Panel on Gravity

DETAILS

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Committee on Environmental Biology

Panel on Gravity

REPORT

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Committee on Environmental Biology

REPORT OF THE PANEL ON GRAVITY

1. Introduction

The Panel on Gravity is a subcommittee of the Committee on Environmental Biology of the Space Science Board.* It was formed to advise the Space Science Board about research on the effects of abnormal gravitational environments on biological matter. The plant and sub-primate animal were to be the organisms of concern to the present panel, with emphasis on low gravitation.

The Panel was convened at Washington, D. C. on November 30, 1962. The meeting was intended to define and discuss the objectives of the study, and to lay a foundation for its completion. Minutes of this meeting are given in Appendix B. As a consequence of the discussions, members were asked for either individual or joint contributions along the following lines:

a. What would be the action of abnormal gravity on biological matter at any level of organization? What are the theoretical and experimental bases for conjectures along these lines?

b. Is the effect of weightlessness on biological material a valid subject for experimental study in space? If so, what experimental approaches can be suggested? What approaches seem inappropriate or undesirable at this time? What ground-based work should be initiated and supported?

* Membership of the Panel on Gravity is given in Appendix A.

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c. Can specific biological experiments be suggested to be conducted in orbiting spacecraft?

The following topics were assigned for individual expansion:

A. Physical Considerations

1. On the nature of gravitational action (Morrison)
2. Physical aspects of low-g biological experiments (Pollard)
3. Acoustico-vibrational considerations in low-g environment (Snodgrass)

B. Plants

1. Embryogenic and histogenic effects of gravity (Torrey-Jensen)
2. Morphological and ecological considerations (Went-Salisbury)
3. Growth phenomena (Gordon)
4. g-detection mechanisms (Thimann-Bogorad)

C. Animals

1. Embryogenesis in invertebrates (Metz)
2. Membrane phenomena (Wilbur)
3. Embryogenesis and regenerative phenomena in vertebrates (Edds)
4. Physiological responses (Prosser)

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Contributions from the panel members are given in full in the body of this report. A resumé of each submission is given below.

2. The Physics of Weightlessness - Phillip Morrison

Gravity is discussed as a flow of momentum into matter. This view is used to differentiate between flotation support and free fall conditions, with particular reference to their respective stress and stress-less states. It is this difference that prevents rigorous transfer of inferences about biological behavior in the compensated or flotation environments to behavior under free-fall. However the momentum "replacement" under flotation makes the conditions of stress (in the physical sense) so close to that of the free fall that many experiments planned for free-fall conditions. could be investigated initially in flotation designs on ground.

It is improbable that molecules can function as georeceptors, and it may be predicted that bacteria, viruses, DNA processes, and the like will not be directly affected by "zero" gravity. Neither will organisms with radial symmetry, but developmental alterations may be expected in asymmetric organisms.

3. Physical Aspects of Low Gravity Biological Experiments - Ernest Pollard

Single cells less than 10μ are unlikely to show direct effects of low gravity, though cell colonies might be affected. In larger cells there may be a redistribution of enzyme-forming systems, which would give rise to polarization. The low surface tension of the cell membrane lends itself to hydrostatic stress distortion, implying an alteration in permeability and hence an almost certain alteration of cell properties under low gravity conditions.

Pollard considers the plant will probably prove to be the more significant responder to low gravity environments; hence priority should be given to experiments on the developing plant and embryo.

4. Acoustico-Vibrational Considerations - James M. Snodgrass

In the study of vibrational effects, it is desirable to introduce perturbing stimuli into the low gravity environmental test system. This is a concept that is beginning to be expressed in other biological areas. It is cognate to the idea that stimuli intrinsic to the vehicle may either influence or dominate other phenomena under investigation. Snodgrass stresses the need not only for adequate mechanical and acoustic isolation of the experimental environment, but also for knowledge of the qualitative and quantitative aspects of the residual and imposed vibrational accelerations. He proposes as a basis for discussion a maximum vibrational acceleration of 10^{-2}g (10 cm sec^{-2}) for frequencies below 100 cycles . sec^{-1}.

5. Embryogenic-Histogenic Effects - William A. Jensen

By and large, the determinants of plant embryogenesis and histogenesis are still too ill-defined to make sophisticated use of spacecraft for experimental purposes. Simple empirical investigations are recommended along two lines: (1) To find out what happens to multicellular plant material allowed to develop under low gravity. Experimental material for which considerable morphogenetic information is already available should be chosen, such as the germinating grass seed and certain strains of tissue culture. (2) To study the early unequal division which occurs after syngamy. This primary histogenetic event determines to a large extent the subsequent patterns of development, but virtually nothing is known concerning the underlying factors involved in this critical division. If low gravity does have an effect at this point, diversion of all subsequent morphogeneses would be anticipated.

Jensen emphasizes the importance of preliminary ground-based work in straight-forward fundamental biology. He also suggests the potential usefulness of development induced by exogenous enzymatic polarization, experiments that could be implemented with relative simplicity using the root hair of grasses and the guard cells of leaves. Implicit in much of Jensen's ideas is an experimental package that would permit periodic fixation of tissues to enable subsequent histochemical as well as histological examination