

FIGURE 3. Survival curve of control rats in Series 1 (extensive burn, no treatment) is represented by solid line. Survival curve of rats in Series 5 (extensive burn treated only with intraperitoneal injection of THAM, pH 7.7) is shown by dashed line.

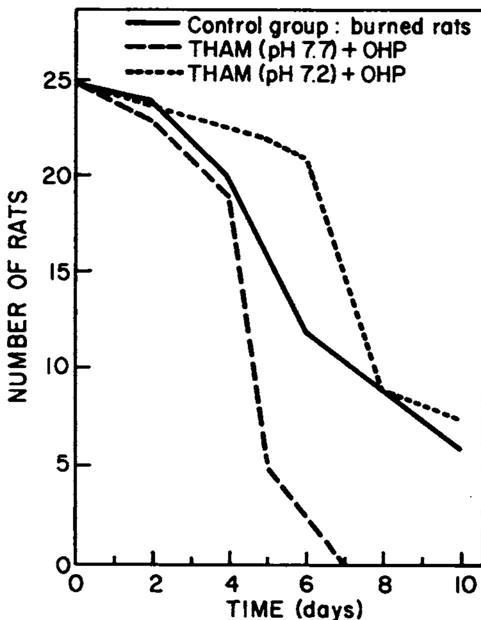

FIGURE 4. Survival curve of control rats in Series 1 (extensive burn, no treatment) is represented by solid line. Survival curve of rats in Series 6 (extensive burn treated with intraperitoneal injection of THAM, pH 7.7, plus OHP) is shown by dashed line. Survival curve of rats in Series 7 (extensive burn treated with THAM, pH 7.2, plus OHP) is shown by dotted line.

ment. Hyperoxygenation seemed to help cutaneous vascularization and produced more healthy granulations. It may be that increased oxygen in some way prevented loss of plasma. In addition, OHP may have resulted in better cicatrization because of its protection against infection due to anaerobes.

The pathogenesis of shock in cases of extensive burns has been subdivided by Simonart[5] into (1) early circulatory shock due to complete dilatation of the capillaries, (2) systemic dehydration because of edema in the burned area, and (3) autointoxication (this last has been questioned by some investigators).

Shock, due to an extensive burn, can produce a marked acidosis.[1] It is known that unless care is taken, OHP treatment can also produce acidosis.[6] Thus the increased mortality observed in Series 2 could perhaps be explained by the additional acidosis due to OHP. In Series 3, rehydration may have temporarily prevented the acidosis. These results, in part, agree with those of Markley *et al.*[7] In our experiments, it seems that the acidosis should have been most effectively corrected with THAM at pH 7.7. However, the best results were obtained in Series 7 which received THAM at pH 7.2 (Figure 5). Whether these results are attributable to less irritation from THAM in the peritoneal cavity at a pH of 7.2 or to administration of OHP only every other day is unclear.

Studies by Sanger *et al.*[8] and Nahas[9] and previous investigation from this laboratory[4,10] have also indicated good results with the combined treatment of THAM and OHP. It should be noted, however, that although this treatment gave the best survival curve in the present experiments, by the eighth day the mortality rate was

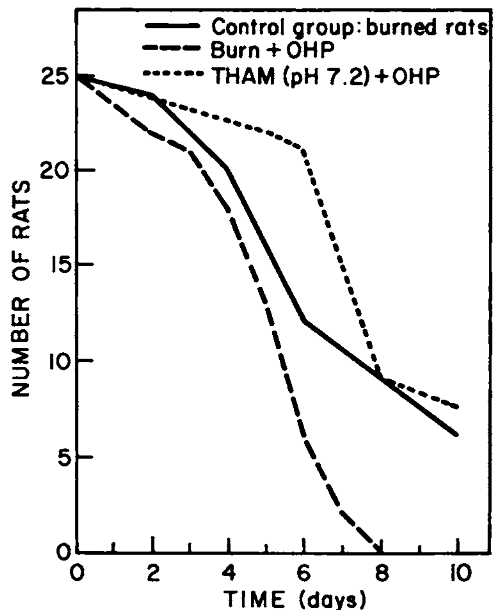

FIGURE 5. Survival curve of control rats in Series 1 (extensive burn, no treatment) is represented by solid line. Survival curve of rats in Series 2 (extensive burn, treated with OHP) is shown by dashed line. Survival curve of rats in Series 7 (extensive burn treated with THAM, pH 7.2, plus OHP) is shown by dotted line.

the same as for the control animals. The survival time was longer in Series 7, which had the greatest mortality from the sixth to eighth day, whereas the greatest mortality in the control group was between the fourth and sixth day.

In conclusion, there was only a slight beneficial effect from treatment with OHP in hooded Long-Evans rats after experimental burns. Since we know that sensitivity to OHP varies widely with different breeds of rats, further investigation will have to be carried out with other animals before OHP can be recommended as a form of therapy for humans with extensive burns.

ACKNOWLEDGMENTS

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DISCUSSION

Discussion of papers by Nelson et al. (*pp. 604–605*) *and Marchal* et al. (*pp. 606–610*).

DR. I. McA. LEDINGHAM, *Session Chairman* (*Glasgow, Scotland*): Dr. Nelson, have you done any studies on the hematocrit readings of your animals which were exposed to high pressure oxygen but not burned? In other words, does simple exposure to high pressure oxygen change the hematocrit?

DR. B. S. NELSON: The only thing we did to control the effect of hyperbaric oxygen alone was a bit speculative, and the control series that you suggest might have done it better. We were concerned with what effect the spleen might have in this situation, as it is capable of altering hematocrit rather dramatically. For this reason, we ran a series with splenectomized animals to show that there was no effect of hyperbaric oxygen on the spleen. We found that it did not produce contraction of the spleen or any apparent alteration in its function, because the curves could be superimposed.

DR. LEDINGHAM: I had thought that hyperbaric oxygen might work on the hematocrit through the peripheral circulation rather than having any effect on the spleen, but this can be left for further studies.

UNIDENTIFIED SPEAKER: Dr. Nelson, did you find any difference in survival between the OHP-treated animals and untreated animals?

DR. NELSON: All animals were sacrificed at the end of 2 hours. There were no survivors.

DR. T. IWA (*Chicago, Ill.*): This might be a new field for the application of hyperbaric oxygen therapy. I am particularly interested in these two papers, since we at the Presbyterian–St. Luke's Hospital in Chicago are carrying out similar experimental work on the effects of hyperbaric oxygen therapy on burns. My experience on this subject goes back to 1964 in Japan, when we treated several patients with carbon monoxide intoxication. Fifty-one victims of coal-mine explosions were treated for carbon monoxide intoxication in our single-tank chamber at the Sapporo Medical College. Many of these patients were also severely burned in the explosion, as is evident in Figure 1. When the patients were treated in the chamber for carbon monoxide intoxication, it was found rather incidentally that these burns (primarily second degree) dried up and healed more quickly than did those which were treated outside of the chamber by ordinary methods. The rapid drying also helped to lower the incidence of infection. Figure 2 shows the same patient of the first slide, only 8 days after the explosion. He had been treated with 100% oxygen at 3 ata for five 2-hour periods. We noticed that even skin which was burned all the way through was saved and healed very nicely. We treated 30 such patients. In contrast, burns treated with standard techniques took at least a month to heal completely.

This experience led me to perform an experimental study, now in progress, of OHP on burns in mice. The studies done to date are quite similar to those of Dr. Thibaut. I believe that OHP has two effects on burn. One is the effect on shock soon after burn, and the other concerns the healing process of burned tissue. Our data to date, which

FIGURE 1. Burned patient, 24 hours after coal-mine explosion. All exposed skin was burned, mostly second degree.

FIGURE 2. Same patient 8 days after receiving the burn. The wound has already dried.

include several species of animals, suggest that OHP has a beneficial effect on both phases. Figure 3 illustrates an example. This

FIGURE 3. Paws of two dogs shown 1 month after experimentally inflicted burn. Right, treated with OHP. Left, no treatment.

photograph shows the paws of two dogs 1 month after identical burns were inflicted experimentally by immersing the paws in 70°C water for 10 seconds. The paw on the right was treated with OHP; the other was untreated.

DR. D. J. D. PERRINS (*London, England*): It might have been advisable for Dr. Thibaut to do arterial oxygen tensions on his subjects before treating them. We have found that in severely burned patients tensions were in the lethal range. This, of course, raises the question whether ambient oxygen is indicated in burns.

Hyperbaric Oxygenation of Ischemic Skin Flaps and Pedicles

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Queen Mary's Hospital, Roehampton
London, England

Plastic surgery is a battle between beauty and blood supply.—SIR HAROLD GILLIES

Blue skin flaps and pedicles are a constant problem in the practice of plastic surgery, as they often precede the death of the transposed skin. If worthwhile results are to be achieved, large flaps have to be fashioned; despite meticulous planning, they may be partially or even totally lost. In this specialty, multiple procedures are commonly performed, and should even one fail it may involve the patient in several more weeks or months in the hospital.

When failure is imminent, the skin becomes acutely congested and cyanosed. At first, blanching on fingertip pressure occurs momentarily, but as the condition progresses the interval lengthens until there is no active circulation. Ultimately, there is gross extravasation of blood and blistering of the surface, a line of demarcation forms, and sloughing or mummification of the affected area ensues.

The underlying pathology of the condition is disputed (Figure 1), some authors implicating a deficient arterial supply

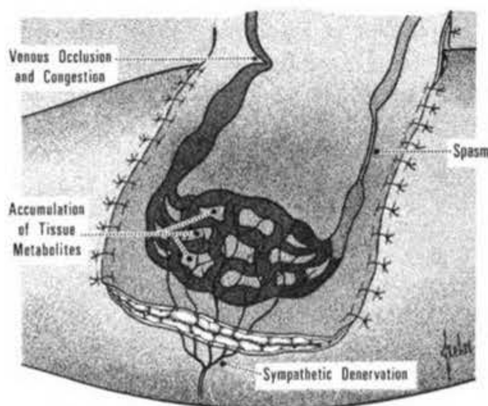


FIGURE 1. Suggested mechanisms of necrosis in transposed skin.

and others inadequate venous drainage with resulting congestion. "Tagged" red-cell techniques have shown that several hours elapse in the immediate postoperative period before labeled cells are detected in the pedicle bed, implying spasm of the larger vessels,[1] but exact clearance studies using ^{24}Na suggest that inadequate blood supply is rarely the primary cause of failure.[2] It has been postulated that the vessels in the distal portion of the flap become denervated during surgical

development and thus become toneless dilated channels containing stagnant blood that eventually coagulates.[3]

This view is contested by those who hold that local metabolic control predominates over the influence of vasoconstrictor nerves, that a period of ischemia produces changes similar to reactive hyperemia, and that vasodilatation is brought about by the accumulation of tissue metabolites.[4] The mechanisms of tissue reaction to hypoxia were reviewed at the Second International Congress.[5]

For skin to survive, it requires a flow of only 1–2 ml of blood per 100 ml of tissue each minute, contrasting markedly with the flow in the normal skin of 90 ml/min. Consequently, the arterial supply can be cut to a minimum before its vitality is affected.[6]

Convincing evidence has been presented that venous congestion is the primary cause of flap and pedicle failure. The application of a loose tourniquet for 24 hours, well above the attachment of pedicles in the limbs of experimental animals, resulted in the necrosis of all preparations.[7] Thus, "ischemic" necrosis would appear to be a misnomer.

That hypoxia plays a fundamental role in the death of transposed skin is evidenced by the progressive cyanosis. Measures to cope with this surgical disaster have previously been directed at maintaining an adequate circulation (Figure 2). Gentle massage, low molecular weight dextran, anticoagulants, and even the application of leeches have been advocated. Hypothermia has been shown to improve survival rates by decreasing the metabolic needs of the skin.[8]

Skin flaps in jeopardy have been treated with OHP by the Amsterdam workers since 1963,[9] and for the past year we have had the use of a small transparent chamber in our plastic surgery center.[10] Despite reports of disappointing results in experimental flaps in animals,[11] our initial experience was encouraging.

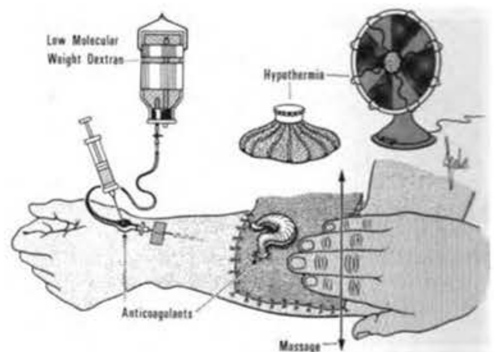


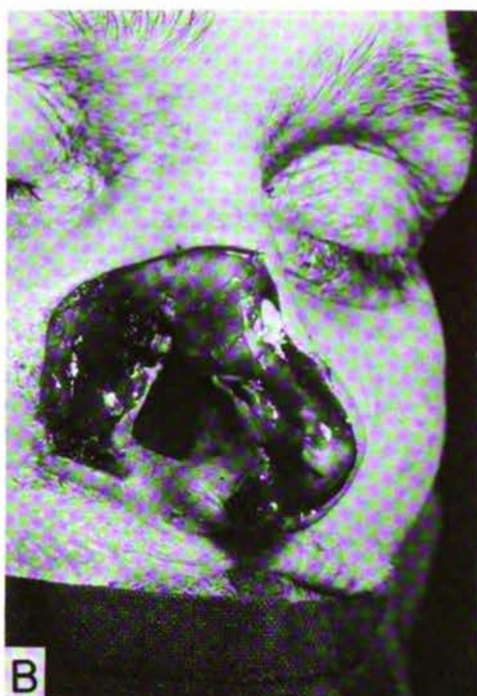
FIGURE 2. Measures advocated to limit necrosis in skin flaps.

ILLUSTRATIVE CASES

Case 1. A 57-year-old woman with a large rodent ulcer on the alar aspect of her nose had eventually agreed to surgical excision, which left a large defect (Figure 3A, 3B). This was repaired by a forehead flap that had previously been prepared. Within a few hours, the flap was dark blue, and OHP treatment was commenced 8 hours after operation. After 20 min at 2 ata, no visible response had occurred so the pressure was gradually increased in steps. After 5 min at 2.5 ata, the flap suddenly came to life and turned a livid pink. Treatment was continued intermittently over the next 4 days, an average of 1.5 hours of exposure being given four times in 24 hours. After 48 hours, less intense exposure was found necessary, as judged by the color of the flap (Figure 3C, 3D). Ultimately, there was minimal superficial epithelial loss at the extremity of the flap, which healed without scarring (Figure 3E).

Subsequent patients have taught us that it is essential for treatment to be commenced early and continued intensively until the flap is no longer in peril and, if necessary, until a collateral circulation has developed. The results of inadequate exposure are instructive.

Case 7. A 73-year-old man had a basal cell carcinoma excised from the flank, and



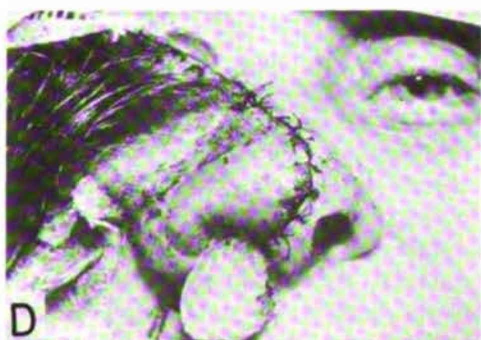


FIGURE 3. Case 1. A, basal cell carcinoma of ala nasi. B, defect created by surgical excision. C, ischemic changes in the forehead flap 60 hours after operation and just before the eleventh exposure to OHP. D, appearance of flap after a 1.5-hour exposure to OHP at 2.5 ata (22 psig). E, appearance of patient on discharge from hospital. Note complete survival of split skin graft to the donor site on the forehead. (Reproduced from Brit. J. Plast. Surg. 19:110, 1966.)

after excision the defect was closed with a rotation flap. When marked ischemic changes became apparent 24 hours later, OHP treatment was commenced immediately. Again, no change was noted until after 2.5 ata had been reached, but this time 20 min elapsed before response was apparent. He was treated intensively for 2 days, but then the prototype console we were using at that time broke down and could not be repaired until next morning. Treatment was recommenced, and again the flap turned pink. The schedule was followed for another 2 days, when the flap started to turn black.

It can thus be inferred that extravasated blood pigment can be adequately oxidized by transcutaneous perfusion, and indeed it has been shown that such changes occur in transposed flaps on pigs that are already dead.[11] That the appearance of pigmentation in a flap does not necessarily imply that the underlying tissue is doomed is illustrated by the next case.

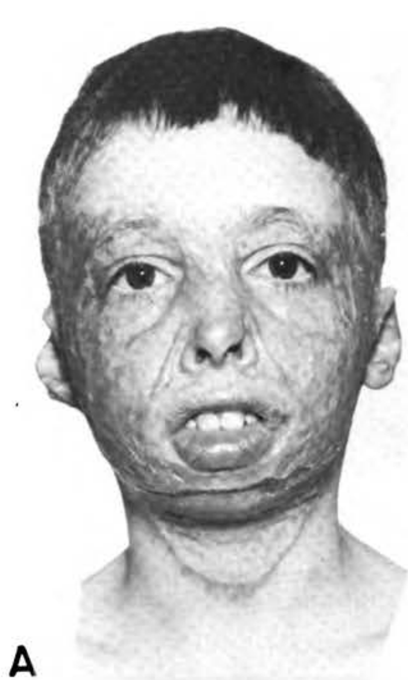
Case 9. An 8-year-old boy was severely burned about the face in 1962, and a series of operations to resurface the skin was started later that year (Figure 4A). An attempt to cover one cheek ended in disaster (Figure 4B), and on this occasion the pedicle turned dusky, with extravasated pigment apparent in the flap by the morning after operation. A second attempt was then made to cover the cheek with an acromiothoracic tube pedicle. Again, extravasated pigment began to appear in the flap (Figure 4C). Treatment was commenced at 2.5 ata and continued intermittently for 2 days. Despite the severe discoloration, complete survival occurred (Figure 4D).

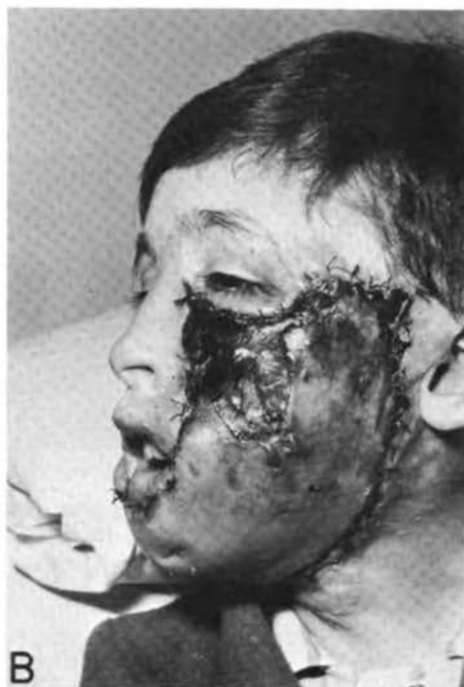
Our routine now is to start treatment as soon as any doubt exists about the viability of the flap. The time required for blanching on fingertip pressure to disappear is a useful guide to the need for treatment (Figure 5). We have arbitrarily chosen 7 sec as the point when hypoxia needs correction. After about 1.5 hours of treatment, the blanching time has usually been reduced to 3 or 4 sec.

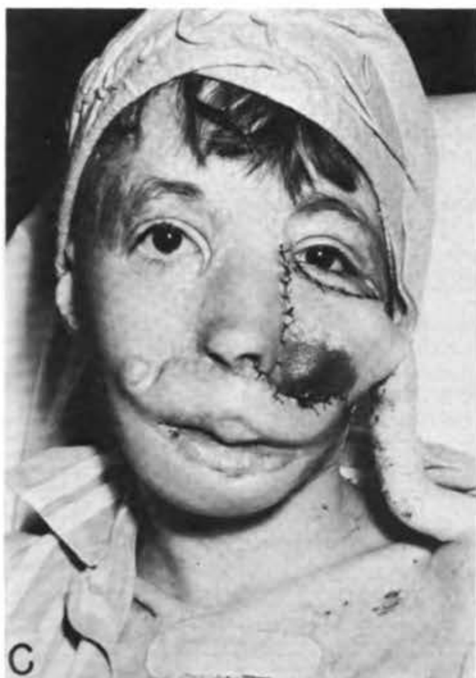
In the past 12 months, 11 cases of possible necrosis in flaps and pedicles have been treated by hyperbaric oxygen (Figure 6). Only once was OHP therapy judged unsuccessful (Case 7), although four pedicles were lost in other patients—two in an old man who refused treatment, one in a patient with a pedicle inserted into the palate, and one in a patient with a pedicle inserted into the vagina. In the latter two patients, failure was not diagnosed until they were examined later under anesthesia.

To establish whether hyperbaric oxygenation has value in the routine practice of plastic surgery, we compared the results presented above with those of the preceding 5 years, when other methods of resuscitation were routinely employed (Table 1). Operations were designated as "at risk" if a flap or pedicle was constructed, transposed, inset, or "delayed" (a maneuver employed in plastic surgery to encourage the development of an adequate blood supply, but occasionally associated with loss of tissue). Significant necrosis was recorded if further operative treatment was required to correct the loss, or if the expected results of the operation were not achieved. Although our series of cases was admittedly small, the overall results suggest that in clinical practice hyperbaric oxygenation significantly improves the chance of survival of surgically transposed skin.

Indeed, the advent of hyperbaric oxygenation is an important advance in plastic surgery. If used correctly, OHP should save the majority of properly designed flaps and pedicles that might otherwise have succumbed. In addition, more ambitious surgical procedures can now be contemplated.







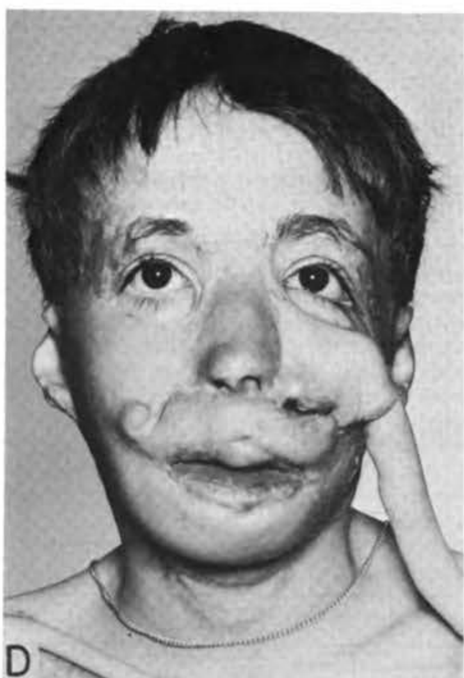


FIGURE 4. Case 9. A, burn scarring of face before commencement of plastic surgery. B, necrotic abdominal tube pedicle 4 days after insetting into cheek. Nasal reconstruction had previously been performed. C, appearance of acromiothoracic tube pedicle 24 hours after inset to repair results of previous loss. D, appearance after intensive resuscitation with OHP.

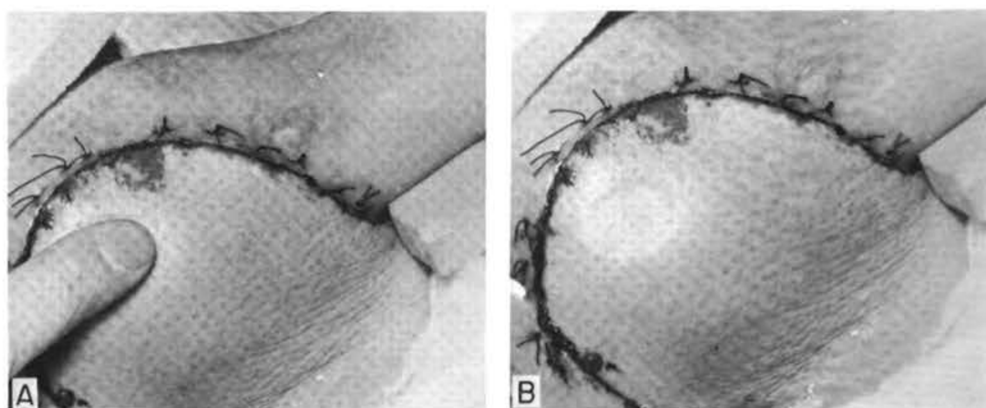


Figure 5. A, cross-leg flap 3 days after inset (Case 8), with incipient marginal necrosis. B, blanching from fingertip pressure, requiring 8 sec to clear and thus indicating need for OHP treatment.

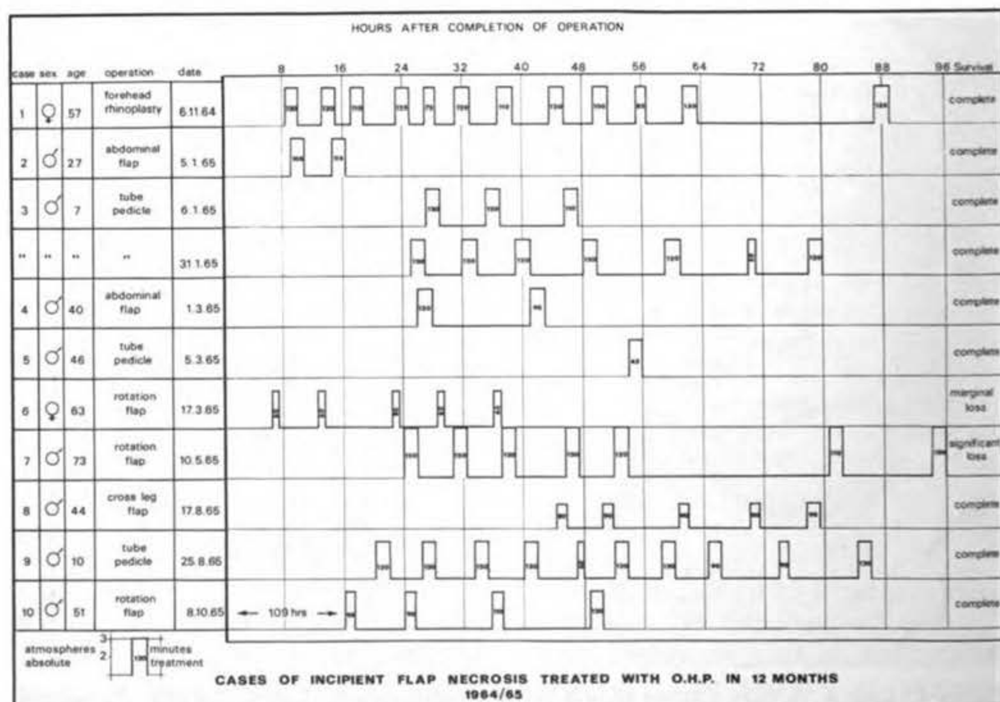


Figure 6. Cases of incipient flap necrosis treated with hyperbaric oxygenation between November 1964 and October 1965. In only one patient (Case 7) was treatment unsuccessful.

TABLE 1. Results of Flap and Pedicle Operations in Past 6 Years

12-month period	Patients: At risk	Patients: With significant necrosis	Operations: At risk	Significant necrosis: Incidents	Significant necrosis: Percentage
1959–60	82	20%	185	17	9.2%
1960–61	42	28%	134	14	10.4%
1961–62	77	20%	176	15	8.5%
1962–63	68	21%	153	15	9.8%
1963–64	65	22%	161	19	11.8%
1964–65	51	8%	111	5 [a]	4.5%

[a] Representing one inadequately treated and four untreated incidents.

ACKNOWLEDGMENT

I am indebted to the surgeons at Queen Mary's Hospital, Roehampton, for their permission and encouragement to treat their patients.

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DISCUSSION

DR. H. J. ALVIS (*Buffalo, N. Y.*): How deep and how long was the treatment routine?

DR. PERRINS: Treatment routine must be adapted to each individual case, as no two pedicles are the same. Treatment must be started early and the pressure adjusted until the flap becomes pink. That is the beauty of this treatment; you can see what is happening. If any of you are entering this

field, please do not form your conclusions unless you or your colleagues are prepared to get up at night. You have got to work hard on some of these cases, and keep going right through, perhaps for as long as 4 days.

DR. I. McA. LEDINGHAM, *Session Chairman* (*Glasgow, Scotland*): What is your feeling, Dr. Perrins, about the length of exposure? I noticed that most of your cases are treated intermittently with 2-hour, or possibly 3-hour, sessions. What would you think about reducing the pressure and leaving these patients in the chamber for a longer period of time? Our feeling is that if you wait long enough, skin-color changes take place even at a lower pressure.

DR. PERRINS: We have noticed this effect too, but we feel that it is due to percutaneous oxygenation which is probably inadequate to nourish the tissue. Judging from our experience, 90 minutes of exposure seems long enough to bring the blanching time back to normal, *i.e.*, to within 3 or 4 seconds. There does not appear to be any point in continuing longer. Furthermore, patients do not like to remain in a small chamber very long.

DR. H. A. SALTZMAN (*Durham, N. C.*): Do you feel sufficiently convinced of the merits of this approach that you do not contemplate doing an alternate-case type of study?

DR. PERRINS: All we can say is that we have not lost any of the cases that we have treated in the past year, with the exception of one flap which we did not treat properly. As all cases behaved alike when treated identically, control trials would appear to be unnecessary.

DR. SALTZMAN: In general, clinically controlled studies with an inadequate therapeutic regime for treated cases will yield negative results even though the therapy has value. Thus, this is the least satisfactory approach to the problem. In the last analysis, we believe what clinical experience shows us to be true over a period of time. Without controls, however, it does take a little longer to convince people.

DR. PERRINS: Could you honestly expect me to use the first case I presented as a control?

DR. SALTZMAN: I appreciate the problem fully.

UNIDENTIFIED SPEAKER: We had a boy whose ear was torn off, leaving only a 1-inch pedicle in the posterior aspect of the ear. A plastic surgeon sewed the ear back on. The edges of the ear were black, and the rest was cyanotic. Under hyperbaric oxygen, the ear pinked up beautifully. We treated the patient twice a day for a week, and the ear was completely saved.

DR. SALTZMAN: We observed very satisfactory improvement with what we hoped would prove to be a reversible ischemic problem in a youngster with purpura gangrenosa, in a similar uncontrolled situation.

DR. LEDINGHAM: Dr. Perrins' comment about the percutaneous oxygenation of the skin flaps is very interesting. It reminds me of a 5-day-old infant who was sent to our chamber in Glasgow with an ischemic limb which was very blue from about the midleg down. Every time this infant was put into the big chamber in an incubator, the leg pinked obviously within half an hour. As long as the infant remained at that pressure, the leg remained pink, although at no time did skin pressure produce blanching. We continued treatment for 48 hours and then took the patient down. I inserted a knife into the anterior aspect of the leg right down to the bone, and found that there was absolutely no viable tissue present.

Hyperbaric Oxygen in the Treatment of Trauma, Ischemic Disease of Limbs, and Varicose Ulceration

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Since 1964, we have used hyperbaric oxygen in the treatment of a number of patients with degenerative ischemic disease of the lower limbs, traumatic ischemia, and varicose ulceration. This therapy was instituted on the rationale that tissue ischemia from vascular insufficiency caused by degenerative disease or trauma should benefit from OHP. All our patients have received OHP treatment at 2.5 ata in a one-man transparent oxygen chamber of the type first described by Emery *et al.*[1] In all cases, treatment has been in 1- or 2-hour daily sessions until maximum benefit was obtained or until it was obvious that no improvement was going to occur.

Because of the variability of the conditions treated and the differing stages of disease at which the patients were first sent for treatment, no scientific or statistical evaluation of results has been possible. The opinions expressed below are based entirely on clinical impressions.

Ischemic Disease of Lower Limbs

Other workers, notably Illingworth,[2] have reported beneficial effects of hyperbaric oxygen in the treatment of limb ischemia. It is pertinent, however, that Bird and Telfer[3] demonstrated 18.9% reduction in blood flow in the normal limbs of patients breathing oxygen at 2 ata. Although these authors stated that the same might not occur in the ischemic limb, their finding does suggest that any alleviation of the ischemia must be due to the high partial pressure of oxygen in the plasma, with the associated plasma-to-tissue gradient overriding the reduction of blood flow.

Acute Nontraumatic Ischemia of Lower Limbs

We have treated 18 cases of acute nontraumatic ischemia of the lower limbs, most cases consisting of thromboembolic phenomena in patients with pre-existing chronic vascular degeneration. Only four patients improved after OHP therapy, and it is possible that they had vascular spasm rather than full organic block. In all the patients in this group, ischemic areas have almost invariably improved in color during compression in the oxygen chamber, but this improvement was lost after depressurization.

One patient, a 38-year-old woman, developed a femoral and popliteal artery thrombosis after she had been taking con-

traceptive pills for 2 months. Although removal of the thrombus was performed, followed by hyperbaric oxygenation, gangrene of the foot resulted and amputation was necessary.

Generally, the results of OHP treatment in these patients have been disappointing. Hyperbaric oxygen may have a place, however, in the treatment of a patient with an embolus in a main artery to a limb while awaiting embolectomy.

Chronic Ischemia of Lower Limbs

Twenty-six patients with chronic ischemia of the lower limbs have been treated (none with Buerger's disease). All were elderly patients with arterial insufficiency; some had frank gangrene, some had pregangrenous changes, and some had ischemic pain or ischemic ulceration.

Only one patient of the group had lasting benefit from OHP therapy. Five others had temporary relief of ischemic pain or healing of ulcers; one old man had periods of relief from ischemic pain of up to 13 hours following treatment.

Most patients showed improved color in the affected area, and some experienced paresthesia in the ischemic areas during hyperbaric oxygenation, but the improvement was not maintained on removal from the oxygen chamber. We noted, however, that areas of wet gangrene tended to dry up under hyperbaric oxygen therapy, and we think this may be due to the antibacterial properties of OHP. Also, we noted that the line of demarcation between gangrenous and healthy tissue became more clearly defined under continued hyperbaric oxygen treatment. This clinical finding has been substantiated experimentally by Stansell.[4]

After some hesitation on our part, one patient with severe Raynaud's disease was treated. She developed increasingly severe pain and treatment had to be stopped. This clinical result supports the experimental work of Bird and Telfer[3] showing reduction of limb blood flow under hyperbaric oxygen.

Our results with hyperbaric oxygenation in chronic ischemic disease of the lower limbs have been disappointing. Perhaps, however, our treatment schedule of 1 or 2 hours daily has been insufficient; it may be that, if patients could be kept pressurized continuously for days rather than hours, ischemic areas might be kept alive until collateral circulation was established.

Traumatic Ischemia

Smith *et al.*,[5] Maudsley *et al.*,[6] and Illingworth[7] have reported good results from hyperbaric oxygen therapy in traumatic ischemic lesions, and our experience is consistent with this. We have treated 22 patients, some with degloving injuries, some with skin flaps of doubtful postoperative viability, and some with limb injuries involving a main blood vessel. Thirteen of these patients did well, improvement being most frequent in those with skin flaps, grafts, and degloving injuries. That traumatic ischemia should respond to treatment better than ischemia from degenerative arterial disease is to be expected, since in trauma the lesion usually occurs in healthy tissue.

Although it has been impossible in this series to match our patients with conventionally treated controls, we have the clinical impression that hyperbaric oxygen therapy is a worthwhile supplement to the treatment of some injuries and further trial is indicated.

Varicose Ulceration

Hyperbaric oxygen therapy in ischemic ulceration of limbs has been reported by Ledingham.[8] At our institution, 17 patients with varicose ulceration of the legs have received OHP therapy after more conventional methods of treatment failed. In five cases healing occurred, and six

more were much improved. Four remained unchanged, and in two others treatment was abandoned at an early stage.

Most leg ulcers are chronically infected with a mixed flora of staphylococci, pyocyaneus, and proteus, all of which have been shown to suffer inhibition of growth when exposed to OHP.[9,10] Clinically, we noticed that the ulcers often became cleaner and less purulent after a number of exposures to hyperbaric oxygen, and islands of granulation could be seen. We feel that the antibacterial effect of OHP helps resolve these leg ulcers; we agree with the suggestion of Ross and McAllister [11] that the exposure of such surface-infected lesions to high pressure oxygen (easily done in the one-man chamber) helps eradicate infection and thus assists healing.

Once the area has healed, provided care is taken, further breakdown may not occur because intact skin makes few metabolic demands. However, relapse is of the nature of the disease in varicose ulceration, and two of our cases have required repeated courses of treatment to keep their ulcers in a state of reasonable healing.

We feel that hyperbaric oxygen therapy is a worthwhile aid in the treatment of varicose ulceration and that a further trial is indicated.

Summary

Using a one-man high pressure oxygen chamber, we applied OHP in the treatment of 18 patients with acute nontraumatic ischemia of the lower limb and in 26 patients with chronic vascular insufficiency of the legs. The results of treatment of these patients were disappointing. Twenty-two patients with traumatic ischemic lesions also received high pressure oxygen therapy, 13 of whom responded well; we feel that a further trial is indicated in this condition. Of 17 patients with varicose ulceration who received high pressure oxygen therapy, five were healed and six improved. These results are encouraging, and here again we suggest that further trial is indicated.

ACKNOWLEDGMENTS

We are grateful to Vickers, Ltd., for lending the hyperbaric oxygen chamber to our unit, and we especially wish to thank Dr. K. G. Williams and Dr. Ian Hopkinson for their help. We also thank the many colleagues who referred patients to us, Mr. D. Lang Stevenson for providing the installation facilities for the hyperbaric oxygen equipment in his surgical ward, and Miss D. English for her secretarial assistance.

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Response of Ischemic Leg Ulcers to Hyperbaric Oxygen

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Rabbits with ischemic foot lesions were intermittently exposed to hyperbaric oxygen to determine its effect upon wound healing, infection, and pulmonary toxicity. We undertook this study after the report of Smith *et al.*[1] indicated that OHP had a considerable clinical influence on the outcome of severe traumatic ischemia. The object of our experiments was to study a series of animals with standardized ischemic limb ulcers and to compare the response of a group of treated animals to that of a group of untreated controls. Since OHP has been reported to produce a reduction in peripheral blood flow, we particularly looked for differences in healing rates of control ulcers placed on the normal foot of each rabbit.

Materials and Methods

Adult New Zealand white rabbits of both sexes (average weight 2.5–3.0 kg) were anesthetized with intravenous thiopental sodium. The skin of the thighs and feet was shaved, prepared, and draped with sterile towels. An incision was made over the right inguinal ligament and carried longitudinally through the skin, subcutaneous tissue, and deep fascia distally to the knee. This exposed all of the femoral vessels, including the circumflex branches (Figure 1). These arteries and veins were ligated, including the popliteal vessels. The resulting ischemia was sufficient to make the extremity appear grossly pale and cyanotic. The incision was then closed in layers with continuous 4–0 chromic catgut suture.

A 2-cm^2 full-thickness specimen of skin was then excised from the dorsum of each foot, thus producing a normal ulcer and an ischemic ulcer. On the ischemic side, the tissues did not bleed. On the normal side, brisk bleeding occurred at the skin edges. The rabbits were given no specific treatment to the ulcers. Animals in the control series were kept in cages containing two animals each, and they were fed the standard pellet diet.

Treated animals were exposed to oxygen environments at different pressures and on different time schedules. The rabbits were placed in a 124 × 77 cm hyperbaric chamber within 2 hours of the surgical procedure, by which time they had nearly recovered from anesthesia. The chamber was flushed with 100% oxygen

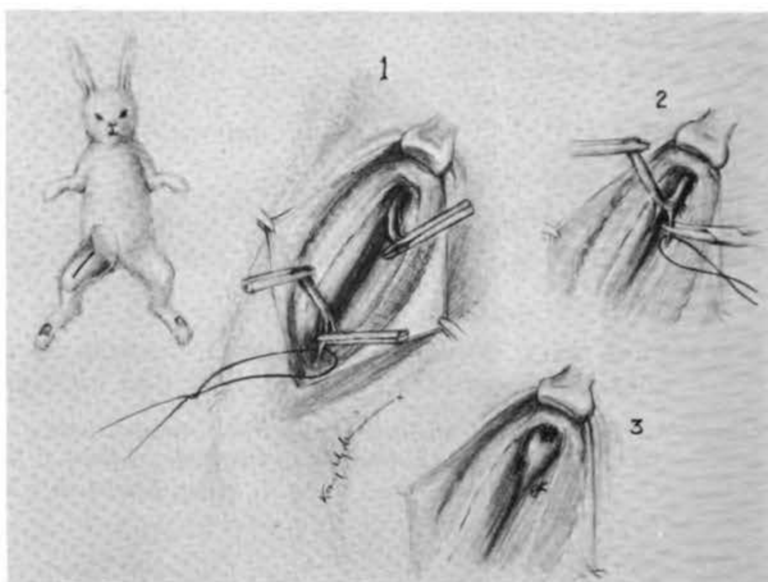

FIGURE 1. Schematic representation of surgical procedure: (1) longitudinal incision exposing all femoral vessels, (2) ligation, and (3) appearance of ligated vessels before wound closure.

and the pressure increased to the desired value. A sufficient constant flow was maintained to provide a 98% oxygen concentration, as measured on the Beckman C analyzer, and a carbon dioxide concentration below 0.5%, as measured on the Scholander apparatus. Temperature remained at 24–25.5°C. The oxygen was humidified. For each session, compression time was 5 min and decompression time 8 min.

Treated Series

Group 1. Eight rabbits with ischemic ulcers were placed in the hyperbaric chamber within 2 hours of the surgical procedure and exposed to a single 2-hour course of 98% oxygen at 30 psi. All of these animals died during this first exposure. They displayed hyperpnea, gasping respiration, and finally respiratory failure. At autopsy, the lungs were grossly red, and diapedesis of red cells into the alveoli was seen microscopically.

Group 2. Twelve surgically prepared rabbits were placed in the chamber and exposed to six 1-hour sessions in 98% oxygen at 30 psi, with a 3-hour interval between sessions at normal atmospheric pressure in room air. Subsequently, six 1-hour exposures to oxygen at 15 psi were given at equal intervals around the clock. Nine of these rabbits died in the first 9 days (at the rate of one per day). At autopsy, the lungs of each showed hemorrhagic changes with patchy atelectasis and edema. Convulsions did not occur, and no intravascular gas was detected. The three surviving rabbits were continued on the around-the-clock exposures at 15 psi (six 1-hour sessions in 24 hours) for 20 days. These animals were sacrificed and autopsies performed.

Group 3. Twelve surgically prepared rabbits were exposed to a 20-day schedule of the same six 1-hour sessions every 24 hours around the clock at 15 psi for 20 days. These animals were never exposed to 30 psi of oxygen, and all survived. Autopsies were performed.

Group 4. Twenty surgically prepared rabbits were exposed to 15 psi of 98% oxy-

gen on a different schedule, consisting of six 1-hour sessions with 1-hour intervals between sessions for 12 hours, followed by an overnight rest. This schedule was continued for 20 days, and all rabbits survived. These animals were sacrificed on consecutive days following the completion of oxygen exposure, and the lungs were examined histologically over a span of 10 days.

RESULTS

Group 1. All eight rabbits died during the initial 2-hour exposure to 30 psi of 98% oxygen. Cause of death was acute pulmonary hemorrhage, congestion, and atelectasis.

Group 2. The initial six 1-hour sessions at 30 psi exceeded the tolerance of these rabbits for oxygen, and only three of 12 survived. Microscopic sections of the survivors' lungs demonstrated an increased cellularity of the alveolar septa, patchy hyaline material in the alveoli, and atelectasis. The ischemic ulcers in each of these animals remained dry and had healed in 3 weeks. In one groin, suppuration had occurred initially and *Staphylococcus aureus* was cultured. This wound healed spontaneously and completely. The three normal ulcers healed completely in 2 weeks and remained dry during the healing process.

Group 3. All of these animals exposed to six 1-hour sessions at 15 psi around the clock survived. The average healing time of the normal foot was 15 days. All of the normal lesions healed, with the early appearance of a dry eschar and subsequent epithelialization. The ischemic feet healed in nine of 12 rabbits, with an average healing time of 21 days (a 6-day delay). The span of healing times was 14–26 days in the treated ischemic ulcers.

At autopsy, in the animals with the longest healing times there was a correlation with a greater degree of gross atelectasis. The feet on the three animals which did not heal remained dry, and the involved feet eventually mummified, with a distinct and narrow line of demarcation between the leathery brown dead tissue and the adjacent healthy tissue (Figure 2). The wounds in the groin in these animals all healed, although some suppuration occurred in four during the first several days, with subsequent healing of the wound. Cultures contained *S. aureus*, *Escherichia coli*, and some aerobacter; in two animals, clostridia were cultured for the first 4 days. These wounds then healed spontaneously and remained dry.

When the animals were sacrificed, lung sections demonstrated proliferation of large round cells in the alveoli and cells mixed with some eosinophils, producing a thickening of the alveolar septa.

Group 4. These 20 surgically prepared rabbits were exposed for six 1-hour sessions during the daytime, with an overnight rest. This cycle was repeated for 20 days, and the animals were then autopsied in sequence in order to determine the stages of resolution of pulmonary toxicity in the animals after chronic exposure to oxygen. All of these animals survived. Three had some suppuration in the groin wound in the early postoperative period, which subsequently cleared. All of these wounds healed completely. The cultures revealed a random incidence of gram-negative and gram-

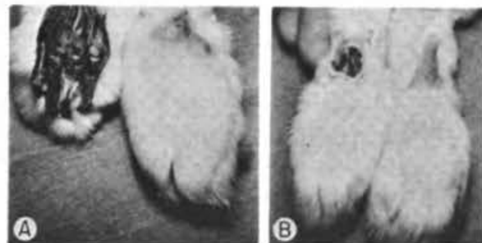

FIGURE 2. Typical appearance of rabbits' feet after 20 days of OHP treatment, showing variation in response. A, dry mummification of ischemic limb. B, healing of ischemic limb.

positive organisms, as well as some *Clostridium perfringens*.

Healing of the normal foot again averaged 3 weeks, and the average healing time of the ischemic foot was 2 weeks. Also in this series, two animals developed mummification of the foot, with the characteristic dry eschar and the distinct narrow margin with the normal tissues. The remaining animals had complete healing of the ulcers.

Histologic study of the lungs performed 1–10 days after the last exposure to oxygen demonstrated a serial reduction in the amount of cellularity in the alveoli and extent of patchy atelectasis; at the 10-day examination, the changes were minimal.

Controls

Twenty-five untreated control rabbits were observed. Of these, six died with suppurating gangrenous limbs. These virulent infections were manifest by breakdown of the inguinal wound closures as well as by spreading infections arising at the foot ulcer sites. Blood cultures were positive for *Cl. perfringens* in two cases, pseudomonas in one, and a mixture of pseudomonas, *E. coli*, clostridia, and staphylococci in two others. Among the 19 survivors, the ischemic leg healed in 14 rabbits, the average healing time being 23 days. Five wounds were purulent and did not heal. The normal ulcers healed in an average of 15 days, which was no different from healing of the normal ulcer in the treated animals.

Discussion

In these experiments, the ischemia of the extremities was produced acutely and simultaneously with the skin lesions. This particular model simulated the conditions in an acutely injured patient or a patient who has suffered an acute arterial occlusion in association with disruption of the integrity of the skin. The potential for lethal infections to develop in these wounds is recognized in clinical practice and was clearly demonstrated in the untreated control group of rabbits in this experiment. Although conventional systemic antibiotic therapy and local measures were not tested here, the mortality from infection was reduced to zero in all animals treated in an oxygen environment.

The role played by inspired oxygen as compared to local oxygen-drenching of the wound was not determined. However, since a major source of fatal infection in the untreated controls occurred in the deep groin wounds that had been sutured closed, it appears that the inspired oxygen contributed to the relative resistance to infection displayed by the treated group. One might raise a valid objection to this hypothesis, however, by referring to the chronic pulmonary lesions and the relative oxygen dependence produced in these chronic exposures. The pO_2 affecting the mixed gram-positive aerobic and anaerobic bacteria and the gram-negative organisms may have approached levels comparable to those in the culture plates of reported *in vitro* studies.[2–4] This applied particularly to the superficial foot ulcers. The tissue pO_2 in the depth of the closed wounds is considerably lower, as described by Hunt.[5] We assume that modest differences in tissue pO_2 may have altered tissue resistance to bacterial invasion.

The average healing time of the normal ulcers was not accelerated by the exposure to OHP. The question regarding the possible acceleration versus retardation of normal wound healing rates by OHP was not clarified by these results. In some of the treated animals, the healing time of the ischemic ulcer approached that of the ulcer on the normal side. This may not represent a true acceleration of wound healing in the ischemic ulcer, but rather a reduced susceptibility to invasion by bacteria.

Although the rabbit is particularly susceptible to the pulmonary toxicity of OHP, all animals did survive a prolonged schedule of exposure provided that individual

sessions did not exceed 1 hour and the intervals in air lasted 3 hours. When overnight rests were included, shorter daytime intervals (1 hour) were tolerated. This suggests that the cumulative effects of the pulmonary response to OHP may be partially repaired in relatively brief periods. Atelectasis and reversible alterations in pulmonary surfactant play a major role here. These animals did not deteriorate grossly during the second and third weeks of exposure. At autopsy, all had patchy areas of atelectasis when autopsied immediately after the last exposure. Within 3 days, there was partial clearing of the atelectasis. Histologically, this was accompanied by reduction in the congestion of cells in the alveolar septa, with a more delicate appearance to the cellular structure. By 10 days after exposure, the histologic sections taken from these rabbits were nearly normal on hematoxylin–eosin stain (Figure 3).

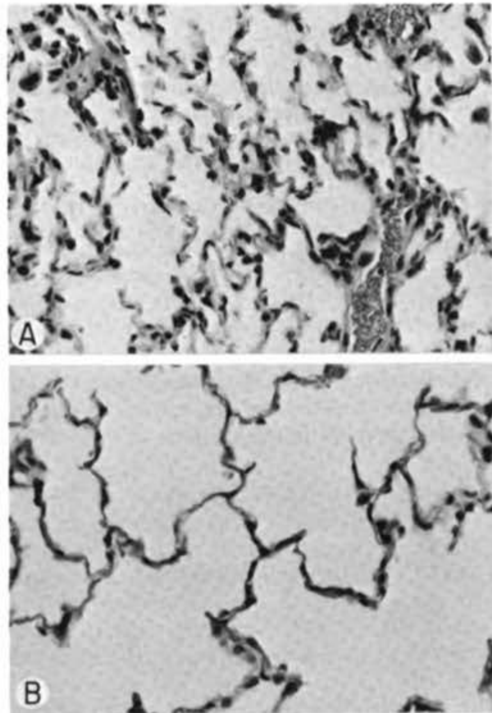

FIGURE 3. A, representative section of rabbit lung 1 day after 20-day exposure to OHP, consisting of six 1-hour sessions every 24 hours (×225). B, lung section 10 days after 20-day exposure to OHP (×225).

SUMMARY

1. Rabbits treated with OHP were protected against fatal infections arising in ischemic lower extremities. Although many gram-positive and gram-negative aerobic and anaerobic bacteria were cultured from wounds in the course of OHP treatment, the wounds typically remained dry, and virulent systemic infections did not occur.

2. There was no difference in the healing of the normally vascularized foot ulcers in animals receiving no treatment compared with those given a prolonged series of hyperbaric oxygen exposures.

3. The average healing time of treated ischemic ulcers was 21 days, compared to the average of 15 days for the normal ulcers. At autopsy, in the animals with the longest healing time there was a correlation with a greater degree of atelectasis. In 10 of 32 rabbits, healing time of the ischemic ulcer was within 2–3 days of that on the normal side.

4. During a 20-day course of intermittent OHP exposure at 15 psi, totaling 6 hours every 24 hours, the rabbits' general condition remained unchanged. At autopsy, there was patchy atelectasis in varying degrees. The postexposure studies of these lungs showed progressive clearing of the atelectasis and alveolar septal infiltration over a 10-day period.

ACKNOWLEDGMENTS

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We are grateful for the technical assistance of Mr. Charles Michielsen during these studies.

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DISCUSSION

Discussion of papers by Slack et al. *(pp. 621–624)*
and Hall et al. *(pp. 625–630).*

DR. I. McA. LEDINGHAM, *Session Chairman* (*Glasgow, Scotland*): One problem common to both of these studies was whether inspired oxygen or locally applied oxygen had effect. This would be a very difficult problem to solve without anesthetizing the animal, but it may be worthwhile to do a controlled trial in the human—perhaps in the small chambers where air can be used as the compressing gas and oxygen plays over just the foot. This would certainly be feasible in the larger pressure chambers.

DR. N. ACKERMAN (*Boston, Mass.*): We have been interested in studying tissue oxygen tensions in an ischemic limb (or, at least, in a limb with marginal blood supply) with the polarographic electrode. Using rabbits, we ligated all the vessels from the bifurcation of the aorta down to the popliteal artery in one leg. The other leg was kept intact and used as a control in some studies. Electrodes were placed in the hind-limb muscle considerably below the knee. Our results showed that, in animals breathing 100% oxygen in a plastic oxygen tent at atmospheric pressure, there was virtually no measurable increase in oxygen tensions in the ischemic limb. In the normal limb, however, there were significant increases in tissue oxygen tension. When the animal was placed in a high pressure chamber and the pressure raised to 15 psig, we obtained results similar to those found in the normal animals. There was an almost immediate increase in tissue oxygen tensions, which reached quite high peak levels, and when the oxygen was discontinued a gradual fall-off of tissue tension levels occurred. Almost identical results occurred in the normal contralateral leg. In most of our animals, demarcation occurred at the knee, and the lower leg turned gangrenous a few days after the study. A spontaneous amputation usually resulted. Thus, it appears that animals with a marginal blood circulation, not sufficient to maintain viability indefinitely, may still be benefited by hyperbaric oxygen. The question is whether 1 to 2 hours of therapy, administered three or four times daily, are adequate in all cases, particularly where results have not been satisfactory. In other words, should we aim for longer and more intensive periods of therapy in treating some of these cases?

DR. W. D. SHOREY (*Chicago, Ill.*): I would like to present a case which indicates the value of high pressure oxygen in severe trauma. A 20-year-old boy was seen in our emergency room and a diagnosis made of sprain of his right knee. Forty-eight hours thereafter, he experienced a sudden tearing sensation in his popliteal fossa. His leg became cold, and there was marked swelling of the popliteal fossa. He stayed at home for 40 hours, then returned to the hospital when his leg began to swell. Exploration of the popliteal fossa showed that there had been complete severance of the popliteal artery at least 44 hours prior to resuture. The popliteal artery was reconstructed and the posterior tibial artery opened with a Fogarty catheter. Incidentally, no clotting mechanism was present in the leg at the time of the operation. After 12 hours, demarcation had occurred at the upper calf. Oxygen was begun at 3 atmospheres absolute. Hyperbaric oxygen treatment was given three times daily for 8 days. At the end of the third treatment, *i.e.*, 24 hours after reconstitution of the artery, the dorsalis pedis suddenly returned to a bounding pulse (almost like an arteritis) as if a marked spasm or intrinsic damage to the artery had been overcome. The patient's leg survived entirely, though a slough of the anterior muscles occurred, and he is now using his leg for partial weight-bearing.

UNIDENTIFIED SPEAKER: Has anyone attempted sympathetic blockade as an adjunct to hyperbaric oxygen?

DR. W. K. SLACK: We have done paravertebral blocks on a number of our patients. We do not really think that it makes any difference in the ones with degenerative vascular disease.

UNIDENTIFIED SPEAKER: This is a most important consideration, because we must remember that at 3 atmospheres pressure in healthy men after a period of time there may be an intense phase of constriction of peripheral vessels—so intense that the individual has what we call a circumoral pallor. There is very little blood supply, so that either carbon dioxide or some adjunct procedure may be of importance. In other words, there is a time here during which oxygen is effective, and then a type of compensatory reaction occurs (presumably in which the sympathetic nervous system is involved) in which there is a cutdown.

DR. LEDINGHAM: We have been pursuing measurements of peripheral limb flow in the abnormal limb similar to those of Telfer and Bird (Lancet 1:355, 1965) with strain-gauge plethysmography, and there are, for reasons which are difficult to explain, quite different responses to hyperbaric oxygen in ischemic limbs. Some of them may show the same 20% reduction in total limb flow which we saw in normal subjects, whereas others show no change in flow. This, of course, offers no solution as to whether there is redistribution from the muscle to skin. However, I think it is going to be interesting if we can predict beforehand whether patients can or cannot be effectively treated with hyperbaric oxygen. We intend to pursue this with sympathetic blockade in various ways.

DR. H. A. SALTZMAN (*Durham, N. C.*): Although ideas always must be tested in the environment in question, studies of blood flow in tissues have shown that, in general, local factors are the primary and systemic factors the secondary determinants of flow to a tissue. That is, with local hypoxia vasodilatation will be present, even though a systemic vasoconstrictive influence may be applied; in such instances, a vasodilator might cause a redistribution of blood flow away from the hypoxic area.

DR. SLACK: We ran a continuous epidural for 5 days on a patient whose leg and foot were crushed in an automobile accident. When his foot was finally amputated, we had the clinical impression that we were able to do a lower amputation than would have been possible with standard treatment. Whether the epidural helped, however, I do not know.

SESSION VII

Hyperbaric Oxygenation and Radiation Therapy

Chairman: JUAN DEL REGATO
Department of Radiology
The Penrose Cancer Hospital
Colorado Springs, Colorado

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Effects of OHP on Dynamics of Cell Replication and the Calculation of Oxygen Effect Factors for X-Radiation *in vitro*

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The oxygen concentration $[O_2]$ in the extracellular fluid profoundly modifies the sensitivity of cells and tissues to x-radiation. Cellular radiosensitivity expressed as damage to proliferative integrity is quantitatively related to $[O_2]$, in accordance with the Alper–Flanders equation:[1]

$$\frac{S-Sn}{Sn}=(m-1)\frac{[O_2]}{[O_2]-K} \quad (1)$$

where S is the radiosensitivity at an oxygen concentration $[O_2]$ μM/liter, Sn is the radiosensitivity in the absence of oxygen, and m and K are constants. This relationship was originally calculated from data obtained from irradiation of bacterial cells. The constant $m=2.74$ represents the maximum oxygen enhancement factor, and K is the oxygen concentration at which radiosensitivity is 50% of the maximum value. Values of K have also been calculated for mammalian cells to be of the order 5–10 μM $[O_2]$ per liter.[2]

This relationship indicates that the presence of a relatively small quantity of oxygen (pO_2 5–10 mm Hg) is sufficient to enhance radiosensitivity of cells to near maximum values. Further very large increases to hundreds of millimeters of mercury produce little more enhancement of radiosensitivity. This is the rationale for the use of hyperbaric oxygen in radiotherapy: the radiosensitivity of most normal tissues, being almost fully oxygenated in this respect under ambient conditions, would increase only slightly, while the radiosensitivity of any anoxic tumor cells would increase twofold to threefold. While the oxygen tension in the extracellular fluid essentially determines the radiosensitivity of cells in respect to oxygen effect, recent experiments by Howard[3] have shown that the oxygen effect acts at an intracellular level. Oxygen was produced intracellularly by photosynthesis in the fresh water alga *Oedogonium cardiacum* held under anoxic extracellular conditions during x-irradiation in a stream of nitrogen. This endogenously produced oxygen was shown by Howard to sensitize the cells with an oxygen enhancement factor of 2.5. Howard was also able to show that carbon dioxide failed to modify the oxygen effect in this system.

Most quantitative studies of oxygen enhancement ratios have been made with suspensions of bacterial and mammalian cells, equilibrated *in vitro* with oxygen over a range of concentrations during

irradiation. The studies of Deschner and Gray [4] with a strain of Ehrlich ascites tumor cells have yielded the most comprehensive information regarding the validity of the Alper–Flanders relationship for mammalian cells. In these studies, Ehrlich ascites tumor (EAT) cells were harvested from mice, equilibrated with oxygen at a particular concentration *in vitro*, and irradiated. After irradiation, the cells were inoculated into the peritoneal cavities of mice and removed after incubation for various times, and the percentage of radiation-induced chromosomal aberrations was scored in terms of abnormal telophase mitoses, *i.e.*, telophase figures with acentrics or dicentrics producing fragments and bridges. The postinoculation time was standardized at 15 hours when aberration rates produced by irradiation were found to be maximal for all radiation doses administered *in vitro*. This peak aberration rate at 15 hours was also found to be independent of the gas phase (oxygen or nitrogen) used during irradiation. The aberration coefficient (α) was expressed as:

$$\alpha = \frac{\log_e F_C - \log_e F_R}{D} \qquad (2)$$

where F_R and F_C are the percentage abnormal telophases in irradiated and unirradiated (control) samples, respectively, and D is the dose in rads. For values of $[O_2]$ over the range from complete anoxia (pure nitrogen) to 1 ata pure oxygen, Deschner and Gray found that the concentrations of oxygen used did not increase the "spontaneous" incidence of abnormal telophases or chromosomal abnormalities. Their irradiation results clearly showed that the oxygen-effect relationship in Equation 1 also applied to mammalian EAT cells.

Our own studies were performed initially to determine whether higher concentrations of oxygen of several atmospheres resulted in an oxygen enhancement ratio similar to that of 2.7 determined by Deschner and Gray for lower pO_2 values. Similar experimental techniques and analysis were used, except that: (1) the tumor used in our studies was a hyperdiploid Ehrlich ascites tumor (ELD Lettré) with a 46 chromosomal mode, that of Deschner and Gray being a hypotetraploid subline (ELT); (2) cells were harvested and equilibrated with oxygen at 5 ata and 35°C in a special thermostatically controlled pressure vessel (Figure 1); and (3) aberration coefficients for cells equilibrated with pure nitrogen at 1 ata and oxygen at 5 ata, respectively, during irradiation were measured for postinoculation times (T) over the range 4–24 hours.

The results of these experiments have been published recently [5] and are summarized in Figure 2. The aberration coefficient α varied with postinoculation time T for both anaerobic (N_2) and hyperbaric oxygen (OHP) irradiations. Furthermore, the peak aberration coefficient values (α_p) for N_2 and OHP irradiations, respectively, occurred at different postinoculation times $\alpha_{p(N_2)}$ occurring approximately 2 hours earlier than $\alpha_{p(OHP)}$, *i.e.*, at $T=8$ and $T=10$ hours, respectively. Calculation of the oxygen enhancement ratio E for various postinoculation times is given by:

$$E = \frac{\alpha_{(OHP)}}{\alpha_{N_2}} \qquad (3)$$

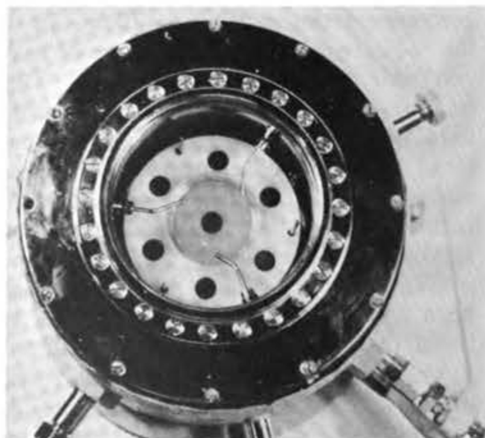

FIGURE 1. Pressure vessel (top view) used for irradiation of cell suspensions *in vitro*, showing three angled microjets directed to surface of suspension.

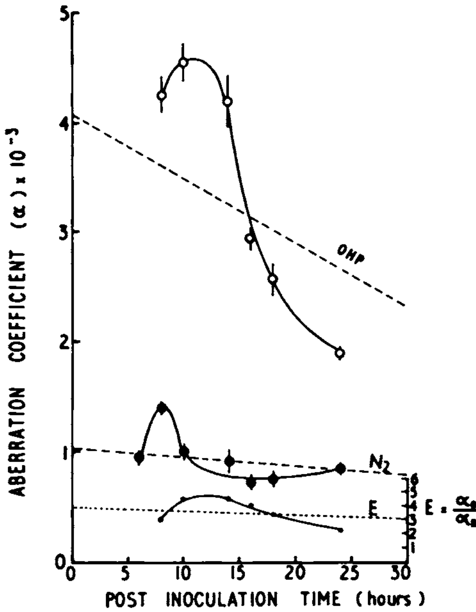

FIGURE 2. Curves for aberration coefficients due to x-irradiation of ELD cells *in vitro* in OHP and anoxia for different postinoculation times *in vivo*. [Reproduced from Nature (London) 207:267, 1965.]

E was highly dependent on T (Table 1). If the value of E was based on the ratio of a_p for N_2 and OHP, the oxygen enhancement ratio (E_p) was given by:

$$E_{p(OHP)}=\frac{a_{p(OHP)}}{a_{p(N_2)}}=\frac{4.6}{1.4}=3.3 \quad (4)$$

This value is somewhat higher than the maximum oxygen enhancement value $m=2.74$ obtained by Deschner and Gray. During these experiments, the observation of Deschner and Gray that exposure of cells to high oxygen concentrations did not significantly increase chromosomal aberrations was confirmed for exposure of cells to oxygen at 5 ata. This observation is pertinent to hypotheses that fundamental analogies exist between oxygen toxicity and irradiation damage in biological systems.[6] Investigation of effects of these agents on chromosomes and cell replication and also studies at a clinical level fail to support such analogies.

TABLE 1. Radiation-Induced Aberration Coefficients in OHP and Nitrogen for Postinoculation Times from 6 to 24 Hours

	Time (hours)						
	6	8	10	14	16	18	24
a_{N_2}	0.95	1.40	1.00	0.91	0.72	0.75	0.84
a_{OHP}	—	4.25	4.55	4.19	2.94	2.57	1.90
$E=\frac{a_{OHP}}{a_{N_2}}$	—	3.03	4.55	4.60	4.08	3.43	2.26

In our experiments, however, if cells were exposed for 1 hour to OHP *in vitro* during irradiation, recovered 4 hours after inoculation in mice, and fixed immediately, the mitotic rate was very low. Indeed, for radiation doses over 20–250 rads, the number of mitoses present in smears was too low to score statistically significant numbers of telophases and aberrations. In this population of cells in log phase of asynchronous growth, OHP appeared to inhibit mitosis by causing a delay at one of the premitotic phases of the cell replication cycle.

It seemed that the variation in a with T, for both nitrogen and OHP exposures, might have been an expression of changes in radiosensitivity occurring during the cell replication cycle ($G_1 \rightarrow S \rightarrow G_2 \rightarrow M$) and that these variations in radiosensitivity differed under anaerobic and aerobic conditions during irradiation. The postinoculation times of $T=8$ and $T=10$ corresponding to $a_{p(N_2)}$ and $a_{p(OHP)}$, respectively, suggested that aberrations scored at mitosis for these times corresponded to cells in late S period or G_2 during irradiation. The delay in $a_{p(OHP)}$ could also be reconciled with this hypothesis, since the OHP exposure allowed fewer mitoses to appear for early postinoculation times than nitrogen exposures, the mitotic rate recovering with increase in postinoculation time.

To elucidate the irradiation phenomena, further experiments have been performed with ELD cells exposed to oxygen or nitrogen *in vitro* but not irradiated during exposure.

EFFECT OF SHORT OHP EXPOSURES *in vitro* ON MITOTIC RATES IN ELD CELLS

Freshly harvested 5-day ELD ascites tumor cells were suspended in normal saline solution and immediately equilibrated at 35°C for 1 hour (50 min at pressure) with the chosen gas phase in the pressure vessel. An aliquot of cells was removed and fixed in acetic methanol (1:3 v/v), smears were prepared and stained with acetic orcein, and mitoses were scored. Immediately after the gas exposure, a second aliquot was injected intraperitoneally in mice, and 6 hours later the mice were sacrificed; the cells were recovered, similarly fixed, and stained and mitoses were scored.

Results from these experiments (Table 2) are summarized as follows:

1. OHP for 50 min *in vitro* halved the mitotic rate, and this depression persisted for at least 6 hours in mice *in vivo*. Oxygen at 1 ata caused similar depression but a somewhat better recovery after 6 hours *in vivo*.

2. Air at 5 ata (*i.e.*, a pO_2 similar to oxygen at 1 ata) did not depress mitotic rates *in vitro*. Apparently the simultaneous presence of nitrogen (at approximately 4 ata) protected the cells against the mitotic inhibition produced by high concentrations of oxygen.

3. In cells exposed to pure nitrogen (complete anaerobiosis) for 1 hour, mitotic rates were only slightly depressed, but if these cells were incubated for 6 hours in the oxygenated peritoneal cavity of a live mouse, mitotic rates increased significantly above normal values; this did not occur, however, in dead (anoxic) mice kept at 35°C in an incubator.

4. If the pO_2 was kept at ambient air values but the pN_2 increased (4% oxygen + 96% nitrogen at 5 ata), the "nitrogen effect" was produced, and mitotic rates rose above normal after 6 hours in live mice.

5. The immediate mitotic depression produced by OHP *in vitro* could be largely abolished if followed by treatment in nitrogen for 1 hour *in vitro*, and the "nitrogen effect" also reappeared after the cells were incubated for 6 hours in the oxygenated peritoneal cavity of mice.

TABLE 2. Effect of Gas Phase *in vitro* on Mitotic Rate of Ehrlich Ascites Tumor Cells and Postinoculation Modification *in vivo*

Treatment *in vitro* (35°C) *	Mitotic index	Post-treatment Inoculation 6 hr in:	Mitotic index
None	4.3 ± 0.05	Live mice	4.3 ± 0.14
Air 1 ata	4.9 ± 0.20	Live mice	4.5 ± 0.10
	4.1 ± 0.17	Live mice	4.1 ± 0.15
O_2 1 ata	2.5 ± 0.05	Live mice	3.0 ± 0.07
O_2 5 ata	2.3 ± 0.03	Live mice	2.4 ± 0.03
O_2 5 ata	—	Dead mice	2.7 ± 0.14
Air 5 ata	4.0 ± 0.15	Live mice	4.6 ± 0.73
N_2 1 ata	3.8 ± 0.10	Live mice	5.6 ± 0.07
N_2 5 ata	3.5 ± 0.11	Live mice	6.7 ± 0.20
N_2 5 ata	—	Mice breathing O_2 1 ata	7.3 ± 0.09
4% O_2 + 96% N_2 5 ata	3.7 ± 0.18	Live mice	7.4 ± 0.34
O_2 5 ata, then N_2 1 ata	3.2 ± 0.29	Live mice	6.2 ± 0.34

* Gas phase was applied for 50 min in each case.

While no definite conclusions can be drawn from these results, it is suggested that high concentrations of oxygen prevented the $G_2 \rightarrow M$ transition by prolonging the late S and postsynthetic G_2 phase and that the $G_2 \rightarrow M$ transition was favored by a low redox potential state, possibly associated with relatively higher concentrations of SH and other reducing metabolites. Thus, it has been shown [7–11] that thiol compounds in the reduced SH form are powerful promoters of mitosis, particularly in regenerative phenomena in which the formation of the blastema is characterized by high mitotic rates and associated with increases in reduced glutathione and other SH metabolites, respiration being chiefly glycolytic with increased lactic acid production and drop in pH.[12,13] The "nitrogen effect" may be due to a shortening of G_2 produced by a buildup of reducing metabolites, but that mitosis can occur only if oxygen becomes available to cause rapid oxidation of reduced metabolites to provide the energy or possibly cause alterations in molecular configuration (*e.g.*, SH→SS bonding) associated with the structural changes in premitosis and mitosis.

Effect of OHP on DNA Synthesis

To determine whether a relatively short exposure of cells to OHP or nitrogen caused changes in DNA synthesis, cell suspensions at 35°C were "pulsed" for 10 min with tritiated thymidine in concentrations of 2 μc/ml cell suspension, either before gassing or immediately after removal from the gas phase. The cells were fixed immediately after labeling, autoradiographs were prepared with stripping film or dipping emulsion techniques and (after development for suitable times) stained with hematoxylin, and the percentage of labeled cells was scored (Table 3). Exposure to oxygen at 5 ata or air at 1 ata did not significantly affect the percentage of cells incorporating the tritiated nucleoside and undergoing DNA synthesis. However, nitrogen at 1 ata caused a significant reduction of approximately 25% in the proportion of cells synthesizing DNA.

Table 3. Pulse-Labeling of Ehrlich Ascites Tumor Cells with ^{3}H-Thymidine[a]

Treatment	Labeled interphase nuclei (%)	Mitotic index (%)
Fresh ascites from mouse	23 ± 1.2	4.1
After 5 ata O_2 1 hr *in vitro*	22 ± 0.8	2.3
After 1 ata N_2 1 hr *in vitro*	15 ± 1.2	3.7
After 1 ata air 1 hr *in vitro*	21 ± 0.9	4.0

[a] Two microcuries of ^{3}H-thymidine added per milliliter for 10 min before fixation of cells.

Since oxygen-treated cells appeared more heavily labeled, grain counts were performed on labeled interphases after exposure to air and to OHP. Grain counts were made for 20 cells in each of six such preparations. Figure 3 shows histograms for the distribution of background counts and net cell grain counts (cell grain count minus adjacent background count), excluding cells with a grain count of <5 grains. The mean grain counts for air- and OHP-treated cells were 12.3 ± 0.116 ($n = 116$) and 14.5 ± 0.202 ($n = 119$) for air and OHP, respectively. The distributions appeared different, fewer cells in the OHP group having low grain counts. Since rates of DNA synthesis are considered to vary during the synthesis (S) phase, being slower in the early and late compartments,[14] it is suggested that OHP differentially speeds up DNA synthesis in one or both of these slow-labeling compartments, particularly that of the late S phase. This would tend to accelerate the entry of cells into the postsynthetic G_2 phase.

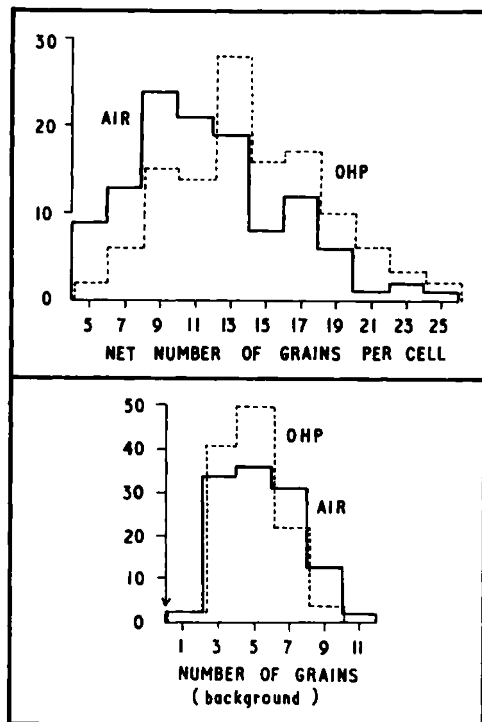

FIGURE 3. Histograms of background grain counts and net cell grain counts for ELD cells exposed 1 hour to air and OHP and immediately pulse-labeled with ³H-thymidine after treatment.

RELATION TO OXYGEN EFFECT ON RADIOSENSITIVITY

Several radiosensitivity studies *in vitro* with partially synchronized populations of mammalian cells have indicated that cellular radiosensitivity alters considerably during the cell replication cycle.[15-17] The various studies have been performed with cells aerated during irradiation, and these reports of variations in radiosensitivity with phase of cell replication are confined to cells radiobiologically oxygenated during irradiation. The data do not provide similar information for anoxic conditions, so that possible variations in the oxygen effect factor with phase of cell replication have not been calculated. Indeed, all available calculations for oxygen effect factor are based on irradiation of asynchronous cell populations. The mean values provided by such data often differ somewhat, perhaps partly because of a variation in the oxygen effect factor for different phases of cell replication—phases which individually may also vary considerably in length for different cell species.

A further analysis of our data has been made on the assumptions that OHP treatment causes some parasynchronization of cells in G_2 and possibly in late S phase, and that a_p values largely represent aberrations produced in cells irradiated in S–G_2 phases, while low aberration coefficient values (a_{min}) at $T = 18$–24 hours result from cells irradiated in the presynthetic phase (G_1). The respective values of E are given by:

$$E_p \text{ (late S–}G_2) = \frac{a_{p(OHP)}}{a_{p(N_2)}} = \frac{4.55}{1.40} = 3.3$$

$$E_{min} \; (G_1) = \frac{a_{min(OHP)}}{a_{min(N_2)}} = \frac{1.90}{0.72} = 2.6 \qquad (5)$$

The ratio of oxygen effect factors is:

$$\theta = \frac{E_p}{E_{min}} = \frac{3.3}{2.6} = 1.3$$

The system provides no accurate estimate of the change in oxygen effect factor with phase of cell replication; more accurate information may be available if synchronized *in vitro* systems are studied. However, considerable differences in radiosensitivity with phase of cell replication have been reported for various cell lines (*e.g.*, HeLa, L-strain fibroblast, and certain hamster cell lines studied under aerated conditions [15-17]). Furthermore, the duration of the various phases of replication for cells in the log phase of growth appears to vary considerably among cell species. Thus, the ELT Ehrlich strain used by Deschner and Gray appears to have a very long S phase and short G_1 phase.[18,19] The difference in the post-inoculation timing of peak aberration coefficients (for both aerobic and anoxic irradiations) between the findings of Deschner and Gray and our results using

the ELT and ELD variants, respectively, may reflect differences in the phases of cell replication for the two strains. Thus, in the ELD strain, the S phase is shorter ($S + G_2 = 6$–8 hours), while G_1 (absent or very short in hypotetraploid ELT strains of Ehrlich tumor cells) contributes the major compartment of the cell replication cycle in the ELD variant.

CONCLUSIONS

Preliminary results obtained with irradiation of ELD Ehrlich ascites tumor cells *in vitro* in OHP at 5 ata and in pure nitrogen at 1 ata are described.

The oxygen enhancement factor (E) for x rays varied from 2.3 to 4.6 for different postinoculation times. This variation may have been largely due to the fact that E was lowest for cells in the presynthetic (G_1) phase of replication and highest in the late synthesis (S) phase and the postsynthetic (G_2) phase.

A short OHP treatment in the absence of irradiation did not cause chromosome aberrations but did cause differential changes in the kinetics of cell replication, consisting of: (1) an increase in rate of DNA synthesis during S phase (particularly in the slower-labeling compartments), and (2) an inhibition of mitotic rate attributed to more rapid entry of cells from $S \rightarrow G_2$ phase and arrest in G_2 phase and delay in the transition of cells from $G_2 \rightarrow M$.

We suggest that metabolic events during the G_2 phase may be largely glycolytic in ELD Ehrlich cells and associated with a low redox potential and an accumulation of reducing substances (*e.g.*, SH groups), with a transition to aerobic metabolism and an oxidative state setting in just prior to mitosis. This arrest in G_2 produced by OHP may thus be related to auto-oxidative changes—changes which can be largely reversed to shorten G_2 time by a short post-oxygen exposure of cells to anoxic conditions.

The higher oxygen effect ratio for cells in S–G_2 phase may possibly have clinical significance in irradiation of tumors during hyperbaric oxygenation by raising their radiosensitivity.

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Radiation Therapy with Hyperbaric Oxygenation

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When my colleagues and I started using hyperbaric oxygen combined with radiotherapy late in 1954, we first tried to see whether the expected increase in radiosensitivity was affirmed by subsequent microscopic examination of the tumors. Eight large tumors were chosen for a trial: four carcinomas of breast and four carcinomas of bronchus. In each case, one half of the tumor was given a single noncurative dose (1000–1500 rads) while the patient was breathing oxygen at 3 ata (29.4 psig), and the other half received a similar dose while the patient breathed room air at normal atmospheric pressure. After an interval, a pathologist (who did not know how each part had been treated) examined the tumors and found recognizably more damage in the oxygen-treated half in seven of the eight cases. In the eighth case, an oat-cell carcinoma of the bronchus, both parts showed such severe damage that no distinction could be made.[1]

Since we thought that no more conclusive evidence could be obtained by proceeding on these lines, in mid-1955 we started a trial using potentially curative doses to whole tumors. As we hoped to demonstrate an improved radiation response quickly and knew not fully what complications to expect, we selected for treatment only those patients whose tumors, although at the time without known distant metastases, were considered too advanced locally to allow cure by conventional radiotherapy. We have continued to select patients in this way, and our results should be assessed with this in view.

Over the years we have made a number of improvements in apparatus and technique. All patients now receive a maximum tissue dose of 3500–3750 rads in six treatments over 18–19 days. Most are treated while conscious, breathing oxygen at 3 ata. If anesthetized, they breathe oxygen at 4 ata (44.1 psig).

We have been unable to run a randomized controlled trial, for two main reasons: (1) a relatively small number of patients has been available to us with similar tumors at similar sites, and (2) the radiation response is so obviously improved in the patients treated in oxygen. In an effort to obtain a control series, however, we have treated a number of patients with equally advanced local disease, who for various reasons unrelated to their disease were unsuitable for OHP treatment, with similar dosage and fractionation during air-breathing at normal atmospheric pressure.

By April 1, 1965, we had treated 235 patients in hyperbaric oxygen and 81 patients in air; tumors in a large number of

different sites have been treated. (Details of treatment results appear in Tables 1–4). As expected in the selection of patients with such advanced disease, the rate of distant metastasis has been high. It has been similar for patients treated in oxygen and in air (26% and 30%). More patients treated in oxygen have subsequently developed a second primary tumor, but this is probably due to their having survived longer (Table 1).

Tables 2 and 3 show the recurrence-free survival rates at periods after treatment of up to 9 years in the oxygen series and 7 years in the air series. The rates have been approximately twice as good in the patients treated in oxygen; whereas there are a number of long-term survivors among oxygen-treated patients, no patient treated in air has yet survived 5 years without recurrence. Some of the patients treated in air have, however, done unexpectedly well considering the way in which they were selected; this we attribute to the method of dose fractionation used, which has already been shown to improve the results in the treatment of animal tumors.[2]

Although the difference in the recurrence-free survival rates between patients treated in oxygen and those treated in air

TABLE 1. Clinical Results of Radiotherapy in OHP and in Air

	No. patients	
	OHP	Air
Total no. patients treated	235	81
Patients with later distant metastases	60 (26%)	24 (30%)
Patients with later second primary tumor	11 (5%)	1 (1%)

has been marked, the difference in the rates of tumor sterilization in the irradiated area between the two series has been even more impressive (Table 4). Especially good results have been obtained in tumors of the tongue, floor of mouth, tonsil, nasopharynx, larynx, and uterine cervix. The one tumor site in which the response has been disappointing has been the brain. Although most cases have had good palliation, none of the eight cerebral tumors treated has been sterilized. A particularly encouraging feature, however, has been the good response of secondary squamous carcinoma in lymph glands (Table 5), a form of malignancy which is notoriously resistant to conventional radiotherapy.

The one serious complication resulting from hyperbaric oxygen combined with radiotherapy (occurring early in our series) was radionecrosis of the laryngeal cartilage, usually appearing 6–9 months after treatment. This occurred in 16 of 96 patients whose larynx had to be irradiated. Cartilage in other sites has been unaffected. There seems no doubt that the necrosis was at least partially due to the use of oxygen, as no necrosis has occurred in 36 patients treated with similar dosage in air. Fortunately, a small dose reduction appears to have eliminated this complication without reducing the tumor sterilization rate, and we have not had a case of laryngeal cartilage necrosis for over 3 years (Table 6).

In more than 850 exposures of conscious patients, four oxygen convulsions have occurred, after 28, 29, 39, and 48 min at 3 ata. In more than 450 exposures of anesthetized patients, one convulsion has occurred after 38 min at 4 ata. No persisting effects have been noted in any of these patients.

TABLE 2. Patients Treated in OHP Surviving Without Apparent Recurrence in Irradiated Zone[a]

Tumor site	Survival time after treatment									
	6 mo.	1 yr.	2 yr.	3 yr.	4 yr.	5 yr.	6 yr.	7 yr.	8 yr.	9 yr.
Bronchus	11/21	5/21	3/21	2/20	1/14	0/10	0/10	0/10	0/10	0/10
Larynx:										
Supraglottic	3/5	1/4	0/4	0/4	0/4	0/3	0/2	0/2	0/2	—
Glottic	4/5	2/4	1/3	1/2	1/2	0/1	0/1	—	—	—
Subglottic	2/2	1/2	0/2	0/2	0/2	—	—	—	—	—
Laryngopharyngeal margin:	7/10	5/10	2/10	0/7	0/5	0/4	0/3	0/3	0/2	—
Mid-part	11/16	5/14	2/12	1/11	0/10	0/9	0/8	0/8	0/6	0/2
Sinus pyriformis	3/8	2/5	0/5	0/5	0/4	0/1	—	—	—	—
Hypopharynx:										
Postcricoid	2/4	1/4	1/4	0/3	0/3	0/3	0/2	0/2	0/2	0/1
Posterolateral wall	5/5	3/5	0/5	0/4	0/3	0/2	0/1	0/1	0/1	—
Nasopharynx										
Vallecula	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Palate	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
Pharynx	—	—	—	—	—	—	—	—	—	—
Esophagus	2/4	0/4	0/4	0/4	0/3	0/3	0/3	0/1	0/1	0/1
Tongue and floor of mouth	17/25	13/25	5/20	4/16	3/10	3/10	3/9	3/9	2/6	1/2
Tonsil	8/9	5/9	1/7	1/6	1/6	1/6	1/5	1/5	0/2	0/1
Brain	5/8	3/8	2/8	1/8	0/8	0/7	0/2	0/2	0/2	—
Cervix and vagina	5/8	3/8	1/5	1/5	1/5	1/5	1/4	1/4	1/2	—
Bladder	25/42	13/32	5/17	3/15	2/14	0/13	0/9	0/2	0/1	—
Bone	5/10	4/9	2/8	2/8	2/8	2/8	2/6	1/6	1/4	0/1
Breast	4/4	3/4	0/4	0/4	0/1	—	—	—	—	—
Other	5/10	2/8	1/7	0/4	0/2	0/2	0/2	0/1	0/1	—
Total no. patients	198	178	148	130	106	89	69	58	44	20
Patients without recurrence	126	72	27	17	12	8	8	7	5	2
Percent without recurrence	64%	40%	18%	13%	11%	9%	12%	12%	11%	10%

[a] Thirteen patients have been excluded: five with carcinoma of breast who had mastectomies performed after treatment, three with carcinoma of bladder who had cystectomies performed after treatment, four with laryngeal neoplasms who died from coronary thrombosis (two cases) and staphylococcal enteritis (two cases) within 3 months of treatment and in whom the irradiated area was found to be histologically clear, and one with carcinoma of nasopharynx who was clinically clear of disease when lost to follow-up 3 months after treatment.

TABLE 3. Patients Treated in Air Surviving Without Apparent Recurrence in Irradiated Zone [a]

Tumor site	Survival time after treatment							
	6 mo.	1 yr.	2 yr.	3 yr.	4 yr.	5 yr.	6 yr.	7 yr.
Bronchus	2/20	1/19	0/17	0/7	0/7	—	—	—
Larynx:								
Supraglottic	1/1	—	—	—	—	—	—	—
Glottic	—	—	—	—	—	—	—	—
Subglottic	—	—	—	—	—	—	—	—
Laryngopharyngeal margin:								
Mid-part	2/5	1/5	1/4	1/4	1/4	0/2	0/1	—
Sinus pyriformis	4/7	2/6	2/5	0/4	0/4	0/3	0/2	0/1
Hypopharynx:								
Postcricoid	3/3	1/3	1/3	1/3	1/3	0/2	0/1	—
Posterolateral wall	0/2	0/2	0/2	0/2	0/2	0/1	0/1	—
Nasopharynx	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Vallecula	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Palate	1/1	0/1	0/1	0/1	0/1	0/1	0/1	—
Pharynx	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Esophagus	—	—	—	—	—	—	—	—
Tongue and floor of mouth	0/7	0/7	0/7	0/5	0/4	0/3	0/2	0/2
Tonsil	1/4	1/4	1/4	0/3	0/3	0/1	0/1	0/1
Brain	1/1	0/1	0/1	0/1	0/1	—	—	—
Cervix and vagina	—	—	—	—	—	—	—	—
Bladder	7/17	5/14	0/13	0/9	0/5	0/4	0/2	0/2
Bone	—	—	—	—	—	—	—	—
Breast	0/2	0/2	0/2	0/2	—	—	—	—
Other	2/5	1/4	0/4	0/2	0/1	0/1	0/1	0/1
Total no. patients	78	71	66	46	38	21	15	10
Patients without recurrence	26	12	5	2	2	0	0	0
Percent without recurrence	33%	17%	8%	4%	5%	0%	0%	0%

[a] Three patients have been excluded: one with carcinoma of breast who had a mastectomy performed after treatment, one whose carcinoma of parotid gland failed to regress completely after ^{60}Co teletherapy and who subsequently had a ^{198}Au grain implant carried out, and one with carcinoma of bladder who refused to return for follow-up.

TABLE 4. Comparison of Primary-Tumor Sterilization with OHP and with Air

	No. patients	
	OHP	Air
Total no. patients treated	235	81
Assessable	211	74
No growth clinically	118(56%)	10(14%)
No growth histologically proven (to date)	42(20%)	2(3%)

TABLE 5. Comparison of Secondary-Tumor Sterilization in Lymph Glands [a] with OHP and with Air

	No. patients	
	OHP	Air
Total no. patients treated	90	38
Assessable	84	28
No growth clinically	61(73%)	7(25%)
No growth histologically proven (to date)	21(25%)	2(7%)

[a] In patients with head and neck cancer.

TABLE 6. Incidence of Laryngeal Cartilage Necrosis Compared with Tumor Sterilization

	No. patients		
		Tumor sterilization	
^{60}Co dose [a]	Cartilage necrosis	Primary	Lymph glands
4500 rads	3/11(27%)	8/11(73%)	7/8 (88%)
4000 rads	1/10(10%)	5/10(50%)	5/7 (71%)
3750 rads	0/4 (0%)	3/4 (75%)	4/4 (100%)
3500 rads	0/30(0%)	25/30(83%)	23/27(85%)

[a] In each case, six treatments were given over 18–19 days.

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Epidermoid Carcinoma of the Cervix Treated by ^{60}Co Therapy and Hyperbaric Oxygen

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From December 1963 to April 1965, 25 hitherto untreated patients with stages III and IV epidermoid carcinoma of the cervix were treated with ^{60}Co teletherapy and hyperbaric oxygen at the Manitoba Cancer Foundation, as a pilot study. To date, nine patients have died, all within 7 months of treatment, and autopsy has been performed in six cases. Six of the nine had clinical evidence of distant metastases. Five of the autopsies showed widespread metastases. Because of our concern that OHP might have increased the incidence of metastases, we attempted to evaluate the possible role of hyperbaric oxygen in modifying the natural history of the disease by comparing the results in this series with those from a retrospective series of 25 patients treated in a similar manner, but without OHP, before 1963 (Figure 1).

Generally speaking, carcinoma of the cervix is primarily a local problem. It spreads by direct local invasion, involving the pelvic viscera in a mass of cancer tissue. Most often, it results in obstruction of the ureters and death of the patient in uremia. Distant metastases are not uncommon, however, particularly in the higher grades of tumor, and the sites affected may be widespread and unexpected. If it is true, as has been suggested, that any cancer can metastasize anywhere, then this is particularly true of

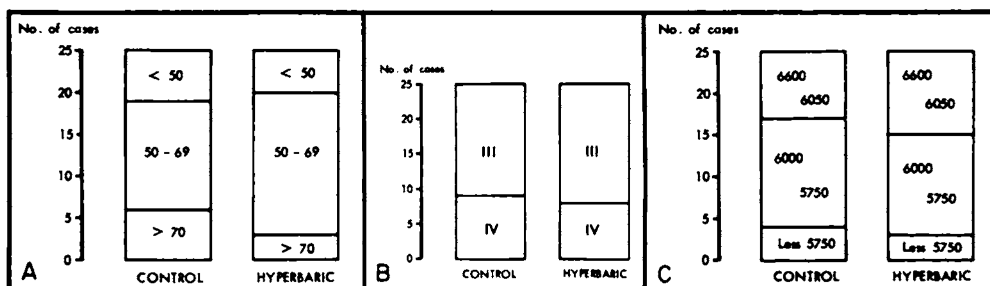

FIGURE 1. A, comparison of ages in control and hyperbaric groups. (One patient in the hyperbaric group was over 80.) B, comparison of proportions of stages III and IV disease in the two groups. C, total dose of ^{60}Co teletherapy (rads) given over 6 weeks in 30 fractions.

epidermoid carcinoma of the cervix. The mere fact of metastasis is not an occasion for surprise; only if the metastatic rate is increased, or the distribution more widespread, or the mode of growth significantly altered is there cause for suspicion that the course of the disease has been significantly modified.

RESULTS

The present series of cases offered no evidence of any qualitative change in the growth and distribution pattern of the cervical cancer. What was suggested was a quantitative increase in distant as opposed to local disease. In none of the six cases in which autopsy was performed did the regime of ^{60}Co teletherapy combined with OHP fully cure the local disease, but it did keep it under control. The emphasis shifted away from parametrial involvement to disease in other parts of the body, and, in particular, to the transport of tumor by the blood vascular and lymphatic systems. Only one of our patients (Case 4) died the classic death from ureteric obstruction and uremia, and she, too, showed numerous metastatic deposits in the lung and pleura.

The findings may be coincidental, but there are some features, both clinical and pathologic, which lead us to believe that OHP may be associated with an important quantitative modification of the growth pattern of cervical cancer. Histologically, one of these features is the preponderance of intravascular dissemination of tumor. Lymphatics, veins, and even arteries are involved in this transport system. Figure 2 illustrates some examples of the kinds of spread we have encountered in the hyperbaric group.

Case 2 in our series offered the most dramatic example of intravascular tumor growth. Large-scale intravascular clotting occurred, which was sometimes intimately associated with tumor cells in the fibrin. So marked was this tendency to intravascular clotting that the woman developed a hypofibrinogenemia with a blood fibrinogen level of only 70 mg/100 ml. Death in this case was an immediate result of the clotting and hemorrhagic tendency (*i.e.*, an acute subarachnoid hemorrhage).

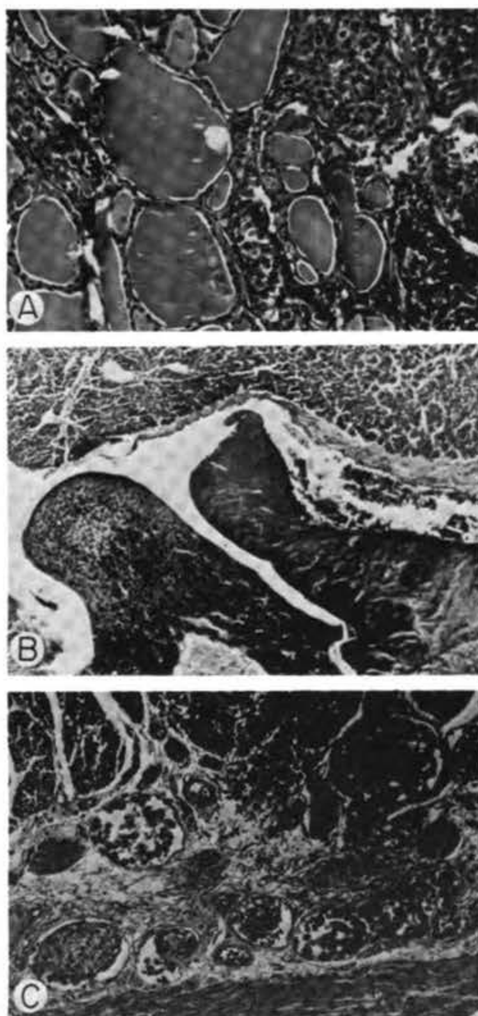

FIGURE 2. A, metastatic tumor in thyroid, an unusual form of metastasis. (Henriksen[1] reported a 0.6% incidence of thyroid metastases in 202 autopsied cases of conventionally treated cervical cancer.) B, thrombus on wall of left ventricle, with tumor cells present in thrombus. (Henriksen reported a 3% incidence of cardiac metastases in his series.) C, network of lymphatic-borne tumor between muscle layers in esophagus.

Another patient (Case 3) showed a plethora of blood- and lymphatic-borne metastases, two sites being of particular interest. First, the right renal pelvis had warty tumor excrescences growing on the surface, with a complete lack of hydronephrosis. (Hydronephrosis was absent in three of our autopsied cases.) Second, the esophagus contained an almost incredible network of lymphatics (Figure 2C). Henriksen,[1] in his report of 420 autopsied cases of cervical cancer, did not list the esophagus as a site of metastasis in either his treated (irradiation without OHP) or untreated groups, but presumably metastasis to the esophagus does occur on occasion.

Establishment of the significance of these results obviously cannot be based on as small a series as ours. Consequently, we have considered other means of investigating the implications of our autopsy findings. We are currently studying the influence of hyperbaric oxygenation on the growth and metastasis of transplanted tumors in mice, but we have been unable to confirm a direct effect in our initial experiments. In view of the prevalence of intravascular thrombosis in our cases, culminating in one case in hypofibrinogenemia and hemorrhage, we have also been considering the role of the clotting and fibrinolytic systems, and we are currently attempting to measure some of the factors involved (together with steroid estimations) in OHP-treated patients.

An evaluation of the tumor-doubling time in the OHP-treated patients, as determined by measurements of pulmonary metastases (assuming them to be spherical) showed that the time taken for a given tumor to double in volume varied among patients, ranging from 8 to 45 days, and this time lengthened as the size of the tumor increased. This led us to believe that some metastases, at least, were present at the time of our initial therapy. Figure 3 illustrates the progression of pulmonary metastasis in a patient treated for a stage III carcinoma of the cervix, in whom the pulmonary lesions were missed on the films taken prior to treatment.

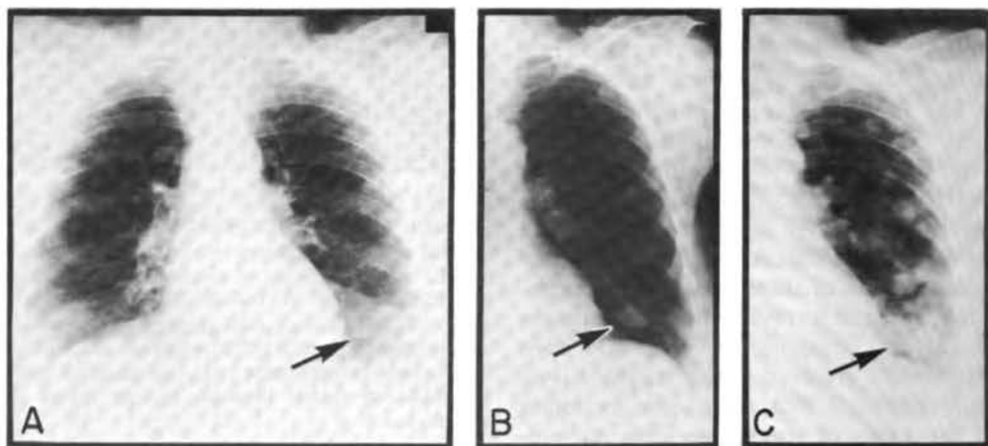

FIGURE 3. Progression of pulmonary metastasis in a patient with stage III disease, between commencement of ^{60}Co irradiation with OHP (A), termination of the 6-week course of radiation therapy (B), and 6 weeks after completion of therapy (C). The time required for the tumor to double in volume was approximately 21 days, both during and after irradiation in OHP. Unfortunately we cannot assume this doubling time to be constant from initial seeding to commencement of OHP therapy. It is possible, of course, that the pulmonary metastases were well enough oxygenated between seeding and the time when tumor was first detected on x-ray film (just before treatment) that no change in doubling time occurred.

Discussion

There is evidence supporting the occurrence of iatrogenic modification of the natural history of some cancers. Sherlock and Hartmann,[2] for example, reported a significant increase in metastatic lesions from carcinoma of the breast in patients receiving adrenal steroids, with an added tendency to splenic metastases in those who had undergone adrenalectomy or hypophysectomy (or both), with subsequent hormone therapy. We considered it at least possible that hyperbaric oxygenation may trigger an "acute" metastasizing reaction in some patients, perhaps mediated by an increased intravascular survival and growth of tumor.

In support of this, Wildermuth [3] has observed an apparent increase in the number of patients with widespread and unusual metastases when their primary tumor was treated under high pressure oxygen. Also, McCredie and Inch [4] have demonstrated that cell generation proceeds more rapidly with raised oxygen concentrations, and mice breathing oxygen at 1 atm have a greater rate of tumor progression than animals breathing air. Alexander and Altemeier [5] found that rabbits given an intra-aortic injection of tumor suspension (via a previously inserted cannula) and given oxygen to breathe in decreasing, graded concentrations from time of injection to sacrifice 3 weeks later had almost double the amount of metastases (as determined at autopsy) as their controls kept in room air. In a second experiment, these workers found that when a tumor suspension was injected into the gastrocnemius muscles of both legs of rabbits, and ischemia was induced in the left leg by ligation of the left femoral artery, the tumors in the ischemic limbs of these animals were considerably smaller at autopsy 15 days later than the tumors in the normal limbs. Similar results were obtained when the left femoral vein was ligated.

On the other hand, Churchill-Davidson [6] and van den Brenk [7] have reported no increase in metastases in their two large series of patients treated with irradiation during hyperbaric oxygenation. They have been using no more than six hyperbaric sessions per patient, however. Similarly, in a recent animal study in which tumor transplants were used, Suit [8] found no difference in tumor growth rate or incidence of metastasis between animals exposed to 100% oxygen at 30 psig 60 min daily, 5 days a week for 6 weeks, and control animals kept in room air. It must be remembered, however, that such transplanted tumors have a doubling time of 2 days, in contrast to 8–40 days for human tumors.

Conclusions

Our results suggest that daily treatment with OHP causes earlier distant metastases, but because our experience has been limited to so few patients, no definite conclusions can be drawn. Possibly this is an unfortunate, purely chance finding. Six patients in the hyperbaric group died from distant metastases within 7 months, whereas only one of the five deaths in the control group occurred before 15 months post-treatment (at 1 month). The remaining four control patients died between 15 and 48 months post-treatment (Figure 4).

The local tumor control rate at 6 months in the hyperbaric group was 45%,

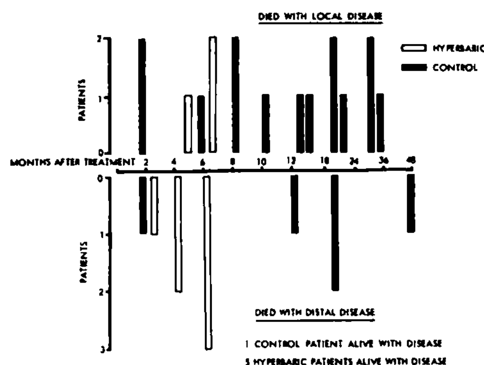

FIGURE 4. Comparison of causes of death and intervals between treatment and death in control patients and OHP-treated patients.

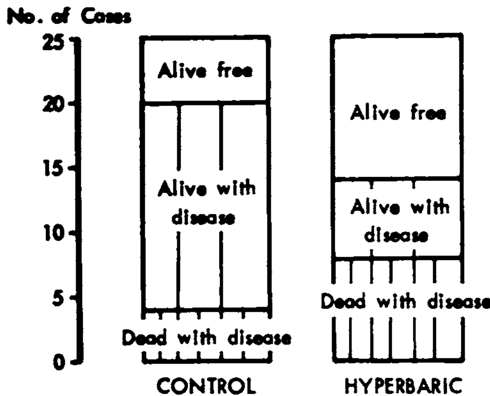

FIGURE 5. Comparison of local tumor control rates at 6 months in control patients and in OHP-treated patients.

compared with only 20% in the control group (Figure 5). In addition, the OHP-treated survivors have enjoyed a remarkable improvement in general health.

We shall continue to use OHP combined with ^{60}Co teletherapy for patients with carcinoma of the cervix, with the possible addition of supplementary irradiation to the primary tumor mass in stage III tumors. We hope, in the future, to join with other North American hyperbaric radiation centers in cooperative randomized trials, for it is vitally important to have well-documented data on the clinical effects of hyperbaric irradiation.

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Experience with OHP in the Megavoltage Irradiation of 614 Patients with Advanced Malignant Disease

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Investigations of the potential of hyperbaric oxygen in radiotherapy have been largely inspired by the fundamental studies of the late L. H. Gray and his colleagues [1] in England and pioneered at a clinical level by Churchill-Davidson *et al.*[2]

Since 1961, when we began using the Melbourne hyperbaric oxygen unit clinically, 614 patients with advanced malignant disease have been treated with 4-million-volt x rays delivered during exposure to pure oxygen at 45 psig (4 ata) during irradiation.[3,4] The case material has been purposely selected from recurrent and essentially incurable malignant disease to evaluate different techniques, the incidence of oxygen toxicity, and local response of tumors in various sites, with particular reference to the effects of fractionation and protraction of radiation dose on tumor responses and tissue reactions. Following is an attempt to summarize our experience to date and particularly to draw attention to new information and any departures from views expressed in our report at the First International Conference.[4]

Hyperbaric Oxygen Techniques

The techniques for anesthesia and intubation, for performing bilateral myringotomies before each treatment, and for carrying out general mock-up procedures on a simulator have not been changed significantly from those reported previously;[4] this routine has proven safe, expedient, and free from complications.

Equilibration Time

After flushing, compression rates of 15 psig/min and decompression rates of 7 psig/min are now used routinely. Over the past 6 months, we have maintained the patient for a full 30 min at 4 ata of oxygen before irradiating the tumor. This doubling of equilibration times was based on results of measurements of tumor oxygen tension in both experimental animals and humans,[5,6] which suggested that large avascular and necrotic tumors are slow to oxygenate even at 4 ata and hence tend to recur after irradiation. Recent experimental studies [7] have also suggested that a more prolonged exposure of a rep-

licating cell population to high pO_2 may cause some synchronization of cells in DNA synthesis and post-DNA synthesis (S–G_2 periods) and possibly further enhance tumor radiosensitivity. However, the main purpose of more prolonged equilibration is to overcome tumor anoxia. Naturally, this measure prolongs oxygen exposure and treatment times and would be associated with a higher risk of overt convulsions if attempted in conscious patients.

Myringotomies

The simple technique of puncturing both eardrums before each compression has proved generally trouble-free with $<5\%$ temporary deafness or infection and no serious aftereffects. However, where the external auditory apparatus is included in the megavoltage beam and receives radical dosage, deafness, delayed healing of myringotomies, and effusions are not uncommon, but usually resolve without significant sequelae.

Tumor and Tissue Oxygen Tension Measurements

The techniques, their limitations, and results in both humans and experimental animals obtained in this laboratory have been published elsewhere [5,6,8–10] and support the results of Evans and Naylor [11] that OHP at 4 ata produces rises in tumor pO_2 sufficient to modify radioresistance due to anoxia.

Chamber Maintenance and Personnel

The pressure chamber and ancillary equipment (manufactured by the Commonwealth Aircraft Corporation, Melbourne) used in our unit [12] have given excellent service over the years, with only occasional minor adjustments to the three-dimensional couch movements and their calibration. A logbook of dose received by the removable Perspex radiation portals is kept, and these portals are replaced when the calculated maximum radiation dose to any portion of the Perspex exceeds 1.5 megarads and darkening of the Perspex becomes noticeable.

The unit is run on a routine daily sessional basis by a team consisting of a trained radiotherapist, an anesthetist, two radiotherapy technicians, and a nursing sister. This team is responsible for all treatments of patients, including radiotherapeutic planning and dosimetry, the performance of myringotomies, biopsies, endoscopic inspection before treatments, and elective tracheostomies; the team also participates in clinical research. Currently, over 200 new cases are treated per year and the unit operates 4 days per week.

Oxygen Toxicity

We have experienced zero mortality attributable to hyperbaric oxygen in our first 614 patients and there has been no evidence of lung or central nervous system damage. These first 614 cases received a total of 2569 exposures at 4 ata of oxygen (2–12 exposures per patient), with mostly three to six exposures per patient being given over 3 weeks and each exposure (compression to decompression) taking place under anesthesia averaging 40 min.

Pulmonary Damage

There has been no evidence of significant pulmonary disturbance or aftereffects from the hyperbaric treatment. Post-treatment radiographs have shown no oxygen-induced lesions, and repeated vital capacity measurements in consecutive patients given six exposures in 17 days (Figure 1) actually tended to improve.

Cardiovascular Effects

No significant electrocardiographic changes have been observed during or after treat-

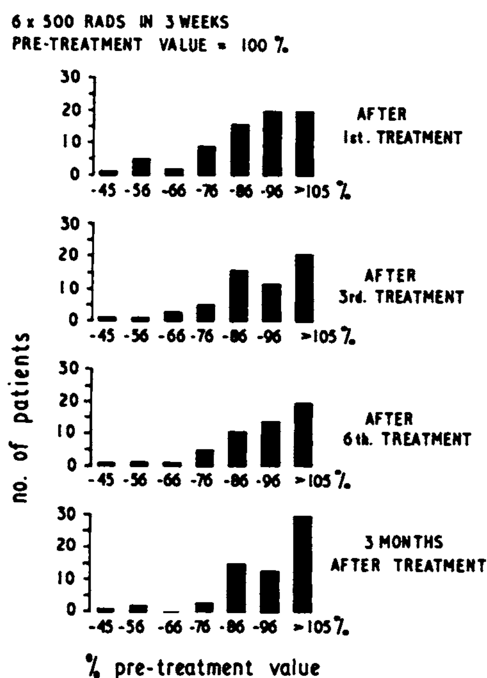

FIGURE 1. Vital capacity survey in 68 cases of advanced malignant disease receiving 6×500 rads tumor dose (on days 0, 4, 7, 11, 15, and 17), 4-Mev x rays under hyperbaric oxygen (4 ata). Patients were anesthetized during each OHP exposure.

ment. Many of the 614 patients have suffered from senility or from severe cardiorespiratory or other disease, including coronary sclerosis, pulmonary fibrosis and emphysema, congestive cardiac failure, and diabetes mellitus. In no such case has the hyperbaric oxygenation produced untoward effects. In several cases, an apparent improvement in well-being appeared to be associated with OHP exposures.

Central Nervous System Damage

No instance of paralytic brain damage produced by OHP under barbiturate anesthesia in these patients has occurred similar to that reported for OHP in anesthetized rats from this laboratory.[13-15] Earlier, we reported the occurrence of nine convulsions for 670 exposures of patients to 4 ata during anesthesia.[4] Since then, two further convulsions have occurred at this pressure after delayed treatments. Recently, a conscious patient treated at 3 ata for gangrene due to frostbite convulsed after 45 min at pressure. There have been no adverse sequelae in the few patients who convulsed in OHP.

Other Somatic and Genetic Effects

There has been no evidence of hemopoietic depression or other effects associated with damage to cell replication in our patients, a finding which casts grave doubt on the analogy proposed by some workers between oxygen toxicity and the effects of ionizing radiations.[16] The report by de Almeida [17] and Matteo and Nahas [18] that OHP causes testicular atrophy in rats has not been substantiated by observations in humans, although a thorough evaluation of testicular function has not been made. Preliminary results of experiments by Jamieson and one of us (v.d.B.) on rats exposed to repeated near-lethal pressurizations in oxygen also suggest that OHP has no significant effect on spermatogenesis, but further studies are required to evaluate this clinically vital point. Female and male patients have reported no loss of libido, and menstruation appears unaltered in females. Several patients treated in their eighth and ninth decades of life have also tolerated treatments without apparent aftereffects from OHP.

CLINICAL MATERIAL

Like Churchill-Davidson [19] in London, we have accepted for treatment only patients with histologically proven advanced malignant disease with a very poor prognosis according to generally accepted radiotherapeutic standards, a policy which was reported at the First International Congress in Amsterdam [4] and has remained unchanged since. All patients have been referred from other specialist radiosurgi-

cal and radiotherapy clinics. Where a further assessment in the hyperbaric unit suggests a clinical tumor staging less advanced than $T_{3,4}N_{2,3}M$, the patient is rejected and returned for conventional therapy. Most terminal patients with disseminated metastases have also been refused, but a large proportion of patients (over 30%) were treated who had received one or more radical treatments by surgery, radiotherapy, chemotherapy, or a combination of these. Most patients treated have had cancer of one or more of the following regions: upper digestive and respiratory passages, bladder, rectum, lung, and lymph nodes (metastatic).

Selection has not been restricted by site or histology, all referrals for radical treatment being gratefully received by the unit, provided attempts to dictate policy, follow-up, and after-care have not governed referral. Failures in adequate follow-up and after-care have often resulted, however. In a considerable proportion of patients referred from other hospitals and from interstate and overseas clinicians, the follow-up has been so poor (or absent) that results for these cases cannot be determined and they have been excluded from analyses. The opportunity to perform autopsy examinations (with histopathologic study) has always been taken, but it has not been possible in most cases. In some of the available autopsy reports, the examinations were so cursory and uninformative that little reliance could be placed on the findings. On the other hand, biopsy and surgical material from all new cases referred for treatment has been reviewed by experienced pathologists of the Pathological Department, Cancer Institute Board. The diagnosis in all cases treated was adequate for registration by the Central Cancer Registry, under the auspices of the Anti-Cancer Council of Victoria.

Radiotherapeutic Techniques

Radiation Dosimetry

Because we believe that sophisticated field planning and dosimetry are essential to proper evaluation of radiotherapeutic gains from OHP, the following procedures are routinely followed as a part of the radiotherapeutic program for each patient.

1. Careful field planning and isodosing are done for every patient treated, using multifield techniques, accurate rigid positioning during treatment with beam alignment, wedge filters and shielding where necessary, accurate tumor–treatment distances, dose corrections for bone and lung where applicable, and skin and tumor buildup for superficial tumor extension.
2. Dose checks are made both on patients under actual treatment in the vessel and on whole-body phantoms placed in the pressurized vessel by means of ionization chambers.[20]
3. Modal and mean tumor doses are specified in rads together with minimum and maximum tumor-dose values.
4. Dose to vulnerable structures is specified (*e.g.*, spinal cord, rectum, esophagus, and orbit).

In the advanced cases, however, the clinical extent of the disease has created a problem because of the lack of uniformity possible in the wide fields requiring irradiation. Previous experience [4] showed that in advanced ($T_{3,4}N_{2,3}$) head and neck cancer, treated with fields to "cover" primary and involved nodes, a distressingly high percentage of recurrences appeared outside the irradiated volume, despite tumor control and regression within the treated zone. This has led to routine wide-field treatments in such cases to the whole neck, to include both ipsilateral and contralateral lymph node chains from the base of the skull to the second intercostal space (Figure 2). In cases of multiple bilateral node metas-

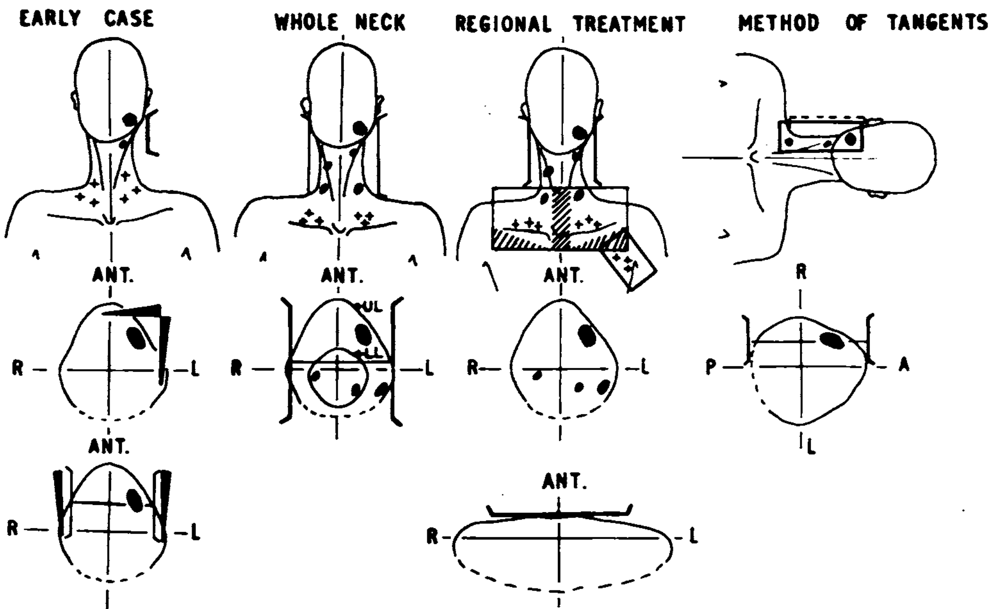

FIGURE 2. Examples of field arrangements used in treatment of cancers of the upper digestive and respiratory passages in hyperbaric oxygen under anesthesia. Regional treatment, as illustrated, is the technique most commonly used.

tases, both axillae and mediastinum are now also frequently irradiated to tolerance. Such wide-field irradiation for head and neck cancer (and also for chest and pelvis) to radical dosage invariably results in substantial lack of tumor-dose uniformity, difficult to overcome without exceeding tissue tolerance and producing high doses to vulnerable tissues and high integral doses.

A particular problem in field arrangements is the dose fall-off in the posterior cervical tissues with single fields. This results in recurrences due to underdosage, more particularly where the posterior edge of the upper opposing fields cannot be extended sufficiently posteriorly to cover the posterior cervical (occipital group) lymph nodes, owing to the radiation dose delivered to the neuraxis in such cases. Other regions present similar dosimetric and tissue-tolerance problems which are not limited to hyperbaric radiation techniques.

Naturally, the selection of earlier and smaller tumors for treatment greatly simplifies problems of dose uniformity and tolerance. Results achieved with advanced cancers only provide a most incomplete picture of the radiotherapeutic potential of hyperbaric oxygenation.

Fractionation and Protraction

We believe that the practical radiotherapeutic potential of OHP is greater when the number of fractions given to a patient can be limited. Indeed, if overall results with six or fewer fractions under OHP are not significantly better than those provided by a conventional course of 20–30 fractions in air, hyperbaric oxygenation would seem unwarranted. While persistent attempts are being made to "formalize" radiation in OHP by giving prolonged courses of small fractions to tolerance levels, we wonder whether these attempts are constructive and warranted on a scientific basis, or whether the "oxygen effect" and other modern concepts of radiobiological effects are sacrificed to conservatism. It seems that multifraction-

ated courses in a hyperbaric environment may impose limitations to dosimetric accuracy and to hyperbaric pressures and possible equilibration times—limitations never constructively justified in terms of radiation physics or oxygen physiology, and even less in terms of the parameters of cell survival. It seems a pity that the results of these techniques must be interpreted on a purely empirical basis, or at best on the basis of a clinical trial (a procedure difficult to devise at any time) involving multiple variables ranging from the patient and his disease to the therapist and the many technical factors.

It must also be stressed that radiobiological theory generally favors large-fraction therapy as most effective in promoting tumor-cell sterilization and cancerocidal effect, and even the radiotherapeutic literature is including some reports of favorable experiences with larger-fraction therapy in air (an experience also gaining ground in our own center).

Radiotherapeutic experience has shown, however, that fractionation and protraction of dose are essential in most regions to improve the therapeutic ratio effect, to provide acceptable tissue tolerance, and perhaps not least to curtail or prolong treatment if excessive tissue reactions appear. Our own work so far has concerned assessing the effect of courses of two to six fractions delivered over similar overall periods of time. Progress results were reported at the First International Congress[4] for two to three fractions, and the radiobiological basis for the project was discussed. This has been continued to include four- to six-fraction treatments, calculating n/n_0 survival fractions on an empirical assumption that experimental results provide parameters of "multihitness" and cell radiosensitivity and that $m = 4$ and $D_0 = 130$ rads, respectively, under oxygenated conditions are fairly reliable estimates which also apply to human tissues *in vivo*. The fractionation regimes most frequently used in this unit are shown in Table 1.

TABLE 1. Fractionation Regimes

Modal tumor dose (rads)	Spacing (day nos.)	n/n_0
2000 (2 × 1000)	0, 7, *or* 0, 14	3×10^{-6}
2400 (3 × 800)	0, 7, 21	6×10^{-7}
3000 (3 × 1000)	0, 7, 21, *or* 0, 14, 28	5×10^{-6}
3000 (6 × 500)	0–17*	3×10^{-7}
3600 (6 × 600)	0–17*	4×10^{-6}
2900 (4 × 725)	0, 7, 14, 21	5×10^{-6}

* Six treatments, given on days 0, 4, 7, 11, 15, and 17.

At each treatment, all fields are irradiated, with the patient maintained in a single posture (supine, prone, or lateral) throughout the irradiation. This position is previously arranged on the simulator, subsequently checked in the vessel after pressurization, and checked again under the accelerator with the light-guide or back-pointers, appropriate adjustments being made for any slight changes in posture. Suitable tungsten alloy blocks arranged in the path of fields outlined by the accelerator light beam are used to shield out specific areas as required. No patients received concurrent adjuvant cancer therapy such as cytotoxic agents and hormones. Treatments were never designed to be primarily preoperative or postoperative.

THERAPEUTIC RESULTS

The following analysis of results applies only to patients initially treated, in whom adequate follow-up was obtained to calculate the cumulative survival of at least 12 months.

Cancer of Upper Digestive and Respiratory Passages

The suspected sites of primary origin are numerically classified, according to the *International Classification of Diseases, Adapted,*[21] as 140 (lip), 141 (tongue),

142 (salivary glands), 143 (floor of mouth), 144 (alveolus, palate, buccal mucosa), 145 (oropharynx, tonsil, vallecula, posterior third of tongue), 146 (nasopharynx), 147 (hypopharynx), 150 (esophagus), 160 (nasal cavities, associated paranasal sinuses, middle ear), and 161 (larynx). Lymph node metastases (primary unknown) are classified as 198. Histologic diagnosis was determined before treatment in all cases, most representing various grades of squamous cell carcinoma, adenocarcinoma, transitional cell carcinoma, and melanoma. Disease was staged according to the TNM classification, all cases treated being in the category of advanced stages $T_{3,4} \pm N_{2,3}$. Previous treatment by surgery, radiation, chemotherapy, or various combinations of these had been administered to 73/178 (41%) patients before treatment in OHP (Table 2).

Tumor Clearance Rates. Tumor clearance rates were assessed as both "optimistic" and "pessimistic," with most emphasis placed on the latter. Pessimistic clearance rate assumes that: (1) all patients dying within 6 months of treatment, but not available for autopsy study, are not free of tumor in the treated zone despite any clinical assessment; (2) cases with microscopic evidence of tumor at autopsy are recurrent; (3) failure of a primary or secondary lesion in the treated zone to have undergone marked and persisting regression at the time of analysis and for more than 6 months after treatment represents recurrent disease; and (4) any evidence of clinical regrowth of tumor at any time after treatment represents recurrent disease. Optimistic clearance rate refers to all cases which failed to show active regrowth in the treated areas after an initial marked clinical regression, but it excludes cases with tumor found at autopsy.

Residual nonhealed ulceration or radionecrosis is excluded from both clearance rate assessments, as are modest residual radiation-induced fibrosis and edema (*e.g.*, submental "dewlap"), recurrences outside the treated zone, and those tumor deposits not treated in OHP.

Pessimistic clearance rates for primaries and nodes are shown in Tables 3 and 4 for radical and palliative doses. Node metastases were cleared somewhat more frequently (70%) than primary disease (64%). A dose of 3 × 1000 rads (days 0, 7, and 21) produced the most consistent clearance of both primary and nodal disease, but its protraction to days 0, 14, and 28 led to substantial reductions in clearance rates. A dose of 6×500 rads (given in 17 days, on days 0, 4, 7, 11, 15, and 17) produced excellent clearance rates at the time of this analysis, a better survival, and improved tolerance (with only moderately acute reactions and a lower incidence of necrosis). A recent survey clearly indicates, however, that this regime of fractionation has produced an increase in later recurrences (mostly 9–12 months after irradiation) and that a revised pessimistic clearance rate is about 50% for primary disease as opposed to the present estimate of 70%. This was initially predicted by us on the basis of "multihitness" of effect (extrapolation values) for cell survival, a cumulative dose of 3000 rads given as 3×1000 rads ($n/n_o = 5 \times 10^{-9}$) being more effective biologically than 6×500 rads

TABLE 2. Previous Treatment of Patients with Cancer of Upper Digestive and Respiratory Passages

	No. pts.
Surgery	
1 operation	23
> 1 operation	12
Radiation therapy	
1 course	19
> 1 course	2
Surgery + irradiation	12
Irradiation + chemotherapy	1
Surgery + irradiation + chemotherapy	4
Total	73(41%)

TABLE 3. Relation of Pessimistic Tumor Clearance to Dose Fractionation in OHP [a]

Dose fractionation	Disease stage								Total [b]	
	$T_{3,4}N_1$		$T_{3,4}N_{2,3}$		$T_{3,4}N_0$		$T_{T0}N_{2,3}$			
	P	N [c]	P	N	P	N	P	N	P	N
Radical irradiation										
3 × 1000 rads (days 0, 7, 21)	5/7	—	12/16	12/16	8/9	—	—	5/6	25/32(78%)	17/22(77%)
3 × 1000 rads (days 0, 14, 28)	1/3	—	11/23	14/23	7/9	—	—	6/9	19/35(54%)	20/32(62%)
3 × 800 rads (days 0, 7, 21)	1/3	—	4/10	6/10	1/2	—	—	1/3	6/15(40%)	7/13(54%)
3 × 800 rads (days 0, 14, 28)	1/1	—	2/3	3/3	—	—	—	1/2	3/4	4/5
6 × 500 rads (days 0–17) [d]	7/8	—	10/16	12/16	2/3	—	—	6/6	19/27(70%)	18/22(82%)
6 × 600 rads (days 0–17) [d]	2/3	—	0/2	1/2	4/4	—	—	—	6/9 (67%)	1/2
"Palliative" irradiation										
2 × 1000 rads (days 0–14) [e]	0/3	—	5/18	6/18	0/3	—	—	0/2	5/24(21%)	6/20(30%)
3 × 1000 or 1200 rads (days 0–48) [f]	—	—	2/3	2/3	—	—	—	—	2/3	2/3

P, primary tumor.
N, nodal disease.

[a] In patients with cancer of upper digestive and respiratory passages.

[b] Overall clearance rate of primary tumors, excluding those of "palliative" group, 78/122(64%). Clearance rate for nodes, excluding N_1 and palliative group, 67/96(70%).

[c] Overall clearance rate for all doses in N_1 nodes, 25/28(89%).

[d] Doses given on days 0, 4, 7, 11, 15, and 17.

[e] Doses given either on days 0 and 7 or 0 and 14.

[f] Doses given either on days 0, 14, and 28 or 0, 21, and 48.

TABLE 4. Pessimistic Clearance Rates in Patients with Cancer of Upper Digestive and Respiratory Tracts Receiving Radical Treatments in OHP [a]

	Clearance		
Stage	Primary	Nodes	Both
$T_{3,4}N_1$	17/25 (68%)	25/28 (89%)	17/25(68%)
$T_{3,4}N_{2,3}$	39/70 (56%)	48/70 (68%)	39/70(56%)
$T_{3,4}N_0$	22/27 (82%)	—	—
$T_{T0}N_{2,3}$	—	19/26 (73%)	—
Total	78/122(64%)	92/124(75%)	56/95(59%)

[a] 148 consecutive cases.

($n/n_0 = 3 \times 10^{-7}$). Indeed, the rate of tumor clearance for 6×500 rads appears little different from 3×800 rads ($n/n_0 = 6 \times 10^{-7}$), and the tissue reactions and tolerance for the two fractionations also appear similar.

In our experience both 3×1000 rads and 6×600 rads ($n/n_0 = 4 \times 10^{-9}$) treatments in OHP cause severe reactions and tissue destruction which exceed acceptable tolerance in head and neck disease. This prompted our recent change in fractionation policy to the administration of 4×725 rads ($n/n_0 = 5 \times 10^{-8}$) at weekly intervals. Protraction of 800- and 1000-rad fractions from 7- to 14-day intervals appears to allow tumor cell repopulation between fractions and reduces clearance rates. Furthermore, such protraction appears limited in its effectiveness in reducing mucous membrane reactions in mouth and throat tumors for 1000-rad fractions.

Time of Recurrence in Treated Zones. Most recurrences were obvious before 6 months after treatment for lower biologically effective doses (2×1000 rads, 3×800 rads), but a substantial number occurred in the 6- to 12-month period. Table 5 shows an analysis of cases clear of tumor made in a consecutive series of patients surviving at 6 months after radical treatments (83/106 = 78%) and at 12 months after treatment (34/37 = 92%). It can be seen that both estimates are higher than the 64% (Table 3) for the series assessed at all survival times. The higher estimate at 6 months is due to cases subsequently showing recurrence clinically or at autopsy (recurrence which is mainly determined from 6 to 12 months after treatment). The high estimate for 12-month survivors is due to exclusion of patients dying before 12 months having a higher recurrence rate than patients living beyond 12 months. Thus, local cure is more certain in cases free of recurrence at 12 months or longer, than sooner after treatment. Because the 6×500 rads regime has been introduced more recently (in 1964), a more realistic appraisal of its results awaits the further passage of time—but since this analysis was made, a substantial increase in delayed recurrence rate has appeared which may indicate that the local "curative" results are much lower for 6×500 rads, particularly in primary disease.

While our optimistic clearance rates for both primary disease and node metastases were high (75–94% in $T_{3,4}N_{2,3}$ tumors for various radical dose regimes), this estimate appears less valuable in dose-effect determinations. On the other hand, the experimental studies of Suit and Gallagher[22] have clearly shown that a histopathologic evaluation of residual tumor may be fallacious, and even autopsy findings are sometimes of dubious worth.

Comparison with Treatment (3×1000 Rads) in Air. Tables 6 and 7 compare results of therapy in OHP (Tables 4, 5) with those for 51 consecutive similar ad-

TABLE 5. Pessimistic Estimate of Primary Tumor Clearance (T_{2-4}) in Upper Digestive and Respiratory Passages [a]

	Classification of sites [b]										
	140	141	142	143	144	145	146	147 & 150	160	161	All sites
Clearance											
6-month survivors	1/1	8/14	8/10	6/8	6/7	25/28	3/3	7/15	10/11	9/9	83/106(78%)
12-month survivors	—	5/5	4/4	2/3	4/5	10/10	2/2	3/3	3/4	1/1	34/37 (92%)

[a] In 106 patients surviving at 6 months and 37 surviving at 12 months after radical irradiation in OHP.
[b] See text for site classification.

TABLE 6. Comparison of Tumor Clearance Rates in Advanced Disease [a] with Irradiation in Air and OHP

Irradiation in:	Dosage	No. cases tumor clearance
Air	3 × 1000 rads	18/34 (53%)
Air	4500–7000 rads [b]	4/51 (8%)
OHP	Radical [c]	59/148(40%)

[a] Cancer of upper digestive and respiratory passages, $T_{3,4}N_{2,3}$. All patients in this group had had one or more forms of previous radical therapy (surgery, irradiation, chemotherapy).

[b] Total dose, given in daily fractions over 3–6 weeks.

[c] Radical dosage consisted of 3×1000 rads, 3×800 rads, 6×600 rads, or 6×500 rads.

vanced cases treated in air with conventional fractionation (4500–7000 rads megavoltage, as daily fractions over 3–6 weeks) and 34 cases treated with 3× 1000 rads in air (days 0, 7, and 14, or days 0, 7, and 21). Clearance rate was significantly better (doubled) in OHP. Indeed, our results for OHP and air are very similar to those of Churchill-Davidson,[19] with primary clearance rates of 56% in OHP and 14% in air and node clearances of 73% in OHP and 25% in air.

Cumulative Survival. Tables 8 and 9 summarize cumulative survival at 12 months for the first 159 cases treated in OHP. Survival was improved in cases receiving radical courses of treatment. In this group, however, fractionated dose schedules of lower biological effectiveness, calculated in terms of cell survival (n/n_0) and based on a "multihit" effect equation (see below), gave the largest percentage of patients surviving 12 months. Thus, survival for 6×500 rads (56%) and 3×800 rads (53%) was better than that for 3×1000 rads and 6× 600 rads. This effect is undoubtedly attributable to the greater damage to normal tissues occurring in the latter two dose groups (Table 13). Furthermore, the survival rate was also higher in cases in which the primary site was previously cleared of disease by irradiation or surgery (T_T) but which presented with node metastases ($N_{2,3}$) treated in OHP. Such improved survival was also obtained in cases presenting with advanced inoperable node metastases (histologically proven by biopsy) but where no primary tumor (T_0) was detected despite extensive investigations.

Survival varied greatly with site of primary origin, being highest in alveolus, palate, and buccal mucosa (45% for all treatments, radical and palliative) and lowest in hypopharynx and esophagus (13%). The overall 12-month survival rate for radical doses was 43%, the corresponding 2-year survival rate only 16% (16/102 cases at risk).

Cumulative Survival for Patients Treated in Air. The comparative 12-month cumulative survival rates for patients treated in air and for patients treated in OHP are shown in Table 10. The difference in survival is not significant. On the other hand, comparable figures from Churchill-Davidson [19] show 46% (38/48) in OHP and

TABLE 7. Summary of Pessimistic Clearance Rates in $T_{3,4}N_{2,3}$ Disease of Upper Digestive and Respiratory Passages

	Treatment		
	Conventional radical irradiation in air	3 × 1000 rads in air	Radical irradiation in OHP
Primary	—	8/28(29%)	78/122(64%)
Nodes	—	5/29(18%)	92/124(75%)
Primary and nodes	14/51(27%)*	9/34(26%)*	56/95 (59%)

* Comparison of radiation treatment in air with radiation+OHP treatment, $\chi^2 = 10.2$, $P<0.01$.

TABLE 8. Relation of 12-Month Cumulative Survival to Dose Fractionation in OHP [a]

Dose fractionation	Disease stage				Total
	$T_{3,4}N_1$	$T_{3,4}N_{2,3}$	$T_{3,4}N_0$	$T_{T0}N_{2,3}$	
Radical irradiation					
3 × 1000 rads (days 0, 7, 21)	3/7	3/16	6/8	3/6	15/37 (41%)
3 × 1000 rads (days 0, 14, 28)	1/3	6/23	3/9	5/8	15/43 (35%)
3 × 800 rads (days 0, 7, 21)	2/3	5/9	1/2	1/3	9/17 (53%)
3 × 800 rads (days 0, 14, 28)	0/1	1/2	—	1/2	2/5
6 × 500 rads (days 0–17)[b]	3/5	5/13	1/2	5/5	14/25 (56%)
6 × 600 rads (days 0–17)[b]	1/1	0/1	0/1	—	1/3
"Palliative" irradiation					
2 × 1000 rads (days 0–14)[c]	0/3	2/18	0/3	0/2	2/26 (8%)
3 × 1000 or 1200 rads (days 0–48)[d]	—	0/3	—	—	0/3
				Overall survival	58/159(36%)
				Excluding "palliative" group	56/130(43%)

[a] In 159 patients with cancer of upper digestive and respiratory passages.
[b] Doses given on days 0, 4, 7, 11, 15, and 17.
[c] Doses given either on days 0 and 7 or 0 and 14.
[d] Doses given either on days 0, 14, and 28 or 0, 21, and 48.

16% (5/31) in air, which is a statistically significant difference ($\chi^2=5.3$, $P<0.05$).

The early survival results in our series for lower dose regimes (6×500 rads and 3×800 rads) suggest that these modifications may produce significant improvements. However, in $T_{3,4}\pm N_{2,3}$ disease it is unlikely that significant improvements in prolonged survival rates are ever obtainable with radiotherapy or ablative treatments, poor prognosis being largely due to irreparable destruction of normal tissues by tumor invasion and metastases. OHP treatments were significantly better than fractionated air treatments as palliative measures and in improving shorter-term survivals (87% at 6 months with OHP). This is attributed to (1) more complete and earlier regression of tumors

TABLE 9. Cumulative Survival at 12 Months After Radical Radiation Therapy in OHP [a]

Disease stage	Pts. with distant metastasis pretreatment	Survivors at 12 months
$T_{3,4}N_1$	7%	10/20 (50%)
$T_{3,4}N_{2,3}$	13%	20/64 (31%)
$T_{3,4}N_0$	7%	11/22 (50%)
$T_{T0}N_{2,3}$	10%	15/24 (62%)
	Total	56/130(43%)

[a] In 159 consecutive cases of cancer of upper digestive and respiratory passages.

TABLE 10. Comparison of Cumulative Survival for Patients Treated in Air and in OHP

Treatment	12-month survival
Conventional daily fraction in air (4500–7000 rads total)	20/51 (29%)
3 × 1000 rads in air	12/32 (37%)
Radical doses in OHP	56/130(43%)

from OHP treatment and (2) more debilitating and prolonged reactions produced by 4- to 6-week courses of daily radiation fractions. In this respect alone, hyperbaric oxygen in radiotherapy can play a role for which any alternative such as additive chemotherapy is considered a poor substitute at present.

Dysfunction and Other Causes of Death. In advanced primary disease $T_{3,4}$, postradiation changes in the respiratory and digestive tracts are the main causes of death (Table 11). These changes include residual mucous membrane defects and intractable ulceration at the site of tumor regression, resulting in exposure, infection, and necrosis of deep structures (*e.g.*, cartilage and bone), strictures in muscular tubular organs (*e.g.*, esophagus), fistulae, exposure of large blood vessels, dysfunction of the muscles of mastication, respiration, and phonation, and pain due to interstitial neuritis. A majority of such residual ulcerations are free of residual tumor but the latter sometimes complicates the picture. Few such cases are amenable to a limited surgical repair or reconstruction, since the irradiated normal tissues do not support an effective regenerative process of cell migration and replication, and spreading necrosis often develops. These changes are not peculiar to hyperbaric treatments, however, and occur at a rate essentially equal to that with radical treatments in air (Table 12). The greater the destruction of normal tissues by tumor before radiation, the higher the incidence of residual defects produced. Furthermore, the higher the biologically effective dose (n/n_0) used, the higher the incidence of tissue damage despite better tumor clearance. Thus 6×500 rads produced much less dysfunction than 3×1000 rads in OHP (Table 13), but, of course, it produced a higher incidence of residual neoplasm. Although recovery processes due to protraction of fractions may favor tissue healing to a limited extent, healing of large primaries in which there is little destruction of epithelium and deep fibrosis is much more frequent. Thus, dysfunction resulting from clearing of large lymph node deposits ($T_{T_0}N_{2,3}$ group, Table 12) is low. Experience indicates that dental trauma, persistent excessive alcoholism, and smoking after radiation are prominent causes of tissue breakdown, and every precaution is needed to prevent alveolar ridge necrosis from poorly fitting dentures or simple tooth extractions.

Distant metastasis is also a prominent cause of death (Table 11), accounting for about 40% of all deaths. In our series, 5% of patients died from a second primary cancer (the same incidence as that reported by Churchill-Davidson [19]).

Normal Tissue Reactions in OHP. After performing animal studies on normal tissue reactions in OHP,[23] we studied

TABLE 11. Causes of Death in OHP-Treated Patients with Cancer of Upper Digestive and Respiratory Passages

Cause of death	Disease stage			
	$T_{3,4}N_1$	$T_{3,4}N_{2,3}$	$T_{3,4}N_0$	$T_{T_0}N_{2,3}$
Dysfunction or recurrence at primary site	44%	45%	40%	—
Metastasis	33%	28%	30%	80%
Other disease (nonmalignant)	6%	19%	15%	8%
Second primary cancer [a]	11%	4%	—	6%
Undetermined	6%	4%	15%	6%

[a] Overall incidence of a second primary cancer in these patients was 5% (6/128 patients).

TABLE 12. Incidence of Radiation Complications [a] in OHP and in Air [b]

Irradiation in:	Radiation Dose	Disease stage	Radiation injury incidence
OHP	Radical [c]	$T_{3,4}N_1$	29%
		$T_{3,4}N_{2,3}$	35%
		$T_{3,4}N_0$	40%
		$T_{T_0}N_{2,3}$	7%
Air	3 × 1000 rads	All stages	16/34(47%)
Air	4500–7000 rads [d]	All stages	12/40(30%)

[a] Includes nonhealing tumor necrosis, residual ulceration, tissue breakdown, stricture, and dysfunction. Excludes "dry mouth," acute radiation reactions and immediate sequelae, tissue necrosis precipitated by surgical intervention, moderate postirradiation atrophy, and fibrosis.
[b] In patients with $T_{3,4} \pm N_{2,3}$ disease of upper digestive and respiratory passages.
[c] Radical dosage consisted of 3 × 1000 rads, 3 × 800 rads, 6 × 600 rads, or 6 × 500 rads.
[d] Given as daily fractions over 3–6 weeks.

skin reactions in a series of patients irradiated with ^{90}Sr beta rays in graded doses in air and in OHP at 4 ata.[24] These results indicate that doses of 500–1000 rads produce significantly more severe reactions in OHP than in air and that approximately a 20–30% increase in dose is tolerated by skin in air. These results are of considerable importance in the design of clinical trials which attempt to assess the value of OHP in radiotherapy. Regardless of fractionation regimes used, we suggest that air doses be increased by 20–30% relative to OHP to ensure that comparable tissue damage is produced before assessing relative tumor sterilization and therapeutic ratios.

TABLE 13. Relation Between Radiation Injury [a] and Dose in $T_{3,4}N_{1-3}$ Disease[b]

Dose		Radiation injury incidence
3 × 1000 rads		30/59 (51%)
3 × 800 rads		6/18 (33%)
6 × 500 rads		5/28 (18%)
6 × 600 rads		5/10 (50%)
	Total [c]	46/115(40%)

[a] Dysfunction, nonhealing of primary, and necrosis.
[b] Cancer of upper digestive and respiratory passages.
[c] Excludes 17 patients treated with 2 × 1000 rads, four of whom (24%) had radiation injury.

Conclusions. Our experience with advanced cancer of the upper digestive and respiratory passages has led us to the following general conclusions:

1. OHP significantly improves tumor clearance (doubles the rate for air treatments) for a similar degree of normal tissue damage. Our results in this respect almost duplicate the experience of Churchill-Davidson in London.

2. OHP treatments significantly improve short-term survival in advanced disease, but the effect on survival beyond 12 months has not been significant in our series. It did, however, appear significant in that of Churchill-Davidson. This may be related to 41% of our patients having been referred for OHP after previous surgical x-ray therapy or chemotherapy, as opposed to only 5% in Churchill-Davidson's series.[25]

3. In OHP (and in air), 3 × 1000 rads tumor dose to primary disease exceeds acceptable morbidity and tolerance; 6 × 600 rads also exceeds tolerance, but 6 × 500 rads modal tumor dose is well tolerated by very large volumes of tissue, producing an estimated 50% clearance rate of tumors, a 56% survival rate at 12 months for advanced ($T_{3,4}N_{2,3}$) disease, and an 18% incidence of nonhealing and dysfunction attributable to radiation in the primary zone. However, 3 × 800 rads in the same overall time produces similar

results and has made possible several 3-year and longer survivals free of tumor and free of necrosis.

4. Dosage regimes in OHP in respect to both tumor effects and tissue damage are related to cell survival data based on the "multihit" equation,

$$\frac{n}{n_0} = 1 - \left(1 - e^{-\frac{D_0}{D}}\right)^m$$

and equivalent biologically effective fractionated courses of radiation can be fairly accurately calculated using a $D_0 = 130$ rads and an extrapolation value of $m = 2$–4 to provide predictable orders of tissue reactions and tumor clearance rates. It appears that tumor sterilization rates in advanced disease in excess of 50–60% in OHP (not possible in air from available data for these stages) are precluded by an unacceptable incidence of normal tissue reactions and damage.

5. The usefulness of OHP appears proven in the eradication of cervical (and other) lymph node metastasis. It is suggested that all cervical lymph node metastases ($N_{2,3}$) that have been proven should be treated by radical radiation in OHP, regardless of their histology, and that surgery is contraindicated in such stages. It is also suggested that block dissections for supraclavicular chain lymph node metastases, in which pathologic involvement of nodes is proven and in which postoperative recurrence rate has been shown to be high in most centers, should be followed by a postoperative course of radical irradiation in OHP.

Cancer of Lung

In the 21 patients with lung cancer referred for treatment, results indicate that both survival and tumor clearance by irradiation may be improved by high pressure oxygen (Table 14), although radiation damage to pulmonary tissues presents a major problem.

The patients treated were all considered inoperable on the grounds of mediastinal involvement, vocal cord paralysis, chronic pulmonary insufficiency, esophageal invasion, cervical node metastases, or postoperative recurrence. Yet, 45% of patients survived 1 year, three are alive over 2 years, and one is alive at 4 years—a result comparing more than favorably with other treatments (Table 15). As in the previous experience with head and neck cancer, 3 × 1000 rads is not tolerated to the large volumes of lung, mediastinum, and adjacent cervical tissues which require irradiation. Radiation pneumonitis is invariably produced, usually with onset 3 months after irradiation and causing death despite absence of tumor in the chest in most of these cases examined at autopsy. A dose of 3 × 800 rads has been better tolerated, and, indeed, all three patients surviving 2 years received this dose. However, pneumonitis and pulmonary fibrosis are still frequent at this biologically reduced level of dosage and appear to be perhaps more frequent for 6 × 500 rads.

Claims for radiotherapy in treatment of carcinoma of the bronchus show great variation, however. Guttman[26] has recently obtained a survival rate over 50% at 12 months for nonresected cancer of the lung after thoracotomy (7.4% 5-year survival rate), no patients having survived 12 months after radiation in

TABLE 14. Carcinoma of Lung: Case Material and Survival [a]

	Cases
Extent of disease	
Extrathoracic metastases	11
Esophageal invasion	3
Recurrent after resection	2
Inoperable stages	5
Survival	
12-month survivors [b]	9/20(45%)
24-month survivors [c]	3/17(18%)

[a] Twenty-one patients made up this group; all received radiation therapy in OHP.
[b] Twelve-month survivors included 2/9 treated with 3 × 1000 rads, 4/5 treated with 3 × 800 rads, and 3/6 treated with 6 × 500 rads.
[c] All three 24-month survivors had been treated with 3 × 800 rads.

TABLE 15. Comparison of Survival Statistics in Patients with Carcinoma of Lung

Source	Irradiation in:	Survival 1 year	Survival 2 years
Peter MacCallum Clinic (Melbourne)[a]	Air (conventional therapy)	49/331(14%)	15/331(5%)
van den Brenk *et al.* (Melbourne)	Air (3 × 1000 rads)	0/7 (0%)	
	OHP	9/20 (45%)	3/17 (18%)
Churchill-Davidson (London)	Air	1/19 (5%)	0/17 (0%)
	OHP	5/21 (24%)	3/21 (14%)

[a] Information gathered from clinic records for 1954–1959.

which resection was attempted but unsuccessful. On the other hand, Belcher and Anderson [27] reported a 2-year survival rate of only 3% for 238 cases of carcinoma of bronchus, nonresectable at thoracotomy, the 5-year survival rate being only 0.5%.

It appears that OHP may well produce improved survival in properly selected lung cancer, and this disease, which is becoming more common, appears suitable for a controlled clinical trial.

Carcinoma of Bladder

The results obtained in our initial 19 cases of advanced and recurrent carcinoma of the bladder (Table 16) are shown in Table 17. In Table 18 a few statistics are shown for comparison with the results of Churchill-Davidson,[19] Minty,[28] Laing and Dickinson,[29] and van der Werf-Messing.[30]

Our own results indicate that OHP improved survival, although the effect was not statistically significant. Our results are poor in comparison with those of Churchill-Davidson in both OHP and air.

TABLE 16. Carcinoma of Bladder: Case Material[a]

	Cases
Stages	
T_2 (2 cases)	
T_3 (6 cases)	19
T_4 (11 cases)	
Prior surgery [b]	7/19(37%)
Renal failure or severe cystitis [c]	9/19(47%)

[a] Of the 19 patients treated by radiation in OHP, 16 were male and three female.
[b] Transurethral resection or partial cystectomy.
[c] All nine of these patients died within 12 months after radiotherapy.

TABLE 17. Results of Radiation Therapy with OHP in Patients with Carcinoma of Bladder

	Cases
Survival	
12-month survivors [a]	6/17(35%)
24-month survivors	2/15(13%)
Radiation necrosis (bowel, bladder) or severely contracted bladder [b]	7/19(37%)
Bladder tumor-free after radiation (by cystoscopy or autopsy)	14/19(73%)
Causes of death	
Metastases	5/13(38%)
Pancreatic carcinoma [c]	1/13(8%)
Radiation damage (bowel, bladder, kidney)	7/13(54%)

[a] At the end of 12 months, 2/12 patients treated with 3×1000 rads survived, 3/3 patients treated with 6×500 rads survived, and 1/2 patients treated with 3×800 rads survived.
[b] Of the patients treated with 6×500 rads, only one showed such evidence of radiation injury.
[c] Second primary tumor.

TABLE 18. Comparison of Survival Statistics in Patients with Carcinoma of Bladder

Source	Irradiation in:	Survival	
		1 year	2 years
Peter MacCallum Clinic (Melbourne) [a]	Air (conventional therapy)	66/134(49%)	45/134(34%)
Minty (Melbourne) [b]	Air (conventional therapy)	30/111(27%)	—
van den Brenk *et al.* (Melbourne)	Air (3 × 1000 rads)	2/9 (22%)	1/9 (11%)
van der Werf-Messing (Holland)	Air (telecobalt)	37% [c] 18% [d]	18% [c] 5% [d]
Laing and Dickinson (London)	Air (megavoltage)	90% [e] 10% [f]	32% [e] 0% [f]
Churchill-Davidson (London)	Air (conventional therapy)	5/14 (36%)	0/13 (0%)
van den Brenk *et al.* (Melbourne)	OHP (radical therapy)	6/17 (35%)	2/15 (13%)
Churchill-Davidson (London)	OHP (radical therapy)	13/32 (41%)	5/17 (29%)

[a] Information gathered from clinic records for 1954–1959.
[b] Peter MacCallum Clinic, 1957, late local and extravesicular disease.
[c] Results for T_3 bladder disease.
[d] Results for T_4 bladder disease.
[e] Results for T_2 bladder disease.
[f] Results for $T_{3,4}$ bladder disease.

However, our cases were mostly very advanced recurrent disease, often with renal failure, and among the earlier cases receiving doses of 3×1000 rads, many patients died from acute radiation damage to intestine and bladder. A change to 6×500 rads has improved tolerance markedly. Radiation cystitis is mild and reversible, and intestinal symptoms and sequelae from the large-volume pelvic irradiation are quite tolerable and less distressing than from conventional radical treatments.

In many cases with T_4 fixed masses involving abdominal wall, rectum, and extravesicular tissues, complete clearance of tumor has been noted clinically and at autopsy, but many patients (43% of deaths) succumbed from metastases.

Conclusions. Our results indicate that: (1) less advanced tumors ($T_{2,3}$) are more successfully treated, with longer remissions and survival; (2) severe cystitis and renal failure, if present before irradiation, almost invariably result in poor radiation tolerance and a high mortality; (3) protraction of larger fractions (800–1000 rads) from 7- to 14-day intervals greatly enhances radiation tolerance of intestinal mucosa; and (4) bladder distention with water during pressurization and irradiation is often effective in displacing intestines upwards and improving radiation tolerance.

The results also indicate that the use of OHP in bladder cancer, including earlier disease, should in no way detract

from radiotherapeutic results but almost certainly bring about additional improvements, provided modal tumor doses of the order of 6×500 rads in 3 weeks (or biologically equivalent fractionations) are prescribed.

Brain Tumors

An early series of 50 cases of supratentorial gliomas were treated with 2×1000 rads on days 0 and 7. These patients were referred from neurosurgical clinics as having advanced disease; some gliomas had only been decompressed, others had been partially resected, and in others no decompression was performed. Restrictions were placed on the hyperbaric personnel (and accepted) to reduce irradiated volumes to a minimum to cover the tumor volume outlined by the neurosurgeons on skull diagrams. The results, summarized in Table 19, were considered poor, and further material has not been offered on the grounds that the treatment produced a high incidence of brain necrosis associated with residual disease.

In retrospect, we consider that many recurrences were due to restriction of the irradiated volume, gliomas being essentially tumors of diffuse and possibly multifocal origin. Second, these tumors are themselves often necrotic (a process which tends to be enhanced by any radiation which efficiently destroys the tissue) and therefore palliative doses only tend to produce temporary "fringe encapsulation" and retention rather than destruction. Third, a large space-occupying lesion in a most vulnerable and important tissue as the brain, enclosed in a rigid cranium and devoid of drainage, presents an irreparable situation. Since focal tissue repair in the central nervous system is extremely limited *per se*, and gliosis itself produces pathologic effects, effective radiotherapy in radical short courses aggravates the situation. Finally, radical irradiation of the ineffectively decompressed brain may produce acute pressure sequelae (*e.g.*, herniation of brain through the tentorium) and sudden death.

Our lack of success certainly does not preclude a modification of OHP techniques, perhaps associated with the addition of CO_2 to produce cerebral vasodilatation and oxygenation. The use of smaller (500 rads) fractions protracted to weekly intervals and the irradiation of large volumes of brain to 3000 rads appear worthy of consideration.

TABLE 19. Supratentorial Gliomas: Case Material[a]

	Cases
Disease	
Glioblastoma multiforme	28
Astrocytoma (grades II, III)	18
Other	4
Dose	
2×1000 rads on days 0 and 7 to limited volumes of brain	46
Survival	
12-month survivors	15/50(30%)
24-month survivors	4/48(8%)

[a] Fifty patients constituted this group; all received radiation therapy in OHP.

Carcinoma of Rectum

Results in the first 13 cases of recurrent rectal cancer (stages III, IV) appear promising (Tables 20, 21), 58% of patients surviving at 12 months and 33% at 2 years. In 62% of the cases, massive fixed perineopelvic masses have cleared

TABLE 20. Carcinoma of Rectum: Case Material[a]

	Cases
Previous surgery	12/13(92%)
Distant metastases	4/13(31%)
Histologic diagnosis	
Recurrent adenocarcinoma or anaplastic rectal carcinoma	12
T_4N_3 squamous cell carcinoma of rectum[b]	1

[a] Thirteen patients with stage III and IV disease constituted this group; all received radiation therapy in OHP.

[b] This patient had undergone no previous surgery.

TABLE 21. Results of Radiation Therapy with OHP in Patients with Carcinoma of Rectum[a]

	Cases
Survival	
12-month survivors	7/12(58%)
24-month survivors	4/12(33%)
Tumor clearance	8/13(62%)
Radiation damage (necrosis of sacrum, bowel damage, or fistula)[b]	6/13(46%)
Causes of death	
Metastasis	5/10
Radiation damage	3/10
Other	2/10

[a] Patients treated either with 3×1000 rads or 3×800 rads in OHP.
[b] Of the group with tissue damage, 5/13 received 3×1000 rads on days 0, 7, and 21.

on both clinical grounds and from examinations performed at laparotomy and autopsy. Doses of 3×1000 rads to large tissue volumes produced a high incidence of tissue damage, morbidity, and mortality, despite the effective tumor clearance. The recent prescription of 6×500 rads is well tolerated and produces effective early clinical regressions.

In this clinic, over the period 1954–1959, 60 cases of rectal cancer were treated with conventional irradiation in air, and this resulted in 1- and 2-year survival rates of 20/60 (33%) and 6/60 (10%), respectively. The OHP results appear to be an improvement and worthy of further pursuit, not only for massive recurrences but as a prophylactic measure after operation in cases of suspected or certain residual disease.

A limited series of large-volume pelvic irradiations with 3×1000 rads in air at weekly intervals produced acute and chronic radiation sequelae comparable to those in OHP.

Carcinoma of Cervix and Vagina

The results in six patients treated with 3×1000 rads in OHP are shown in Table 22. Similar radiation sequelae were experienced with these doses in either OHP or air, as in other pelvic disease, but the rate of tumor clearance (5/6 cases) was high. Recommendations regarding dose and fractionation for this disease are similar to those for bladder and rectal disease, and should improve survival figures and palliation.

TABLE 22. Carcinoma of Cervix and Vagina: Case Material[a]

	Cases
Previous surgery and x-ray therapy	3/6
Tumor clearance by radiation + OHP	5/6
Bowel damage, fistula, other radiation damage	4/6
12-month survival	2/6[b]
Causes of death	
Metastasis	1
Radiation damage	2
Renal failure	1

[a] All six patients were treated with 3×1000 rads in OHP on days 0, 7, and 21. All had stage IV disease, one with distant metastases.
[b] Of these two patients, one has remained alive 13 months and the other 42 months.

Carcinoma of Breast

Results for six $T_{3,4}$ cases treated with 3×1000 rads (two cases) and 3×800 rads (four cases) are shown in Table 23. It is doubtful if OHP, even at 4 ata, can

TABLE 23. Carcinoma of Breast: Case Material[a]

	Cases
Radiation therapy in OHP	
3 × 1000 rads	2
3 × 800 rads	4
Tumor clearance	2/6[b]
12-month survivors	5/6[c]

[a] Of the six patients in this group, all had T_3 or T_4 tumors with fixed node masses; three had metastases.
[b] Active recurrence in two patients, and clearance uncertain in two remaining patients.
[c] Of these five survivors, three died at 13, 16, and 21 months, and two have remained alive for 21 and 32 months. All deaths were due to metastases with or without recurrence.

effectively oxygenate large fixed multi-quadrant scirrhous disease of the breast to produce permanent local eradication of the tumor, late recurrences (at 12 months or more) being common despite most impressive early regression. The prognosis associated with this disease so frequently depends on such factors as the production of metastases and hormone influences that local control becomes a secondary and relatively insignificant feature in the overall results. OHP would therefore appear less valuable in this disease unless one adopts the McWhirter philosophy [31] in the preference of radiation to surgery in the primary treatment of regional lymph nodes—a preference which requires careful pathologic substantiation with an analysis of lymph node recurrence and its origin, following either surgery and/or irradiation in air, for its further justification.

Miscellaneous Neoplasms

Our results with soft tissue and bone sarcomas (to be published elsewhere) indicate that regression of massive tumors is produced more consistently and thoroughly by irradiation in OHP than in air, irrespective of histologic characteristics of the tumor. Survival is not improved, however, since the poor prognosis in these patients arises from the high early incidence of distant metastases. As a palliative measure, though, irradiation in OHP appears clearly superior to irradiation in air, being predictable in causing a high rate of rapid tumor regression which usually persists until death.

General Conclusions

The experience accumulated by this unit since 1961 in treating advanced malignant disease with 4-Mev x rays delivered to tumors in patients exposed to oxygen at 4 ata pressure before and during irradiation has shown that:

1. Tumor regression and clearance in most sites produced by irradiation in OHP is clearly superior to that resulting from treatment in air, being more predictable and complete, irrespective of histologic and other pathologic features purported to represent tumor radioresistance. These results support those of Churchill-Davidson.[19]
2. In advanced disease, survival rates at more than 1 year appear only slightly better in OHP-treated patients—a result to be expected on biological grounds and probably largely dependent upon factors other than the effectiveness of local treatments.
3. Early survival rates are generally better and should be further improved with modified dose schedules.
4. Predictable effects of irradiation in OHP in terms of dose and fractionation, in respect to tissue tolerance and tumor effects, can be determined by the implementation of cell dose–survival parameters in clinical prescriptions. Equivalent biologically effective doses for three to six fractions delivered in 3–4 weeks are calculated for a $D_0 \approx 130$ rads and $m \approx 2$–4. We considered that further allowance of a 20–30% dosage reduction factor (DRF) for OHP relative to air should be made for normal tissues (particularly for skin and mucous membranes).
5. OHP has particular advantages in the palliative or curative treatment of lymph node metastases and in neoplastic disease recurrent after surgery (*e.g.*, rectal cancer).
6. A dose of 3×1000 rads exceeds tolerance in both OHP and air, and dose-fractionation prescriptions equivalent to 6×500 rads or 3×800 rads (or perhaps somewhat higher) provide acceptable tolerance by most tissues for large-volume irradiation.
7. Prolonged courses of daily fractions did not improve results in advanced disease treated in air, and there is little evidence that results with fractionation should differ in OHP. Indeed, improved early survival results obtained in OHP-treated cases may be partly related to reduced

stress on patients (shorter courses under anesthesia, more rapid tumor response, and earlier mucosal recovery).

8. No mortality, significant morbidity, or aftereffects have resulted from 2569 exposures to OHP at 4 ata during anesthesia in the first 614 cases of advanced malignant disease, most patients receiving four to six exposures within 17 days and each exposure averaging 40 min.

9. Finally, it is stressed that the exploitation of "oxygen effect" in radiotherapy has not resulted in sufficient radiosensitization of tumors to preclude the necessity for prescribing radical levels of dose which tax tissue tolerance, nor has it removed the necessity to invoke all possible refinements of radiotherapy in respect to radiation dosimetry, its planning, and its execution in practice. Failure to abide by these principles will offset the potential advantages provided by hyperbaric oxygen and related techniques.

Addendum

A cumulative analysis of the first 95 patients surviving over 12 months (from all groups in the series), in a total of 267 cases at risk (36% cumulative crude survival rate) was done to determine the rate of tumor recurrence in the irradiated zone before and after 12 months. The findings appear in Tables 24 and 25. (It should be borne in mind that 45% of patients in the total series already had recurrent disease after previous surgery, x-ray therapy, and/or chemotherapy by the time they received irradiation in OHP). Tables 26 and 27 show the results of a similar analysis of the first 31 patients surviving over 2 years (crude survival rate 18% at 2 years).

TABLE 24. Tumor Recurrence in Irradiated Zone in First 95 Patients Surviving over 12 Months [a]

	Cases
Recurrence	
Clear at 12–24 months	68(72%)
Uncertain (U) or definite (D) recurrence 12–24 months	27(28%)
Appearance of U or D recurrence	
Before 12 months	21/27
After 12 months	6/27

[a] Counted from the time of irradiation in OHP.

Comparison of this analysis with the pessimistic clearance rates obtained for tumors of the upper digestive and respiratory passages suggests that recurrences first appearing at any time after 12 months following irradiation in OHP are few at all dose levels, and are least likely to appear for doses with the lowest n/n_0 values (*i.e.*, 3 × 1000 rads). No local recurrence has so far appeared later than 2 years from the time of irradiation in OHP in this early series of cases, despite a high

TABLE 25. Relation Between Recurrence in Irradiated Zone and Dose in First 95 Patients Surviving over 12 Months [a]

Dose	Total cases	Clear cases [b]	U–D cases [c] after 12 months
2 × 1000 rads	14 [d]	5(36%) [e]	2(14%)
3 × 1000 rads	44	34(77%)	1(2%)
3 × 800 rads	22	16(73%)	1(5%)
6 × 500 rads	15	13(85%)	2(15%)
	95	68	6

[a] Counted from the time of irradiation in OHP.
[b] Clear of tumor in irradiated zone at 12–24 months.
[c] Uncertain or definite recurrence appearing only after 12 months.
[d] Includes nine cases of cerebral tumors.
[e] Includes four cases of cerebral tumors.

TABLE 26. Tumor Recurrence in Irradiated Zone in First 31 Patients Surviving over 24 Months [a]

	Cases
Recurrence	
Clear after 24 months	26(84%)
Uncertain (U) or definite (D) recurrence after 24 months	5(16%)
Appearance of U or D recurrence	
Before 12 months	4/5
Between 12 and 24 months	1/5

[a] Counted from the time of irradiation in OHP.

death rate due to distant metastases and other causes (*e.g.*, necrosis and nonmalignant diseases). However, although treatment with 6×500 rads has been instituted too recently to assess, the 15% rate of recurrences first appearing between 1 and 2 years in patients surviving more than 1 year suggests that late recurrences may appear more frequently with this dose. This is attributed to the lower biological effectiveness for this fractionation ($n/n_0 \approx 10^{-7}$).

TABLE 27. Relation Between Recurrence in Irradiated Zone and Dose in First 31 Patients Surviving over 24 Months [a]

Dose	Total cases	Clear cases [b]	U-D cases [c] after 24 months
2× 1000 rads	4 [d]	4(100%) [d]	0
3 × 1000 rads	19	17(89%)	0
3 × 800 rads	8	5(63%)	0
	31	26	0

[a] Counted from the time of irradiation in OHP.
[b] Clear of tumor in irradiated zone at 24 months.
[c] Uncertain or definite recurrence appearing after 24 months.
[d] All four were cases of cerebral tumors.

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Clinical Exploration into the Value of Hyperbaric Oxygen with Radiotherapy of Cancer

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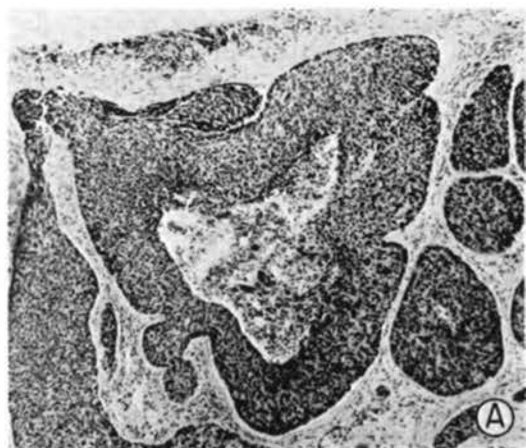
Squamous cell carcinoma tends to grow away from its blood supply in the same fashion as its normal counterpart. The supply of oxygen furnished by the capillary bed is completely depleted by diffusion through 150 microns of living cell mass. Beyond this, all cells are dead. Between these extremes exists a zone of cells which are viable but relatively anoxic. These are critical cells in radiation response, as indicated by the curve in Figure 1. This illustration demonstrates that, in studies of cell survival after radiation, the anoxic cell requires 2.7 times as much radiation for the same effect as those cells at the pO_2 found in the capillary bed (40 mm Hg). An added and most attractive dividend is that overcorrection of the anoxia, or superoxygenation of cells, does not increase the radiation response over that obtained at normal capillary bed oxygen pressures.

By applying Henry's law, can we, then, correct the tissue anoxia? With the increased oxygen-carrying capacity of the circulation under 30 psig of 100% oxygen, a pO_2 of 1600 mm Hg has been measured in the antecubital vein.[1] Will hyperbaric oxygenation correct the loss of radiation sensitivity that results from the growth pattern of epithelial cancer cells? The answer must be sought in clinical trial of a laboratory fact. This research should also answer questions of tolerance versus complications, to avoid adding another burden to those already carried by the patient with cancer.

For hyperbaric oxygenation to gain a place in the medical armamentarium, it must be uniquely beneficial, practical in its use, and applicable to a reasonable number of patients. We also might well require that it be not just another stopgap in the decline of a patient with uncontrollable cancer. The benefit, even when obvious, must not be so rare that it is difficult to justify application of the procedure to a large number of patients in order to find an instance of such salutary effect.

In January 1963, we initiated a clinical exploration at the Tumor Institute of the Swedish Hospital, Seattle, into the feasibility, tolerance, and value of hybaroxic radiotherapy for cancer patients. To date of this report, 489 patients with cancer have been exposed to 3 ata of 100% oxygen during irradiation, for a total of 5041 pressure cycles (Table 1). Their ages have ranged from 16 to 91 years, and their physical condition from vigorous health to preterminal states.



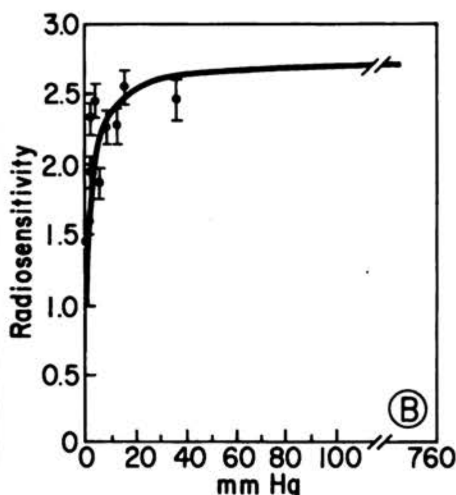


FIGURE 1. A, photomicrograph showing large island of squamous cell carcinoma, the central part of which is necrotic due to anoxia resulting from its isolation from the capillary bed and the limitation of oxygen diffusion. B, graph showing the relationship between radiosensitivity of squamous carcinoma cells and the pO_2 of the cellular environment.

With the one-man chamber, no team of technicians, physicians, and scientists is necessary. One modestly trained, intelligent departmental aide can attend the patient in the oxygen chamber, with the gentle understanding, coaching, and watchfulness that gives these patients their necessary psychological support. The remainder of the procedure, radiotherapy, is carried out by the usual departmental staff. The initial cost of the one-man chamber is approximately that of a deep x-ray therapy machine of 1940-vintage capacity. With justifiable modifications of the treatment schedules, the cost of therapy can be covered by private medical insurance. Since treatment can be given to 10 patients in a normal working-day, it is applicable to a reasonable number of patients.

Patients with various forms of malignant disease were chosen for hybaroxic irradiation therapy (Table 2) for reasons ranging from inoperability to advanced

TABLE 1. Patients Treated by Hyperbaric Oxygenation and Radiotherapy 1963–1965 [a]

	No. patients	Total no. treatments
1963	185	1336
1964	200	2311
1965 [b]	104	1394
Total	489	5041

[a] An additional 82 patients with cancer were given a total of 429 OHP exposures (for a variety of reasons), but not in combination with radiotherapy. At the beginning of our work with the one-man chamber in 1963, 28 healthy volunteers also underwent pressurization, one exposure each.

[b] Through September 1965.

TABLE 2. Hybaroxic Radiotherapy for Cancer in 377 Cases [a]

Site	No. patients
Skin (includes melanomas)	28
Oral cavity structures	57
Upper respiratory and digestive tracts	83
Thoracic esophagus	26
Lung	25
Gastrointestinal tract and organs	34
Genitourinary tract and organs	20
Connective tissue (sarcomas)	12
Lymphoid tissue (lymphomas)	27
Lymph nodes (metastases)	52
Miscellaneous (includes central nervous system)	13

[a] Includes only patients treated through June 1965, in order to provide some follow-up period.

age, beyond the tolerance of ordinary conventional therapy. In most instances, only patients were selected who, in the judgment of their therapists, had less than a 20% survival chance with conventional means. In the early days of the study, even more drastic selection criteria were applied, but it soon became apparent that some of the patients must have an expected survival of a year or more. With only short-term survivals, the therapist would have no opportunity to study the complications and recurrences that might require a year or more to become evident.

Following is a report of our results, discussed according to the type of disease, in the hybaroxic irradiation treatment of patients with malignant lesions. Any conclusions regarding the success of this form of treatment would, of course, be premature at this time.

Carcinoma of the Tongue (16 Patients)

We had great hopes of improving the expected survival statistics in patients with this disease. Carcinoma of the tongue is a squamous cell carcinoma often showing the growth pattern that can be explained by epithelial cells outgrowing their blood supply (Figure 1). It has a fairly early response to radiation and a high recurrence rate, and surgical treatment is destructive to the normal contours and function. Attainment of a cure with hybaroxic radiotherapy was, however, unsatisfactorily infrequent (Table 3). Of the 16 patients treated in hyperbaric oxygen, five are living and well, three surviving for 27 months, one for 9 months, and one for 8 months. The appearance of the last two is such that we expect them to survive or to succumb from other causes due to advanced age, without recurrence. Two more died at 3 and 5 months after treatment, with no evidence of disease at autopsy. Three are living, but at surgery 6, 3, and 3 months after hybaroxic radiotherapy, cancer was recognizable in surgical specimens. Of the six who died with cancer in the treated area, five had little or no evidence of healing of massive late-stage disease, while one had good early response, only to have recurrence at 3 months.

One might claim that the advanced disease of these patients precluded a high cure rate, but the fact that five of the six who died with their disease locally evident showed little or no healing indicates that, in at least a third of the cases, the radiation resistance of the tumor could not be eliminated by hyperbaric oxygen, or by the way it was applied.

Cancer of the Floor of the Mouth (8 Patients)

Our results in this group of patients (Table 4) were little better than those ob-

TABLE 3. Results of Hybaroxic Radiotherapy in Carcinoma of the Tongue (16 Cases)

	Clinical diagnosis		Surgical diagnosis		Autopsy diagnosis	
	No disease [a]	Disease	No disease	Disease	No disease	Disease
No. patients	5	—	—	3	2	6
Survival times [b] (months)	27	—	—	6	5	8
	27			3	3	7
	27			3		5
	9					5
	8					2
						2

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.

TABLE 4. Results of Hybaroxic Radiotherapy in Carcinoma of the Floor of the Mouth (8 Cases)

	Clinical diagnosis		Surgical diagnosis		Autopsy diagnosis	
	No disease [a]	Disease	No disease	Disease	No disease	Disease
No. patients	3	1	1 [c]	2	2 [c]	—
Survival times [b] (months)	18 18 27	24	6 [c]	7 7	8 24 [c]	—

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.
[c] The patient in whom no disease was found at operation (for necrosis) 6 months after hybaroxic radiotherapy is also listed as free of disease at autopsy 24 months after therapy. Thus, this patient is listed twice in this table.

tained in patients with cancer of the tongue. While this lesion is seldom as large when originally seen as cancer of the tongue, its tendency to cross the midline anteriorly tends to make surgical procedures even more deforming. Of the eight patients treated with hybaroxic radiotherapy, three are living and well at 18, 18, and 27 months; one with mouth lesions controlled had a positive neck dissection at 24 months. Two had positive biopsies which led to surgery at 7 and 4 months after hybaroxic irradiation therapy. One had surgery at 6 months for necrosis, to die 18 months later without evidence of recurrence. Two are dead without evidence of disease (one at 24 months) and probably represent cures.

Gingivobuccal Cancer (8 Patients)

Of our eight patients with gingivobuccal cancer (Table 5), one is alive at 30 months, with mandibular necrosis. One died at 3 months without evidence of cancer at autopsy. Three died with persistence of inoperable disease despite hybaroxic radiotherapy. One is living 24 months after resection for necrosis at 2 months, with a question of cancer cells present in the specimen. Two are living 27 and 30 months after surgery performed 6 months after hybaroxic radiotherapy for osteonecrosis, with no evidence of malignancy. In this group, then, where invasion of the mandible by squamous cell cancer is frequent, a high rate of

TABLE 5. Results of Hybaroxic Radiotherapy in Gingivobuccal Carcinoma (8 Cases)

	Clinical diagnosis		Surgical diagnosis		Autopsy diagnosis	
	No disease [a]	Disease	No disease	Disease	No disease	Disease
No. patients	1	—	2	1	1	3
Survival times [b] (months)	30	—	6 6	2	3	8 3 3

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.

radionecrosis and/or tumor-induced necrosis persisted after treatment. Surgical intervention for removal of the necrotic tissue was successful in three instances, in only one of which was there even a suspicion of residual disease. One patient died at 3 months from other causes, due to advanced age, without evidence of cancer at autopsy.

One might claim, therefore, effective elimination of the tumor in five of eight cases. It has been hypothesized that bone invasion by epithelial cancer is not controllable by conventional radiation therapy because the vascular system of the bone is unable to respond to the demands of the advancing cancer, resulting in relative or complete anoxia of the tumor. By our experience, hyperbaric oxygenation combined with radiotherapy does not appear to have repaired the defect.

Cancer of the Tonsil (13 Patients)

Of the 13 patients with cancer of the tonsil (Table 6), seven have remained alive and well without evidence of residual disease for periods ranging from 5 to 27 months. Of the six who succumbed from persistent disease, one died from spread of disease at 5 months after hybaroxic radiotherapy which was given 6 months after laryngectomy for carcinoma. Another died 1 month after attempted resection performed 6 months after hybaroxic radiotherapy. Of the seven who are living and well, four had involved nodes at the time of treatment, and one had extension of the disease involving the soft palate, the anterior tonsillar pillar, the adjacent tongue, and lateral pharyngeal wall.

Cancer of the Soft Palate (4 Patients)

All four of these patients were treated because of advanced age or other considerations which made surgery undesirable and conventional radiation of the usual duration a serious burden. All are living and well 9 months after hybaroxic radiotherapy.

Cancer of the Nasopharynx (6 Patients)

Treatment results with these patients were disappointing (Table 7), with only one patient living and well at 9 months. Of the remaining five, three are living with disease, one at 6 months with local control but extensive disease in the mediastinum, another at 12 months with recurrent local disease and extensive metastases, and the third at 15 months with no evidence of local recurrence but extension into the brain. (This last patient seemed to respond transiently to methotrexate after radiotherapy.) Two are dead

TABLE 6. Results of Hybaroxic Radiotherapy in Cancer of the Tonsil (13 Cases)

	Clinical diagnosis		Surgical diagnosis		Autopsy diagnosis	
	No disease [a]	Disease	No disease	Disease	No disease	Disease
No. patients	7	—	—	—	—	6
Survival times [b] (months)	27	—	—	—	—	12
	24					8
	18					6
	9					5
	8					5
	7					4
	5					

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.

TABLE 7. Results of Hybaroxic Radiotherapy in Cancer of the Nasopharynx (6 Cases)

	Clinical diagnosis		Surgical diagnosis		Autopsy diagnosis	
	No disease [a]	Disease	No disease	Disease	No disease	Disease
No. patients	1	3	—	—	—	2
Survival times [b] (months)	9	15 12 6	—	—	—	11 4

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.

with disease, one at 4 months and the other at 11 months with extensive necrosis and recurrent disease. The latter patient had been treated over the previous 5 years with radiation therapy to the nasopharynx (5500 rads in 3 months with 250-kv x rays). He had an ipsilateral radical neck dissection for extension into that region, followed by radiation therapy to the neck for local recurrence. When seen at our institute for hybaroxic radiotherapy, his nasopharynx and oropharynx were almost completely obstructed by recurrent disease extending well past the midline from his left side and massive metastasis at the angle of the mandible. This patient demonstrated the futility of hyperbaric oxygenation with radiotherapy for postradiation recurrences. The scar was relatively ischemic and its response to radiation therapy seemed intensified by the hyperbaric oxygen, an observation made otherwise only in cartilage and the cornea at this time.

Cancer of the Larynx (19 Patients)

Of the 19 patients exposed to hybaroxic radiotherapy (Table 8), 11 are living and well with no evidence of disease for 6–28 months. One is living at 3 months with persistent inoperable local disease after irradiation in hyperbaric oxygen. Two had surgery for edema and necrosis at 3 and 15 months after therapy, but no cancer was found in the surgical specimens. One had laryngectomy, and cancer was found in the specimen. Three died of intercurrent disease at 5, 11, and 20 months, with no evidence of cancer at the time of death. One died 9 months after hybaroxic radiotherapy given for recurrence in the anterior neck 1 year

TABLE 8. Results of Hybaroxic Radiotherapy in Cancer of the Larynx (19 Cases)

	Clinical diagnosis			Surgical diagnosis		Autopsy diagnosis	
	No disease [a]		Disease	No disease	Disease	No disease	Disease
No. patients	11		1	2	1	3	1
Survival times [b] (months)	28 24 23 18 16	12 12 12 9 6 6	3	15 3	5	20 11 5	9

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.

TABLE 9. Results of Hybaroxic Radiotherapy in Cancer of the Maxillary Antrum (11 Cases)

	Clinical diagnosis		Surgical diagnosis		Autopsy diagnosis	
	No disease [a]	Disease	No disease	Disease	No disease	Disease
No. patients	6	—	—	—	2	3
Survival times [b] (months)	30	—	—	—	15	7
	27				14	3
	14					3
	12					
	9					
	9					

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.

after laryngectomy. Considering the material, we felt that hyperbaric oxygen combined with radiotherapy proved to be as curative as any other method of managing cancer of the larynx and that it offered many advantages (as reported previously [2]).

Cancer of the Maxillary Antrum (11 Patients)

In these 11 cases (Table 9), the disease was thought to have arisen in the maxillary antrum, but in most instances it had extended to involve the ethmoids as well. Six patients are living and well after 9–30 months, four of these over 12 months. Of the dead, three had positive persistent disease in the maxillary sinus at 3, 3, and 7 months. The last of these three was a teenager with highly anaplastic disease. At the time of her death, the disease had completely disseminated, with massive liver, lung, and brain involvement. The breasts and adrenals were bilaterally involved. A nodule in the left breast was evident at the original examination. Two more died with no evidence of local disease; one was found to have either metastasis to the lung or a new primary as the cause of death. The judgment of the pathologist was that it was a new primary. Eight of the 11 are living and well or died with local disease controlled.

TABLE 10. Results of Hybaroxic Radiotherapy in Supraglottic Cancer (15 Cases)

	Clinical diagnosis		Surgical diagnosis		Autopsy diagnosis	
	No disease [a]	Disease	No disease	Disease	No disease	Disease
No. patients	6	1	—	1	2	5
Survival times [b] (months)	18	12	—	7	18	9
	15				8	8
	14					5
	13					4
	13					4
	12					

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.

Supraglottic Cancer, Including Epiglottis, Vallecula, and Adjacent Structures (15 Patients)

Of the 15 patients in this group (Table 10), six are living and well for more than 12 months. One is living with evidence of recurrence at 12 months. Five are dead with persistent disease, all with less than 9 months of survival. Two of the patients who died had no evidence of disease at autopsy at 18 and 8 months, although the 8-month case had extensive necrosis of the cartilage.

Cancer of the Piriform Sinus and Cervical Esophagus to the Sternal Notch (14 Patients)

Of these 14 patients (Table 11), six are living and well at 6–24 months after hybaroxic radiotherapy. One is living at 6 months with disease recurrent in the radiation field. One had no disease in the surgical specimen at 11 months. Five are dead of disease, and one other survived 18 months, to die of complications of transverse myelitis, with no evidence of local recurrence.

Carcinoma of the Thoracic Esophagus (21 Patients)

Thirteen of these 21 cases were squamous cell carcinomas, the remainder adenocarcinomas (Table 12). The four living and well had squamous cell carcinomas. Nine patients were judged at the time of treatment to have local disease encompassed by the radiation field, and 12 had involvement beyond the extent of the radiation. Of the 17 dead, 10 had locally persistent or recurrent disease at death. Of the remaining seven, one died at 5 months with no evidence of local disease but extensive lung and liver metastases, one at 5 months (at 77 years of age) without clinical or x-ray evidence of local disease, one at 7 months of acute alcoholism, one at 15 months with the complications of transverse myelitis, one at operation at 15 months with no evidence of cancer in the surgical specimen, one at 17 months of massive liver metastasis and involved abdominal nodes but no evidence of local recurrence, and one at 16 months with no evidence of local disease but extensive abdominal metastases.

Cancer of the Lung (21 Patients)

Despite the observation that at autopsy more than half of the patients dying of cancer of the lung have no demonstrable metastases outside of the thoracic cavity, it is a well-known fact that the longer the patients survive their local disease, the more evidence of metastasis is discovered. In this series (Table 13), all patients

TABLE 11. Results of Hybaroxic Radiotherapy in Cancer of Piriform Fossa and Cervical Esophagus (14 Cases)

	Clinical diagnosis		Surgical diagnosis		Autopsy diagnosis	
	No disease [a]	Disease	No disease	Disease	No disease	Disease
No. patients	6	1	1	—	1	5
Survival times [b] (months)	24	6	11	—	18	15
	17					12
	14					9
	9					3
	8					3
	6					

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.

TABLE 12. Results of Hybaroxic Radiotherapy in Carcinoma of the Thoracic Esophagus (21 Cases)

	Clinical diagnosis		Surgical diagnosis		Autopsy diagnosis	
	No disease [a]	Disease	No disease	Disease	No disease	Disease
No. patients	4	—	—	—	7	10
Survival times [b] (months)	21	—	—	—	17 7	13 5
	15				16 5	12 3
	15				15 5	7 2
	4				15	6 2
						5 1

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.

were inoperable, either because of disease extending beyond the scope of surgery, inoperability at the time of thoracotomy, or microscopic invasion found beyond the resected margins. Five of these patients are living and well without evidence of disease more than 12 months after irradiation in hyperbaric oxygen. Of the 16 dead, four survived over 12 months. A total of nine of the 21 survived over 1 year (41%). This is considerably better than the 22% 1-year survivals recently published in the literature.[3]

Epidermoid Carcinoma Metastatic to Cervical Nodes (13 Patients)

Although earlier workers in radiotherapy combined with hyperbaric oxygenation obtained excellent results in the treatment of this disease,[4,5] only three of our 13 patients with inoperable metastatic cervical nodes have remained alive and well at 8, 15, and 27 months (Table 14). One died at 5 months without evidence of local recurrence; one died at 11 months at surgery for cartilage necrosis, with no evidence of residual disease. The remaining eight failed to respond or the disease recurred and was judged to cause their death.

SUMMARY

The procedure of hyperbaric oxygenation was well tolerated, even in debilitated patients of all ages. The responses of 170

TABLE 13. Results of Hybaroxic Radiotherapy in Cancer of the Lung (21 Cases)

	Clinical diagnosis		Surgical diagnosis		Autopsy diagnosis	
	No disease [a]	Disease	No disease	Disease	No disease	Disease
No. patients	5	—	—	—	—	16
Survival times [b] (months)	18	—	—	—	—	18 5
	15					15 5
	14					15 4
	14					12 3
	12					7 3
						7 3
						6 1
						6 1

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.

TABLE 14. Results of Hybaroxic Radiotherapy in Epidermoid Carcinoma Metastatic to Cervical Nodes (13 Cases)

	Clinical diagnosis		Surgical diagnosis		Autopsy diagnosis		
	No disease [a]	Disease	No disease	Disease	No disease	Disease	
No. patients	3	—	—	—	2	8	
Survival times [b] (months)	27	—	—	—	11	14	6
	15				5	9	5
	8					8	5
						6	3

[a] Living and well.
[b] After termination of hybaroxic radiotherapy.

patients to OHP combined with radiotherapy for several kinds of cancer are presented. Most of the patients were judged to have less than a 20% chance of survival with conventional means.

The results were somewhat disappointing. Anoxia seems to be only one of the deterrents to successful control of cancer with radiotherapy. Results in 13 patients with inoperable or recurrent metastatic cancer in nodes in the neck were poor, as were those obtained in cancers of the tongue, floor of mouth, and gingivobuccal areas. On the other hand, the results in cancers of the tonsil, supraglottic region, larynx, cervical and thoracic esophagus, and lung were gratifying.

Two conclusions are evident: (1) further study into methods of the application of hyperbaric oxygen to radiotherapy of cancer is necessary to determine if all the benefits possible are being obtained, and (2) in those areas where there is an improved effect of radiotherapy on certain cancers, a study with proper controls to demonstrate the rate of effectiveness should be carried out.

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Regional Oxygenation and Its Effect Upon Radiation Therapy and Isotope Localization

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The development of a technique for regional oxygenation by the intra-arterial route was motivated primarily by a desire to bypass the lungs and their inherent problems of toxicity and to avoid the need for the extensive equipment used in hyperbaric oxygenation.

Our original work involved the use of gaseous oxygen introduced into the arterial system, as reported by Clark and Ross,[1] but experience soon showed the advantages of using oxygen already in solution. Consequently, in 1961, Finney[2] developed combinations of concentrations of hydrogen peroxide (H_2O_2) and buffering solutions which could be successfully employed. Concurrently, Balla *et al.*[3-6] modified a catheter mobile pump system so that long-term intermittent infusion techniques could be accomplished with a minimum of complications. The most salient points of these studies will be presented below, along with the clinical results obtained in the treatment of malignant tumors with H_2O_2 infusion in conjunction with external irradiation and in the diagnosis of malignant tumors using H_2O_2 concomitantly with selected isotopes for scanning studies.

Jay *et al.*[7] demonstrated supersaturation of human blood with oxygen when dilute solutions of H_2O_2 were introduced, both *in vitro* and *in vivo*. Finney[8] demonstrated the rapid dissociation of H_2O_2 when it was introduced into human blood, predominantly due to the action of catalase. In addition, he established some base-line findings for concentrations and flow rates to achieve optimum arterial oxygen concentrations; this was done to avoid vessel spasm and bubbling which can be produced with this technique.

Regional Oxygenation and External Protracted Radiation Therapy

By the therapeutic technique developed at our center,[4-6] a catheter is introduced into the artery under direct surgical exposure. For tumors in the head and neck, the external carotid system is ordinarily used; the common carotid system can also be used, but considerable attention to the infusion response of the patient must be given in order to avoid complications. For treating the extremities, the main arterial supply is usually used, *i.e.*, the femoral artery. In the abdominal and

pelvic areas, we have found that retrograde placing of the catheter into the abdominal aorta is most successful. The desired level of distribution determines placement of the catheter.

We have found that each area has its own limitations, in terms of optimum H_2O_2 concentration and flow rates, and therefore treatment must be individualized. Individual adjustments must be made on each patient until maximum concentration and flow rates are established. The infusions and standard protracted external irradiation are given daily to a predicted 100% tolerance dosage. Irradiation therapy is carried out during the latter portion of the infusion, and infusion is stopped after irradiation is complete.

Considerable attention to detail in handling patients by a small, experienced, and dedicated team is necessary to achieve efficient care and avoid possible complications.

Response of Normal Tissue

Because of significant differences between the vascular anatomy of most animals and that of humans, animal values are not comparable to human values and thus could not be used as guidelines for dosage in our patients. For the past 3 years, we have been using full 100% normal protracted daily doses of radiation in conjunction with H_2O_2 infusion. The only parameters of tolerance in normal tissue have been early skin and mucous membrane reactions and late evidence of fibrosis, osteitis, necrosis, *etc.* To date, we have been unable to detect any significant decrease in tolerance of normal tissue with this system, and no late complications have occurred.

Complications from Treatment

We have encountered very few complications using this method of treatment, but the infusion of H_2O_2 solutions can cause severe vessel spasms and bubbling, resulting in mechanical vessel obstruction. In addition, all of the common complications encountered with insertion of catheters are experienced on occasion, such as infection or clot formation. With careful individualization and well-controlled experienced care, all significant complications have virtually been eliminated.

Illustrative Case

A 29-year-old white man with a recurrent grade II squamous cell carcinoma of the lower jaw had undergone surgical resection of the left alveolar ridge and had a left radical neck dissection 2 years before referral to us. When we saw him, he had a recurrence in the lower jaw, 50 cm^2 on the surface and 6 cm deep, with fixation of the base of the tongue. Osteomyelitis of the mandible was present, a pin remained in the mandible, and there was necrosis and infection (Figure 1A).

A catheter was inserted in the right external carotid artery, dye was infused, and satisfactory distribution of the dye was apparent, so the patient received 3 weeks of regional oxygenation by daily infusion with simultaneous external opposing-field 2-mev irradiation (4500 rads delivered in 15 treatments over 19 days). No other treatment was given. The therapy was started in July, and by August the tumor had regressed quite well (Figure 1B). In September, the pin was removed, 20 biopsy specimens were taken, and all were negative (Figure 1C). A sliding graft was performed, with complete success. By December, healing was complete (Figure 1D). The patient is alive and well to date of this report, with no further apparent activity of his osteomyelitis.

Results

From July 1961 through June 1965, 131 patients were treated by this technique. We selected patients who, in our opinion, had less than a 10% chance of surviving 3 years with conventional forms of therapy. Tables 1 and 2 show the results of therapy in terms of survival. Although the number of patients is small and the

TABLE 1. Malignancies of Various Sites Treated with Intra-Arterial H_2O_2 and External Irradiation

Site	Years treated	Patients treated	Died within 60 days	No. patients surviving and % survival (*see note below*)								
				2–6 mo	0.5–1 yr	1–1.5 yr	1.5–2 yr	2–2.5 yr	2.5–3 yr	3–3.5 yr	3.5–4 yr	4–4.5 yr
Head and neck	1961–65	44	4 (9%)	40 (91%)	33 (75%)	27 (69%)	24 (65%)	19 (56%)	18 (55%)	14 (54%)	8 (57%)	2 (50%)
Ovary	1962–65	8	2 (25%)	6 (75%)	6 (75%)	4 (43%)	3 (50%)	2 (40%)	1 (33%)	1 (50%)	1 (100%)	—
Liver	1961–65	14	4 (29%)	10 (71%)	8 (57%)	4 (29%)	3 (23%)	3 (30%)	3 (33%)	2 (29%)	—	—
Lung	1963	1	—	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	—	—	—
Cervix	1961–65	14	2 (14%)	12 (86%)	11 (79%)	5 (45%)	4 (50%)	3 (50%)	3 (75%)	2 (100%)	2 (100%)	—
Colon	1961–64	8	—	8 (100%)	5 (63%)	4 (50%)	2 (40%)	2 (50%)	2 (50%)	1 (50%)	1 (50%)	—
Brain	1961–63	5	1 (20%)	4 (80%)	2 (40%)	1 (20%)	—	—	—	—	—	—
Bladder	1961–65	14	3 (21%)	11 (79%)	9 (64%)	5 (38%)	5 (42%)	4 (36%)	3 (33%)	3 (38%)	1 (20%)	—
Extremity	1963–65	3	—	3 (100%)	3 (100%)	1 (50%)	1 (100%)	1 (100%)	1 (100%)	—	—	—
Eye	1963	1	—	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	—	—	—
Prostate	1964	1	—	1 (100%)	1 (100%)	1 (100%)	—	—	—	—	—	—
Uterus	1964–65	2	—	2 (100%)	2 (100%)	1 (100%)	1 (100%)	—	—	—	—	—
Breast	1962–63	3	1 (33%)	2 (67%)	1 (33%)	1 (33%)	1 (33%)	—	—	—	—	—
Spinal cord	1964	2	—	2 (100%)	2 (100%)	2 (100%)	—	—	—	—	—	—

Stomach	1963–64	4	2 (50%)	2 (50%)	—	—	—	—	—	—	—	—
Pancreas	1963–64	5	2 (40%)	3 (60%)	1 (20%)	—	—	—	—	—	—	—
Abdomen: liposarcoma	1964	1	—	1 (100%)	1 (100%)	1 (100%)	1 (100%)	—	—	—	—	—
Abdomen: mesothelioma	1963	1	1 (100%)	—	—	—	—	—	—	—	—	—

Note: The percent survival values for each column were computed using only those patients whose treatment was started before the time period shown in the column heading at the top. Thus, in viewing survival figures and percentages, the reader must bear in mind that *a decrease in survivors* between one interval and the next *does not necessarily imply that deaths occurred.* In many cases, therapy was initiated in one of the later treatment years, so that survival could be recorded only up through a given interval. Where sufficient numbers of patients were treated in the early years of this study, the percentages give a better picture of the success of therapy in terms of survival.

TABLE 2. Summary of All Cancer Patients Treated with Intra-Arterial H_2O_2 and External Irradiation from July 1961 to June 1965

Year treated	During months	Patients treated	Died within 60 days	No. patients surviving								
				2–6 mo	0.5–1 yr	1–1.5 yr	1.5–2 yr	2–2.5 yr	2.5–3 yr	3–3.5 yr	3.5–4 yr	4–4.5 yr
1961	7–12	7	2	5	3	3	3	2	2	2	2	2
1962	0–6	24	6	18	16	13	12	11	11	11	11	—
	7–12	21	4	17	13	11	11	10	10	10	—	—
1963	0–6	23	1	22	15	13	11	10	10	—	—	—
	7–12	11	1	10	8	3	3	3	—	—	—	—
1964	0–6	17	5	12	10	7	7	—	—	—	—	—
	7–12	16	3	13	11	9	—	—	—	—	—	—
1966	0–6	12	—	12	11	—	—	—	—	—	—	—
Totals		131	22	109	87	59	47	36	33	23	13	2
% survival *				83%	66%	50%	46%	42%	44%	44%	42%	29%

* Percent of patient survival at bottom of each column was computed by calculating the percent still alive of the total number treated at or before the beginning of that interval (shown at top of column).

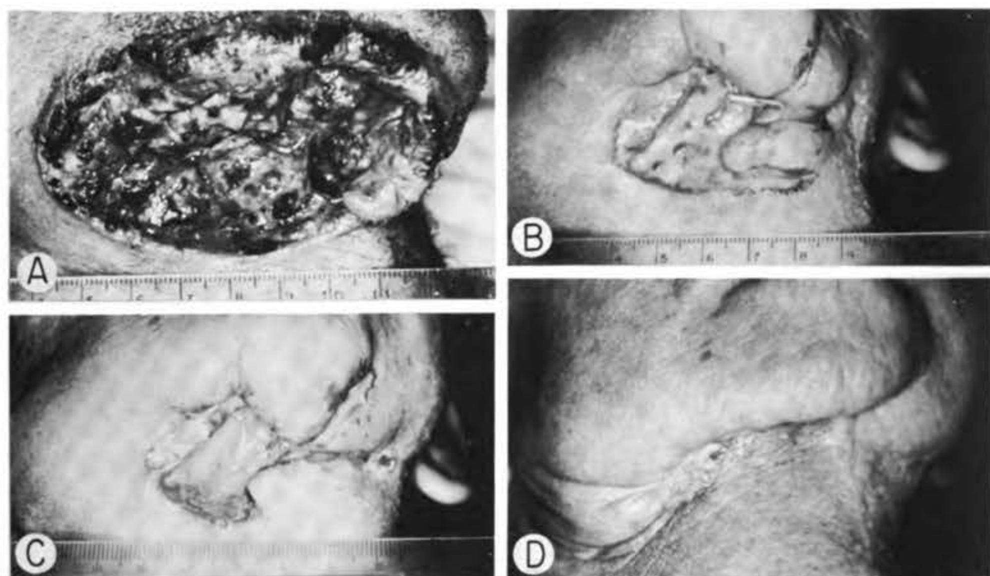

FIGURE 1. A, jaw of 29-year-old man with recurrent grade II squamous cell carcinoma before treatment. B, appearance of jaw 6 weeks after completion of therapy. C, appearance 40 days later (note size of ulceration); the pin has been removed and there is no evidence of tumor. D, appearance of jaw after closure of the defect by a sliding graft; healing is complete.

variables numerous, the 44% 3-year survival rate in these patients with advanced disease appears significant to us. We are especially encouraged by the results in patients with head and neck tumors, advanced disease of the cervix and ovary, recurrent carcinoma of the colon, and carcinoma of the bladder. The results in carcinoma of the bladder appear specifically significant when one considers that the irradiation dosage was 4500 rads in 4 weeks to the entire pelvis.

Comments

Because of the need for individualizing treatment when using this technique, the problems involved in the selection of patients when applying experimental techniques, and the number of significant variables, we believe that randomization studies are not currently feasible, except perhaps in specific areas such as stage III and regional stage IV cervix and bladder carcinomas.

REGIONAL OXYGENATION AND ISOTOPE LOCALIZATION

The cellular localization of certain isotopes has also been possible by the adjunctive use of regional oxygenation with H_2O_2.[8-11] Using HeLa cells and KB cells, we have been able to demonstrate selective uptake of radioiodinated human serum albumin (RIHSA) under the direct influence of H_2O_2 (Figure 2). The process is reversible, so that the application of H_2O_2 and its withdrawal before adding the isotope reveal virtually no uptake of the large-molecule isotope. Similar but as yet incomplete studies using hyperbaric conditions of 2–6 atm demonstrate the same phenomenon, which also appears to be reversible. Further studies are in progress. The mechanism is still unknown, but the selective reaction of normal cells compared to tumor cells appears to be in a ratio of approximately 1:5. In addition, the effective half-life of the isotope is prolonged because of the considerable increase in time that the isotope remains intracellular.

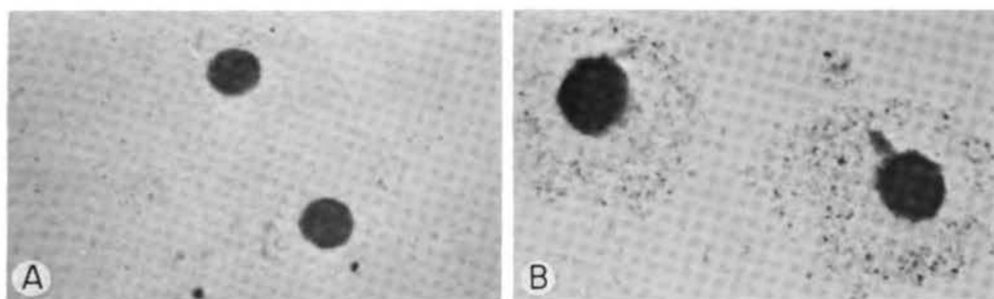

FIGURE 2. A, HeLa cells exposed to RIHSA without H_2O_2 (control), with no selective uptake apparent. B, HeLa cells exposed to RIHSA and H_2O_2; cellular uptake is demonstrated by black dots.

Autoradiographic studies with the electron microscope at ×40,000 show the distribution to be predominantly at the cell wall and within the nucleus (Figure 3).

Subsequent studies were performed using VX-2 symmetrical hind-leg tumors in rabbits. Both iliac arteries were individually catheterized, the left iliac artery was flushed with 7 ml of carrier solution, 10 μc of RIHSA, and finally with 3 ml more of carrier solution. Little or no RIHSA was demonstrated at 24, 48, 72, or 96 hours. The right leg was infused in the same manner, except that 0.12% H_2O_2 was included in the carrier solution.

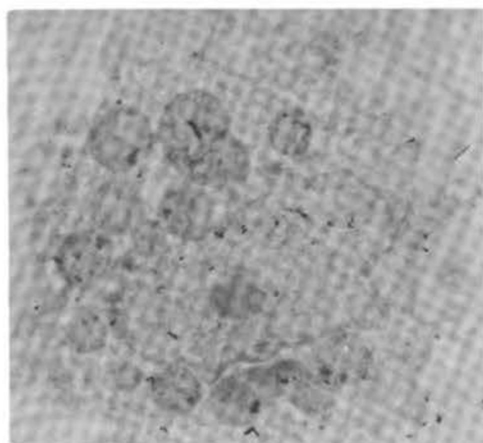

FIGURE 3. Isotope tracks shown by autoradiographic study with the electron microscope demonstrate distribution primarily at the cell wall and in the nucleus.

In contrast to the control experiment, the right leg showed considerable concentration of the isotope within the tumor at 24, 48, 72, and even 96 hours (Figure 4). Forty animals were tested and all demonstrated the same phenomenon.

Human scanning studies were then carried out (78 scans have been done). The procedure entailed a percutaneous puncture of either the femoral or the carotid artery with a 19-gauge Cournand needle. A catheter was then threaded through the needle until the tip was at the level of the diaphragm, and 200 ml of 0.12–0.48% H_2O_2 in electrolyte carrier solution (Ionosol T) with dextrose was infused. The infusion was then stopped and 250 μc of RIHSA injected

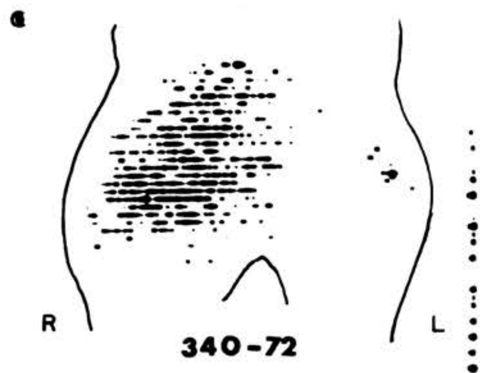

FIGURE 4. Positive scan of tumor in right leg of rabbit, in which H_2O_2 and RIHSA were used. compared to negative scan of tumor in left leg, in which RIHSA alone was used.

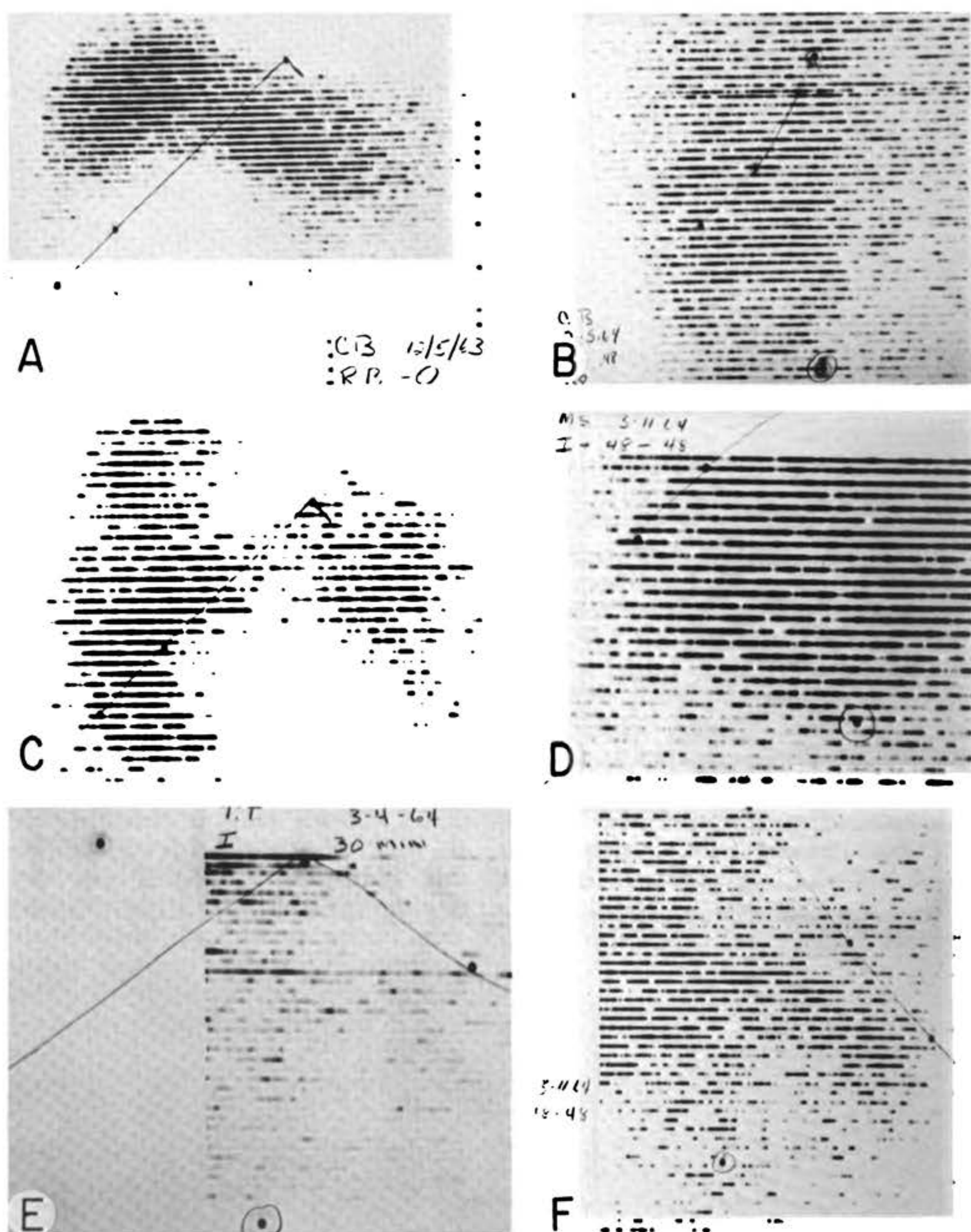

FIGURE 5. A, primary hepatoma defect demonstrated by rose-bengal scan; B, positive tumor localization (reversal of A) by use of H_2O_2 and RIHSA. C, metastatic carcinoma of colon to liver, with defect demonstrated by rose-bengal scan; D, positive tumor localization (reversal of C) with H_2O_2 and RIHSA. E, negative control scan of pancreatic tumor, with intra-arterial infusion of RIHSA in carrier solution; F, positive scan made several days later with RIHSA and H_2O_2.

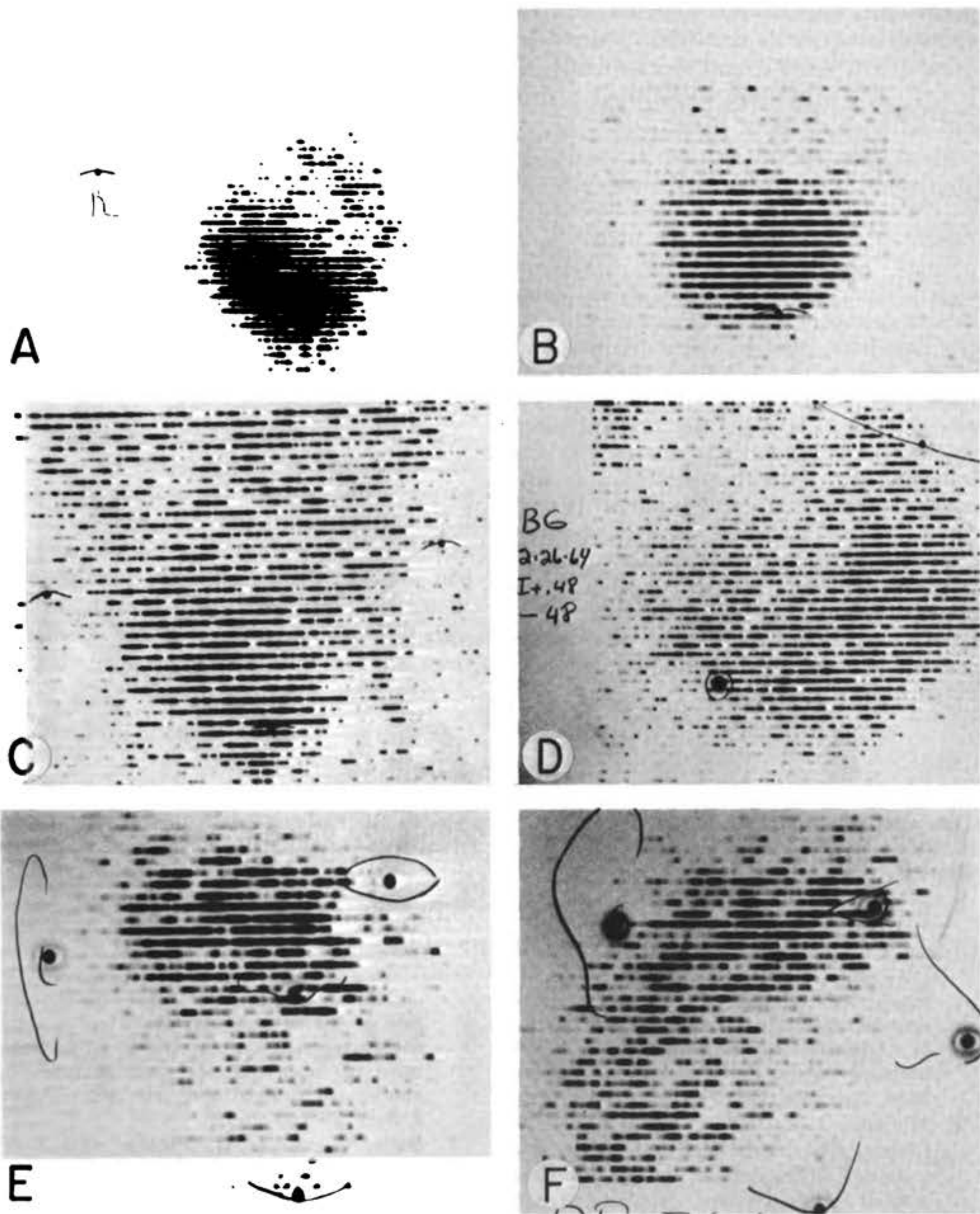

FIGURE 6. Positive scans with RIHSA and H_2O_2 showing: (A) carcinoma of uterus, (B) recurrent carcinoma of rectum in pelvis, (C) carcinoma of ovary, (D) upper abdominal retroperitoneal liposarcoma, (E) recurrent squamous cell carcinoma in the retro-orbital pterygoid space, (F) lateral scan of tumor shown in E, demonstrating involvement of neck nodes.

into the catheter. Following this, 50 ml of the H_2O_2 solution was infused. The procedure was then completed and the catheter and needle removed. Scans were done at 24, 48, 72, and, occasionally, 96 hours. The procedure was always carried out on an outpatient basis.

Illustrative Cases

Patient 1 had a pathologic diagnosis of primary hepatoma, and the defect was clearly demonstrated by a standard intravenous rose-bengal scan (Figure 5A). Several days later, after complete disappearance of the isotope, a combined H_2O_2–RIHSA scan was performed. The catheter was placed retrograde from the femoral artery up into the lower thoracic aorta. The scan following this procedure showed a positive isotope localization in the tumor (Figure 5B).

Patient 2 had metastatic carcinoma of the colon to the liver. A rose-bengal scan was done, showing the defect (Figure 5C). Later, the H_2O_2–RIHSA scan showed the reverse, with the isotope outlining the tumor (Figure 5D).

Patient 3 had a pathologic diagnosis of pancreatic carcinoma. For this study, the catheter was passed into the lower thoracic aorta. The control scan was done by the intra-arterial infusion of carrier solution with RIHSA. No localization of the isotope was demonstrated (Figure 5E). Several days later the same procedure was carried out with H_2O_2 added to the carrier solution, and the tumor was well outlined at this time (Figure 5F).

Other scans demonstrating the successful outlining of tumor by this technique appear in Figure 6. To date, positive localization has not been achieved in lesions other than malignant tumors. We have had three false-negative scans and one false-positive scan using this procedure. The false-negative scans occurred in two cases of pulmonary metastasis and probably reflect an inability to adequately infuse this area as yet. One false-negative scan was obtained in a case of poorly vascularized recurrent postsurgical carcinoma of the thyroid. The false-positive tumor occurred in a patient with an extremity rhabdomyosarcoma. Pelvic metastases were diagnosed by the scan, but at surgery these were not found.

ACKNOWLEDGMENTS

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DISCUSSION

Discussion of papers by van den Brenk and Sparrow (pp. 635–642), Churchill-Davidson (pp. 643–647), Johnson and Lauchlan (pp. 648–652), van den Brenk et al. *(pp. 653–675), Wildermuth (pp. 676–685), and Mallams* et al. *(pp. 686–695).*

DR. D. STEINHARDT (*Los Altos Hills, Calif.*): It is surprising, in view of the emphasis on oxygen to make cancers radiosensitive, that no attempt seems to have been made to use the increased solubility of oxygen at lower temperatures during hyperbaric radiation to bring a richer supply of oxygen to avascular foci. Lowered temperature also would depress oxygen consumption sharply and allow a buildup of oxygen tension. To test this, we at the Santa Clara Kaiser Foundation Hospital used grafted carcinoma in C3H Bitner mice and beta-emitting strontium plaques. These small concave plastic plaques have strontium granules active enough for the time intervals that we use. Figure 1 shows our refrigerated hyperbaric chamber for mice. Mice were grouped as follows: untreated, radiated, radiated with hyperbaric oxygen, and radiated with hybaroxia with simultaneous hypothermia. We also are currently trying the same experiment at various intervals in the thaw phase after chilling. In another experiment, without radiation, one group of animals was untreated, a second was twice given 30 minutes of compression with oxygen, a third was given hypothermia twice, and the fourth group was given either four or two successive combined "barothermal" treatments. Both control and treated animals were sacrificed after the same period of growth. We bisected the total fixed tumor specimens and examined them grossly and microscopically for relative extent of necrosis. There was moderate deceleration of tumor growth rate in the group radiated with the addition of hyperbaric oxygen. With further addition of deep hypothermia, the bisected specimens evidenced profound and extensive necrosis.

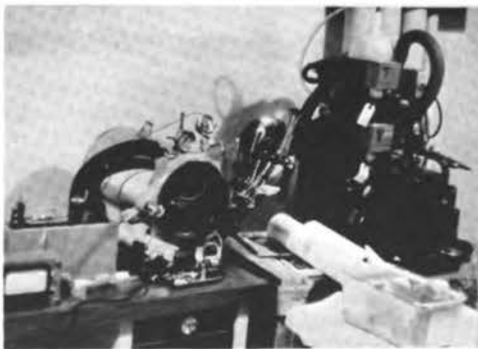

FIGURE 1. Refrigerated hyperbaric chamber for rodents.

In the second type of experiment, without radiation, using serial compressions of 30 minutes with deep hypothermia (barothermal treatment), massive necrosis of the tumor appeared in otherwise unimpaired animals. Figure 2A shows a control tumor, a mammary carcinoma. The changes in this type of tumor 4 weeks after two barothermal treatments can be seen in Figure 2B. Figure 2C illustrates the anoxic area away from the blood vessels in the type of tumor that we were using. In Figure 2D, complete necrosis 4 weeks after two barothermal treatments has occurred. Both groups on compression alone and on chilling alone showed slight irregularities in growth curves, but the tumors receiving two or four combined treatments exhibited much greater irregularities. Many of the curves show very sharp peaks in volume followed by a drop, indicating a massive necrotizing phenomenon. Figure 3 summarizes the results. First, control tumors were bisected and fixed in formalin. Second, two of the bisected specimens presented massive necrosis (the lighter area in the figure shows necrosis and growth at the rim of the tumor). The darker specimen in the figure was inhibited and liquefied by the treatment. These middle three specimens exhibited marked effects of barothermal

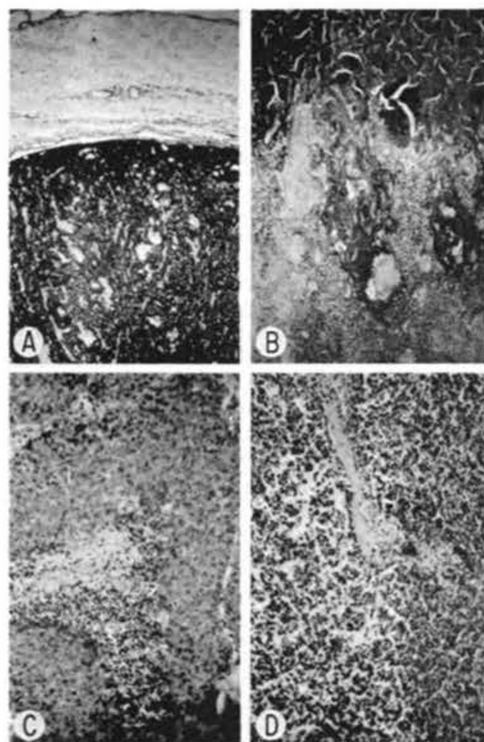

FIGURE 2. A, control mammary carcinoma. B, mammary carcinoma, showing necrosis after two barothermal treatments. C, control mammary carcinoma at 6 weeks; necrosis from anoxia has occurred in areas remote from vessels. D, mammary carcinoma, showing nearly complete necrosis 5 weeks after two barothermal treatments.

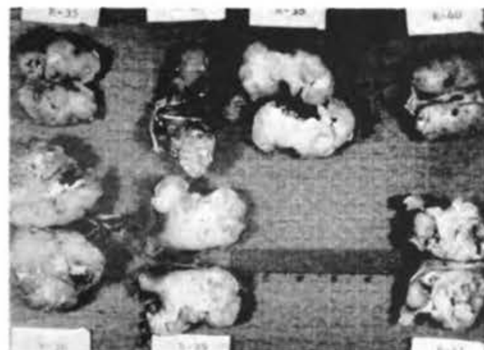

FIGURE 3. Bisected fixed specimens. Left, typical controls. Middle, two with massive necrosis after barothermal treatment. Right, top treated by hyperbaric radiation and bottom by addition of hypothermia.

treatment, without radiation. Third, the upper tumor was treated with hyperbaric radiation; the lower was treated with hyperbaric radiation and hypothermia in the average range of 20°C.

In summary, a new effect is demonstrated in which hybaroxia at very low temperature has an apparent necrotizing effect on the tumors studied.

DR. B. ANDERSON, JR. (*Durham, N. C.*): I would like to ask Dr. Mallams how he localizes the peroxide distribution in head and neck cases with the technique he presented.

DR. J. T. MALLAMS: We use the standard fluorescein dye techniques, which we check before and after surgery and also during infusion. This work is simply an extension of that done with methotrexate many years ago.

DR. H. P. PLENK (*Salt Lake City, Utah*): I would like to ask Dr. Churchill-Davidson about the complication rate in carcinoma of the larynx, which, I believe, was about 20%. At what dose schedules did these complications arise, and how did you change these schedules to deal with them?

DR. I. CHURCHILL-DAVIDSON: It's a bit complicated. There were six treatments with carbon dioxide irradiation over 18 to 19 days. With 4500 rads, cartilage necrosis occurred in three out of 11 cases (27%); with 4000 rads, it occurred in one out of 10 (10%); with 3750 rads, the incidence was none out of 4; and, with 3500, it was none out of 30.

DR. PLENK: Would you comment on local recurrences?

DR. CHURCHILL-DAVIDSON: I have the sterilization rates. At 4500 rads, the sterilization of the primary was eight out of 11 (73%); for glands, it was seven out of eight (88%). At 4000 rads, primary sterilization occurred in five out of 10 (50%) and, for glands, in five out of seven (72%). At 3750 rads, the primary was sterilized in three out of four cases (75%) and in four out of four glands (100%). At 3500 rads, the primary was sterilized in 25 out of 30 cases (83%);

in the glands, sterilization occurred in 23 out of 27 (85%).

DR. PLENK: Do you always use opposed cobalt heads?

DR. CHURCHILL-DAVIDSON: Not necessarily. In carcinoma of the tongue, for instance, we often use just one head and probably an anterior wedged field plus compensating filter, a lateral similar field with wedge and compensating filters, and a third open field between the two, to a relatively low dose.

DR. PLENK: Dr. van den Brenk, would you comment on the relative merits of 3 and 4 atmospheres and also translate dosages from 4 to 3 atmospheres?

DR. H. A. S. VAN DEN BRENK: We have had no clinical experience with 3 atmospheres. All irradiation treatments have been carried out at 4 atmospheres. I can only refer to some of the experimental work in animals that we did before we began using oxygen clinically. We could find no differences in mice irradiated with 3 and 4 atmospheres. I would not alter the dosage at all for 3 atmospheres, since this would be illogical from a radiobiological point of view.

DR. PLENK: Dr. Wildermuth, have you noticed excessive edema of the larynx after treatment of carcinoma of the larynx?

DR. O. WILDERMUTH: As with radiation therapy without oxygen, edema is very dose-dependent. It is also related to the extent of infection present at the time of treatment, whether the infection clears well, and whether there is early re-epithelialization of the tumor-bearing area in the larynx. My personal opinion is that more submental, subcutaneous edema occurs with the short higher-dose treatments in the first 6 months than in conventional radiation.

DR. E. GRADY (*Atlanta, Ga.*): I have a question relating to the radioisotopes that Dr. Mallams spoke of with scanning and enhancement of the pickup by hydrogen peroxide. At Georgia Tech, some of our colleagues have made some nice particulate isotopes for intra-arterial injection, and we have had success with local treatment of cancers with the arterial administration of the isotope. Could the concentration and radiation effect be improved with the addition of hydrogen peroxide? The radiation effect might especially be increased in the liver through the hepatic artery, as there is 100% retention there. Would the radiation effect in this instance be increased by increasing the oxygen saturation of the tumor cell?

DR. MALLAMS: The first case I presented was carcinoma of the pancreas. The patient had a prior control scan of exactly the same type, eliminating only the peroxide in the arterial infusion. This scan was negative in contrast to the other in which the hydrogen peroxide was utilized. In addition, we do have an entire series in which we used ^{203}Hg intra-arterially for scanning brain tumors. We have done both of these with and without the hydrogen peroxide and isotope, using one as the control and the other with intra-arterial hydrogen peroxide with the isotope. These experiments were controlled, using both intravenous and intra-arterial infusions to check these two variables. The series definitely indicated enhanced uptake when peroxide was added to the isotope, either intravenously or intra-arterially. In addition, the fixation of the isotope was increased significantly. For example, one patient with a glioma had a positive scan for 20 days after the injection of the isotope.

DR. GRADY: If you inject ^{32}P into the hepatic artery, there is nearly 100% retention in the liver. Perhaps ^{32}P in conjunction with the peroxide might improve therapy to the liver by increasing the oxygen saturation of the tumor cells there.

DR. MALLAMS: The isotopes we have used to date have been ^{59}Fe, ^{203}Hg, and the RISA. We have done preliminary work in our *in vitro* series using peroxide and ^{32}P. The suggestion is that the ^{32}P does not act in the same manner as the other isotopes mentioned. At the moment, work indicates that the peroxide-enhanced localization refers only to large molecules. Therefore, it may be due to the size of the molecule and not the isotope itself. The parameters, of course, are very exciting, but to date I cannot be definite about the mechanisms.

DR. O. WILDERMUTH: The most exciting thing about isotope localization is pancreatic carcinoma localization. I know of no other dependable way to localize or diagnose a carcinoma of the pancreas short of open biopsy. If this is real, I think Dr. Mallams should abandon everything else he is doing and begin localizing a series of carcinoma of the pancreas by this technique.

DR. J. DEL REGATO, *Session Chairman (Colorado Springs, Colo.)*: Dr. Wildermuth, have you seen any cases of cartilage necrosis in your series?

DR. WILDERMUTH: We have seen cartilage necrosis early with original dose pattern of 600-rad exposure to the tumor in six sessions.

DR. DEL REGATO: Do you think that it is directly related to the intensity of the treatment?

DR. WILDERMUTH: It is somewhat different. It is definitely dose-related as far as the surviving cell percentages are concerned. We ought to improve this, but, of course, in these advanced cases, there is sure to be some necrosis.

DR. MALLAMS: Has anyone seen cartilage necrosis in patients who have not had invasion of cancer into the cartilage? I think this is an important differential.

DR. CHURCHILL-DAVIDSON: We have seen it in cases without invasion of the cartilage.

DR. C. L. LEWIS *(Buffalo, N. Y.)*: I would like to see a really controlled series done in this area, and we at Roswell Park would be happy to join in a corporate controlled study, if we could solve some of these problems. I am concerned that all four of these studies seemed to have utilized different dose schedules. Is there a common factor in the dose schedules? Are you doing nearly the same thing, but in slightly different ways?

DR. DEL REGATO: I wanted to ask that question myself. What are the acceptable circumstances for randomization?

DR. VAN DEN BRENK: Randomization is going to take a great number of patients and a tremendous amount of effort. I feel that one would hesitate to start if the results are not generally acceptable. Dr. Lewis' question focuses attention on the fact that we have all used different dosage schedules, and I think that, before randomization and trials in hyperbaric therapy are begun, we should ascertain the best form of fractionation by conventional methods. As yet, there is no clear answer on this. In Great Britain particularly, efforts are being made to find what fractions other investigators use, what reactions they get, and what rates of tumor control result from different fractions. Now, if we are going to add this variable to hyperbaric oxygen radiotherapy, or, for that matter, the use of hydrogen peroxide or any other modality, I think that any answer that comes up will be most equivocal. I would, on my side, be prepared to start a randomized trial with my own techniques. Of course, as to the results obtained, I'm quite sure that various people would say, "Oh, yes, but we're not going to do it your way; we can do much better by another system." In fact, they may, and I feel that is the great difficulty. What randomization trials should really attempt to show is whether, indeed, there is a significant oxygen effect factor in human tumors. Our data have convinced me that this is so, as have the sporadic attempts being made to treat alternate cases in air and oxygen. It is very disheartening to see tumors actually keep on growing while you are treating them in air with a dosage which, if given in oxygen, would almost guarantee resolution of the tumor. Great ethical problems are involved here. After all, we are not entitled to sacrifice patients purely to satisfy what amounts to idle curiosity if the answer that will come from one of these trials is going to be equivocal.

DR. DEL REGATO: Dr. van den Brenk, I am not sure of this, but I doubt that the committee could devise a randomization study that would be satisfactory to you. Would you randomize your own cases by simply doing the same thing on half of the patients, but without hyperbaric oxygen?

DR. VAN DEN BRENK: We intend to start this next year, particularly in stage T_3 and T_4 cancer of the bladder.

DR. DEL REGATO: Dr. Johnson, what would be the minimal conditions that you would require for randomization?

DR. R. J. R. JOHNSON: We should be willing to randomize cases at the Manitoba Cancer Treatment and Research Foundation provided that: (1) we agreed on the method of treatment for the control group in a cooperative randomized trial, and (2) the material from this Foundation, together with that of other radiotherapy centers, would be made up of enough patients to be of statistical value in a reasonable period of time. I think that advanced carcinoma of the cervix and bladder would be good material and could be randomized using conventional fractionation. Sites which do not include bowel could be chosen for short fractionation studies.

DR. WILDERMUTH: There are a good many attempts at fractionation underway that have nothing to do with hyperbaric oxygen, and I am at a loss to know of one that is successful. Until those have been run, I do not see why we should confuse this particular study. Furthermore, I don't know the best way to use hyperbaric oxygen in the treatment of each variety of cancer. I am quite certain it is not the same in one area as it is in another, and limitations exist in one area that we need not be concerned with in another. If we can explore and find an area where change is significant and obvious, then it becomes worthwhile to randomize that area and determine the numerical effect, if you will, or get a large number of cases by any method you want. Randomization of something as artistic as radiation therapy is tough enough. Then, to completely complicate things by adding oxygen techniques seems to me to present a problem almost beyond the capacity of any group to set up a worthwhile program that will be acceptable to a reasonable number of people who have this modality available to them.

DR. DEL REGATO: You are recommending, beyond randomization, a cooperative study with the same technique acceptable to everyone, but I do not expect that this would be acceptable to the main protagonists. I think that one could postulate a randomized study in which each individual will simply divide his cases between hybaroxic and conventional radiotherapy with the same technique and without everyone accepting the same procedures.

DR. WILDERMUTH: I am pretty skeptical of the advantage of randomizing, even in my own cases, on such a complex organism as a human being with a cancer. It is difficult enough with the controlled animal.

DR. DEL REGATO: Well, radiotherapists themselves are pretty complicated, but hyperbaric radiotherapists are more so. Dr. Bloedorn, do you care to comment?

DR. F. BLOEDORN (*Baltimore, Md.*): I would like to make some comments as a member of the audience, because I have no experience with hyperbaric oxygen in radiotherapy. I think that, even if the work presented today is excellent, it should not be taken as the final answer to the problem, but rather should be considered as preliminary work on the clinical applicability of the procedure. I agree that the results are interesting, but they confuse me when I see a mixture of good results which are sometimes better than expected with radiation alone and other times equal to or lesser than results with conventional radiation procedures. I see no serious objection as was raised here in applying randomization for future studies. However, I do object to the introduction of unusual protraction in the cases treated with hyperbaric oxygen. They represent a new variance, probably an important one, which should be studied independently. I would like to suggest that any future studies for the assessment of hyperbaric oxygen in radiotherapy be done on the basis of conventional protraction irradiation with and without oxygen. There is enough evidence today to know that such a study is ethically acceptable. There is the experience of Dr. Johnson and others applying daily treatment for 4 to 6 weeks with oxygen without untoward effect.

DR. DEL REGATO: Dr. Wildermuth, Dr. van den Brenk mentioned his observation of radioresistant tumors which became radiosensitive under hybaroxic radiotherapy. Have you seen instances of this?

DR. WILDERMUTH: Postradiation persistence?

DR. DEL REGATO: No. He mentioned that there were instances of radioresistant tumors, or reputedly radioresistant tumors, which became radiosensitive under hybaroxic radiotherapy.

DR. WILDERMUTH: Why don't you speak on that instead of me?

DR. VAN DEN BRENK: I think Dr. del Regato is trying to determine whether you have seen instances of tumors you would normally classify as radioresistant, which, when irradiated in oxygen, respond in such a way that you conclude that the results are better than you could have expected in air.

DR. DEL REGATO: Such as on the question of osteosarcoma?

DR. VAN DEN BRENK: Yes, osteosarcoma, for example.

DR. WILDERMUTH: Did you get any?

DR. VAN DEN BRENK: Yes, I did.

DR. WILDERMUTH: In what?

DR. VAN DEN BRENK: In osteosarcomas, in chondrosarcomas, certainly in melanomas, but of course there is a spectrum of radiation response in melanomas. We may consider that the secondary squamous cell nodes are radioresistant by conventional standards. This was certainly reiterated in many textbooks, and here again, I feel that the improved irradiation responses might be included in this respect.

DR. WILDERMUTH: I have instances of so-called internal control of malignant melanoma that did not respond to conventional therapy, and then, when another node was treated in oxygen, we saw complete, permanent regression. But I think we have all seen malignant melanomas that do not act the same in the same patient, so I am not so sure about even that observation as being hyperoxic-dependent.

DR. DEL REGATO: I have seen melanomas that were as radiosensitive as a lymphosarcoma without hybaroxia.

DR. MALLAMS: I think we all have already shown that when you consider volume (and one must certainly consider dose and sensitivity with volume) increased radiosensitivity has occurred in large squamous cell tumors. We are perhaps beginning to see evidence of it in certain adenocarcinomas, and certain resistant tumors in the parotid have markedly responded to radiation. *In addition, we have several patients in the sarcoma group. We obtained an excellent response in a large retroperitoneal liposarcoma that was recurrent. We have had it in lipomyosarcoma and also in fibrosarcoma, so it is hard to see a pattern. I think, however, if you see enough of them, you are going to recognize them. They are clear-cut when they occur.

DR. DEL REGATO: What are those parotid tumors that are radioresistant?

DR. MALLAMS: Adenocarcinoma.

DR. DEL REGATO: Malignant tumors of the parotid are not infrequently radiosensitive. Whether they are curable by irradiation depends on the adequacy of the treatment, but radiosensitive they are. They are not as dramatic in their regression as a squamous cell carcinoma or lymphosarcoma, but adenocarcinomas anywhere, in the endometrium, as well as in the rectum or in the parotid, are not really radioresistant.

DR. WILDERMUTH: Dr. Mallams talked me into treating a man with fibrosarcoma, post-amputation, with metastasis to the mediastinum. When the patient arrived in Seattle, however, he also had a good deal of lung metastasis. Could we overcome the difficulties in perfusing the mediastinum and the lung with oxygen by using hyperbaric oxygen? I looked at our material (we have explored this area, as I indicated earlier), but I was disappointed to see that only an occasional sarcoma responded. I remembered that our mesenchymal tumors had done fairly well, but I only remembered the good ones apparently, because when we looked them all over, only an occasional patient (something less than one in three) had a good response. Even in osteosarcoma, you often see an early response with conventional radiotherapy. What we have completely ignored here, I think, which is

important as a part of the art, is that the patient treated in hybaroxic radiotherapy certainly tolerates the treatment much better. He does not have the long continuous ordeal of 6 weeks of daily radiotherapy. If we obtained no better than conventional results but got them with the alacrity achieved by Dr. van den Brenk, it would represent an advancement in our art.

DR. MALLAMS: I would like to compliment Dr. Steinhardt on his presentation, and I agree that there is another parameter that we should study. I think the problem with this, though, is that we have too many parameters already. However, in our own experience over the last 10 years, I am now totally unimpressed with the use of pathologic specimens as a parameter to evaluate any type of a response. The only thing I know that really helps in the long run is survival without disease.

DR. JOHNSON: Yes, I would agree with Dr. Mallams. I gather from Dr. Steinhardt that the tumors initially continued to grow following hypothermia, and that later they regressed. Could this not be due to spontaneous regression?

DR. DEL REGATO: It is paradoxical, for hypothermia diminishes circulation and this diminishes metabolism; one would not expect the uptake of oxygen to be greater.

DR. WILDERMUTH: Did Dr. Steinhardt mention what the increased solubility of oxygen in water was at these temperatures?

DR. STEINHARDT: I think, in the range of hypothermia available to human beings at the present time, there is a 20 or 25% increase in solubility in physical solution.

DR. VAN DEN BRENK: I must agree with Dr. Mallams' criterion of effectiveness of tumor eradication. Certainly, in most experimental systems, one must show that the tumor actually disappears, and this is the endpoint which should be adopted. I would like to point out that when we adopt certain treatments, we must be very careful that we do not misinterpret benefits. For example, the fact that hypothermia may decrease oxygen consumption and even increase the amount of oxygen in the blood may be completely offset by other factors, such as poor blood flow and vascular spasm. This is something that we have found in experimental systems, not actually dealing with tumors but with normal tissues. It is a well-known fact that hypothermia actually causes radioprotection in the whole animal, and the main reason for this is, of course, that it produces anoxia by indirect vascular mechanisms. Similarly, if your skin is cold, there is hardly any blood flowing through it, and there is a reduction in metabolic rate. However, this is often completely offset by the fact that although some blood gets to the tissue even when high concentrations of oxygen are used, the increased pO_2 is also insufficient to overcome reduced blood flow, and the advantages which may be considered from a biochemical view to result from hypothermia or change in temperature cannot compensate for reduced blood flow.

DR. P. CAVANAUGH (*Durham, N. C.*): This question, or comment, relates to the observation of Dr. Wildermuth and Dr. Johnson that metastatic disease may grow faster and more frequently in these patients. Would one of these gentlemen comment on the possible role of this mechanism of nonspecific stress? I think surely 30 compressions might be equivalent to one car wreck.

DR. JOHNSON: It was suggested by Dr. van den Brenk that possibly stress may affect the extent or rate of growth of metastases. I think this is one area where a randomized cooperative study could, in a short period of time, yield results showing whether the extent or growth rate of metastases is affected by daily hyperbaric oxygen.

SESSION VIII

Hyperbaric Oxygenation in the Treatment of Certain Toxemias and in Tissue Preservation

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Effect of Hyperbaric Oxygen in Cyanide Poisoning

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Ivanov[1] showed that hyperbaric oxygen quickly restored normal electrical activity to the cerebra of white mice given a nearly lethal dose of cyanide. He also showed that most animals poisoned by lethal doses of potassium cyanide were saved by early and continuous inhalation of 2.5–2.8 atm of pure oxygen. Because this observation suggested that oxygen could bypass an apparently blocked metabolic pathway, we decided to repeat this work, to carry it further, and to investigate the mode of interaction of oxygen and cyanide at the cellular level.

METHODS

Experiment 1

One hundred fifty C3H mice from an inbred strain maintained in the department (average weight 26.5 gm) were divided into three groups of 50, with 25 males and 25 females in each group. Potassium cyanide, approximately 0.01 M in normal saline solution, was injected intraperitoneally in a dose of 8.5 mg/kg. The solution was prepared fresh 1 hour before use by diluting a 0.2 M stock solution of potassium cyanide in water. The stock solution was standardized before use by titration with silver nitrate.[2]

A control group (Group 1) was observed in air following cyanide administration.

Group 2 was exposed to 4 hours of oxygen at 1 ata in the following manner. The 50 mice were divided into 10 subgroups of five mice each, and the animals in each subgroup were injected with cyanide and immediately placed in a Perspex box, 1100-ml capacity, which had been flushed with oxygen. To ensure a rapid return to 100% oxygen within the box, oxygen was then administered at a flow rate of 10 liters/min for 5 min. It was reduced to 4 liters/min for the rest of the 4-hour treatment period, after which the mice were returned to air. The time interval between injection of cyanide and commencement of oxygen therapy was between 15 and 60 sec.

The animals in Group 3 were treated identically to those in Group 2, except that injection was administered in a large pressure chamber at 2 ata while the mice breathed air. The mice were then treated with oxygen at 2 ata for 4 hours in identical Perspex boxes as those used in Group 2. At the end of the treatment period, the surviving animals were returned to air and decompressed to normal atmospheric pressure over a 15-min period.

The surviving mice in Groups 1, 2, and 3 were observed for 2 weeks. Each mouse

was examined for central nervous system damage by observing general activity, gait, and performance of simple tests of agility such as climbing down a pole and crawling along a ruler edge.

Experiment 2

The endogenous respiration of rat brain homogenate was studied along with the effect of 1 ata oxygen on the oxygen consumption of homogenate poisoned by cyanide. Oxygen consumption experiments were performed on the Warburg apparatus, with reaction flasks of 12.5–16.5 ml capacity and single sidearms. Rat brain homogenate was prepared immediately before use as a suspension in Krebs-Ringer phosphate solution at pH 7.4.[3] Analytical grade chemicals were used throughout.

Calcium cyanide was prepared in the laboratory according to the method of Robbie,[4] kept refrigerated, and standardized before use by titration with silver nitrate; the appropriate center-well solution was prepared by dilution with 10% calcium hydroxide suspension. Cyanide inhibition was achieved using the method of Robbie.[5] The calcium cyanide solution in 10% calcium hydroxide suspension was placed in the center well of the reaction flask to give a constant tension of hydrogen cyanide in the gas phase and, after equilibration, a constant concentration of cyanide in the test homogenate. In the control flasks, 10% calcium hydroxide suspension was used to absorb the carbon dioxide given off during respiration.

At the end of each experiment, the cyanide concentration in the test homogenate was assayed by a modification of the phenolphthalein method of Robbie.[6]

For each experiment, two rats weighing 220–270 gm were lightly anesthetized with ether and decapitated, and the brains were rapidly removed. The brains were homogenized in 10 ml of Krebs-Ringer phosphate solution at 4°C with an M.S.E. micro-emulsifier. The resulting homogenate was brought to 40 ml with cold Krebs-Ringer solution. Then 0.6 ml of 10% calcium hydroxide suspension was added to the center wells of six flasks as controls. Six test flasks were prepared by adding 0.6 ml calcium cyanide in 10% calcium hydroxide suspension to the center wells. To each flask was added 2.5 ml homogenate, and the flasks were attached to the manometers and placed in a water bath at 37°C. Humidified oxygen was then flushed through three of the test flasks and three of the control flasks for 10 min. The gas phase in the remaining six flasks was air. All 12 flasks were then sealed, and, after a 40-min period for equilibration of the cyanide system, the oxygen consumption in each flask was measured for 60 min. The flasks were then unsealed and the six test flasks assayed for cyanide content.

This experiment was performed 12 times, varying the concentration of calcium cyanide in the center-well mixture from 0.0046 to 1.07 M to give a cyanide concentration in the test homogenate from 1×10^{-5} to 0.46×10^{-2} M.

Results

Experiment 1

The effect of hyperbaric oxygenation on cyanide poisoning in mice is summarized in Table 1, which shows the mortality in

TABLE 1. Mortality and Survival Time of Mice Poisoned with 8.5 mg/kg Potassium Cyanide and Exposed to Various Tensions of Oxygen

	1 ata air	1 ata O_2	2 ata O_2
Mortality [a]	48/50	28/50	10/50
Survival time [b] (hours)	0.2	51	31
(Range)	(0.07–0.57)	(0.25–240)	(1.0–72)

[a] Mortality in each group at the end of 2 weeks.
[b] Average survival time of the mice which died before termination of the experiment.

each group at 2 weeks and the average time of survival of the mice which died before the end of the experiment.

All 50 control animals exposed to air at normal atmospheric pressure (Group 1) lost consciousness within 30 sec of injection and convulsed; 48 became apneic after 2–6 min and no heart beat was discernible in these animals after 4–30 min. The two survivors in this group regained consciousness 50 and 60 min after injection and survived for 2 weeks, until sacrifice. Of these two, one showed minor central nervous system damage as evidenced by a loss of normal agility and change in gait. The other surviving mouse appeared normal in all respects.

In the group treated with oxygen at 1 ata for 4 hours (Group 2), 22 mice (16 females and 6 males) survived until sacrifice at 2 weeks. All mice showed the same initial reaction to cyanide as Group 1, with rapid loss of consciousness and convulsions. Four of the 50 died without regaining consciousness while receiving oxygen treatment. The remaining 46 recovered consciousness and were returned to air after the 4 hours of oxygen-breathing. Of these, 40 showed varying degrees of central nervous system damage, the signs being similar to those described in Group 1. In 24 of these 40, death occurred between 3 hours and 10 days following treatment. In nine of the 40, the central nervous system damage resolved and they appeared normal at the time of sacrifice. The remaining seven had persistent neurologic damage at the time of sacrifice. Six mice showed no neurologic signs whatsoever during the 2-week observation period.

Group 3, exposed to oxygen at 2 ata for 4 hours, had a mortality rate of only 10/50 at 2 weeks. Initial reaction to cyanide was identical to that seen in Group 2. Three mice died while breathing oxygen at 2 ata without regaining consciousness; one of these showed congestion and consolidation of the lungs similar to that seen in oxygen poisoning. The remaining 47 recovered consciousness, and 20 of these showed some signs of neurologic damage. In seven of the 20, death took place between 4 and 72 hours after treatment. In seven others, the central nervous system damage resolved and they appeared normal at the time of sacrifice. The remaining six had persistent signs of neurologic damage. Of the 40 surviving mice, 27 showed no signs of central nervous system damage at any time.

Experiment 2

Experiment 2 was performed to determine the effect of 1 ata oxygen on the respiration of rat brain homogenate poisoned by cyanide. Table 2 shows the oxygen consumption in mm^3/2.5 ml homogenate. When cyanide concentrations were greater than 0.0001 M, the oxygen consumption increased in the homogenate exposed to oxygen. The cyanide concentrations in the test flasks at the end of each experiment appear in Table 3. Figure 1 shows the oxygen consumption of the homogenate in the test flasks expressed as a percentage of the oxygen consumption of the homogenate in the control flasks and plotted against the cyanide concentration as assayed.

Discussion

The results of Experiment 1 confirm the finding of Ivanov [1] that oxygen at high pressure can protect mice from a lethal dose of cyanide. The mortality dropped from 96% in the control group to 56% in the group breathing oxygen at 1 ata, and to 20% in the group breathing oxygen at 2 ata. A marked difference was also seen in the incidence of neurologic sequelae when oxygen at 2 ata was breathed; in this group, 27 mice were free of central nervous system damage whereas only six mice in the group treated with oxygen at 1 ata showed no neurologic damage. Several authors have demonstrated the protective effect of oxygen

TABLE 2. Mean Oxygen Consumption (mm³ O_2) of Rat Brain Homogenate Poisoned with Cyanide at Various Concentrations and Exposed to Air or Oxygen

		Air				Oxygen			
		Test		Control		Test		Control	
Exp. no.	CN^- conc. (M)	mm³ O_2	SD	mm³ O_2	SD	mm³ O_2	SD	mm³ O_2	SD
6	0.00001	77.6	0.2	78.3	1.9	73.3	2.2	75.6	0.7
10	0.000022	71.1	2.4	74.1	1.8	70.3	1.7	69.7	1.3
3	0.000046	58.4	3.4	66.1	1.8	57.7	1.7	61.2	0.4
7	0.0001	48.0	1.1	64.6	3.2	52.1	3.2	60.4	1.0
4	0.00022	41.5	3.1	70.7	0.9	49.4	1.5	64.9	0.9
11	0.00046	34.6	0.3	81.4	3.4	46.5	2.3	77.9	4.9
2	0.00046	33.5	0.6	95.0	1.7	49.3	0.9	86.5	0.9
8	0.001	23.2	1.2	71.5	1.6	34.6	1.5	66.7	1.0
9	0.001	25.2	1.9	70.3	1.9	32.6	0.2	63.7	1.4
1	0.0022	15.5	1.2	117.1	3.2	30.7	1.3	106.1	1.7
12	0.0022	4.8	1.2	81.7	2.5	14.0	0.1	80.9	0.1
5	0.0046	6.2	0.3	79.9	1.6	11.4	0.3	70.8	1.4

SD, standard deviation.
[a] Each value represents the average of readings from three flasks.

at 1 ata in cyanide poisoning whether the cyanide was inhaled as hydrogen cyanide gas or injected as a cyanide salt.[7]

The mechanism of the protective effect of oxygen is still obscure but it is evident that the protective effect is a function of the tension at which oxygen is delivered to the tissues, as there is no decrease in the oxygen-carrying capacity of the blood. The mode of action of cyanide as a poison is the reversible inhibition of the respiratory enzyme cytochrome oxidase according to the reaction:

$$\text{cytochrome oxidase} + CN^- \rightleftharpoons \text{cytochrome oxidase cyanide}$$

Inhibition of this widely distributed enzyme produces a generalized inhibition

TABLE 3. Mean Molar Concentration of Cyanide Assayed in Homogenate in Test Flasks Compared to Value Predicted by Robbie[4]

Exp. no.	CN^- conc. (M) (Robbie)	Air CN^- conc. (M)	Oxygen CN^- conc. (M)
6	0.00001	—	—
10	0.000022	—	—
3	0.000046	—	—
7	0.0001	0.00017	0.00017
4	0.00022	0.00032	0.00038
11	0.00046	0.00079	0.00054
2	0.00046	0.00089	0.00077
8	0.001	0.0015	0.0014
9	0.001	0.0015	0.0014
1	0.0022	0.0028	0.0026
12	0.0022	0.0033	0.0032
5	0.0046	0.0074	0.0072

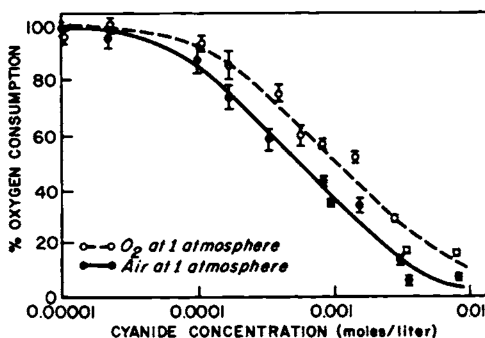

FIGURE 1. Oxygen consumption of cyanide-poisoned rat brain homogenate, exposed to 1 ata air or oxygen, expressed as a percentage of the oxygen consumption of control homogenate and plotted against the cyanide concentration as assayed.

of cellular respiration (so-called "histotoxic anoxia"). The clinical effects are unconsciousness, anoxic convulsions, apnea from poisoning of the respiratory center, and, finally, cardiac failure (as shown in Experiment 1).

In 1931, Barcroft [8] showed that cyanide is not a cumulative poison; it is detoxified within the organism by conversion to a nontoxic thiocyanate under the influence of the enzyme rhodanase (thiosulfate transsulfurase) according to the reaction:

thiosulfate + cyanide→sulfite + thiocyanate

Provided that the rate of detoxification is greater than or equal to the rate of absorption of cyanide, poisoning does not occur. If cyanide absorption exceeds the rate of detoxification, however, poisoning takes place, the time to death depending on the difference between the absorption and detoxification rates. Barcroft was of the opinion that oxygen exerted its therapeutic effect by increasing the rate of detoxification, but he gave no experimental evidence to support this hypothesis.

If oxygen at increased tension has no effect on the rate of cyanide detoxification, it is possible that it exerts its effect by disturbing the equilibrium between cytochrome oxidase + CN^- and cytochrome oxidase cyanide, driving the reaction to the left and freeing increased amounts of cytochrome oxidase for the continuance of cellular respiration. Cytochrome oxidase is also inhibited by carbon monoxide, the degree of inhibition depending on the ratio of the partial pressures of carbon monoxide and oxygen. (Carbon monoxide combines more stably with the reduced form of cytochrome oxidase.) Unlike carbon monoxide, cyanide combines more stably with the oxidized form of cytochrome oxidase.[9] On this evidence, it seems unlikely that high pressures of oxygen exert their effect in cyanide poisoning by competing with cyanide at a receptor site in cytochrome oxidase.

A third possible explanation of the protective effect of oxygen in cyanide poisoning is that sufficient cellular respiration continues by some cyanide-insensitive pathway to enable continuance of life until the cyanide has been detoxified. Paulet [10] showed that oxygen at 1 ata had some protective effect in dogs poisoned with intravenous sodium cyanide, and he concluded that cyanide-insensitive respiration was the mode of action of oxygen; he gave no experimental proof to support his hypothesis, however. Zelitch [11] demonstrated a cyanide-insensitive respiratory system in spinach leaves involving the oxidation and reduction of glycolic and glyoxilic acids with peroxide as an end-point. Dixon [9] pointed out that tissues low in cytochrome *c* have been observed to contain relatively more peroxidase, the enzyme acting on hydrogen peroxide.

No definite conclusion can be drawn from our results regarding the mode of action of oxygen at high pressure in the treatment of cyanide poisoning; further studies must be undertaken to elucidate this question.

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Oxygen at High Pressure in Acute Nitrogen Tetroxide–Nitrogen Dioxide Poisoning

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Acute pulmonary edema induced by exposure to inhaled nitrogen dioxide (NO_2) causes not only hemoconcentration and decreased circulating blood volume,[1,2] but also impairment of ventilatory gas exchange.[1,3,4] Using white mice, Boren[5] demonstrated a decreased mortality when the animals were placed in 100% oxygen at atmospheric pressure after NO_2 exposure. Oxygen has been used with varying success as a part of the therapy of NO_2 poisoning in men.[6–9] Moskowitz *et al.*[10] noted no improvement in oxygen saturation of arterial blood when his patient was given 100% oxygen at ambient pressure. Nichols[2] commented on the ineffectiveness of oxygen in relieving cyanosis when it was given by nasal catheter to the victims of NO_2 inhalation in the Cleveland Clinic fire. He stated that oxygen given by mask or tent appeared to be of benefit. Grayson[11] suggested that oxygen at high pressure (OHP) might be beneficial during the period of pulmonary edema.

The pulmonary syndrome of the newborn is remotely similar to this disorder in that there is a diffusion defect of the lung. OHP has been investigated in these cases,[12,13] but the results have been somewhat discouraging. Experimental kerosene pneumonitis has been treated with OHP by Schwartz *et al.*[14] Haldane *et al.*[15] used OHP in treatment of nitrite poisoning in mice and rabbits.

This report describes our experiments conducted because of the need for information concerning the efficacy of oxygen at high pressure in increasing survival chances in cases of acute pulmonary edema developing after exposure to vapors of the rocket propellant oxidizer nitrogen tetroxide (N_2O_4). This substance is in equilibrium with nitrogen dioxide (NO_2), and concentrations of the mixture are customarily expressed in terms of NO_2.

The report consists of two parts, in which both NO_2 and OHP, as well as a combination of the two, were used. The first presents a simple lethality experiment using mice as the biological indicator. The second reports experiments with dogs, in which the packed-cell volume, the hemoglobin concentration, the oxygen tension, the carbon dioxide tension, the pH, the base deficit of arterial blood, and the incidence of convulsions were observed.

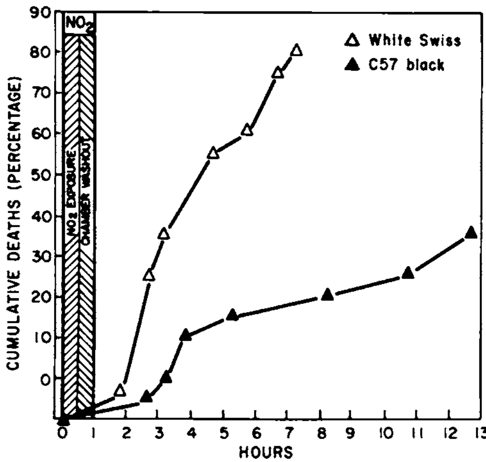

FIGURE 1. Mortality (percent) in 40 mice exposed to NO_2 (164 ppm).

NO_2 Vapor Inhalation in Mice

Preliminary experiments demonstrated a difference in the susceptibility of the white Swiss and C57 black strains of mice to the lethal effect of NO_2 (Figure 1). This difference was seen not only in acute lethality but also when animals were kept under observation for several weeks after the exposure.

The influence of total atmospheric pressure and partial pressure of oxygen on survival in the two strains was observed.

Methods

Mice were randomly divided into groups of 30, each group was placed in a wire cage, and treatment was given as outlined in Table 1. The eight cages containing mice for NO_2 exposure were placed in a stainless-steel dynamic-flow gassing chamber in which 99% of the equilibrium concentration of the NO_2 is reached in about 12 min. The time of gassing was 30 min, followed by a 30-min chamber washout period. Soon after removal from the gassing chamber, the survivors, as well as their counterparts that had not received NO_2, were placed either in air at ambient pressure, in a plastic box equipped for use as an oxygen tent at 1 atm, or in the USAFSAM high–low pressure chamber (previously described[16]) used for compression with air or with 100% oxygen.

TABLE 1. Treatment Groups of Mice for NO_2-Exposure Studies

Treatment	No. animals exposed			
	Air 1 atm	O_2 1 atm	Air 3 atm *	O_2 3 atm *
No NO_2 (controls)	30	30	30	30
NO_2 105 ppm	30	30	30	30

* High pressure treatment was given for 2 hours.

Of the latter group, those that were to receive OHP were placed in a separate small chamber contained within the larger pressure chamber. The small chamber was flooded with oxygen throughout the periods of pressurizing, holding at pressure for 2 hours, and depressurizing.

The mice were pressurized twice. An interval of 6 hours at 1 atm elapsed between the two periods of pressure exposure. The animals receiving oxygen at ambient pressure were kept in the tent for a period corresponding to the time from the beginning of the first period of pressurizing to the end of the second for the animals receiving OHP (11 hours). Deaths were recorded immediately after removing the animals from NO_2, immediately before putting them under pressure, and at 30-min intervals thereafter for 12 additional hours, except when the animals were under pressure. Observations were made at 3-hour intervals for the remainder of the 24 hours.

Results

Many of the mice exhibited convulsions, particularly those previously exposed to NO_2. All mice that died after having been exposed to NO_2 had gross pulmonary pathology typical of acute NO_2 poisoning.[5,17] In the few mice that died after receiving only OHP, gross pulmonary pathology was minimal or at least considerably less spectacular than the

classical picture[18-20] in animals exposed somewhat longer.

Figure 2 shows that 2–25% of the white mice died during the period of exposure to 105 parts per million (ppm) NO_2. The treatment with OHP did not decrease the mortality below that occurring in animals given other treatments nor, indeed, below that in the mice merely kept in room air at normal pressure. In fact, it appears that the initial treatment with OHP led to slightly greater mortality, which may have been an additive effect of OHP alone. Note the deaths occurring in the white mice that received only OHP. The results of this experiment also indicated that abrupt removal of NO_2-exposed animals from supplementary oxygen, such as supplied in an oxygen tent, might increase the probability of subsequent death.

Figure 3 shows that although 105 ppm NO_2, when breathed for 30 min, was not lethal by itself to C57 black mice, treating the exposed animals with OHP caused significant mortality.

Effects on Blood Constituents and Occurrence of Convulsions

Concurrent with the lethality experiments with mice, other work was done to gather information on the effects on constituents of blood. The specific effects of OHP might be strikingly apparent in indicators of circulating blood loss (such as packed-cell volume and hemoglobin concentration), of respiratory gas exchange (arterial pO_2 and pCO_2), or of acidosis (arterial pH and base excess or deficit). In these experiments, we planned to use a standard dosage of NO_2 which would produce an unequivocal pulmonary edema and which would be fatal to 50% of the animals within 24 hours. We did not succeed in this simple design because of the large variation in susceptibility to NO_2 among mongrel dogs. In retrospect, the pronounced and consistent differences in susceptibility between two purebred strains of mice should have made wide differences in individual susceptibility in a randomly bred population entirely expected.

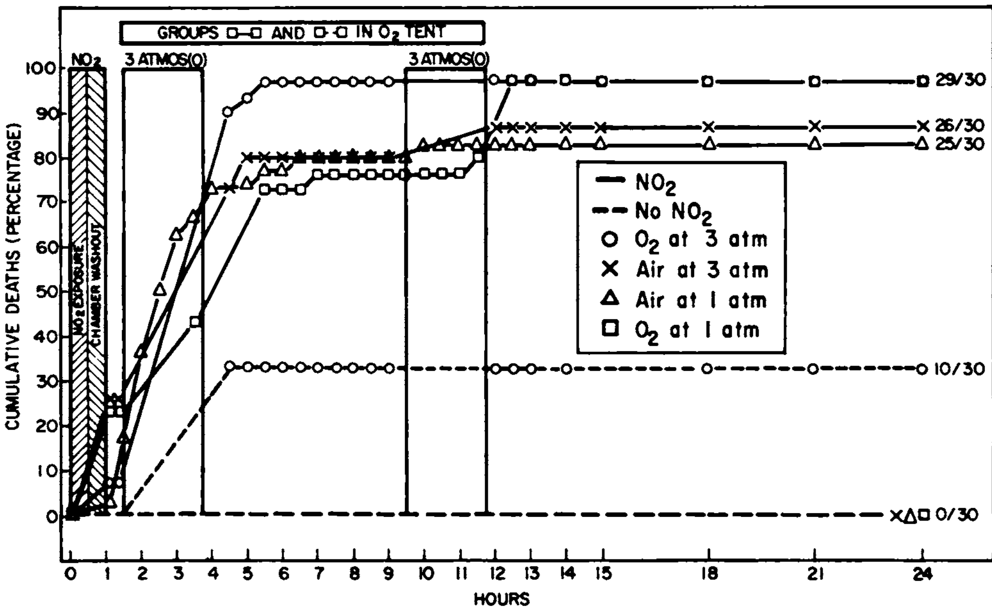

Figure 2. Influence of various treatments on mortality of white Swiss mice exposed to NO_2 (105 ppm).

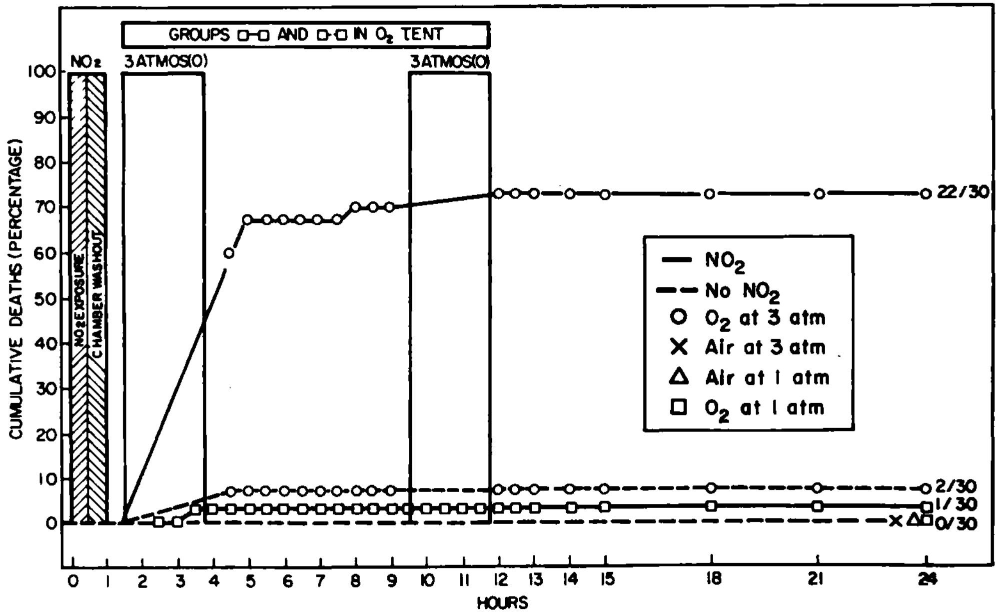

FIGURE 3. Influence of various treatments on mortality of C57 black mice exposed to NO_2 (105 ppm).

Methods

Five individual dogs were given OHP only, and 10 matched pairs were used for NO_2 exposure. On the day before OHP treatment or gassing, a sterile catheter for blood sampling was placed aseptically under thiopental anesthesia in a femoral artery. Since the desired effect of exposure to NO_2 was appearance of acute pulmonary edema, stethoscope microphones were secured to both sides of the chest of the conscious dog to permit remote auscultation. Exposure to NO_2 (172–225 ppm for 15 min/hour) was terminated promptly for a given dog when moist rales were unequivocally audible.

Gassing of the other dog of the pair continued until it also developed these same signs. A double-blind procedure was used for selecting which dog of the pair would receive OHP and which would be kept on air at normal pressure. Samples of blood were collected anaerobically within the hour before placing the dogs in the NO_2 exposure chamber. A second sample was collected immediately upon removing the animal from the NO_2, after which the dog was subjected to oxygen at 3 ata for 2 hours. During this 2-hour period, third and fourth samples were collected in the pressure chamber after 30 min and 90 min had elapsed. Subsequent samples were collected within 30 min after decompression and at 1, 2, 7, and 14 days thereafter. At the same time periods, samples were drawn from the animals given no OHP. Hemoglobin was determined as cyanmethemoglobin; pH and arterial pO_2 and pCO_2 were determined on an Instumentation Laboratories apparatus. Base deficit was derived using the nomogram of Siggaard Andersen and Engel.[21]

Results and Discussion

Table 2 shows not only the experimental protocol but also the numbers of survivors at each of the periods in which blood samples were collected. There appeared to be a prolongation of survival during the period immediately following NO_2 exposure in animals given OHP, on the

TABLE 2. Experimental Schedule Showing Dogs Surviving NO_2 Exposure at Each Period When Blood Samples Were Collected

Treatment	No. dogs	Survivors								
			Day 2							
				O_2 at 3 ata [c] 2 hours						
		Day 1 [a]	NO_2–N_2O_4 exposure, then back to rm. air [b]	½ hr	1½ hr	Back to rm. air	Day 3	Day 4 [d]	Day 7	Day 14
NO_2 + OHP	10	10	10	10	10	7	3	3	3	3
NO_2 alone	10	10	9	8 [c]	7 [c]	7	5	4	4	4
OHP alone	5	5	— [b]	5	5	5	5	—	—	5

[a] On Day 1, femoral artery cannulation was done and base-line blood samples collected.
[b] NO_2–N_2O_4 exposure was given only to animals in the first two treatment groups.
[c] OHP exposure was given only to animals in the first and third treatment groups, but samples were drawn at the same intervals in room air in the second group (NO_2 alone).
[d] On Day 4, the femoral artery cannula was removed from dogs in the first two treatment groups.

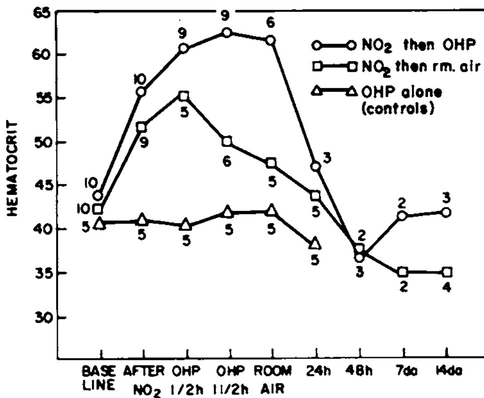

FIGURE 4. Hematocrit of dogs surviving at each period after having developed pulmonary edema while breathing NO_2 (172–225 ppm) at room temperature and pressure. Number at each point on the curves is number of animals surviving.

basis of this small population. Two of the dogs alive at the 90-min OHP sampling period died shortly thereafter, while still under OHP; another died during depressurizing. In any event, the eventual lethal outcome appeared unaltered by OHP.

Audible rales, dyspnea, and hemoconcentration all confirmed the presence of pulmonary edema after the NO_2 exposure. Figure 4 shows that OHP itself did not give rise to an increased hematocrit. Nor, on the basis of the results, can it be stated that OHP intensified the hemoconcentration caused by NO_2. These data might just as logically be interpreted as showing that OHP allowed increased survival times of animals that probably would have died early if merely kept in room air.

There is no doubt that a significant inverse correlation exists between increased hematocrit and survival following NO_2 exposure. This relationship holds whether or not the animals received OHP. For instance, Figure 5 illustrates the hematocrits of surviving and nonsurviving dogs kept in room air after developing pulmonary edema. Note that, with the exception of dog P45, animals whose post-exposure hematocrits reached 55–60% had a poor prognosis for survival.

Figure 6 is the same type of presentation, except that these dogs received OHP. Again, there was a relationship between high hematocrit and death. Notice, however, that two of the three survivors transiently experienced a hemoconcentration sufficient to produce hematocrits of approximately 60%. The use of hematocrit and also pH, as will be shown later, would probably have been a better indicator of the severity of the NO_2 damage than pulmonary edema. Many of the animals died early, so that the pairing attempt was essentially futile.

There is no doubt that effective alveolar ventilation-perfusion was disturbed by the NO_2. As shown in Figure 7, the exposure to NO_2 decreased arterial pO_2 immediately from 80–90 mm Hg to 40–50 mm Hg. In the animals which survived that were kept on room air, the arterial pO_2 gradually improved over the next few hours. However, even after 24 hours the partial pressure was still quite low, returning to normal within the following week as the chest sounds and general clinical impression indicated progressive recovery.

Dogs given OHP may be separated into three groups. First, those that received only OHP attained an arterial pO_2 on the order of 1750 mm Hg, which decreased to base-line values immediately after decompression. Second, those that received NO_2 and survived attained an arterial pO_2 on the order of 1450 mm Hg during OHP. Immediately upon removal from OHP, the arterial pO_2 of this group decreased to, and subsequently followed the same pathway as, the arterial pO_2 of the survivors which had been kept continuously in room air. One can infer that, in this case, the hyperbaric excursion was merely a side trip for animals that probably would have survived without OHP. Third, those that received NO_2 and did not survive attained an arterial pO_2 of only about 750 mm Hg during OHP. Two of the seven animals in this group exhibited massive pulmonary edema and respiratory failure during the last hour of

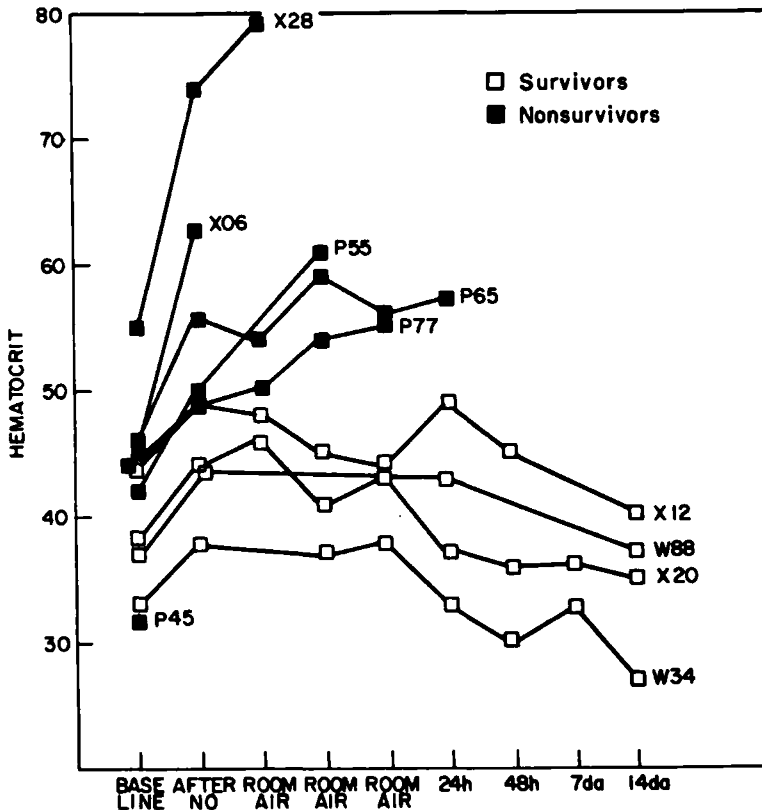

FIGURE 5. Hematocrit values of dogs either surviving or dying after developing pulmonary edema from exposure to NO_2. Animals were kept in air at normal atmospheric pressure after exposure to NO_2.

OHP, and their arterial pO_2 dropped to 63 and 158 mm Hg just before death. Immediately after being depressurized, three of the remaining five members of the nonsurviving group exhibited an arterial pO_2 of only 25–30 mm Hg and died within minutes. The remaining two animals exhibited an arterial pO_2 of approximately 50 mm Hg after decompression. These died within 24 hours.

It is obvious that alveolar capillary gas exchange was impaired by NO_2 and that OHP sustained some animals that would have died much earlier otherwise. Certainly, the dramatic respiratory arrest and death in three of these animals immediately after depressurization implies that a "crutch" had been removed. Other deviations from normal base-line values occurred under OHP, which are reasons for proceeding cautiously in interpreting the efficacy of this therapy in NO_2-induced pulmonary edema.

Figure 8 shows that the impeded respiratory gas exchange induced by NO_2 produced a moderate elevation in arterial pCO_2. In animals subsequently kept in room air, this soon returned to approximate base-line values. In animals placed in OHP, there was apparently a further elevation of pCO_2. In the survivors, pCO_2 decreased immediately upon removal from OHP, but in animals which did not survive, the pCO_2 increment was greater, on the average, and did not decrease after they were removed from OHP.

As might be expected, arterial pH showed changes more or less reflecting the changes in pCO_2 (Figure 9). The acidosis

FIGURE 6. Hematocrit values of dogs either surviving or dying after developing pulmonary edema from exposure to NO_2.

FIGURE 7. Arterial pO_2 of dogs that developed pulmonary edema while breathing NO_2 and were then exposed to OHP or air at normal atmospheric pressure. Numbers in parentheses are numbers of animals remaining at each point.

was not due entirely to CO_2 retention, however. There was a perceptible metabolic component which, in the animals kept in room air, tended to correct itself, or at least got no more severe, even in animals that subsequently died (Figure 10). Placing the edematous animals in OHP allowed the "base deficit" to intensify, however. Since OHP alone did not cause a metabolic acidosis, we cannot yet explain its appearance or its intensification in animals exposed to OHP after exposure to NO_2. One reasonable hypothesis, of course, is that OHP increased peripheral resistance to the flow of the highly concentrated blood to the degree that tissue hypoxia occurred. Another hypothesis is that the site of systemic toxicity is the same for NO_2 and OHP.

Table 3 shows the incidence of convulsions in the 25 dogs. The most striking feature of the seizures in dogs exposed to NO_2 and OHP was the short time interval preceding their onset.

TABLE 3. Incidence and Time of Convulsions in Dogs Receiving OHP Treatment

Treatment	No. dogs	No. convulsing	Onset time of seizures
NO_2 + OHP	10	4	14–110 min[a]
OHP alone	5	1	115 min
NO_2 alone	10	0	—

[a] One dog had seizures at 20, 49, and 110 min; the other three animals had single seizures.

SUMMARY

1. OHP treatment exerted a detrimental effect on survival of C57 black mice which had breathed an equilibrium mixture of nitrogen tetroxide and nitrogen dioxide.

2. Neither oxygen at high pressure nor oxygen at normal pressure was superior

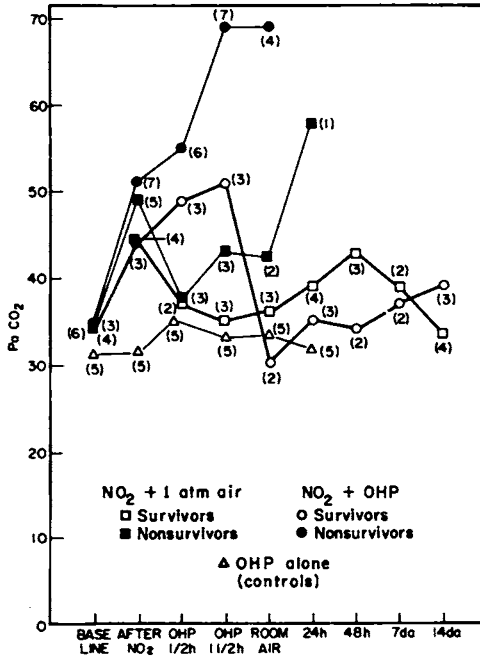

FIGURE 8. Arterial pCO_2 of dogs that developed pulmonary edema while breathing NO_2 and were then exposed to OHP or air at normal atmospheric pressure. Numbers in parentheses are numbers of animals remaining at each point.

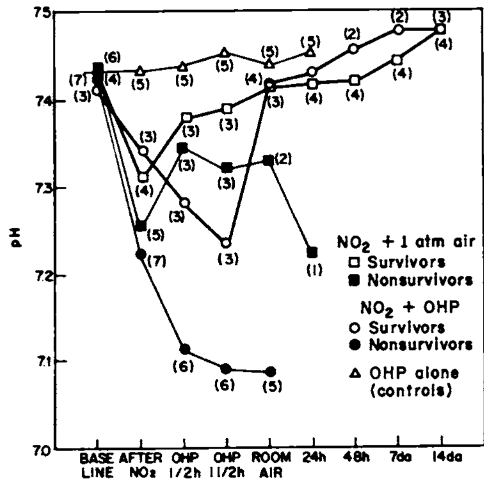

FIGURE 9. Arterial pH of dogs that developed pulmonary edema while breathing NO_2 and were then exposed to OHP or air at normal atmospheric pressure. Numbers in parentheses are numbers of animals remaining at each point.

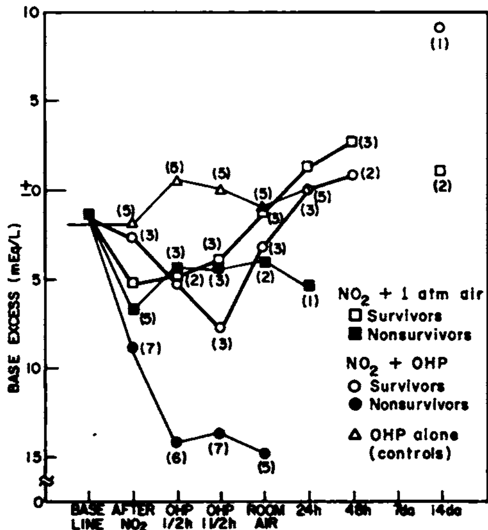

FIGURE 10. Base excess in arterial blood of dogs that developed pulmonary edema while breathing NO_2 and were then exposed to OHP or air at normal atmospheric pressure. Numbers in parentheses are numbers of animals remaining at each point.

to room air in influencing survival in white Swiss mice exposed to the mixture of nitrogen tetroxide and dioxide.

3. OHP treatment appeared to delay death in dogs that developed acute pulmonary edema from nitrogen tetroxide-dioxide exposure; however, the overall mortality was not decreased.

4. Several questions involving tissue perfusion, ventilatory control, prevention of metabolic acidosis, and the interaction of NO_2 and OHP in these *in vivo* studies must be investigated before a meaningful recommendation can be made concerning OHP or other forms of oxygen administration in NO_2 poisoning.

5. The OHP temporarily corrected the hypoxic element of NO_2 poisoning in dogs, but did not alleviate the carbon dioxide retention or the respiratory acidotic and metabolic acidotic components.

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DISCUSSION

Discussion of papers by Skene et al. *(pp. 705–710) and Reeves* et al. *(pp. 711–720).*

DR. F. DICKENS (*London, England*): I was interested in Dr. Skene's paper and I congratulate him on such a clear-cut result, but I was doubtful about his interpretation. It is a pity that Britton Chance is not still with us, as he would have certainly been able to say definitely whether there is likely to be competition between oxygen and cyanide for cytochrome oxidase. My own view is that this is rather improbable because of the high affinity for cyanide. The other explanation suggested was that there might be a cyanide-insensitive pathway revealed by the high oxygen in the brain tissue, and I think that this again is not very probable. The evidence for a cyanide-insensitive pathway, which was put forward some years ago, was based on measurements in phosphate buffer. A good many years ago Otto Warburg showed that, if you used a bicarbonate buffer where the pH was better maintained inside the cells and where hydrogen cyanide was not removed by potassium hydroxide used as carbon dioxide absorbent, tissue respiration was practically 100% cyanide-sensitive. I wonder whether Dr. Skene and his colleagues have considered whether cyanide might be removed during the exposure to oxygen, perhaps by oxidation to cyanate or by combination with a keto-acid, since we and others have produced some evidence that deficient oxidation of keto-acids, as produced in carbohydrate metabolism, might be caused by oxygen toxicity.

DR. J. N. NORMAN: I did not conclude that there was a mass-action type of effect. In fact, your suggestion that cyanide metabolizes to thiocyanate in some way is the line of investigation which we are hoping to pursue.

DR. C. HITCHCOCK, *Session Chairman* (*Minneapolis, Minn.*): Dr. McIver, when you were discussing nitrogen dioxide poisoning, were you referring to what we clinically recognize as "silo-filler's disease"?

DR. R. G. MCIVER: Silo-filler's disease is brought about by nitrogen dioxide inhalation. It is actually a chronic disorder of slow onset which occurs from multiple exposures to extremely low dosages of nitrogen dioxide, and it is more of a fibrous obliterative disorder than the acute caustic thing which we produced. We have tried to induce silo-filler's disease in animals, but it is very difficult to do.

DR. HITCHCOCK: Have you tried to induce that disease in primates? It might be worthwhile.

UNIDENTIFIED SPEAKER: I was of the impression that silo-filler's disease was a pneumoconiosis associated with dust. We are now talking about farm silos, rather than aircraft silos, are we not?

DR. MCIVER: Silo-filler's disease is caused by inhalation of nitrogen dioxide fumes, a product that is found in both farm silos and missile silos.

UNIDENTIFIED SPEAKER: We had an interesting experience that would be pertinent to chambers. A power line in a tunnel went out and burned about 18 inches of insulation from a heavy power line about 3 inches in diameter. Some rather sophisticated gases are produced by this, and they create problems similar to yours. In this case, 10 men developed severe pulmonary edema. We gave them oxygen, and they all recovered.

DR. MCIVER: I was hoping someone would mention clinical treatment methods for this type of disorder. We have produced a disorder in animals that apparently does worse with OHP than with the unsatisfactory treatment that we formerly had. It may be that

if we can control the pCO_2 and acidosis oxygen may be beneficial. We plan to repeat these studies and try to control the acidotic factor.

UNIDENTIFIED SPEAKER: We have been treating severe burns with hyperbaric oxygen and have found that patients who have inhaled the hot products of combustion are quite uninfluenced by this method. In fact, our mortality is exactly the same as in those patients who have burns around the mouth. They go into acute pulmonary edema and die in a very short time.

DR. HITCHCOCK: In other words, in your experience, hyperbaric oxygen has not helped the pulmonary problem secondary to inhalation of hot gas?

UNIDENTIFIED SPEAKER: That is correct.

Harvesting of Human Cadaver Kidneys Under Hyperbaric Conditions

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If homograft rejection were to be solved today, obtaining enough donor organs of good quality would still remain a problem. In the case of paired organs with excess reserve function, such as the kidney, living human donors may be used and the ischemic time for the donor organ can be minimized. However, it is always undesirable to jeopardize the future health of a live donor, and organs such as the lung cannot be removed without some residual crippling. In the future, when single organs such as the heart, liver, and pancreas are transplanted, human cadavers must be used as the source unless heterografts or artificial organs become feasible.

Hypothermia as a method for preserving living tissues has been studied intensively for the past two decades. In 1959, Levy [1] showed that hypothermia reduced oxidative metabolism of the kidney and that the effect was reversible. He also found a reduction in the arteriovenous oxygen difference, approximately proportional to the temperature decline. Hypothermia alone has been shown to protect the canine kidney during several hours of extracorporeal storage.[2-7] After reimplantation of the cooled kidneys, stored extracorporeally up to 22 hours,[3,4] function is satisfactory and the animal survives for prolonged periods following contralateral nephrectomy. Couch *et al.*[8] stated that, when cold perfusion of the kidney was not utilized in homograft and isograft kidneys from living human donors, acute tubular necrosis occurred in 33% of the cases. In contrast, when homograft and isograft kidneys from human living donors were protected by cold perfusion, function was much better and no kidney developed acute tubular necrosis.

Kiser *et al.*[9] developed an effective perfusate for dog, baboon, and human kidney perfusion which protected the organ during prolonged extracorporeal storage at 6–10°C. To 500 ml of low molecular weight dextran (Rheomacrodex), they added 30 mg of sodium heparin and 50 ml of 1% procaine hydrochloride. The perfusate was gently washed through the kidney by means of a polyethylene cannula inserted into the severed renal artery, and, with a pressure not exceeding 165 mm Hg, the flow was continued until the renal vein effluent was clear and the temperature of the renal

parenchyma had been reduced to 10–15°C.

Telander[10] demonstrated that the baboon and sheep kidney tolerated up to 7 hours of extracorporeal normothermic perfusion; after reimplantation of the perfused kidney, function was satisfactory, permitting contralateral nephrectomy and subsequent survival. Manax *et al.*[11] found that, with hypothermia of 6–10°C and hyperbaric oxygen at 3 ata, the canine kidney tolerated 24 hours of extracorporeal storage. These kidneys functioned after reimplantation, and the dogs survived contralateral nephrectomy performed 1–4 weeks later. These authors stated that hypothermia alone, or normothermic hyperbaric oxygenation at 3 ata for 24 hours alone, failed to protect the dog kidney and that it did not function following reimplantation. Marchioro[12] was disappointed in the function of human renal homografts obtained from cadavers when pump oxygenator support and hypothermia were used during the harvesting of the kidney under normal atmospheric conditions.

Ideally, a cadaver should furnish more than one good organ for homotransplantation. In the future, better means of organ storage must be developed, using methods such as cooling, hyperbaric oxygenation, prolonged organ perfusion, or a combination of these. Theoretically, perfusing a hypothermic cadaver with a pump oxygenator during harvesting and providing the hypothermic organs with an additional oxygen supply during the ischemic period of transplantation should protect the cells. This method forms the basis for this report. The techniques for harvesting human cadaver kidneys in the hyperbaric chamber and evaluation of the early function following homotransplantation are reported. Immediately after death, five donors were operated upon by this method, and one kidney from each was transplanted to recipient human patients with chronic glomerulonephritis.

Procedures

Upon death of the donor (each of whom was an accident victim), heparin (3 mg/kg) was injected intravenously and the body was taken into the hyperbaric chamber. In the first two cases, closed- or open-heart massage and assisted mechanical ventilation were used during transfer of the deceased accident victim to the chamber, during the preparation of the body for surgery, and during the harvesting of the kidney. In the three subsequent cases, however, the attending physician first declared each of the patients officially dead and the body was then transferred to the chamber without cardiorespiratory support.

During pressurization of the chamber to 3 ata, the anterior surface of the body was prepared for aseptic surgery. Both common femoral veins were cannulated and used to return blood to the bubble oxygenator. One femoral artery was cannulated for perfusion of the cadaver from the pump oxygenator; the opposite common femoral artery was clamped. The pump oxygenator was primed with low molecular weight dextran and dextrose in water. Extracorporeal circulation was started and the cadaver cooled by means of a heat exchanger in the arterial line.

The abdomen was opened with a midline incision extending into the left chest, and the aorta was clamped just above the diaphragm. The kidney was harvested by the technique described by Marchioro.[12] When the kidney was removed, it was immediately perfused with 500 ml of low molecular weight dextran which had been cooled to 4°C and contained heparin and procaine. During the harvesting procedure, 100% oxygen had been bubbled through this perfusate. A physician who could be rapidly decompressed then took the cooled kidney through the chamber lock to the recipient's operating room which was at 1 ata of pressure.

Each kidney was transplanted into the right iliac fossa of a recipient, using an

end-to-side anastomosis of the renal artery to the external iliac artery and an end-to-side anastomosis of the renal vein to the external iliac vein. The ureter was implanted into the urinary bladder as a neocystostomy. Bilateral nephrectomy and splenectomy were also performed on each recipient during the implantation operation.

Immunosuppressive therapy in the recipients consisted of administration of azathioprine (Imuran), prednisone, and cobalt irradiation to the homografted kidney. The transplantation data on the kidneys harvested from the five human cadavers are listed in Table 1.

Case 1. The first cadaver donor was a 4-year-old boy admitted unconscious after being struck by a car. He had a left pneumothorax, multiple fractures, and a severe head injury with a right basilar skull fracture. Bilateral trephination was done for localizing signs, which revealed a moderate subdural hematoma on the right and a minimal hematoma on the left. Supportive therapy included steroids, hypothermia, and fluid restriction. The hospital course was characterized by the development of decerebrate rigidity, papilledema, irregular respirations, dilated fixed pupils, and no response to pain.

Seven days after injury, the patient developed hypotension which required vasopressors and apnea which necessitated constant ventilatory support via a tracheostomy tube. Respiratory support was continued for 12 hours, at which time the attending physicians decided that it should be discontinued. Electrocardiographic wave activity became flat 32 min later.

The lungs were ventilated with 100% oxygen through the endotracheal tube, external cardiac massage was started, and the chamber was pressurized to 3 ata. Both kidneys and ureters were removed with a segment of the aorta and vena cava. An aortic pO_2 measured during the procedure was 2052 mm Hg.

The recipient was a 40-year-old white man. The kidneys were implanted in the right iliac fossa in a tandem manner. The time from death until the kidneys were revascularized was 3 hours and 23 min. There was no urine output for 2 hours. For the next 6 hours, urine output was scanty due to hypovolemia; when corrected, output rose to 200 ml/hour. The urine flow remained at a satisfactory level of 1500–3000 ml/day. The blood urea nitrogen was maintained in the range of 34–50 mg%, and the creatinine remained normal except during a period of rejection. The creatinine clearance rose from 8 ml/min immediately after transplantation to a high of 55 ml/min at 3 weeks.

Case 2. The second donor was a 27-year-old white man who fell 70 ft and was admitted to the hospital comatose and areflexic with a dilated fixed right pupil. Bilateral trephination revealed only cerebral edema. Episodes of hypotension responded to vasopressors. Apnea required ventilatory assistance via a tracheostomy. On the third day after injury, cardiac arrest occurred which did not respond to external cardiac massage or to intracardiac and intravenous drugs. External cardiac massage was continued for 1 hour on the ward and then for 15 min in the chamber while the body was prepared for aseptic removal of the left kidney. Ventilation with 100% oxygen was maintained through the tracheostomy tube, and aseptic internal cardiac massage was started. Cooling to 31°C was accomplished by ice bags placed beneath the cadaver. The pO_2 in the aortic blood was 1840 mm Hg during the procedure.

After removal, the left kidney did not perfuse well with low molecular weight dextran. The right kidney was removed and perfusion was satisfactory. The total time from death to revascularization was 4 hours and 31 min. Urine output commenced 24 min after revascularization in the recipient, a 29-year-old man. When the recipient was moved from the operating table to the recovery room cart, however, cardiac arrest occurred which did not respond to external and internal cardiac massage and drug therapy.

Case 3. The donor was a 21-year-old white man who was struck by a car and was semicomatose on admission to the hospital. Papilledema developed, and after bilateral carotid angiograms were done the patient developed apnea, bilaterally dilated fixed pupils, and areflexia. Ventilation was ac-

TABLE 1. Human Kidneys Harvested from Five Cadavers

Case no.	Donor	Recipient	Death until cardioresp. support begun (min)	Kidney harvest (min)	Perfusion transf. to recipient (min)	Implantation (min)	Total time (min)	Start urine output (min)
1	R.P., male, age 4, 18 kg, type O+	L.O., male, age 40, 59 kg, type A+	32	106[a] (30°C)	7	58	203	120
2	M.L., male, age 27, 82 kg, type O+	H.B., male, age 29, 55 kg, type O+	75[a]	145[a] (31°C)	11	40	271	24
3	D.P., male, age 21, 75 kg, type O+	A.L., male, age 27, 82 kg, type A+	50	48[b] (31°C)	14	56	168	5
4	T.D., male, age 4, 15 kg, type O+	P.D., male, age 24, 66 kg, type O+	105[c]	134[b] (10°C)	15	58	312	6
5	E.J., male, age 19, 75 kg, type B—	D.W., female, age 24, 68 kg, type B+	33	93[b] (20°C)	18	50	194	6

[a] Closed-heart massage and ventilation with oxygen.
[b] On pump oxygenator.
[c] Ventilation with oxygen.

complished on a respirator through a tracheostomy tube. The angiograms were negative, and a posterior fossa hematoma was suspected. A posterior craniotomy was negative and a laminectomy of C-2, C-3, and C-4 was done. The neurologic status remained unchanged for 3 days. A drop in the systolic blood pressure to 45 mm Hg required a vasopressor drip. The attending physicians felt that further cardiorespiratory support was not indicated.

The donor was taken to the chamber, pressurization was started, and support by respirator was discontinued. There was no respiratory activity, but electrocardiographic activity continued for 50 min. After 40 min of apnea at 3 ata, the femoral artery blood had a pO_2 of 36 mm Hg, a pCO_2 of over 100 mm Hg, and a pH of 6.93 (Table 2). The left kidney was removed during hypothermic extracorporeal perfusion. The changes in the systemic arterial blood gases are shown in Table 2.

Urine output started 5 min after revascularization of the donor kidney in the recipient (a 27-year-old man), and it continued at the rate of 5000–12,000 ml/day for the first 5 post-transplant days. Urine output remained at about 3000 ml/day until discharge from the hospital on the twelfth postoperative day. Normal blood urea nitrogen and creatinine were maintained, and the creatinine clearance was consistently around 100 ml/min.

Case 4. The donor was a 4-year-old white boy who aspirated a piece of apple and was apneic for 8–12 min. He was resuscitated with mouth-to-mouth breathing, intracardiac epinephrine, and external cardiac massage. One hour after hospital admission, decerebrate rigidity was present. The hospital course was characterized by several episodes of hypotension requiring vasopressors and a second episode of cardiac arrest with successful resuscitation. On the fourth postadmission day, the parents requested that in the event of death the patient's corneas and kidneys be used for transplantation. The hypotension became more refractory to vasopressors, and apnea was treated by mechanical ventilation with a tracheostomy tube. On the seventh day, an electroencephalogram showed no cortical activity. During the next 105 min, the patient was ventilated with 100% oxygen and a blood pressure could be only intermittently measured. The body was moved into the hyperbaric chamber upon death.

The kidneys and both ureters were removed with segments of the lower aorta and vena cava and transplanted in the right iliac fossa of a 24-year-old man. After completion of the vascular anastomoses, urine flowed in 6 min and continued at the rate of 3000–4000 ml/day. The blood urea nitrogen stabilized at about 50 mg%. The creatinine dropped from 11.1 mg% to normal and remained at approximately 1.6 mg%. The creatinine clearance was 20 ml/min at the end of the first week and then gradually rose to 60–120 ml/min after 1 month.

TABLE 2. Blood Gas and pH Data on Donor, Case 3[a]

	At death,[b] femoral art.	During extracorporeal perfusion: Aorta	During extracorporeal perfusion: Inf. vena cava
pO_2 (mm Hg)	36	2325	120
pCO_2 (mm Hg)	> 100	39.0	52.5
pH	6.93	7.30	7.15

[a] All samples were drawn and analyzed at 3 ata. Temperature was 30–31°C.
[b] After 40 min of apnea.

Case 5. The donor was a 19-year-old boy who, after being shot in the head, was admitted to the hospital unconscious, flaccid, and with dilated fixed pupils. Apnea and hypotension developed on the first day after admission and were treated with assisted ventilation and vasopressors. Electroencephalographic wave activity was flat. On death, the body was taken to the chamber without cardiorespiratory assistance, and the left kidney was removed according to the described chamber protocol. Table 3 shows the results of the blood gas analyses before and after extirpation of the left kidney. The pO_2 in the right renal vein was 1660 mm Hg.

Urine flowed 6 min after revascularization of the kidney in a 42-year-old female recipient. Thereafter, the daily urine output ranged between 2000 and 4000 ml. The blood urea nitrogen increased from 47 to

TABLE 3. Blood Gas and pH Data on Donor, Case 5*

	Before extirpation		After extirpation	
	Aorta	I.V.C.	Aorta	I.V.C.
pO_2 (mm Hg)	2132	1720	2180	1660
pCO_2 (mm Hg)	52.0	62.0	43.5	51.5
pH	7.14	7.06	7.17	7.13

I.V.C., inferior vena cava.
* All samples were drawn and analyzed at 3 ata. Temperature was 20°C.

129 mg% in the first postoperative week and then dropped to 38 mg% during the next month. The creatinine clearance was 29 ml/min at the end of the first week and 58 ml/min postoperatively.

DISCUSSION

The frequent failure of the human donor kidneys to function satisfactorily in the days immediately following transplantation has been a serious drawback. When function is seriously impaired in this period, it is almost impossible to know whether this is due to organ rejection, to technical problems, or to ischemic damage which occurred from the time of death of the donor to revascularization of the kidney in the recipient. If the onset of urine flow is prompt and sustained and renal function satisfactory, organ rejection is much easier to evaluate.

The donor kidney usually sustains some damage due to antemortem hypotension, respiratory distress, and possibly episodes of cardiac arrest. These problems were present in various combinations in all of these cases. In Cases 3, 4, and 5, the donor kidneys excreted urine 5–6 min after revascularization in the recipient and subsequently maintained satisfactory function. In the first two instances in which cardiac massage was used, the kidney perfusion was not as satisfactory (Table 1).

A kidney may be harvested quickly and then protected with cold perfusion,[13] but there are distinct advantages to being able to harvest a kidney in a more leisurely manner, using the meticulous techniques and principles advocated by Marchioro *et al.*[12,14] An aortogram to evaluate the arterial supply to the donor kidney is usually not possible in cadavers, but if several small arteries supply the left kidney (making anastomoses difficult) the arterial supply to the right kidney can be easily evaluated at surgery. The kidney in which adequate hemostasis has been accomplished during the harvesting does not require post-transplantation drains—a distinct advantage in minimizing infection. The blood supply to the ureter can be protected. Time is available for the harvesting of both kidneys, which was the situation in three of these cases.

A combination of techniques such as perfusion, hypothermia, and hyperbaric oxygenation allows time in which to ready the recipient. It can also allow harvesting of several organs from the same cadaver, which will become important in the future.

There are a few disadvantages in harvesting cadaver kidneys using this technique. Cautery cannot be used under hyperbaric conditions, and the time of harvesting is prolonged by using suture ligation techniques only. In our cases, heparinization and the dilutional technique caused loss of fluid into the operative wound which sometimes obscured the operative site. Fluid was replaced in the extracorporeal system, and flow rates of 30–70 ml/kg/min were attained. The hemoglobin in the perfusate was about 3 gm% at the end of the procedure.

Satisfactory arterial oxygen tensions can be obtained using the described technique (Tables 2, 3). The aortic blood was always acidotic, due to a metabolic acidosis and sometimes an elevated pCO_2. The acidosis was undoubtedly increased

because of the low pH of the priming fluids of the pump oxygenator. Possibly the pH should be corrected by adding buffering agents, but in our cases it did not seem to adversely affect the immediate urine output. Table 2 illustrates how hypoxia, hypercapnia, and acidosis were definitely improved during use of the pump oxygenator in the chamber.

It is still impossible to say how much hyperbaric oxygenation protected the kidney during the ischemic period, if at all. Manax *et al.*[11] showed that hyperbaric oxygenation enhanced the protective effect of hypothermia in the extracorporeally stored canine kidney. Our various techniques for organ preservation, both short-term and long-term, must be improved. Undoubtedly, in the future a combination of methods will be used.

SUMMARY

Human cadaver kidneys were harvested using hypothermic perfusion with a pump oxygenator in the hyperbaric chamber at 3 ata. When the technique had been adequately worked out, function of the homotransplanted kidney was prompt and continued satisfactorily.

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Effects of Hyperbaric Oxygenation on Renal Ischemia

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Hyperbaric oxygenation has been combined with hypothermia to protect the ischemic kidney,[1-3] but the individual effects of OHP alone have not been well defined. This present study evaluates hyperbaric oxygenation *per se* as a means of prolonging renal tolerance to ischemia.

Materials and Methods

Left nephrectomies were performed in 35 mongrel dogs. Two weeks after nephrectomy, the abdomen was re-entered, using endotracheal halothane and 99% oxygen for anesthesia. The stumps of the left renal artery and vein were dissected free and catheterized with polyethylene tubing (Figure 1). The aorta and inferior vena cava were mobilized in the region of the renal vessels, and the right kidney was completely freed of its perirenal fat. Vascular clamps were placed around the aorta and vena cava at points above and below the renal vessels.

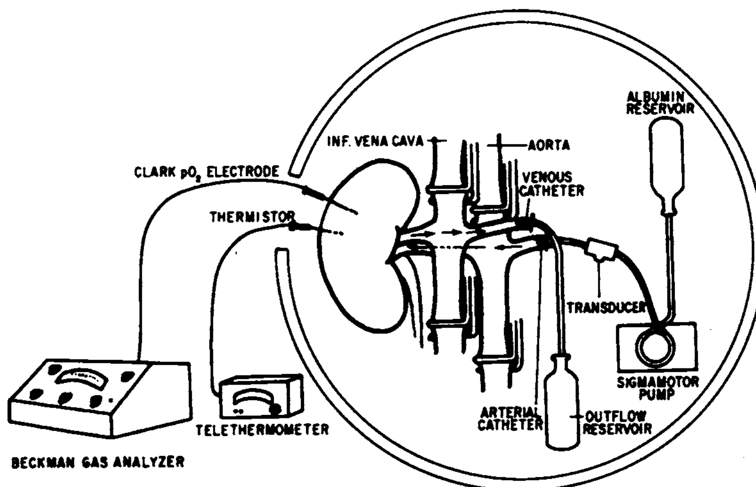

FIGURE 1. Schematic representation of experimental preparation.

The animals were then placed in a small hyperbaric chamber (described elsewhere [4]). The vascular clamps could be applied to the aorta and vena cava from outside of the chamber by remote control. This effectively interrupted the blood supply to the right kidney and established a closed circuit through which the kidney could be flushed. After vascular occlusion, the kidney was flushed with heparinized 4% human albumin in saline at room temperature until the effluent from the renal vein was clear.

Renal temperature was measured by a thermistor inserted into the renal parenchyma. Renal oxygen tension was determined by a Clark oxygen electrode positioned within the cortex of the kidney. This electrode was used with the Beckman 160 gas analyzer. The recorder terminals of the analyzer were coupled to a Sanborn amplifier (Model 350–1500), using a 100-μvolt preamplifier insert. This instrumentation permitted renal pO_2 values greater than 800 mm Hg to be recorded directly on a Sanborn polygraph (Model 350). Electrocardiogram, brachial arterial pressure, and rectal temperature were also monitored.

In each experiment, the vascular clamps were initially applied for 5–10 min at 1 atm and then released. Renal pO_2 was measured during this period and charted on the polygraph. In this way, renal pO_2 before, during, and after clamping was recorded for each animal and served as a control value at ambient pressures.

Renal ischemia was maintained for periods of 1 and 2 hours. In one series of control experiments, the kidneys were ischemic at normal atmospheric pressure (1 atm). In a second and third series, the chamber was pressurized to 3 and 4 atm of oxygen, respectively. The vessels were occluded after 30 min had elapsed, to ensure complete saturation of the renal tissues with oxygen. After the clamps were released, a brief period of re-equilibration was permitted, followed by decompression and closure of the abdominal incision.

Results

Five animals were included in each of the three groups subjected to 1 hour of renal ischemia. All survived. Those animals maintained at 3 and 4 atm before and during renal vascular occlusion for 1 hour had somewhat lower elevations in blood urea nitrogen (BUN) concentrations than control animals (Figure 2). Six animals were studied in each of the 2-hour ischemia groups. There were four deaths in this series, two at 1 atm and one each at 3 and 4 atm. The BUN levels in the survivors did not vary significantly among the three groups (Figure 3).

With the animals breathing 100% oxygen at 1 atm, renal pO_2 ranged from 125 to 350 mm Hg. A typical tracing of the renal pO_2 values for a 2-hour occlusion at 4 atm is depicted in Figure 4. During the control arterial clamping at 1 atm, there was a rapid linear decrease in renal pO_2 to zero, usually occurring within 10 min of clamping. On release of the clamps, the renal pO_2 returned to control values within 2–3 min. With pressurization to 4 atm, the

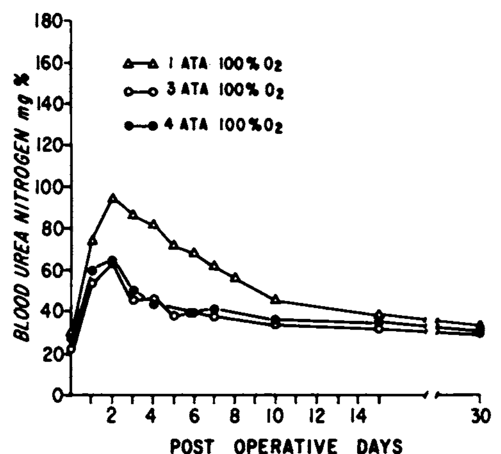

FIGURE 2. Effect of 1 hour of renal ischemia on blood urea nitrogen.

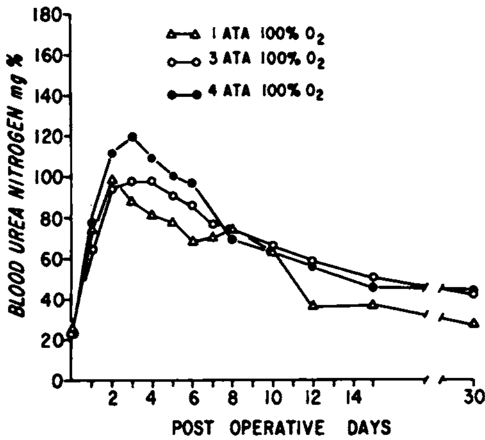

FIGURE 3. Effect of 2 hours of renal ischemia on blood urea nitrogen.

renal pO_2 reached levels of 2000 mm Hg. After equilibration for 30 min, the clamps were applied again, and the pO_2 dropped to zero over a 15-min period. This rapid reduction in renal pO_2 occurred whether or not the kidney was flushed. The renal pO_2 remained at zero for the remainder of the ischemic period, and after the clamps were released it returned to elevated levels.

Renal temperature stabilized at 32–34 °C during the ischemic period. Rectal temperature was maintained at 36–38 °C with a heating pad placed beneath the animal.

The histopathologic changes were comparable for the kidneys in the various experimental groups. The spectrum of pathology ranged from minimal leukocyte infiltration to extensive cortical necrosis with calcification. In general, the most severe damage was found in those kidneys subjected to 2 hours of ischemia.

Discussion

The data make it apparent that a reservoir of oxygen is established within the kidney at increased ambient pressures, but this reservoir is rapidly dissipated when the blood supply to the kidney is interrupted. There also appears to be no transcapsular uptake of oxygen. Thus,

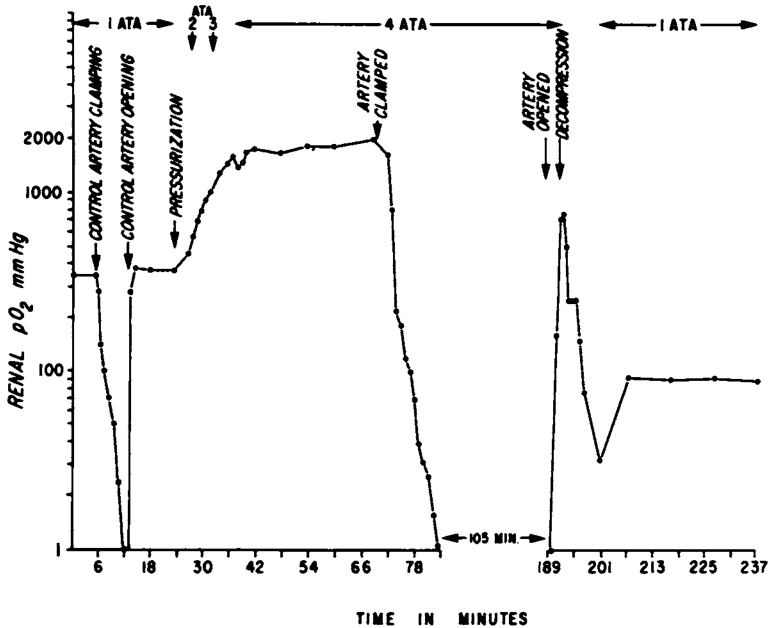

FIGURE 4. Renal pO_2 changes during renal ischemia at 1 and 4 atm of 100% oxygen.

within 15 min after occlusion, the renal pO_2 is reduced to zero, regardless of whether the kidney is at normal or at increased ambient pressures. One might expect, then, that hyperbaric oxygenation would not prolong renal tolerance to ischemia, and this supposition is borne out by the comparable BUN elevations, renal histology, and animal survival in the various experimental groups.

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In vitro Preservation of Canine Kidneys by Hyperbaric Oxygenation and Hypothermia

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The problem of the successful long-term storage of kidneys is closely related to the present experimental and clinical trials in their transplantation. Its solution would facilitate the homotransplantation procedures and would partially resolve practical and ethical problems related to the donors. Previously described procedures for *in vitro* kidney preservation, such as hypothermia and normothermic[1,2] or hypothermic perfusion with blood, plasma, or dextran,[3,4] have provided limited success, and they remain far from the ultimate goal of providing a bank of organs for homotransplantation. Like some other workers in this field,[5] we have felt that a technique combining the well-known advantages of hypothermia and hyperbaric oxygenation should be tried in an attempt to extend the viability of kidneys, and the following text will describe these experiments.

Briefly, the procedure consisted of canine nephrectomy, kidney perfusion, and storage in cold dimethylpolysiloxane fluid (Medical Fluid 360, Dow Corning) under increased oxygen pressure for 1, 3, and 7 days. After that period, the organs were reimplanted into the same animals and their subsequent viability controlled with a laparotomy and ulterior contralateral nephrectomy, together with biochemical analysis of blood and urine.

Materials and Methods

Mongrel dogs of both sexes weighing 14–34 kg were anesthetized with sodium pentobarbital and intubated, and respiration was maintained by Engstrom respirator. Twenty-five animals breathed room air, and the other 20 pure oxygen, and all animals received intravenous infusion of Ringer's solution with 5% dextrose. To this solution, 100 ml of 20% mannitol and 500 ml of 10% low molecular weight dextran in saline (Rheomacrodex, molecular weight 40,000) were added in 23 cases. Through a long midline laparotomy, standard left nephrectomy was performed after injection into the perirenal space of 50 ml of 0.5% procaine. Five kidneys were removed from animals heparinized with 1.5–2.0 mg of sodium heparin/kg body weight, and 16 animals received 10 ml of 2.4% aminophylline intravenously just before the nephrectomy.

Immediately after the nephrectomy, the

renal artery was cannulated and the kidney perfused by gravity from a bottle. The perfusion fluid consisted of 500 ml of Ringer's solution, 500 ml of 10% low molecular weight dextran in saline, 50 ml of 50% dextrose, 50 ml of 1% procaine, and 100 mg of sodium heparin. In 21 cases, 10 ml of 2.4% aminophylline was added to the above solution. The pH of the fluid varied from 7.20 to 7.70, and it was adjusted with 4.2% solution of $NaHCO_3$, or the fluid was buffered with THAM at concentrations of 0.78 or 7.2 gm/liter. The osmolality of the fluid was 484 milliosmoles. The perfusion pressure was 80 cm H_2O, with free outflow from the renal vein, or (in 21 cases) 90 cm, 10 cm of the venous pressure being created by cannulation of the vein. The temperature of the perfusion fluid was between 0°C and 2°C, and 15 min of perfusion time lowered the temperature of the organ to 13–17°C. The total flow during 15 min of perfusion varied from 250 to 700 ml. During the perfusion, the kidney did not change in form or size, nor did it gain in weight. It retained its soft consistency and became grey and pale. The last 20–50 ml of fluid leaving the renal vein was clear. At the end of the perfusion, the renal vessels were interconnected with a polyvinyl chloride cannula, and the kidney was placed in the pressure chamber containing the ice-cold dimethylpolysiloxane fluid.

Dimethylpolysiloxane is a colorless, odorless fluid, known to be chemically inert. Its "biological inertness" was established by the almost complete absence of foreign-body reaction after injections into animals.[6] For the storage of kidneys, fluids having viscosities of 500 and 1000 centistokes were sterilized for 1 hour at 140°C with hot dry air. Ten kidneys were simply dipped into the 0°C precooled dimethylpolysiloxane fluid and then transferred into the empty ice-cold pressure chamber, while in the other 35 cases, the kidneys were placed in the pressure chamber where they were submerged in the 0°C dimethylpolysiloxane fluid for the entire preservation period (Figure 1).

The oxygen pressure in the container was immediately increased to 3.9, 4.8, or 8.7 ata and maintained at this level for 1, 3, or 7 days with the temperature between 0°C and 5°C. At the end of the preservation procedure, the pressure was gradually decreased. The decompression time for 21 kidneys was 2 hours, for two kidneys it was 1 hour, and for 22 kidneys it was only 20 min.

The preservation procedure did not change the external aspect of the kidneys except that there was a small loss of fluid, never more than 3 ml. After the shunt between the renal vessels had been removed and its content taken for pH determination, the kidney was reimplanted into the right iliac fossa of the same animal. End-to-end vascular anastomoses between the renal and iliac vessels were made with 5–0 or 6–0 continuous silk sutures and the ureter was implanted into the bladder. For this intervention the animals received the same anesthesia and infusions as for the nephrectomy, including 10 ml of 2.4% aminophylline just before the vascular clamps were removed.

After re-establishment of the circulation, the external aspect and consistency of the kidney were noted, urine from the reimplanted ureter was recovered for biochemical analysis, and the abdomen was closed. The only postoperative treatment in all animals consisted of daily injections

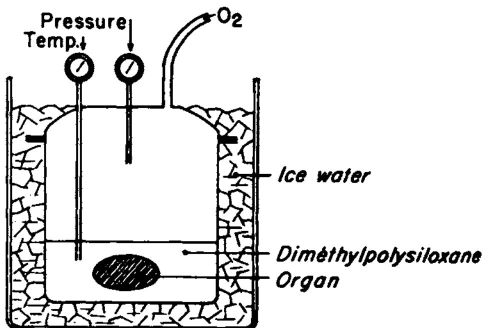

FIGURE 1. The apparatus and method for kidney storage.

of antibiotics for 1 week. The success of the preservation procedure was determined at laparotomy 2–8 weeks after the reimplantation, when the function of the reimplanted kidney was evaluated by the catheterization of its ureter.

In 24 animals the kidney was preserved for 1 day, in 18 for 3 days, and in three for 7 days.

RESULTS

Only two reimplanted kidneys, from the group of 24 kidneys preserved 1 day, were able to maintain the animals in good health for 3 months after the contralateral nephrectomy. During the nephrectomy these animals breathed pure oxygen and received the infusion of low molecular weight dextran and mannitol. These two kidneys had been perfused with solutions containing aminophylline, pH 7.68 and 7.65, and for 15 min flows of 680 and 520 ml were recorded. They were submerged in the dimethypolysiloxane throughout the storage period. One of the kidneys was kept at an oxygen pressure of 4.8 ata and the other at 8.7 ata. Before the reimplantation, the pH values of the liquid from the shunts between renal vessels were 7.60 and 7.45. During the reimplantation procedure, the animals breathed pure oxygen and had infusions of low molecular weight dextran and mannitol, as well as an injection of aminophylline just before removal of the vascular clamps. After the circulation was re-established, both kidneys assumed a bright red color, they remained soft, and the renal veins were enlarged with a large venous return. During the first 30 min, 5 ml of clear liquid came from the ureter of one kidney, while the output in the other kidney was almost nonexistent.

When the exploratory laparotomies were performed 4 weeks later, the size, color, and consistency of the kidneys were the same as at the reimplantation, and 15 and 10 ml of urine passed from the ureters in 30 min. The intravenously injected indigo carmine was excreted slightly later than from normal kidneys. For the first 2 weeks after the contralateral nephrectomies, the animals passed through a transitory uremic state, when the values of blood urea nitrogen increased up to 400 mg%, but without significant changes in serum electrolytes. Subsequently, there was a progressive amelioration of the urinary output and concentrating power.

The failures of preservation in the other animals were manifested by small pale fibrotic kidneys in which the cortex almost ceased to exist; thrombosis of the renal artery was found in more than half of the cases.

The histologic examinations of renal tissue after the perfusion, preservation, and reimplantation showed the following characteristics. After the perfusion, the kidneys were free of blood, with moderate interstitial edema existing in the medulla. The basal membranes of glomeruli were thickened, and there were signs of hyaline degeneration in some of the tubular cells. The preservation did not change this picture except for a decrease of interstitial edema in the medulla. However, after the reimplantation, there was progressive extension of the following: the glomeruli were obliterated by cells and blood; blood was found in desquamated tubules and interstitium; and considerable interstitial edema developed in the cortex and medulla. All blood vessels except the larger arterial branches were occluded with blood clots.

DISCUSSION

The partial success of these experiments indicates that kidney preservation may, at least for short periods, be possible, but that it is attended by limitations which require further investigation and improvement before clinical application becomes feasible. Thus, the following discussion is an attempt to analyze some important aspects of the storage and to draw conclusions for further studies.

Nephrectomy

We noticed in the course of the experiments that the breathing of pure oxygen and intravenous infusion of low molecular weight dextran and mannitol during the nephrectomy resulted in "better-looking" kidneys before the perfusion; they cooled faster, and the fluid leaving the renal vein was clear after 5–7 min. On the other hand, heparinization of the animal and intravenous injection of aminophylline seemed to have no effect.

Perfusion

The 15-min perfusion time was chosen because longer perfusions with ice-cold solutions failed to produce faster or more notable decreases of kidney temperature. This period of time and the hydrostatic pressure of 80 cm H_2O were sufficient to cool and wash the organ with flows higher than those recorded by other authors.[6] Histologic examinations of specimens after the perfusion showed that the pressure employed and the hyperosmolality of the fluid did not induce irreparable morphologic lesions. Higher flows were obtained with fluids of higher pH and higher concentrations of THAM.

The most interesting finding evoked by this procedure was the drop in pH of fluid in the renal vessels after 24 hours of storage (Figure 2). When, in the first five animals, the pH of the perfusion fluid was adjusted to 7.25–7.40 by the addition of $NaHCO_3$, the pH values were around 6.50 in all of them after 24 hours of storage. Slightly smaller but still considerable decreases were found when the perfusion fluid was buffered with THAM, 0.78 gm/liter. The two lowest pH values, 6.00 and 5.80, were recorded in two kidneys preserved for 3 days. Only a high concentration of THAM prevented subsequent changes in the hydrogen ion concentration. The pH of the two viable kidneys did not fall below 7.45.

The possible reasons for the changes of pH might be (1) that the metabolism of

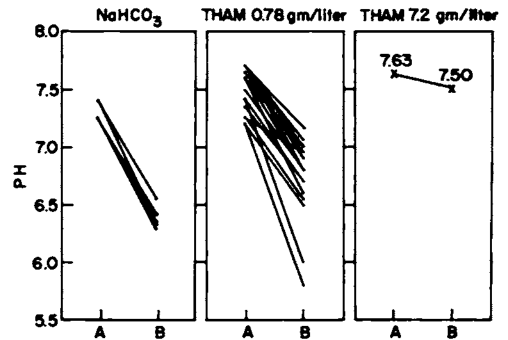

FIGURE 2. The pH drop in fluid in the renal vessels after 24 hours of storage. When the pH of the perfusion fluid was adjusted with $NaHCO_3$ in five animals (left), the pH after 24 hours (B) was around 6.50. The middle panel shows the 24-hour drop when the fluid was buffered with THAM (0.78 gm/liter). The two lowest values were found in two kidneys after 3 days. On the right are shown the mean pH values for 25 kidneys perfused with a high concentration of THAM in the fluid.

the kidney at low temperatures was higher than anticipated, (2) that the low metabolic rate under hypothermia was increased by the pressure *per se,* and (3) that the cellular metabolic effects acted in combination with an equalization effect between the low intracellular pH and that of the perfusion fluid. The cause of the increase of hydrogen ion concentration should be investigated in further experiments, as it may have considerable bearing on the ultimate success of this preservation procedure.

Preservation

Many factors supported the use of dimethylpolysiloxane as a medium for kidney preservation. Besides its chemical and biological inertness, it was assumed, on the basis of data for silicone rubber, that the fluid should have a sufficiently high diffusion rate for oxygen. At present, although exact data on this diffusion rate are unavailable, the bright red color of the blood on the organ leaving the pressure chamber, together with the abundant release of large bubbles of gas from the di-

methylpolysiloxane, are the only valid indications of this property. It might be, however, that the most important advantage of the dimethylpolysiloxane is that it seals the surface of the organ during storage and prevents the humoral changes of its content, while with any known solution of balanced electrolytes, the full control of exchanges between the organ and medium cannot be attained.

In these experiments, dimethylpolysiloxane fluids with viscosities of 500 and 1000 centistokes were used. These have the highest levels of biological inertness, but they are also of higher viscosity, which results in a lower diffusion rate for gases. This fact might suggest the use of lower viscosity dimethylpolysiloxane fluids, or an increase in oxygen pressure, since the diffusion rate through the medium directly influences the length of anoxia of the hypothermic kidney at the very beginning of the storage period.

Hyperbaric Oxygenation

Pressurization of oxygen to 3.9, 4.8, or 8.7 ata was done over about 5 min, and in these experiments reasons could not be found for a slower and more gradual increase.

The absence of exact data on the diffusion rate through the renal tissue makes the higher pressure preferable in order to shorten the time when the organ is without oxygen supply. Differences were not seen between the various lengths of decompression times, the two viable kidneys having been decompressed in 20 min.

Reimplantation

The intravenous infusion of low molecular weight dextran, aminophylline, and mannitol during the reimplantation was supposed to increase the renal blood flow and wash out tubules. Despite the fact that both viable kidneys were treated in this manner, it is difficult to give a definitive judgment about the advantages of this infusion. Immediately after the circulation was re-established, the kidneys became bright red, after which the color was either retained or became dark red or cyanotic. In the group of kidneys preserved 1 day, the organs treated with the perfusion fluid of high buffer concentration and kept under higher oxygen pressure were the only ones which remained bright red. Also, half of the kidneys treated in this manner for 3 days retained their bright red color, while all the kidneys preserved for 7 days immediately became cyanotic. Rarely in this experiment did the kidneys become distended and hard after the vascular clamps were removed; regardless of color changes, they stayed soft. Almost all kidneys passed urine (2–10 ml/30 min) immediately after the reimplantation, the urine being hemorrhagic when the preservation lasted 3 or 7 days, or when the poststorage pH was low.

The appearance of urine after the reimplantation is misleading and should not be a reason for early optimism, because the bright red, soft kidneys that produced clear urine later became reduced to nothing but scar tissue. The analysis of this urine showed values for blood urea nitrogen, creatinine, and electrolytes so close to those of plasma that it would be more appropriate to name it the transudate. Throughout these experiments, the color and consistency of the kidney and the appearance of the urine were not valuable prognostic signs.

On the other hand, the filling of the renal vein with the venous return flow could be closely correlated to the length of the storage. Kidneys stored 1 day had veins distended, with good return; this distention was less marked in the kidneys preserved 3 days. The kidneys stored for 7 days had flat empty veins. It should be mentioned here that the kidneys with decreased or nonexistent venous return were not hard and distended but kept their soft consistency, while the color changed from bright red to cyanotic.

Histology

These phenomena, and also the subsequent discovery of small fibrotic nonfunctional kidneys, may be explained by the findings of the histologic examination of the renal tissue immediately after reimplantation, where a circulatory obstruction appeared to be the first and perhaps principal lesion.

Conclusions

Although only two viable kidneys were able to keep the animals in good health, they still offer valid support to the hypothesis that the combination of hyperbaric oxygenation and hypothermia could be a promising procedure for long-term renal preservation. Apparently the procedure can preserve the viability of the renal tissue, and the failures are primarily related to biochemical imperfections of the perfusion fluid. During the storage, the properties of the capillary membrane were altered either by direct action of the perfusion fluid or in combination with the metabolic products of the renal tissue. When circulation was later re-established, the selective transport across the capillary walls no longer existed, the interstitial edema kept increasing, and a circulatory obstruction occurred at the level of capillaries. The results indicate that further improvements in the storage procedure depend largely upon amelioration in the preservation of adequate function of the capillary membrane.

ACKNOWLEDGMENTS

Dr. Hamra's leave from the University of Oklahoma Medical Center (as Resident in Surgery) and the support of his work on this study were made possible by a Foreign Scholarship of the International College of Surgeons.

We are most grateful for the excellent technical assistance of Miss I. van Berkel, Mr. F. Ganguillet, and Mr. G. Stauffer. Dr. B. Riedel and Mr. A. Fischer performed histologic examinations of specimens.

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Long-Term *in vitro* Heart Preservation with Hyperbaric Oxygenation and Hypothermia

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Our partial success with canine kidney preservation *in vitro* under hyperbaric oxygenation and hypothermia[1] encouraged experiments on prolonged preservation of canine hearts. Following are described the results of these experiments, together with an attempt to explain the present limitations of the procedure and the possibilities for further improvements.

MATERIALS AND METHODS

Mongrel dogs weighing 15–30 kg were anesthetized with sodium pentobarbital (30 mg/kg), heparinized with sodium heparin (3 mg/kg), and respirated with pure oxygen by an Engstrom respirator. The thorax was entered through a longitudinal midsternal incision and the large vessels were dissected free in the following order: the origin of the descending aorta, the left subclavian artery, the brachiocephalic trunk, the superior vena cava, the azygos vein, the inferior vena cava, the pulmonary artery, and the left and right pulmonary arteries and veins. After dissection was completed, all vessels were tied and divided, beginning with the left subclavian artery, and the heart was then removed from the chest.

Immediately after the extirpation, the aorta was cannulated and perfusion with ice-cold fluid started. The perfusion fluid consisted of 500 ml of 10% low molecular weight dextran (Rheomacrodex, molecular weight 40,000) in 5% dextrose, 500 ml of Ringer's solution, 50 ml of 1% procaine, 50 ml of 50% dextrose, and 100 mg of sodium heparin. It was buffered with THAM in concentrations of 0.78 or 7.2 gm /liter, to give a pH of around 7.50. The hearts were perfused by gravity from a reservoir 100 cm above the organ, and during the 4- to 7-min perfusion with 2000 ml of fluid their temperature decreased to around 12°C. At the end of the perfusion, the cannula in the aorta was left in place and occluded, as well as the three cannulas inserted into the right atrium, left atrium, and left ventricle. The pale, brown-grey soft hearts were then transferred to the pressure container and immersed in precooled (0°C) dimethylpolysiloxane (Medical Fluid 360, Dow Corning). The pressure of the pure oxygen introduced into the chamber was increased to 4.8 or 8.7 ata and was main-

tained at this level for 1, 3, or 7 days, while the temperature in the container varied between 1 °C and 4 °C. At the end of the storage period, the container was gradually decompressed over a 20-min period and the heart placed into the test system as follows.

The preserved heart was perfused with the blood of a donor animal by the method illustrated in Figure 1. The donor animal was anesthetized in the same way as the donor of the heart and its femoral vessels were cannulated. The femoral artery cannula was connected to the aortic cannula of the heart, while a precalibrated roller pump returned to the donor all blood from the right half of the heart. A reservoir filled with warm Ringer's solution was branched to the left atrium cannula, the level of the fluid in the reservoir being 15 cm above the left atrium. The left ventricle cannula was connected to the chamber with an artificial valve, and from this chamber the fluid was returned to the reservoir. The pressures in front of and behind the artificial valve and the flow through the system were measured, so that imposed resistance to the myocardial contraction could be calculated (expressed in dynes × sec × cm^{-5}). Electrodes for the recording of the electrocardiogram were sutured directly to the right atrium, left ventricle, and aorta. The temperature of the heart was measured throughout the experiment by a needle probe inserted into the myocardium of the left ventricle.

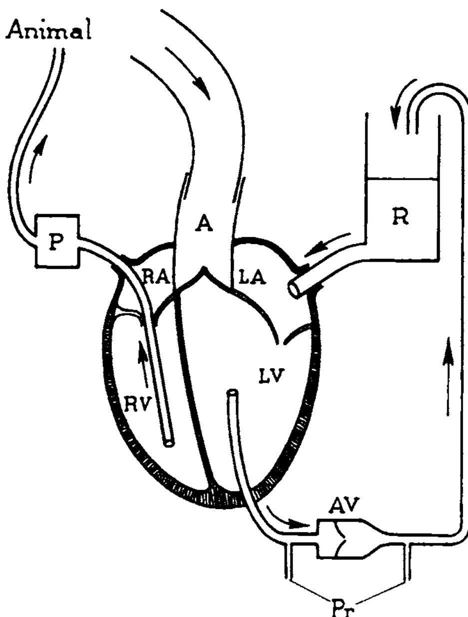

FIGURE 1. The apparatus and procedure for the *in vitro* test of stored canine hearts.

In this manner, the success of the preservation procedure was tested for 12 hearts preserved 1 day, 10 hearts preserved 3 days, and one heart preserved 7 days.

RESULTS

All 22 hearts stored for 1 or 3 days resumed the coordinated beat after being perfused and rewarmed by blood from the donor animal (Figure 2). When the reappearance of the cardiac contractility was manifested by fibrillations, a single shock by the defibrillator was sufficient to convert them to the regular rhythm. The prompt resumption of the heartbeat could not be clearly related to the length of preservation, to the pressure of oxygen, or to the pH of the fluid in the heart cavities after the storage. Hearts perfused with the low concentration of THAM in the fluid had a pH at the end of 24 hours' storage as low as 6.50, but they still resumed strong rhythmic contractions. In many instances, when the hearts started with weak but rhythmic contractions at low temperatures, these contractions degenerated into fibrillations at temperatures between 20°C and 25°C; later, when the hearts were rewarmed to 34°C, defibrillation was easily accomplished.

The differences between the groups of hearts stored for 1 and 3 days could be noted in other properties than the simple resumption of the heartbeat.

Of the hearts preserved for 1 day, half developed atrial contraction, as recorded electrocardiographically in advance of the

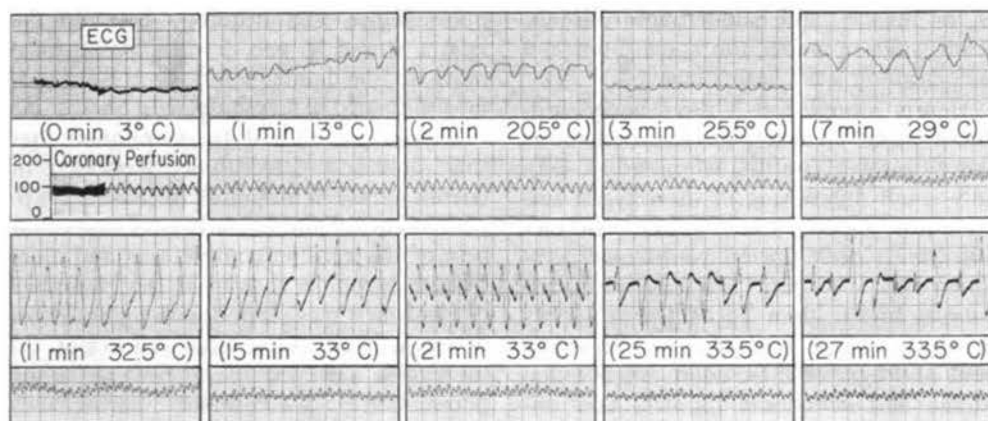

FIGURE 2. The resumption of electrocardiographic activity in a canine heart preserved 3 days. Recorder speed, 1 cm/sec.

ventricular complex. They had better venous return from the coronary sinus (up to 250 ml/min), and the differences in oxygen saturation between the arterial and venous blood were significant. They had stronger contractions producing high outputs through the test system, and they reacted promptly and forcefully after injections of calcium chloride and epinephrine solutions (Figure 3). When the tests were discontinued after 2–3 hours, these hearts showed only moderate edema

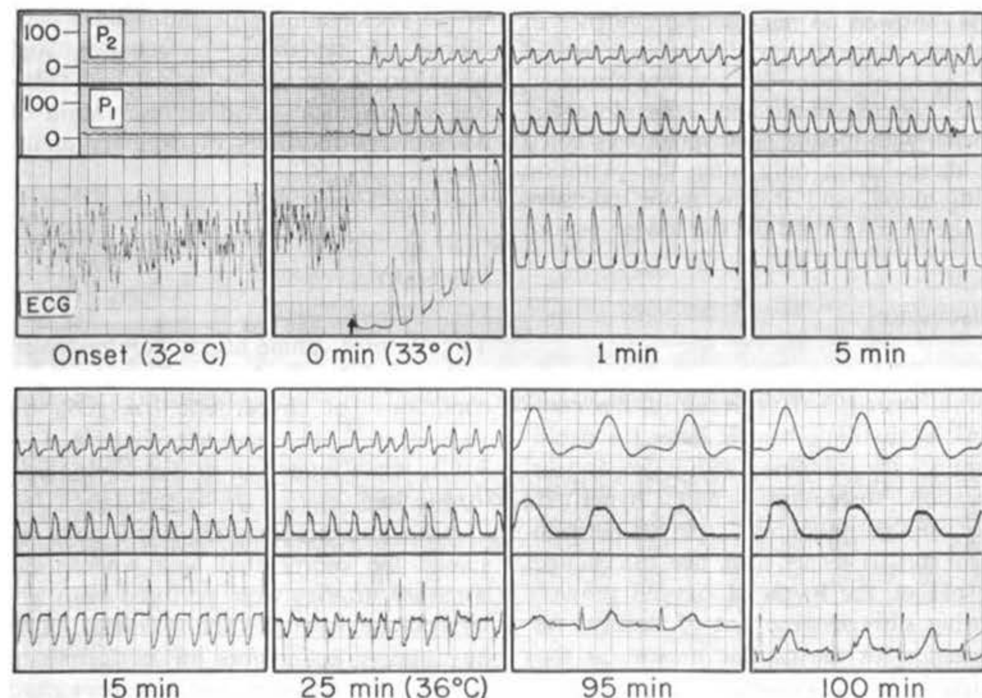

FIGURE 3. Electrocardiographic tracing, ventricular pressure (P_1), and "aortic" pressure (P_2) in a canine heart stored 1 day, defibrillated at 33°C.

of the muscle, and hemorrhages into the atrial walls or under the epicardium and endocardium were not extensive.

Hearts stored for 3 days never produced atrial contractions and had lower venous returns (up to 150 ml/min), with only small differences in the oxygen saturation of blood. Their weaker contractions never built up left ventricular pressures over 100 mm Hg, nor were the outputs higher than 150 ml/min. They showed sluggish reactions to the injections of drugs. In these hearts, myocardial edema and hematoma in atria and under the epicardium and endocardium appeared even after the first 30 min of the test.

The heart preserved 7 days never resumed the beat, nor did it fibrillate. There was no venous return, the veins remained empty, edema appeared immediately, and the myocardium was totally replaced with a hematoma.

Histologic examination of the myocardium after the 1- or 3-day preservations showed no microscopic evidence of damage immediately following removal of the hearts from the pressure chamber. The subepicardial and subendocardial edema with medial layer intact was noted in these hearts only after the perfusion with blood, and it was more extensive in the hearts stored for the longer period.

Discussion

The striking facility with which the hearts stored 3 days resumed the coordinated rhythmic beat is indeed a significant advance obtained with a simple preservation procedure — and hyperbaric oxygenation undoubtedly played the principal role. It seems, also, that the changes in pH or the levels of oxygen pressure during storage were not of decisive importance for the cardiac muscle, as they might be for the kidney.[1]

On the other hand, the differences in performance and histology between the hearts stored 1 and 3 days point once more to the inadequately preserved permeability of the capillary membrane. At this point, it should be mentioned that this increased permeability, resulting in edema and hematoma formation, appeared earlier and caused faster degeneration of the cardiac contractions and electrocardiographic activity if the perfusion of the hearts was started with higher flows of oxygenated blood from the donor. In the canine kidneys,[1] the alterations in the permeability of capillary membranes could be caused by biochemical imperfections of the perfusion fluid used during storage, resulting in nonfulfillment of the metabolic requirements of the organ during storage. While the myocardial cell appears to be morphologically and functionally viable after preservation, it is impossible to answer its metabolic demands when the organ is removed from the pressure container and rewarmed.

This indicates that the real limitations of the preservation procedure under conditions of hyperbaric oxygenation and hypothermia cannot be established without improvements in the preservation of adequate permeability of capillary membranes.

Summary

Twenty-three canine hearts were removed and stored for 1, 3, and 7 days under conditions of hyperbaric oxygenation (4.8 or 8.7 ata) and hypothermia (1–4°C), while immersed in dimethylpolysiloxane fluid.

All hearts preserved for 1 or 3 days resumed the coordinated beat when their coronary arteries were perfused with arterial blood from the donor animal. The quantitative differences in performance between these two groups were presented and their possible causes discussed.

ACKNOWLEDGMENTS

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We are most grateful for the excellent technical assistance of Miss I. van Berkel, Mr. F. Ganguillet, Mr. G. Stauffer, and Miss C. Stauffer. Dr. B. Riedel and Mr. A. Fischer performed histologic examinations of specimens.

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Protective Effect of Hyperbaric Oxygen During Prolonged Renal Ischemia in the Dog

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Studies of renal ischemia in dogs have shown that complete renal artery occlusion for over 2 hours usually damages renal function permanently.[1] Protection from this damage can be afforded by the use of hypothermia during ischemic periods.[2,3] The detrimental effects of renal ischemia are enhanced by: (1) hyperthermia,[4,5] (2) repeated brief unclamping of the renal artery during the ischemic period,[6] and (3) venous occlusion before arterial occlusion.[7]

Moyer *et al.* have shown that subfiltration arterial pressure, as provided in certain experimental situations or in the presence of collateral flow, has a protective effect during periods of renal ischemia.[8] The possible role of circulation through the capsular collateral system of the kidney is considered by Moyer. He states that, "The circulation through the renal capsule to the cortex may be of greater importance than is usually considered to be the case, and may indeed play an important role in renal circulation. The last consideration is based upon the fact that renal arterial occlusion alone for 2 hours does not produce as severe functional damage as when arterial occlusion is combined with aortic occlusion (hypotension of the distal segment); then severe renal damage is produced in every case. This suggests that when the renal artery alone is occluded the systemic blood pressure in the tissues surrounding the kidney remains unaltered, and consequently remains sufficiently high to maintain some blood flow through the renal capsule. This is frequently enough to keep the kidney intact for as long as 2 hours. Conversely, if one occludes one renal artery and the aorta simultaneously, resulting in hypotension in a distal segment, then the blood pressure in the surrounding tissues is reduced to the point at which little or no blood flows through the renal capsule to the cortex, which results in complete renal ischemia and necrosis." [8]

Considering Moyer's demonstration of the protective effect of renal capsular flow during renal artery occlusion, we can postulate enhancement of this protective effect with a change in the physical characteristics of the capsular flow. Therefore, we have attempted to demonstrate the effect of hyperbaric oxygen as an adjunct in protecting the kidney via the renal capsular flow during renal artery occlusion.

Methods

Dogs weighing 15–25 kg were anesthetized with intravenous pentobarbital sodium (12 mg/kg). Before skin incision, a slow drip of 500 ml of 5% dextrose in water, containing 1 million units of penicillin and 1 gm of Chloromycetin, was started and subsequently adjusted so that the termination of the infusion coincided with final skin closure. Through a midline incision, a left nephrectomy was completed, and the right renal artery was dissected approximately 5–10 mm from its aortic origin. An atraumatic completely occlusive vascular clamp with Pilling teeth was then applied to the renal artery and the time noted (Figure 1). In one group of dogs, the clamp was left in place for varying times at 1 atm with the dog breathing room air; in another group, this was done at 3 atm during oxygen-breathing. The kidney capsule was left intact. Oxygen was supplied to the dogs in the chamber by a demand valve via a cuffed endotracheal tube while the animal was under 3 atm of pressure. In animals in the hyperbaric oxygen group, the renal artery was not clamped until the animal was at 3 atm and had been breathing 100% oxygen for 5 min (in addition to oxygen-breathing during descent).

Blood urea nitrogen (BUN) was measured preoperatively and at intervals of 2–3 days postoperatively for the first 2 weeks, then weekly thereafter. Arterial pH, pCO_2, and pO_2 values were also measured in a number of dogs during hyperbaric oxygen treatment. Similarly, arterial blood pressures were monitored continuously with a Sanborn transducer and recorder in a representative group of dogs from the time of the skin incision to the time of skin closure. When animals began to survive the renal artery occlusion under hyperbaric conditions, aortograms were performed to assess the possible presence of accessory renal arteries, but none was found.

Results

A definite protective effect was demonstrated in our animals when the renal artery was occluded during hyperbaric oxygenation (as compared to animals undergoing occlusion at 1 atm). Figure 2 demonstrates the increase in survivors when capsular artery flow carried oxygen-enriched blood during occlusion of the renal artery at 3 atm of 100% oxygen-breathing.

Six of 10 dogs with 4 hours of renal ischemia at 1 atm died of uremia within 10 days. Ten of 11 dogs that had occlusion of the renal artery for 4 hours at 3 atm breathing 100% oxygen survived. Four of the 10 survivors in this group had long-term BUN values between 20

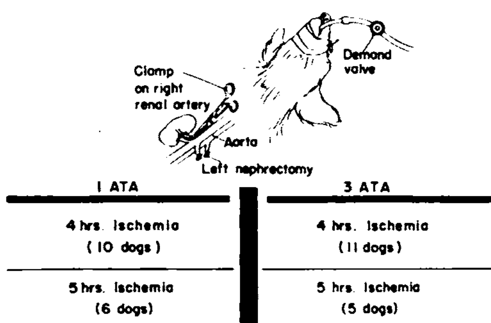

FIGURE 1. Graphic representation of experimental procedure (top) and treatment schedule (bottom).

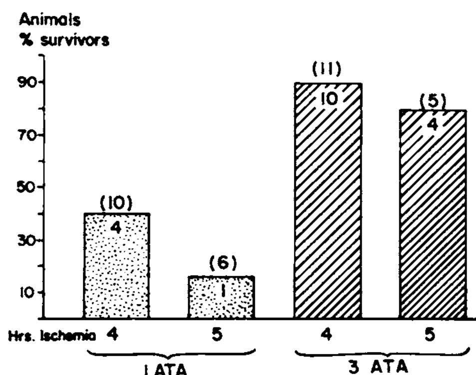

FIGURE 2. Dogs surviving renal ischemia at 1 and 3 atm.

and 30 mg%, and the remaining six had values below 20 mg% (Table 1).

Renal ischemia for 5 hours at 1 atm produced five deaths from uremia and one survival among six dogs. Of five dogs that underwent occlusion for 5 hours at 3 atm during 100% oxygen-breathing, four survived. The four survivors all had normal BUN values on long-term follow-up. Death was judged as due to uremia in this study when: (1) BUN rose to above 90 mg% when last measured before death, and (2) autopsy specimens revealed gross and/or microscopic renal damage, and no other cause of death was apparent (Figure 3).

Survivors that died of surgical complications (*e.g.*, intussusception, sepsis) with normal BUN levels for more than 1 week after renal artery occlusion were considered survivors of the renal ischemia. When an animal died within 5 days after operation, it was removed from the study (since no valid conclusions could be drawn).

Arterial blood oxygen determinations were made on animals treated in the chamber at 3 atm breathing 100% oxy-

TABLE 1. Blood Urea Nitrogen Levels of Dogs Exposed to Renal Ischemia Under Normal or Hyperbaric Conditions

		Blood urea nitrogen (mg%)						
Experimental conditions	Dog no.	Preop.	Day 1–2 *	Day 3–4	Day 5–7	Day 14	Day 21	Long-term
4-hr ischemia 1 atm	297	15	41	50	104	Dead		
	296	16	44	69	90	Dead		
	264	20	66	94	Dead			
	274	7	18	19	18	27	17	12
	291	10	42	74	54	31	18	17
	280	9	45	70	40	28	—	22
	290	6	17	24	21	18	—	15
	308	5	25	120	Dead			
	309	9	33	59	146	Dead		
	201	14	41	79	110	Dead		
5-hr ischemia 1 atm	206	8	72	140	159	Dead		
	313	13	50	76	148	Dead		
	316	11	—	60	129	Dead		
	323	9	48	64	40	—	12	—
	X	9	40	—	129	Dead		
	Y	12	35	—	118	Dead		
4-hr ischemia 3 atm	152	13	55	153	178	57	26	13
	172	18	38	29	26	20	30	13
	203	2	41	81	52	42	38	22
	220	17	17	20	25	40	18	18
	228	8	31	—	21		Sacrif.	
	183	10	—	30	—	—	22	17
	189	8	—	—	—	—	—	13
	216	9	65	82	139	56	36	30
	204	10	38	42	44	32	31	27
	215	9	45	73	82	33	31	17
	70	10	59	163	Dead			
5-hr ischemia 3 atm	252	9	—	14	30	20	—	—
	275	9	—	132	187	Dead		
	283	15	44	37	46	17	20	25
	56	4	22	20	11	15	27	27
	278	9	28	34	21	16	—	18

* Postoperative.

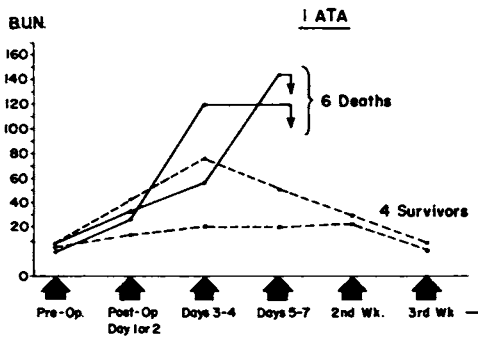

FIGURE 3. Characteristic BUN values of dogs exposed to renal ischemia at 1 atm.

gen by means of a demand valve. The pO_2 values ranged from 1800 to 2050 mm Hg under these conditions. The pCO_2 and pH determinations performed concomitantly were either normal or nearly normal. Abnormalities that did exist were reflected by a mild metabolic acidosis, with a rise in pCO_2 from 40 to 60 mm Hg and a fall in pH from 7.32 to 7.21 generally occurring after 2–3 hours of hyperbaric oxygenation. Blood gas and pH determinations were performed on 30% of the animals studied. Continuous blood pressure monitoring via an arterial cannula in a representative number of dogs showed no significant changes during the experimental period. This included the time of nephrectomy, renal artery occlusion, and, in some groups, hyperbaric oxygen therapy.

A number of interesting problems were noted during the study. Initially, the dogs studied during renal artery occlusion under hyperbaric oxygen conditions were ventilated with a Bird automatic respirator, and they frequently had pulmonary complications suggestive of the Lorrain-Smith effect. Lungs in these animals tended to be wet and hemorrhagic at postmortem examination. Four of these dogs were excluded from the study because of their early pulmonary deaths. We altered our experimental procedure after these observations, with subsequent ventilation done by demand-valve regulator with humidification. Thereafter, lung complications were markedly reduced. Another problem was seizure activity during exposure to hyperbaric oxygen. Two dogs receiving 5 hours of hyperbaric oxygen therapy were found to be decerebrate after exposure; one survived and regained relatively normal neurologic function, but the other died on the fifth postoperative day with increasing evidence of decerebration.

In comparison to our demonstration of an apparent protective effect from damage secondary to renal ischemia with the use of hyperbaric oxygen, recent reports have described detrimental effects of hyperbaric oxygen on renal function.[9] Since the experimental situations are not comparable, however (*i.e.*, low flow versus normal flow conditions), a contradiction does not necessarily exist. Future experiments regarding the protective effect of hyperbaric oxygen during organ ischemia might be better studied using some method to control blood flows at prescribed levels, since the collateral flow used in our study is, by its nature, highly variable from animal to animal. The variability of our results probably reflects these anatomic variations in collateral flow pathways.

ACKNOWLEDGMENTS

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Core Hypothermia and Hyperbaric Oxygenation with Intermittent Blood Perfusion for Organ Preservation: Histologic Evaluation by Electron Microscopy

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If human organ banks are ever to become feasible, a method must be found to store organs over long periods and allow their complete resuscitation. Only a little progress has been made toward the solution of this challenging problem to date. At our institute, we investigated several methods for organ preservation (including freezing the heart in liquid nitrogen), but our experiments were unsuccessful until we applied hyperbaric oxygenation. Canine heart–lung preparations were successfully preserved for as long as 47 hours at 4°C in a cylinder pressurized to 54 psig with oxygen. When the preserved organ was connected to the circulation of a recipient animal, satisfactory heartbeat was restored in a significant number.

In 1964, Bloch *et al.*[1] reported their studies on preservation of the canine heart in which the organ was perfused with a heparinized solution of dextran, stored at 3.3 ata of pressure, and maintained at a temperature of 4°C for 24 hours. Thirty hearts stored in this fashion, when replaced as homografts in the neck of a recipient dog, resumed a "coordinated ventricular beat after revascularization"; 10 required electrical defibrillation. Hearts stored by a combination of hypothermia and hyperbaric oxygenation showed no histologic evidence of damage under 24 hours. Eight additional hearts stored up to 120 hours did not resume contractions after 48 hours. No provision was made for organ perfusion during the storage period.

Materials, Methods, and Results

Three different methods of organ preservation were used in our most recent experiments. The operative technique, however, was the same for all. The superior and inferior vena cava were dissected out. The azygos vein was divided, the dog was heparinized, and the brachiocephalic artery, right auricle, and left ventricle were cannulated. The aortic arch and descending aorta were then dissected from the posterior mediastinum and ligated. The superior and inferior vena cava were also ligated.

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Surface Oxygenation

In the first method, only surface oxygenation was employed. Sixteen adult dogs were used, eight as donors and eight as recipients. The coronary arteries and lungs of the donor dog were perfused for 20 min with 10% dimethylsulfoxide (DMSO) solution containing 20 mg of heparin, after which the heart and lungs were removed as an intact specimen (thus simulating a condition required for human temporary transplant) and placed in the pressurized cylinder.

Five heart–lung specimens were stored under 54 psig of oxygen pressure at 4°C for 24 hours, and three were stored for 48 hours. They were then decompressed for 10 min and removed from the cylinder. The previously cannulated brachiocephalic artery of the preserved heart was connected to the femoral artery of the recipient dog, and the cannulas from the right auricle and left ventricle of the preserved organ were connected to the recipient's femoral vein. A blood sample for enzyme studies was taken from the right auricle as soon as circulation was established.

Gas bubbles were immediately observed on either side of some of the preserved hearts. During fibrillation, solutions of sodium bicarbonate, calcium gluconate, and procaine hydrochloride were injected intravenously into the recipient dog. If normal sinus rhythm was not restored, electroshock was applied to the myocardium. The preserved organs were observed from 3 to 13 hours, during which time electrocardiograms were made; these were interpreted as normal by the cardiologist (Figure 1).

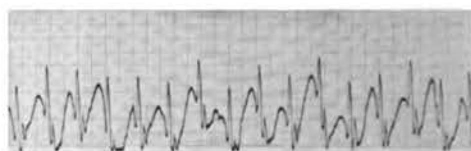

FIGURE 1. Typical electrocardiogram of preserved canine heart (interpreted as normal) after reimplantation in recipient dog.

By surface oxygenation alone, there was a significant increase in enzyme activity (Table 1). Of the five organs stored for 24 hours, four developed a normal sinus rhythm, lasting as long as 13 hours and averaging 5 hours. Of the three organs preserved for 48 hours, regular heartbeat was restored in two. In none of the eight specimens were any pathologic changes found in the myocardium, although some of the lungs showed congestion.

Core Hypothermia and Hyperbaric Oxygenation

In the second method, all of the procedures were carried out in our hyperbaric

TABLE 1. Enzyme Activity and Resumption of Heartbeat in Canine Hearts Stored by Surface Oxygenation [a]

Dog no.	LDH [b]	SGOT [c]	SGPT [c]	Storage (hours)	Resuscitated beat (quality, duration)
1	2000	—	—	24	Satisfactory, 3 hours
2	2000	5000	5000	24	Satisfactory, 4 hours
3	1980	1480	107	48	Satisfactory, 3 hours
4	520	200	39	48	Satisfactory, 3 hours
5	2000+	2200	660	24	Continued fibrillation
6	770	200	39	24	Technical error
7	560	11	—	24	Good, 13 hours
8	1410	510	240	48	None (extensive gas emboli)

[a] Hearts were stored at 54 psig of oxygen pressure.
[b] Measured in Berger-Broide units.
[c] Measured in Reitman-Franklin units.

chamber at 3 ata of pressure. Also, instead of perfusing the organ with DMSO, the dog's own blood was retained in the specimen.

Seven donor animals breathed oxygen for 15 min before removal of the heart–lung preparation. Six specimens were then placed in a pressurized cylinder and kept at 4°C, as previously described. Five were kept for 24 hours and one for 8 days. One specimen was immediately transplanted as a control. The preserved specimens were removed from the cylinder, without decompression, and connected to the recipient dog under 3 ata of pressure in the hyperbaric chamber.

Four of the five hearts preserved for 24 hours resumed beating after circulation was established. Resuscitation occurred more rapidly than in the previous experiment; also, the quality of the contractions was better and they continued for an average of 12 hours. (One heart continued to beat for 24 hours.) The electrocardiograms were interpreted as normal. Gas bubbles were not observed. Enzyme studies on this group showed significantly lower levels of lactic dehydrogenase (LDH) activity than in the previous experiment (Table 2). Histologic examination, even in the one organ stored for 8 days, showed apparently normal cell structure.

Core Hypothermia, Hyperbaric Oxygenation, and Intermittent Blood Perfusion

Throughout these studies, there was a definite correlation between the level of the enzyme activity and the quality of the heartbeat. The failure of the first and second methods to maintain normal enzymatic levels led to our developing the third method, consisting of core hypothermia, hyperbaric oxygenation, and intermittent perfusion with whole blood. To accomplish the organ perfusion, a 1-liter plastic chamber, electronically controlled to oscillate every 2 sec, was devised to oxygenate the blood at 35 psig.

In this plastic chamber, 600 ml of the donor animal's blood was pressurized for 15 min. (Homologous blood from other animals was subsequently used to replenish the supply as needed.) The heart–lung preparation was placed in a porous bag to avoid kinking during perfusion, and it was suspended in a stainless-steel cylinder which was then pressurized to 32 psig. The cylinder was then attached to the plastic chamber, the entire apparatus (Figure 2) was placed in the cold room, and the organ was perfused at a pressure gradient of less than 5 psi (250 mm Hg).

The organ was perfused every 8 hours with the hyperoxygenated blood from the plastic chamber and, before each perfusion, 10 ml of blood was taken from the

TABLE 2. Enzyme Activity and Resumption of Heartbeat in Canine Hearts Stored by Core Hypothermia and Hyperbaric Oxygenation [a]

Dog no.	LDH [b]	SGOT [c]	SGPT [c]	Storage (hours)	Resuscitated beat (quality, duration)
1 [d]	—	—	—	24	Good, 10 hours
2	230	55	29	24	Good, 7 hours
3	450	125	35	24	Good, 8 hours
4	1220	117	54	24	None
5	680	490	98	24	Good, 24 hours
6	2000+	1080	59	192	None
7	—	—	—	0 (control)	None

[a] Hearts were stored at 54 psig of oxygen pressure.
[b] Measured in Berger-Broide units.
[c] Measured in Reitman-Franklin units.
[d] Blood sample lost.

FIGURE 2. Portable assembly showing oxygen tank, oscillating 1-liter pressurized chamber containing blood for perfusion of the organ, and stainless-steel cylinder (which is pressurized to 54 psig).

cylinder for enzyme studies, including serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and lactic dehydrogenase (LDH). After each perfusion, the cylinder pressure was increased to 54 psig.

After 48 hours or more, the organ was decompressed (during perfusion) over a period of 15 min and connected to the recipient dog. We are now using a plastic box to house the preparation during the temporary transplant. By connecting the box to a warm-water tap, the organ is easily kept moist.

To date, five organs have been stored up to 47 hours by this most recent method, with return of strong sustained auricular contractions in three (Table 3). (In the future, we intend to increase the duration of storage by perfusing the organs more frequently.)

Histology

Two heart specimens preserved by the third experimental method (above) were examined histologically by one of us (G.O.), using electron microscopy; they were compared with one specimen preserved by the second experimental method. The results showed only slight damage to the organ in the third group and rather marked changes in the one organ preserved by the earlier method (Figure 3). By the standard technique of histologic examination, all specimens appeared to have a normal cell structure, regardless of the method of preservation.

TABLE 3. Enzyme Activity and Resumption of Heartbeat in Canine Hearts Stored by Core Hypothermia, Hyperbaric Oxygenation, and Intermittent Perfusion [a]

Dog no.	LDH [b]	SGOT [c]	SGPT [c]	Storage (hours)	Resuscitated beat (quality, duration)
1 [d]	340	67	19	24	None
2	1080	67	40	24	None
3	730	49	30	24	Excellent, 1 hour
4	860	90	37	24	Excellent, 8 hours
5	210	198	40	47	Excellent, 15 hours

[a] Hearts were stored at 54 psig of oxygen pressure.
[b] Measured in Berger-Broide units.
[c] Measured in Reitman-Franklin units.
[d] Failure of resuscitation was traced to perfusion with a pressure gradient across the organ of greater than 5 psig (250 mm Hg).

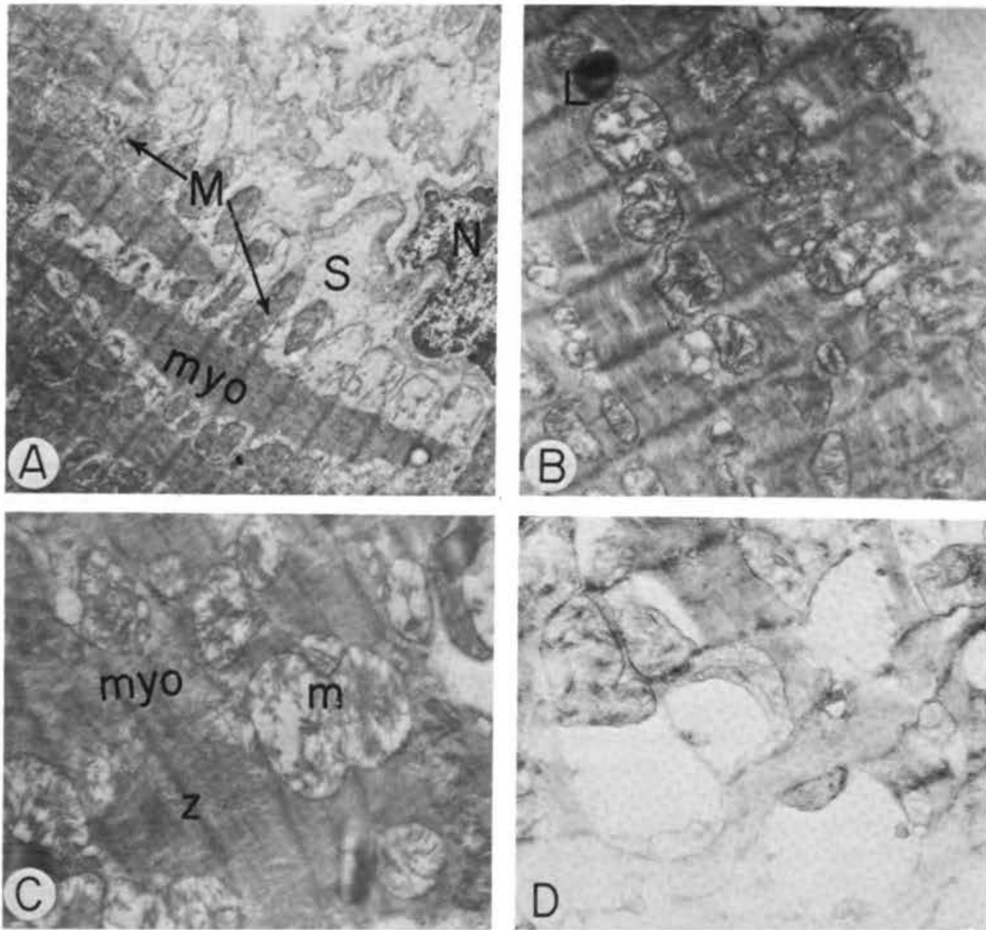

FIGURE 3. A, architecture of normal canine myocardium (×3700): N, nucleus; S, sarcoplasm; M, intact mitochondria; myo, myofibril. B, an example of myocardial response to intermittent perfusion under hypothermic OHP (third method). The mitochondria show edema but cristae are generally intact. Cystic areas are seen around the mitochondria. Note dense lipid accumulations (L) adjacent to mitochondria. The myofibrils are relatively unaffected (×5900). C, another example of myocardium after perfusion with hypothermic OHP: m, mitochondria; z, Z band; myo, myofibril. Greater mitochondrial alterations are observed. There is also some edema of myofibrils (×7400). D, myocardial tissue after 24 hours of storage under hypothermic OHP without perfusion (second method). Marked cystic degeneration is apparent, with severe mitochondrial alterations, as well as myofibrillar pathologic changes (×7400).

Method. Portions of cardiac muscle tissue were cut into small pieces and placed in 1% osmium tetroxide fixative for 1.5 hours. Postfixation was carried out in 1% neutral formalin, dehydration was done in graded alcohols, and finally the specimens were embedded in Maraglas. Cold sections, cut by an LKB microtome, were captured on Formvar-coated wire-mesh grids and counterstained with lead. An RCA EMU-3F electron microscope was used.

SUMMARY

The experiments described above are still in progress, and it is too early to draw

definite conclusions. On the basis of the quality of the heartbeats in the resuscitated organs, however, and the findings by electron microscopy in this limited number of specimens, we believe that the combination of core hypothermia and hyperbaric oxygenation, together with automatic intermittent perfusion with whole blood, has greatly improved the state of preservation of the organ.

ACKNOWLEDGMENTS

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Canine Kidney Preservation and Transplantation with Particular Reference to Clinical Significance

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Perhaps the current decade will be looked back upon as one in which the most impressive surgical investigative effort was focused upon the transplantation of whole organs. Many problems in organ transplantation have been partially solved, but three basic obstacles remain: (1) refinement of transplantation techniques, (2) organ preservation *in vitro*, and (3) understanding of the phenomena of transplantation biology (especially homograft rejection).

Transplanting a vital organ to replace a worn-out or irreparably diseased one requires the same approach as the corrective therapy of any illness. Effective treatment depends upon: (1) removal of the cause of the disorder, (2) substitution of deficient organ function by physical and chemical methods, or by organ transfer from one individual to another, and (3) amelioration of adverse environmental factors.

Kidney Supply, Procurement, and Preservation

At present, the only possible sources of kidneys for transplantation are healthy living donors, fresh cadavers, and suitable living healthy animals. All three have been used to provide kidneys, but most surgeons agree that for the present, at least, heterografting should be confined to the research laboratory.

The use of a live donor involves certain ethical considerations, as well as certain hazards. Already there are documented cases of live donors having suffered severe postoperative infection and hypotension, and at least one case of an identical twin donor who developed glomerulonephritis in the remaining kidney.[1] If the use of living donors continues, fatalities are almost inevitable. The operation is more hazardous to the donor than a routine nephrectomy, since the renal vessels must be mobilized extensively and divided near their origins at the aorta and vena cava.

Distinct advantages attend the use of kidneys from living donors, however. Renal ischemic damage is minimized, resulting in better function. If the donors are relatives, tissue typing is possible, with consequent avoidance of poor donor–recipient combinations. Nevertheless, transplant investigators generally agree that the present long-term results leave much to be desired.[2] Even with the use of identical twins, at least six recipients have had recurrence of their original

disease. Moreover, world statistics reveal that cadaver transplants have fared almost as well as transplants from live unrelated donors.[1] It seems reasonable, therefore, to study the possibility of clinical transplantation of cadaver kidneys to the fullest extent.

Cadaver Kidneys

For a cadaver kidney to be used for clinical transplantation, written legal permission must be obtained from the next of kin, the kidney must be free of disease, the deceased patient must be under 70 years old, death must have occurred after prescribed treatment by the attending physicians, and the heart must have stopped beating for at least 5 min.

The most important consideration, of course, is the prevention of harmful effects from prolonged ischemia in the transplant, which may lead to irreversible tissue damage.[3] The kidney must be kept viable during the terminal stage of the donor's life (*in vivo* preservation), and deterioration of the organ after resection from the donor must be minimized (*in vitro* preservation).

Physiologic Considerations in Kidney Preservation

Any method for *in vitro* preservation of a kidney must protect against the altered physiology resulting from ischemia and hypoxia. Since the basic living unit is the cell, successful preservation must maintain cellular stability despite decreased oxygen supply, to which the cell is extremely sensitive. The maintenance of cellular integrity is a dynamic process resulting from a balance between breakdown and repair reactions.[4] Physiologic interruption of this balance by hypoxia or anoxia may cause irreversible damage, unless protective compensation is afforded. The logical approach is to reduce metabolic activity. It follows, then, that to obtain cadaver kidneys in optimum condition, renal metabolism must be sufficiently reduced before death of the donor (as well as after).[5]

The present investigation was undertaken to determine the advantage, if any, of antemortem treatment in minimizing renal ischemic damage during low organ perfusion before the death of the donor. In addition, studies were performed to evaluate a method of *in vitro* preservation of such kidneys after removal. These experiments were designed to simulate the clinical situation in which cadaver kidneys are used.

It has been shown that cellular hypoxia from any cause results in leakage of enzymes through the limiting lipoprotein membranes of lysosomes, minute subcellular organelles in cells.[6] These lysosomal hydrolytic enzymes (which include proteases, nucleases, and phosphatases) are released by damaging stimuli, especially oxygen deficit, and then act upon cellular proteins, nucleic acids, and polysaccharides.[7] This insult may start a chain reaction, since the enzymes once released affect both extracellular and intracellular structures.[8] With this pathophysiologic background in mind, it is reasonable that a method for organ preservation must protect lysosomal and mitochondrial membranes of the involved tissue cells.[9]

Effect of Total-Body Hypothermia on Kidneys *in vivo*

We designed a series of laboratory experiments to evaluate the effect of protective measures for decreasing renal metabolism preterminally by total-body hypothermia.

Experimental Method

Forty adult mongrel dogs were divided into four groups of 10 dogs each (Table 1). In Group 1, hypothermia was in-

TABLE 1. Experimental Method for Evaluating Protective Effects of Hypothermia on Canine Kidneys

Group	No. dogs	Rectal temp.(°C)	Induction and duration of hypothermia	No. dogs undergoing postmortem nephrectomy at:		
				1 hr	2 hr	3 hr
1	10	28	3 hr ante mortem	4	4	2
2	10	15	Immed. post mortem until nephrectomy	5	3	2
3	10	15	30 min post mortem until nephrectomy	4	4	2
4 (Controls)	10	Normal	None	5	3	2

duced by external total-body cooling with ice to a rectal temperature of 28°C; after 3 hours, all dogs were asphyxiated by tracheal occlusion. In Group 2, no preterminal hypothermia was induced, but immediately after death by asphyxia, external body cooling was induced until a rectal temperature of 15°C was reached. In Group 3, a latent period of 30 min after death by asphyxiation was allowed without treatment; external cooling was then begun and continued until a rectal temperature of 15°C was reached. Group 4 animals were kept normothermic both before and after death.

In all 40 dogs, the following study protocol was adopted: (1) the right kidney was removed at an interval of 1, 2, or 3 hours after death (or, in Groups 2 and 3, after induction of desired hypothermia); (2) each kidney was perfused with 5% low molecular weight dextran (40,000)[10] in a balanced salt solution (Tis-U-Sol) immediately after its removal[11] and then replaced as a cervical homograft by methods previously described[11-14] (Figures 1, 2); (3) the

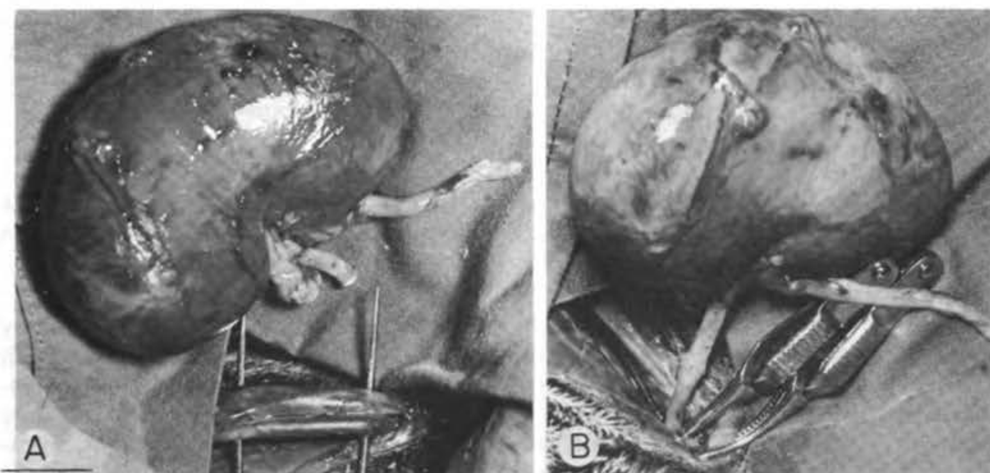

FIGURE 1. A, appearance of kidney immediately after perfusion, just before anastomosis to the external jugular vein and common carotid artery of the recipient. B, after arterial anastomosis. Note the thin film of methyl 2-cyanoacrylate monomer which is used as an adjunct to suture. There is no compromise in lumen size at the anastomotic line. This step prevents leakage at the suture site in the presence of dextrans and anticoagulation. Venous anastomosis is carried out similarly.

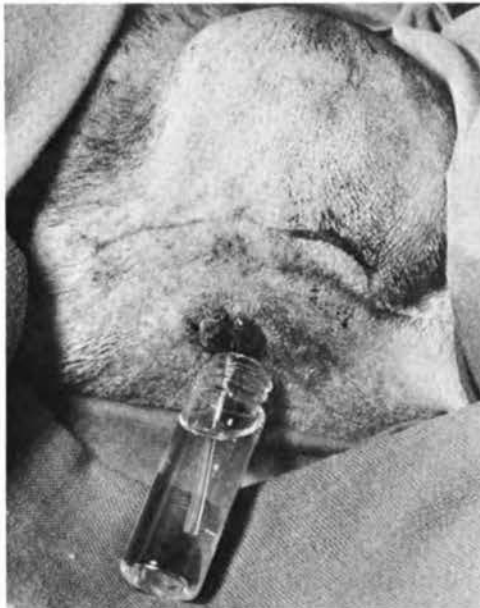

FIGURE 2. Preserved cervical kidney transplant (stored 24 hours at 2°C and 4 ata) excreting clear urine on the first postoperative day.

grafted kidneys were evaluated by appearance (consistency and color), arterial inflow and venous drainage, immediate and delayed urine production, urine osmolarities, and open renal biopsies.

Results

The results are summarized in Table 2 and Figure 3. Generally, external body cooling before death protected canine kidneys for periods up to 2 hours post mortem. Hypothermia immediately after death protected the kidneys from irreversible anoxic damage only when the ischemic period did not exceed 1 hour. After 2 hours of ischemia, definite microscopic signs of tubular damage were present, although all kidneys in this group produced urine immediately, with a gradual increase up to 24 hours (Table 2, Group 2). Hypothermia induced 30 min after death protected a kidney for 1 hour of ischemia, although the damage here was more marked than that noted in Group 2 for the same ischemic interval (Table 2, Group 3; Figure 4A). When renal ischemia was prolonged to 2 hours, more diffuse tubular damage was evident microscopically, and function was poor.

When no treatment was given (Table 2, Group 4), kidney removal immediately after death was mandatory to salvage a functional organ. In our experience, kidney revascularization should be accomplished within 40 min (or less, if possible) after its removal for optimum function.[5]

These results indicate that protective measures to decrease renal metabolism should be instituted before death whenever the use of a cadaver kidney for human transplantation is considered.

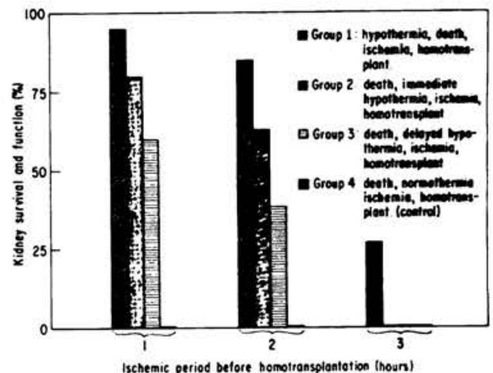

FIGURE 3. Effect of antemortem and postmortem treatment of the donor (by hypothermia) on kidney function and survival.

EFFECT OF TOTAL-BODY HYPOTHERMIA WITH CHLORPROMAZINE ON KIDNEYS *in vivo*

The above data clearly show the efficacy of total-body hypothermia before death to protect against irreversible kidney damage. The addition of chlorpromazine ante mortem (0.5 mg/kg body weight, intravenously) to hypothermia seemed a logical supplement to this, because this drug is a well-known cellular protective agent, due to its metabolic inhibitory effects. Moreover, our previous experience in this laboratory showed that

TABLE 2. Results of *in vivo* Studies on Effect of Hypothermia

Group	Experiment	Time ischemic (hours)	No. dogs	Kidney appearance after homotransplantation	Urine production	Urine osmolarity (mOsm/liter)
1	Hypothermia 3 hr	1	4	Normal (gross and microscopic) until rejection	+++ (immed.)	427–1350
		2	4	Normal (gross and microscopic) until rejection	+++ (immed.)	380–1150
		3	2	Initially soft, purple, 30 min; then tense, congested; hydropic degeneration; loss of "brush border" from tubular cells	Minimal initially then anuric in 4 hr	—
2	Hypothermia immed. after death	1	5	Normal (gross and microscopic)	++ (immed.)	337–1200
		2	3	Initially tense, purple, then normal in 15 min; tubular damage as above	+ to ++	250–1250
		3	2	Tense, discolored, tubular damage marked	None	—
3	Hypothermia 30 min after death	1	4	Initially tense, then normal color and consistency; moderate tubular degeneration	+ to ++	250–1150
		2	4	Initially tense, purple in 3 kidneys without reversal; more diffuse tubular damage	+ in 1 dog ++ in 2 dogs anuria in 1 dog	250–840
		3	2	Initially very tense, purple, without reversal; diffuse tubular damage	None	—
4	Normothermia (controls)	1	5	Initially tense, purple, then grossly normal; hydropic degeneration microscopically, but reversible	+ to ++	250–1150
		2	3	Initially tense, purple; irreversible tubular damage	None	—
		3	2	Tense, engorged; marked irreversible tubular damage	None	—

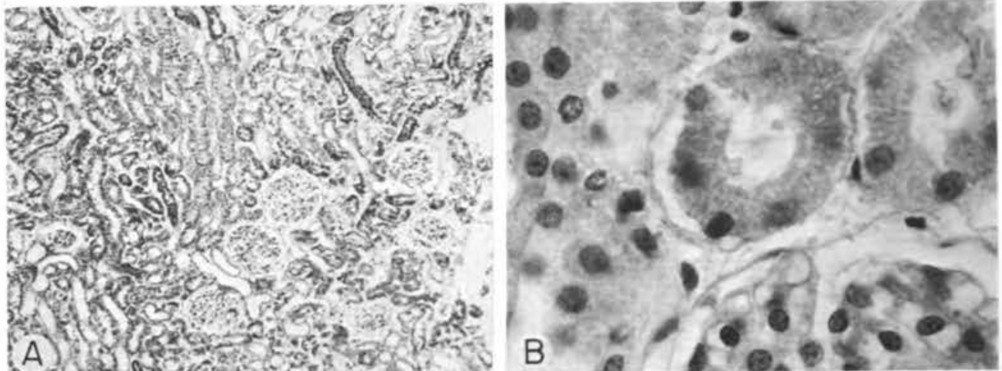

FIGURE 4. A, tubular change (hydropic degeneration) in a cadaver kidney. Hydropic change is the characteristic feature of renal ischemia, the damage depending on the degree and duration of ischemia. B, flattening of the "brush border" of the proximal tubules in a kidney preserved *in vitro*. A change of the degree shown above is reversible.

chlorpromazine and hypothermia, or chlorpromazine, hypothermia, and hyperbaric oxygen ("preservation triad") are successful in preserving whole organs *in vitro* for periods up to 72 hours.[15,16]

Chlorpromazine, a phenothiazine drug, has a general inhibitory effect on cellular metabolism, but more specifically it helps prevent mitochondrial swelling and leakage of lysosomal enzymes during cellular injury.[17] Thus, it can specifically combat the adverse changes that occur during ischemia and hypoxia.[9,18]

This combination was tested experimentally in the laboratory, and the favorable results led to our using chlorpromazine on certain selected preterminal patients designated as potential kidney donors after death.[14]

PRESERVATION OF KIDNEYS *in vitro*

Experimental Method

Previous reports from this laboratory described *in vitro* renal preservation for periods up to 24 hours and longer, with return of satisfactory function after contralateral nephrectomy.[19,20]

In vitro preservation, as described herein, was studied to evaluate kidney function after 24-hour storage, but this investigation differed from previous work in that storage was not begun until 1 hour had elapsed after nephrectomy.[13] The study consisted of two parts: (1) in 15 dogs, a right nephrectomy was performed, immediate organ perfusion was done, and the kidney was then immersed in balanced salt solution (20°C) for periods of 1, 2, or 3 hours before replacement as a cervical autograft; and (2) in another 15 dogs, a right nephrectomy was performed, the kidney was perfused and immersed in balanced salt solution (20°C) for 1 hour, and the organ was then preserved for 24 hours by hypothermia (2°C) and hyperbaric oxygen at 4 ata. After storage, the kidney was replaced as a cervical autograft.

In both groups, kidney function was assessed by initial appearance and urine production, periodic biopsies, urine osmolarities, blood urea nitrogen (BUN) levels, and the ability of the autotransplant to sustain the life and health of the animal after contralateral nephrectomy.

Results

The results are summarized in Tables 3 and 4. Those transplants held *in vitro* for 1 hour before replacement functioned satisfactorily. The gross appearance, consistency, and texture of the kidneys were

TABLE 3. Results of *in vitro* Studies Using 1, 2, and 3 Hours of Ischemia

No. dogs	Time *in vitro* (hr)[a]	Appearance after transplantation		Urine production		Osmolarity (mOsm/liter)	BUN (mg%) after contralateral nephrectomy[b]	
		Gross	Microscopic	Immediate	Delayed		First 5 days	At 2 weeks
9	1	Normal	Mod. tubular hydropic degen., flattening of "brush border" of proximal tubules (3 dogs had acute inflam. filtrate)	+++	Mod. until contralateral nephrect., oliguria 3 days, then mod. again	288–760	>150 (highest 235)	32
3	2	Tense, purple 15 min; then normal	Marked hydropic tubular degeneration	++ to +++	Mod. until contralateral nephrect., oliguria 3 days, then mod. again	250–745	>160	38
3	3	Tense, purple no reversal	Severe tubular damage (necrosis)	+	Anuric after 48 hr	—	Animals died before contralateral nephrectomy	

[a] In balanced salt solution at 20°C.
[b] Within first 5 days after contralateral nephrectomy.

TABLE 4. Results of 1-Hour Delay Between Nephrectomy and 24-Hour *in vitro* Storage [a]

No. dogs	Appearance after transplantation		Urine production		Osmolarity (mOsm/liter)	BUN (mg%) after contralateral nephrectomy	
	Gross	Microscopic	Immediate	Delayed		First 5 days	At 2 weeks
12	Normal	Mod. tubular hydropic changes, flattening of "brush border"	+++	++ until contralateral nephrectomy	245–790	>200	35
3	Tense, congested	Severe tubular hydropic changes, some areas of tubular necrosis	+ (1 dog) ++ (2 dogs)	+ until contralateral nephrectomy	230–560	>265 [b]	—

[a] A total of 15 dogs was used.
[b] These three animals died in uremia; average survival was 26 hours.

within normal limits after revascularization. Urine excretion began immediately and remained within acceptable limits for several days. Urine osmolarity values ranged from 288 to 760 milliosmoles/liter. During the first 4 or 5 days after contralateral nephrectomy (delayed for 2 weeks), most animals showed some oliguria and the BUN level rose, the highest value reached being 235 mg%. After 9–10 days, however, urine output returned to acceptable daily volumes and BUN levels returned to near-normal values. Microscopically, moderate tubular hydropic changes could be visualized, and discrete flattening of the "brush border" of the proximal convoluted tubules appeared (Figure 4B). In three dogs, an acute inflammatory infiltrate was present.

Three dogs undergoing renal autotransplantation after 2 hours of ischemia before cervical replacement showed impaired renal function, the findings in these animals being more marked than those noted above. A striking microscopic difference was the greater degree of hydropic degeneration of the renal tubules.

All three kidneys held ischemic *in vitro* for 3 hours before cervical replacement had scant urine output initially, and by 48 hours the dogs were anuric. Histology of these kidneys again showed severe tubular degeneration with areas of tubular necrosis in some sections.

Twelve of 15 kidneys held ischemic for 1 hour, preserved *in vitro* by hypothermia and hyperbaric oxygen for 24 hours, and then reimplanted as cervical autografts showed immediate clear urine output, and the animals survived after contralateral nephrectomy. During this period of time, the BUN levels were within upper normal limits, and the dogs were healthy. The remaining three dogs died in uremia 26 hours (average) after removal of the opposite kidney. The average BUN level (of the 12 surviving dogs) before contralateral nephrectomy was 15 mg%, it increased to a mean value above 200 mg% within 4–5 days afterwards, and then it gradually decreased over 10 days to levels within upper normal limits. At this time, urine osmolarity was in the normal range. The microscopic changes were essentially the same as those seen in kidneys which had been held *in vitro* for 1 hour but autotransplanted in the neck immediately after this period.

Prolongation of *in vitro* Renal Preservation

Previously we reported prolongation of successful *in vitro* canine kidney preservation from 24 to 48 hours by hypothermia and hyperbaric oxygenation.[14,16,20] Extension of successful storage to 48 hours was accomplished by increasing the hyperbaricity from 3.0 to 8.0 ata while keeping the temperature constant at 2°C. The limit for successful storage at 3.0 ata and 2°C was 24 hours in repeated studies, and kidneys held *in vitro* beyond this period under these conditions uniformly showed irreversible damage both functionally and histologically.

These results prompted further investigation to prolong preservation to 72 hours by raising the pressure even higher.[21] However, we were unable to successfully preserve canine kidneys for 72 hours *in vitro* by combined hypothermia (2°C), hyperbaric oxygen (15 ata), and chlorpromazine.[5,20] The chlorpromazine was included in the perfusate (10 mg/100 ml).

Kidneys so preserved will not sustain dogs in good health after contralateral nephrectomy. The BUN levels have been higher than those in dogs with single autotransplanted kidneys preserved 24 or 48 hours. Also, serial biopsies have shown renal scarring.

Discussion

This study indicates that, for successful function of cadaver kidneys after transplantation, both *in vivo* and *in vitro* renal protection from ischemia are paramount.

Cadaver kidneys undergo longer and more variable ischemic periods than kidneys from living donors. Thus, the solution to the problem requires, first, that kidneys be obtained in the best possible condition and, second, that once the kidneys are removed, measures be taken against further damage until blood flow is re-established in the recipient.

When renal blood flow is interrupted for more than a short time, characteristic histopathologic changes occur which usually are manifested in the renal tubules, since the glomeruli seem to be much less vulnerable. Hydropic degenerative tubular change with flattening of the so-called "brush border" is the usual microscopic appearance in renal ischemia, the degree of damage relating directly to the duration and degree of ischemia. Unduly prolonged ischemia causes tubular necrosis, an irreversible pathologic state.

Unquestionably, lowering of the temperature permits a longer ischemic period because of its inhibitory action on intracellular metabolism; [22-24] the oxygen requirements decrease exponentially with temperature depression.[25,26] It was apparent in our experiments that general profound hypothermia before death allowed a longer period of extracorporeal renal ischemia.

Our ultimate goal is to develop a standardized technique by which cadaver kidneys can be used routinely and successfully. Implicit in such a method are *in vivo* treatment before death of the donor and simplicity of preservation method *in vitro*. Moreover, immediately on pronouncement of death, closed cardiac massage and artificial respiration should be started, and preferably continued until the kidneys have been removed. Heparinization of the donor and, in some cases intravenous administration of low molecular weight dextran may be of some value preterminally.[10]

The cadaver kidney must be chosen with great care. Several reports in the literature describe inadvertent transplantation of unsuitable cadaver kidneys. Kolff has reported two cadaver kidney transplants in which the donor had unsuspected septicemia, only to be recognized after homotransplantation.[27] Both failed. Furthermore, at least one report has appeared of a transplanted kidney containing a malignant tumor.[28]

An important factor contributing to inappropriate selection is the lack of time due to rapidity of demise of the donor. As a result, proper renal evaluation is often lacking. In living donors, complete renal evaluation is possible before transplantation. Perhaps this difference alone accounts for the early reports condemning the use of cadaver kidneys.[29] A kidney obtained after death of a donor during open-heart surgery is usually ideal, because heparinization and cooling have already been done and preoperative renal assessment has been completed.

In the experiments reported herein, those kidneys protected from irreversible ischemia both *in vivo* and *in vitro* still showed functional and histopathologic alterations after transplantation. This consideration necessitates early support by the temporary use of intermittent hemodialysis for patients whose transplanted kidneys have enough renal tubular damage that they are not yet able to function adequately. In dogs, support during this initial stage of repair is afforded by the animal's intact opposite kidney. It is for this reason that contralateral nephrectomy is delayed several days.

The *in vitro* method we employ to preserve kidneys deprived of blood supply has been a combination of cooling and oxygen under pressure, without continuous renal perfusion.[20,30] The mechanism whereby this combination successfully stores a whole organ in an isolated environment is unclear. Generally, canine kidneys preserved by cooling alone and replaced in the same animals, with immediate contralateral nephrectomy, can support life after ischemic periods up to 6 hours.[8,11] Hyperbaric oxygen used alone to preserve an organ *in vitro* at

37°C causes more damage to the organ than no treatment at all.[10] Thus, the effects of these two modalities together are apparently synergistic rather than additive. Detailed theoretical views regarding the respective roles of hypothermia, hyperbaric oxygenation, and chlorpromazine in preserving whole organs in a viable state are described elsewhere.[14,16]

Various perfusates used to cool and wash out the kidney, once removed, have ranged from simple balanced salt solutions, such as Ringer's or Hank's solution, to more elaborate combinations of blood, serum, plasma, and dextrans.[4] In our laboratory, we screened several perfusates, the most satisfactory being cold (5°C) 5% low molecular weight dextran (40,000) in a balanced salt solution, with heparin added, buffered to pH 7.4 just before perfusion.[31] Generally, kidneys perfused with salt solutions such as pure Ringer's solution become edematous, and initial renal function is poor after transplantation. Edema does not occur when colloids are used in the perfusate, and initial function of colloid-perfused kidneys is more satisfactory.

Ideally, freezing of kidneys would offer the greatest protection that could be expected of cooling.[15] However, no one has yet devised a method to freeze and subsequently thaw canine kidneys with preservation of function.

ACKNOWLEDGMENTS

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DISCUSSION

Discussion of papers by Haglin et al. (*pp. 723–730*),
Matloff et al. (*pp. 731–734*),
Mirkovitch et al. (*pp. 735–740 and 741–745*),
Nelson et al. (*pp. 746–750*),
Taheri et al. (*pp. 751–756*),
and Manax and Lillehei (*pp. 757–767*).

DR. B. K. KHASTAGIR (*Cleveland, Ohio*): I would like to ask a question of Dr. Haglin regarding his method of harvesting the kidney, in which he has used the extracorporeal pump along with hyperbaria. We are concerned with cadaver kidney transplantation at the Cleveland Clinic. We have done 78 transplants in 3 years. Our method of kidney collection is by removal, with closed-chest massage of the heart, and then exposure of the kidney to hyperbaria and transplantation as soon as possible. It seems to make no difference whether the cadaver is subjected to hyperbaria before removal of the kidney. Actually, the major consideration is the time between removal and transplantation. I just wondered whether Dr. Haglin has any control data to support his observations.

DR. J. J. HAGLIN: We have no human controls. This project has only started, and as yet we have only five cases. The initial procedures have been modified as mentioned. If I understand you correctly, harvesting the kidney and immediately cooling it has proved very satisfactory in your experience. I think that we all agree that immediate cooling is the most important method so far in preservation (the others being simply adjuncts) and that you can sometimes get a satisfactory kidney with immediate harvesting and cooling. However, it appears that these kidneys do not always function satisfactorily. In fact, they may have unsatisfactory function for as long as 3 weeks, and it is very difficult to evaluate rejection. The other point that I mentioned was that multiple organs can be removed. If one kidney is unsatisfactory, the opposite one can be used. This offers other advantages, which I think we have already mentioned.

DR. C. HITCHCOCK, *Session Chairman* (*Minneapolis, Minn.*): Others in this country (*e.g.*, Marchioro and Starzl, Surgery 54:900, 1963) have attempted to use pump oxygenators alone at normal temperatures to maintain the body during the harvesting of livers and kidneys, and they have been rather unhappy with this as an overall approach. Do all of you agree with Dr. Haglin that hypothermia is the best method so far developed in accomplishing our purpose?

UNIDENTIFIED SPEAKER: I have a comment regarding both Dr. Haglin's and Dr. Mirkovitch's concern about pH. The kidney is well known to be gravely susceptible to anoxia. At the same time, it is peculiar in that it is more sensitive to alkalosis than it is to acidosis, and raising the pH to 7.6 or 7.65 in a functioning kidney will very often produce more of a dysfunction than will lowering it a proportionate amount below the normal physiologic pH. Therefore, I think the amount of buffering might in some way interfere with the function of these kidneys, whereas, in acidosis, going down to 7.3 or 7.25 might not.

The second point is a question of how rigorous a test we should apply to a preserved kidney. Ultimately, we all would like to have a kidney that can be taken from preservation, can be put into a recipient, and can begin functioning immediately. At the same time, many kidneys have transient tubular injury and often will not function for 2 or 3 weeks following implantation. Therefore, one wonders if an immediate contralateral nephrectomy is not too rigorous a test and whether we are not diminishing our selectivity, thereby missing potentially promising techniques for organ preservation. Perhaps we should leave the

contralateral kidney in for 3 weeks and, if the kidney works after this period, do an immediate contralateral nephrectomy to see if we can get function right away.

DR. HITCHCOCK: Before we ask Dr. Mirkovitch and Dr. Manax to answer, I would like to emphasize why we have been pleased with this empirical approach of the harvesting of human kidneys in the hyperbaric chamber with the pump oxygenator. We do have immediate good function within 5 or 10 minutes. The uremic patient immediately begins to improve with the lowering of the BUN. The creatinine clearances have run as high as 90 in these kidneys, and in the one that was shown here it never did fall below about 70 cc per minute. Consequently, we believe that this is one method of getting organs from a cadaver where you cannot always control all of the time factors from the moment of death until harvesting.

DR. V. MIRKOVITCH: Our previous illustration of pH changes showed the simple fact that there was a considerable metabolic rate in kidneys during storage. Your comment about the alkalosis is quite right. The liquids used for perfusion are significantly alkalotic when compared with the intracellular pH, and this might be a cause for many failures. A new series of experiments with fluids of pH 7.0 to 7.05 (closer to the intracellular pH) has been started, but it is still too early to discuss these results.

DR. W. G. MANAX: Do I understand that you think it is a good idea not to do immediate contralateral nephrectomies?

UNIDENTIFIED SPEAKER: Yes, that was my point. I think we may miss some potentially promising techniques for preservation by doing, in the early screening period of evaluating a method, immediate contralateral nephrectomies, because of the very high incidence of malfunction in the early period that you described. I agree with you that this works nicely.

DR. P. WEBB (*Yellow Springs, Ohio*): I am very interested in Dr. Mirkovitch's use of silicone fluid, the dimethylpolysiloxane. What is the oxygen tension in the silicone fluid under high pressure? Have you measured it, or do you know the solubility coefficient, as compared to water, for example?

DR. MIRKOVITCH: Dow Corning did not measure it, and we did not measure it either. On the basis of data for silicone rubber, it has been assumed that dimethylpolysiloxane should have a high absorption coefficient for gases. In the course of experiments, we observed that after the decompression, bubbles of gas were abundantly leaving the fluid, the organ was bright red, and the blood on the organ was highly saturated with oxygen. These observations are of small value, and until Dow Corning performs the measurements of absorption coefficients for various gases under various pressures and of temperatures for dimethylpolysiloxanes of various viscosities, we are bound to stay with our assumption.

DR. WEBB: It would be a fairly simple measurement if you had an oxygen electrode. I would hope that you could use one of these in the future.

DR. MIRKOVITCH: I am not quite convinced that there is a really good oxygen electrode.

DR. WEBB: Did you stir the silicone fluid? Since you are trying to transfer oxygen to the surface of the kidney cortex, stirring would be important for both temperature and oxygen transport.

DR. MIRKOVITCH: We did not do this. However, thinking that perhaps the thickness of the dimethylpolysiloxane layer could impair the oxygenation, we submerged 50% of the organs into the fluid and placed the other half into empty containers after being just dipped into the fluid. There was no difference in results.

DR. WEBB: Apparently you did not want to flush the renal vessels with the silicone fluid, and I wondered why. Actually, this might both transport oxygen to renal tissues and preserve the intima of the vessels. It is a very inert material, and you could presumably flush afterward with saline and then blood and get restored function.

DR. MIRKOVITCH: Dimethylpolysiloxane is, indeed, a very inert material, but to the

best of my knowledge, this material, as well as the other similar inert materials, does not have the acceptable surface properties for the direct contact with the capillary membrane.

DR. A. D. TURNBULL (*Quebec, Canada*): Dr. Haglin, would you elaborate on tandem transplants and also on your use of the pump oxygenator? Dr. McLean has instituted a program whereby chronic dialysis is done in one of the teaching hospitals in Montreal and renal transplantation at another. We are particularly fortunate in our relationship with the Neurological Institute, where many severe trauma cases are received. We usually have an intended recipient in the hospital, and the resident staff of the Neurological Institute is prepared to bring over the hopelessly injured patient once permission for transplantation has been given. Two operating theatres are set up, with two teams working simultaneously. The kidney is transferred rapidly from one room to the other, and in a few instances both kidneys have been given to two recipients at the same time. The ischemic time has been as low as 20 minutes, and Dr. McLean has done over 30 cadaver transplants.

DR. HITCHCOCK: What is the status of the donor at the time that the operation is performed?

DR. TURNBULL: The donor is transported on a respirator. The brain is completely destroyed from massive head trauma, *etc.*

DR. HITCHCOCK: I understand, but what is the status of the donor?

DR. TURNBULL: Organ perfusion may be maintained by cardiac massage.

DR. HITCHCOCK: So the donor is legally alive, is that correct?

DR. TURNBULL: No, I don't think they could be considered legally alive.

DR. HITCHCOCK: This is one of the core problems here. When does one declare the cadaver dead?

DR. HAGLIN: I think we all appreciate the problem, in discussing the harvesting of kidneys from human cadavers, of when death occurs. It is almost an impossible thing to discuss, and I think we will have to modify our thinking somewhat over a period of time.

As far as the tandem preparations are concerned, these are employed when small cadavers (30 or 40 pounds) are used and more kidney parenchyma is needed. In an adult transplant, usually only the left kidney is used. If two recipients are in the hospital and ready, you would certainly want to use both kidneys, and this is feasible. Or, if for some reason there is an adequate perfusion of the extirpated kidney with low molecular weight dextran, the second kidney may be harvested and used. There is something I failed to mention. After the kidney is extirpated with the pump oxygenator, the kidney is also perfused with low molecular weight dextran in saline (Rheomacrodex) which has been cooled to 2 to 4°C. Oxygen, at 3 atmospheres absolute, is bubbled through the Rheomacrodex. The kidney is perfused until the effluent is clear, and this requires less than 500 cc. Exactly what a pressure of 3 atmospheres absolute adds as an additional supply of oxygen to that kidney is difficult to say. We can set up animal experiments, but it is impossible to duplicate the human conditions. I am sure that, in your cases, you encountered the same situations we did. These patients had cardiac arrest either on admission or subsequently. They had periods of hypotension that were treated with vasopressors, periods of hypothermia, and then periods of normothermia. Consequently, it would be almost impossible to duplicate a series or compare one series with another, because each patient was different. I think that there are various ways of getting around this problem, and each of us, with the methods we have at hand, is simply trying to improve the early function of the kidney homograft.

DR. HITCHCOCK: From what has been said, I think we would all have to agree that Dr. McLean's group is really doing controlled living-donor transplants, which, of course, are being done many other places with about the same general ischemia times. We might state for those in the audience who

are not as *au courant* with the general problem of transplantation, that a blood-related, compatible, living donor still provides the best kidney and the best chance for survival.

DR. N. ACKERMAN (*Boston, Mass.*): In reference to Dr. Mirkovitch's studies, I think we should not confuse the difficulties in obtaining accurate tissue oxygen tension recordings with those in obtaining oxygen tension recordings in fluids. Such techniques with fluids are generally well accepted. The studies that have been suggested could easily be performed. Some of us are a little skeptical as to the actual penetration of the oxygen through the fluid into the tissues, and we would welcome the addition of oxygen tension studies.

I would like to compliment Dr. Matloff on his tissue oxygen tension studies and would be very interested in seeing additional studies performed at varying depths in the kidney to find the point to which oxygen penetration occurs.

The studies of Dr. Nelson are extremely interesting, and it appears, on the basis of his studies in the kidney and also our studies in limbs, that even if there is a very marginal blood supply, insufficient to maintain permanent viability under ordinary conditions, there can be protection by hyperbaric oxygen.

DR. D. B. MATLOFF: Our probes were placed on the innermost portion of the cortex, and we did not move them. We were impressed, however, that even though there was a sinus track through the cortex to the positioning point of the probe, oxygen did not leak down through the sinus to the probe. We assumed, on the basis of our studies, that there was minimal, if any, transcapsular uptake of oxygen.

DR. HITCHCOCK: Dr. Nelson, do you care to comment about the practical applications of the collateral flow through the renal capsule?

DR. G. D. NELSON: Of course, one of the clinical applications that initiated our study was the situation of abdominal aneurysms where the renal arteries had to be occluded and reimplanted or merely occluded during repair. Therefore, I think there are many clinical applications of this situation where maintaining the collateral capsular flow with hyperbaric oxygen may broaden our treatment horizons.

DR. MATLOFF: I have several comments, the first directed to Dr. Haglin. Recently at the Surgical Forum, Dr. Arthur Thomas reported that exposure of kidneys to hyperbaric oxygen adversely affects their function by a direct action upon them. He also demonstrated that there were detrimental systemic effects as well, these being hypovolemia and hypotension. What are your feelings about this direct toxic effect of hyperbaric oxygenation on renal function?

DR. HAGLIN: If I recall correctly, the hypotension and the toxic effects, as manifested by reduced function, did not occur for 2 to 4 hours. In other words, there was good function for the first 2, 3, or 4 hours, and then these animals manifested hypotension. When this was corrected, function returned again. It may well be that oxygen is toxic to the cell. In our cases, the perfusions are not usually longer than 2 hours (frequently shorter) so this probably does not apply. In evaluating experiments, one must recognize that these organs differ, depending upon the species of animals used. They differ in their perfusion characteristics for prolonged periods under hypothermia or normothermia. As valuable as animal work is, in many cases we must go to humans and not be too discouraged by our results. It is not always accurate to say that oxygen toxicity affects dog kidneys, and for this reason we should not risk it in the human being. Similarly, we know that the lungs of various species of animals have different tolerances to oxygen. Thus, I think we should progress higher on the species scale in our investigation.

DR. MATLOFF: Dr. Manax, I have followed your work over the past year with enthusiasm and interest. Although we were mainly interested in the problem of the normothermic kidney in hyperbaric oxygenation, we did perform several experiments under hypothermic conditions. Our feeling was that the hypothermic kidney might certainly have a greater affinity for oxygen under increased pressure than the kidney at normothermic temperatures. In two experiments performed at 4 atmospheres, vascular clamps

were applied, and the kidney was cooled to 12°C by continuous perfusion with hypothermic heparinized 4% albumin in isotonic saline. With the albumin reservoir cooled and exposed to 4 atmospheres, enough oxygen was dissolved in the albumin to establish renal pO_2 levels at 200 to 230 mm Hg in the hypothermic ischemic kidney. When perfusion was discontinued, the renal pO_2 decreased to zero within 10 minutes, even though renal temperature remained at 12 to 13°C. We therefore believe that at 4 atmospheres the hypothermic kidney does not necessarily have any greater affinity for oxygen than the normothermic kidney. I wonder if you might have any remarks concerning this observation.

DR. MANAX: The solubility of oxygen increases by 2.3 vol% for each 1-atmosphere increase. Therefore, at 3 atmospheres, the oxygen in physical solution rises to 6.9 vol%. Also, with increased pressure and decreasing temperature, there is increased solubility. Thus, according to the basic gas laws, a situation is created in which you will have more oxygen available under the conditions of our experiments.

PANEL DISCUSSIONS

Moderators: HARRY J. ALVIS
STANLEY MILES
WIRT W. SMITH

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Hyperbaric Chamber Safety, Codes, Fire Prevention, and Insurance

Moderator: HARRY J. ALVIS [a]

Panelists: WILLIAM H. L. DORNETTE,[b] GEORGE GRABOWSKI,[c] W. B. PARKER,[d] GORDON STROPLE,[e] HARLAN TURNER,[f] ROGER WAITE,[g] and CARL W. WALTER [h]

To launch the discussion on chamber safety, the moderator asked Captain Harlan Turner of the Los Angeles Fire Department to show his films and discuss the tests conducted at the Hospital of the Good Samaritan in Los Angeles in cooperation with Dr. Roman Yanda, Director of Hyperbaric Medicine there. Captain Turner explained that they had found that material usually burned brighter and faster in compressed air than at normal atmospheric pressure. Mannequins dressed as doctors and nurses, some wearing treated flameproofed clothing and some wearing untreated clothing were set afire. While it was evident that the "flameproof" clothing did not catch fire as readily under the conditions of the test, some did burn in a chamber compressed with air to 45 psig. It was notable that, even though the outer garment was flameproofed, the untreated clothing beneath burned freely. In addition, any areas of clothing shielded from the direct spray continued to burn after the general fire had been extinguished. Trials of the effectiveness of a hand-held hose indicated that this would be useful. It appears that the best thing for chamber personnel to do when a fire starts is get down on the floor, because the flames spread vertically much faster than they do horizontally. (Published reports of these studies appear in the November 1966 issues both of The Fire Journal and of Fire Technology.)

The members of the panel were called forward and each made a general opening statement concerning the interests of his organization. Dr. Walter explained that he was chairman of the Committee on Hospitals of the National Fire Protection Association. This committee attempts to foresee fire hazards in hospitals. The committee feels some apprehension about the fact that chambers are often located in the central area of the hospital, and an explosion might destroy a major part of the institution. There is also some concern over future developments in hyperbaric medicine. While the pioneers and early workers in the field are cognizant of the potential hazards, the prolifera-

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tion of chambers in hospitals with the passage of time may create a situation where less skill, knowledge, and conscientiousness are applied in the operation of these potentially dangerous installations.

Mr. Strople, Executive Secretary of the Compressed Gas Association, explained that the Association is concerned with anything affecting the safe use of compressed gases, and consequently they participate in writing and they assist others in writing codes related to safe practices. Mr. Parker of the Hartford Steam Boiler Inspection and Insurance Company explained that his company is interested in ensuring the safe fabrication, installation, and operation of various kinds of pressure vessels, for which there is an American Society of Mechanical Engineers Code. Mr. Waite of the Aetna Life and Casualty Insurance Company indicated that he spoke for the casualty insurance industry, not only for his own company. These companies are concerned with coverage for public liability, workmen's compensation, and employee's compensation. He expressed confidence that the insurance industry and hospitals would solve the unique problems presented by hyperbaric chambers, just as they had solved a number of other important problems in the past. These panelists were joined by Dr. Dornette, an anesthesiologist, and Mr. Grabowski of the Fenwall Corporation of Boston, both of whom were members of the Committee on Hospitals of the National Fire Protection Association.

The discussion began with a question concerning the significance of the ASME Code in construction of hyperbaric chambers. Mr. Walter explained that many states and municipalities have regulations concerning the use of pressure vessels within their jurisdiction and require that they be constructed safely. In many instances, this means adherence to the ASME Code. He pointed out that the insurability of a chamber was considerably more sure if it was built according to the Code. In answer to a question concerning the construction of plastic chambers, Mr. Walter said he knew of none which had been constructed to Code specifications because the Code does not include plastic construction.

At this point, a lively exchange ensued among the panelists and several members of the audience. Someone on the panel ventured the opinion that plastic was too flammable for use in chambers. This prompted numerous speakers from the floor to point out that a considerable number of plastic chambers are already in operation and are being used without dissatisfaction. The moderator intervened, calling attention to the improbability of being able to eliminate all risks in chamber use; no matter what is done or what the opinions are concerning materials, the operation of these units involves some calculated risks. Dr. Dornette raised the point that, while seriously ill patients are in a position to run some risks, having much to gain and little to lose, this is not true for attendants and some volunteers. He compared the situation to that of the anesthetist who is exposed to the risk of anesthetic explosions. His feeling was that everything within reason should be done to reduce and eliminate as many of the risks as possible.

The subject of insurability was raised, and it was explained that a company such as the Hartford Steam Boiler Inspection and Insurance Company insured the vessel against failure only when it was being used according to specifications and operated by competent personnel. Their contracts usually include an annual inspection service to be sure that this is being done. On the other hand, casualty insurance companies insure against injury to personnel and property damage, apart from the integrity of the vessel and its construction.

Mr. Strople announced that the Compressed Gas Association had a committee which had been working on a safety code for hyperbaric chambers. The committee had hoped to have completed its work

by the time of the meeting, but some unresolved matters remained. He asked and received permission to read briefly from the preliminary draft of the code. The panel and audience were impressed with the minute and far-reaching details taken up in this study. In response to a question, Mr. Strople explained that such a code involves numerous trades and operating groups, which accounts for the complexity and detail of the prospective document. He assured the listeners that it was the Association's desire only to help the medical profession in areas where their technical skills and experience make them qualified advisors; they are in no way attempting to interfere in treatment policy.

At one point, several comments and questions were raised concerning the fire at the Experimental Diving Unit in Washington. It was announced that an account of this fire had been published in the November 1965 issue of The Fire Journal; as other information became available concerning chamber safety, one could expect to read of it in the Hyperbaric Medicine Newsletter.

The meeting concluded with a showing of colored slides depicting in more detail the studies in Los Angeles which had been shown earlier in moving pictures.

Compression and Decompression Problems

Moderator: STANLEY MILES [a]

Panelists: ALBERT R. BEHNKE,[b] EDWARD H. LANPHIER,[c] P. PAULEV,[d] and ROBERT WORKMAN [e]

Having introduced members of the panel, the moderator suggested that each member say a few words on his own specialty and that thereafter the meeting be open for discussion and questions. It was important to realize that in addition to the therapeutic requirements of high pressure oxygen, considerable skill and knowledge were required to ensure the safe conduct of the procedure, not only for the patient but also for those engaged in the administration of the therapy. Dr. C. J. Lambertsen of Philadelphia and Dr. C. Lundgren of Sweden, who were in the audience, were invited to give advice on oxygen poisoning and alternobaric vertigo. The deliberations of the evening are summarized below.

DECOMPRESSION SICKNESS

Decompression sickness, a hazard which always attends the exposure of man to excess atmospheric pressure, is due to the formation of bubbles in the tissues or in the bloodstream when the pressure is reduced too rapidly. In practice, it is possible to remain at 2 ata of pressure indefinitely and be immediately decompressed without harm. As the pressure is increased, however, the time at which there is no risk on immediate decompression is lessened. At 4 ata, for example, immediate return to surface pressure would be safe up to 30 min. For periods of time and for pressures in excess of this, a decompression schedule is required. This is based largely on Haldane's theory of ratios, which states that a two-to-one reduction in pressure is safe. For example, it would be safe to return to 2 ata immediately after working at 6 ata, and thereafter a calculated period of rest would be required. (These schedules are readily available in many publications.)

It is believed that in many instances bubbles may form without producing symptoms ("silent" bubbles), and these may be the cause of the well-known feelings of fatigue and malaise sometimes occurring after a period of exposure to pressure and decompression. The ideal way to decompress is so slowly that bubble formation would be nonexistent, but this is often impractical because of the time required. In the Sea Lab project, for example, decompression from saturation at 200 ft took 33 hours. Time may be saved in the later stages of decompression by breathing oxygen, which allows the inert gas to escape at a greater rate.

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Decompression sickness is unlikely to be a major problem with the pressures in current use for hyperbaric oxygen therapy, but instances may arise where personnel of a hyperbaric unit are asked to treat decompression casualties from elsewhere. Such treatment normally demands pressures above those usually possible in hyperbaric chambers, and a compromise must be made. This might well be to compress to 3 ata and give oxygen for 40 min and air for 20 min, keeping a careful watch on the patient and doing simple pulmonary function tests. The slightest complaint of sore chest would be an indication to stop oxygen; treatment may be required for 3–4 days.

One should remember, however, that it is the reduction of pressure which causes bubbles in decompression sickness and not the pressure itself; so, with carefully controlled decompression, decompression sickness should never occur.

Air Embolism

Air embolism is a rare but serious condition resulting from the trapping of air in the alveoli during decompression. This may be due to lung pathology, in which case the trapping is often local, or to breath-holding in untrained and frightened personnel, the latter situation generally occurring only in underwater work. As pressure is reduced, the air which cannot escape stretches and ruptures the alveoli and enters the bloodstream, passing through the right side of the heart and producing emboli in the arterial circulation; the dangerous areas are, of course, the cerebral circulation and coronary vessels. Alternative sites for escaping air are the pleural cavity and interstitial spaces. A surprisingly small reduction of pressure may cause this condition; in underwater experience it has been recorded in an ascent of as little as 7 ft.

The treatment of the condition is immediate recompression to reduce the size of the bubble; this must be done without delay. There is no time to make a firm diagnosis. Action must be taken with any patient who, on release from pressure, shows any sensory or motor abnormality or loss of consciousness. A doctor can be locked into the pressure chamber subsequently to confirm the diagnosis. The maximum safe pressure available in the chamber should be used. In diving practice, 6 ata is the pressure of choice. If no pressure chamber is available, the patient should be placed in a position between prone and left lateral, with the head down, and be given oxygen to breathe.

Oxygen Toxicity

This subject was broadly discussed at other sessions but simple guidelines would seem appropriate. Two atmospheres absolute can be safely tolerated for 10–12 hours, but 4 ata is dangerous even for short periods and may be regarded as the absolute limit. Some patients would undoubtedly have convulsions. Clinical judgment is critical, and the risk of oxygen poisoning must be balanced against the need of the patient for hyperbaric oxygen treatment. Drugs are not recommended to control the convulsion, as they may mask underlying irreversible damage. In practice, it is generally believed that to alternate high pressure oxygen with air reduces the risk of oxygen convulsions.

The treatment of the oxygen convulsion is to return the patient to air at atmospheric pressure and to keep him under observation for several hours, being alert for irrational behavior. Barbiturates may be of some help at this stage.

Nitrogen Narcosis

Although serious signs of nitrogen narcosis are unlikely to be encountered in the pressures under consideration, it would not be unreasonable to assume that at

pressures of 3 ata or more there might be some minimal deterioration in mental reactions and delicate skills. The condition, of course, increases with pressure, and 4 ata should possibly be regarded as a limit for surgical techniques.

Ear Problems

A less serious but troublesome problem is that of clearing the ears to equalize pressure. It has been said that 12% of the general population might have trouble even with repeated exposures. In selected persons, such as divers and even hospital staff, the figure may be 5%. Even with an optimal rate of compression between 1 meter in 5 min and 25 meters in 1 min, the majority of complaints occurred within the first 3 meters of equivalent pressure, and all occurred within 10 meters. Thus, when testing ears, a 2–10 meter range is adequate. It would be a normal practice, however, to be sure (as far as possible) that individuals can "pop" their ears before entering the chamber. It may be possible in difficult cases to instruct and coax patients while going to pressure, but this may take up to 2 hours, which is sometimes impractical. For such patients, a myringotomy may be needed, but this practice cannot be recommended routinely; it is thought that 80% of cases where this is done are unnecessary, the exception, of course, being the unconscious patient. The commonest cause for difficulty in clearing the ears is an upper respiratory tract infection. In some cases this may be eased by decongestants such as Neosynephrine. Another important factor is motivation; anybody who wants to go under pressure can usually achieve it.

Other cases occur when oxygen is breathed and the middle ear becomes filled with it. This is later absorbed, giving negative pressure with cupping and congestion of the drum and pain. A simple technique for clearing the ears is to tilt the head to one side and blow the nose hard; this usually clears the lower ear and can be repeated on the other side.

Vertigo

Vertigo is, from time to time, associated with change in pressure and is common in divers who are surfacing. It may be accompanied by nausea and vomiting. It is particularly common in the presence of an upper respiratory tract infection and is relieved by decongestants. It is rarely persistent but should be recognized as a complication of pressure reduction.

Migraine

Migraine has occasionally occurred during or after exposure to increased pressure, but there is no evidence that there is any cause-and-effect relationship between the two.

Aseptic Bone Necrosis

Concern has recently been expressed that aseptic bone necrosis may be a potential hazard for the staff involved in hyperbaric oxygen therapy as a result of repeated pressurization. The condition is almost unknown in divers but relatively common in tunnel workers who spend long periods doing hard work under pressure and frequently neglect their decompression schedules in order to get off duty. It is, however, a condition which must be watched. Certainly, from a medicolegal point of view, regular x-ray examination of shoulders, elbows, hips, and knees of exposed personnel should be carried out, perhaps once a year, or once every 3 years.

Noise

Some pressure chambers may, during operation, produce a noise level (90–120

decibels) high enough to produce hearing loss. Such chambers should be insulated, or the persons within them should wear ear plugs or muffs. There is, however, no evidence that the pressure itself produces any permanent hearing loss.

Decompression Collapse in Aviators

Collapse has occurred in aviators on return from altitude as a result of bubble formation in the circulation, and serious consequences may occur if they are left at ground pressure. Such cases should be treated in the same way as decompression sickness in divers.

Restriction on Flying

After exposure to pressure (*e.g.,* a surgeon in a hyperbaric chamber), one is very vulnerable to altitude and high flying; even a bus ride into the mountains may precipitate decompression sickness. Care should therefore be taken to avoid flying for at least 6 hours after exposure to pressure, unless at very low altitude (many Air Forces put this limit at 12 hours).

Chamber Instrumentation and Physiologic Monitoring

Moderator: WIRT W. SMITH [a]

Panelists: I. S. LONGMUIR,[b] R. G. MCIVER,[c] RICHARD MORIN,[d] and CARL SEM-JACOBSEN [e]

Dr. McIver began the discussion by reviewing his chamber instrumentation and the problems involved. Most of his work involves altitude (partial vacuum) chambers, but he does some work with hyperbaric research facilities. He was impressed with the differences in sampling and analytical techniques between hypobaric and hyperbaric work. As an example, he described a specialized sample loop installed through the wall of an altitude chamber which allows gas analysis by chromatography without the uncertainties or hazards of alternative sampling methods. Investigators in his group do manned chamber experiments which may last as long as 62 days; they are quite concerned about atmospheric contaminants that accumulate in that time and about oxygen depletion of the chamber atmosphere.

Mr. Morin described the instrumentation of the physiologic research hyperbaric chamber at the State University of New York at Buffalo. He reported that they are able to monitor and record many different physiologic and physical measurements, depending upon the requirements for the experiment. He described and discussed a mass spectrometer in use which allows breath-to-breath analysis of respiratory gases. The gas sample is brought through the chamber wall continuously through a 0.008-in. stainless-steel capillary to the spectrometer, which is outside of the chamber. The chamber with which he works is a small recompression chamber with a limited number of penetrations (holes through the chamber wall), and so he must make every hole serve as many purposes as possible. He cited several ingenious examples.

Dr. Sem-Jacobsen, who had recently worked on Sea Lab II directing the electroencephalographic (EEG) monitoring of the aquanauts, was asked to describe his apparatus, methods, and experiences. He showed the group one of his miniaturized EEG machines, an instrument about $15 \times 9 \times 2$ cm, completely encapsulated in plastic. It was a single-channel machine, but he said that he had four-channel recorders in packages about twice the size of the one he had with him. These miniature units write with a very fine ink line on a narrow paper strip to produce a miniaturized, but high-fidelity, EEG tracing. The units, which were originally constructed for a Federal Aviation Agency project, run for about 30 hours on one set of batteries and are intended to be carried in the clothing of the person on whom the EEG recording is being made. They are not yet commercially available.

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Dr. Sem-Jacobsen then discussed the difficulties of obtaining EEG tracings on the aquanauts. The electrodes were applied before the men dived to Sea Lab. The principal problem was that the electrodes could be torn off in the process of getting in and out of the rubber wetsuits. The recorder itself was carried by the aquanaut in a water-tight case. Dr. Sem-Jacobsen pointed out that an aquanaut immersed in salt water presents a very low impedance input to the EEG recorder; this had been a considerable technological difficulty, but it had been overcome. Other technical difficulties were the potentials generated by the ocean water moving in the earth's magnetic field and the voltages induced by magnetic storms over Indonesia (which occurred every day), producing a recording pattern indistinguishable from delta waves and suggestive of deep narcosis or brain tumor. During the first few hours of immersion, EEG wave frequency increased, requiring at least 2 days to return to a base-line frequency of 10–11/sec for the aquanauts. There was a short discussion between him and Dr. McIver about "bending" equipment, such as pO_2 sensors in vacuum or high pressure environments, particularly in helium atmospheres.

Dr. Smith then showed a slide of the Duke hyperbaric chamber's blood-gas measuring equipment. He pointed out that spurious pO_2 calibration values could result if blood tonometered at an elevated pressure was decompressed and then used to calibrate an oxygen tension electrode. Under these circumstances, microbubbles form in the blood which are invisible to the eye but readily pass oxygen to the electrode membrane and result in misleadingly high values. There was an extended discussion between the panel and the audience regarding such phenomena. Dr. Longmuir closed by pointing out that very small oxygen cathodes in a carefully defined layer of saline can give reproducible readings whether the sample is air or gas. These matters depend on geometry and permeability of the membrane.

Dr. Smith raised a question about the extent of monitoring required for a particular clinical case. Dr. McIver remarked that in aerospace research the trend was toward simplicity of instrumentation and pointed out that careful clinical observation can provide much information that would be difficult to get even with elaborate instrumentation.

Dr. Sem-Jacobsen, in comment, said that, while his laboratory for brain research was elaborately equipped with instrumentation, one of his most valuable recording methods was a sound motion picture camera. This is ready at all times, requiring only the push of a button to start it. He thought this was very important, because the sound motion picture captures all events as they occur and is not subject to the preoccupation and distraction that can affect men in stressful situations.

Dr. McIver and Dr. Smith exchanged comments about taking x-ray pictures in the chamber. Dr. Smith had remarked that, in the Duke chamber, instruments were available to do all of the things that were ordinarily possible in a well-equipped physiologic cardiovascular research laboratory, except take x-ray pictures. Dr. McIver reported that the aerospace group at San Antonio was able to do this through a chamber wall, using an aluminum plate as an x-ray port.

The next topic was the measurement of tissue oxygen tension. Dr. Longmuir pointed out that this was an art and not a science. Such measurements are not absolute; they must be calibrated external to the tissue, and the proper values can be gotten only in a homogeneous medium. Tissue capillaries are so close together that the electrode tip overlaps several simultaneously and may affect the capillary blood flow by pressure; changes cannot be recognized which are brought about by spontaneous opening and closing of the capillary. He believes that at present

one cannot measure tissue oxygen tension meaningfully.

An extended discussion then ensued among members of the audience, the consensus seeming to be that tissue oxygen tension measurements are still quantitatively very crude. The difficulty results from the anatomy of the capillaries and tissues compared to the relatively enormous sizes of the sensing electrodes. Dr. Longmuir, in response to comments from the audience about very small electrodes, pointed out that the residual current of such tiny electrodes is enormous compared to the signal and thus cannot provide a useful signal for measuring oxygen tension.

Dr. Smith raised the question of radiotelemetry and directed this topic to Dr. Sem-Jacobsen. He replied that, although he had worked with telemetry, he strongly preferred "hard-wire" systems. Telemetry introduces so many difficulties that there must be an outstanding reason for its employment over a hard-wire system. Dr. McIver agreed with Dr. Sem-Jacobsen; he, too, has worked with radiotelemetry and finds that the complexity and extra care required usually do not warrant its use. Dr. Sem-Jacobsen's response was that this was why he had developed the very small EEG recorders discussed earlier.

The next topic involved the quality of electrical recording of microphysiologic signals, the electroretinogram being particularly difficult to record from within a hyperbaric chamber. Dr. Sem-Jacobsen pointed out that a well-shielded unbroken wire between subject and instrument was the simple solution to these problems. Solid silver or gold-plated connectors do not solve the problem of interconnecting terminal points. Corrosion invariably develops, particularly in hot and humid environments. If such plug and connection systems are used, they must be polished frequently to ensure good signals. A discussion arose regarding a method of making pressure-tight signal-wire leads from within the chamber to the instruments on the outside. Drs. Sem-Jacobsen, Smith, and McIver expanded slightly on this point; such wire penetrations are available commercially or can be made locally with techniques that are not difficult and which do not require special machinery or equipment.

Dr. Smith next discussed the planning of chamber penetrations that must be done in advance of chamber fabrication to ensure adequate access ports for unanticipated needs. He cited the "double rule": after having planned for every conceivable need for penetrations, the number ultimately needed will actually be about double that anticipated. Dr. McIver concurred and related the anxious moments that he had when they had to hydrostatically test a chamber that had required more penetrations after installation.

An unidentified speaker in the audience next asked the panel about the means of measuring arterial blood pressure within the chamber. A many-sided discussion resulted from this question. The methods of adapting electronic strain gauges for hyperbaric chamber use, the techniques of modifying and adapting other blood pressure measuring equipment, and the use of blood pressure cuffs, stethoscopes, and low-frequency microphones all came up for review. The problem of inflating the cuff by remote control from outside the chamber was extensively discussed, and several methods were suggested. The futility of trying to measure the blood pressure of an animal inside the chamber with a transducer referenced to external pressure was brought up.

A question was asked about methods of sampling blood within the hyperbaric chamber and getting outside the chamber under pressure for blood gas analysis. Dr. Smith showed a slide of a screw-operated syringe pipette which collects an accurately defined volume of blood at pressure over mercury. The entire measured quantity of blood is then delivered into the gas analysis equipment (*e.g.*, a Van Slyke machine), thus circumventing the problem of sample fractionation by

bubbling. This device and its use represent a modification of a method proposed many years before by Dr. C. J. Lambertsen.

A member of the audience reopened the question of the proper or adequate amount of physiologic monitoring to be done in hyperbaric chambers in clinical situations. Dr. Smith explained the Duke philosophy of hyperbaric clinical instrumentation. This is: the maximum amount of instrumental data should be gotten from any case, consistent with the clinical situation, the patient's safety, and the needs of the moment. Each case treated in the hyperbaric chamber is unique and requires individual consideration of what is to be done.

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Concluding Statement

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The field of hyperbaric medicine exemplifies many of the changes taking place in the structure of the scientific and academic community. In this field, conventional departmental affiliations have become less marked, as engineers, chemists, physicists, anesthesiologists, oncologists, microbiologists, surgeons, internists, and professional divers all meet on common ground—adding their talents to move the venture forward. Hyperbaria has affected the scene in another way, too. It has caused the commitment of large sums of money to a biological area for buildings, for equipment, and for the training of scientists and their supporting teams. Commitments on this scale have frequently been made in the physical but only rarely in the biological sciences.

The possibility of improving the care of sick patients has provided the impetus to the extraordinarily rapid growth of knowledge in hyperbaric research. The imaginations of both professional and lay people have been stirred by the demonstration that gas gangrene could be controlled and acute carbon monoxide poisoning reversed in the hyperbaric chamber, that animals could breathe water in a hyperbaric environment, and that plasma could replace blood. The hope has been expressed that we might be near another "breakthrough" in the treatment of many major illnesses.

Still, we must recognize that the dramatic successes of hyperbaric medicine have been limited to those achieved in the treatment of bends, carbon monoxide poisoning, and gas gangrene. We must face the fact that biology is complex and that many poorly understood variables influence the course of acute illnesses. The difficulties encountered in the clinical evaluation of individual acutely ill patients have demonstrated our ignorance of many biological problems and have emphasized the need for more studies involving normal men and animals. Study of the integrated behavior of the whole animal, in turn, emphasizes the need for more knowledge of the many phenomena occurring in individual organs and tissues. The study of these

simpler systems has raised the question of the effect of hyperbaria on function of individual cells. This inquiry obviously leads to the study of cell organelles and cell enzymes *in vitro*.

As might be expected, the more simple the system the harder it is to obtain data. On the first day of this conference, the biochemists described their well-designed and controlled experiments on the effects of increased oxygen tension on biochemical systems. The next day, the physiologists looked at oxygen toxicity. The experiments were more difficult to control and the data allowed more alternative explanations. The clinicians had the hardest time; their patients showed great variability and, in general, the problem of good controls was unsolved.

The importance of oxygen toxicity and the need for understanding mechanisms by which biological systems can adapt to different gaseous environments were repeatedly emphasized in the meetings. As deep sea exploration proceeds, the study of the relationship between environment and integrated organ function reaches into a new dimension. The study of hyperbaria and hyperoxia will be catalyzed by the engineering developments in the deep submergence program, just as high-altitude flying catalyzed the study of oxygen transport at normal and rarefied atmospheres. The wide variations in oxygen toxicity among different species show that the problem of oxygen toxicity is an approachable one, but the solution remains for the future.

The physiologists and clinicians have made it clear that oxygen must be delivered to tissues through a capillary network and that hyperbaria will not be useful in the absence of perfusion. The degree of perfusion which must be present has not been established. We need to know over what range of flow, if any, oxygen is the limiting factor in survival.

The studies reported at this meeting make it clear that oxygen storage in tissues can be increased by hyperbaria, but the increase is not enough to permit prolonged interruption of blood flow. The study from the Children's Hospital in Boston suggests that in critically ill "blue babies" enough oxygen may be stored in tissues to increase the survival chances after short periods of circulatory arrest, although this highly competent surgical team has instituted other changes in patient care which make it impossible for them to state with certainty that hyperbaria was the major factor in their success.

The work on survival of skin grafts attracted me particularly, because this system is one in which capillary perfusion is present. The clinical studies combining hyperoxia and radiation in the treatment of cancer and the studies of the effects of hyperoxia on the survival of organs for transplantation open up new areas for future investigations, but the present information does not allow definitive conclusions.

In the foreseeable future, clinical hyperbaric facilities will constitute a regional resource; there are not enough well-defined therapeutic indications to put a clinical hyperbaric facility in each major city. The hyperbaric unit

will be useful in direct proportion to the degree to which it is instrumented and staffed to produce quantitative data. It will have the greatest impact and be most easily financed in those areas where scientists from many disciplines wish to carry on studies in their areas of competence in a hyperbaric environment. It is far easier to teach diverse scientists to work in a hyperbaric environment than it is to teach the staff in charge of the chambers all of the disciplines of science which must be applied in hyperbaric work.

This has been an excellent meeting. Clearly, we have all lived at the right time.

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Afterword

During the conduct of the meetings, a number of participants inquired about the time and place of a Fourth International Conference on Hyperbaric Medicine. In the absence of a formally constituted organization for workers in the field of hyperbaric oxygenation, however, no medium exists for further planning or discussion on an international level. Certainly it would be presumptuous of the Planning Committee of Duke University or the Division of Medical Sciences of the National Academy of Sciences – National Research Council to assume the role of spokesman for the unorganized group. Therefore, as President of the Third International Conference, I suggested at the conclusion of the meetings (and I reiterate that suggestion now) that we all recognize and endorse the leadership of Dr. I. Boerema, who organized the First International Conference. It is my recommendation that those interested in a Fourth International Conference on Hyperbaric Medicine forward their suggestions to Dr. Boerema in Amsterdam. I am sure we will all be grateful to him for his wisdom in this matter.

Ivan W. Brown, Jr.
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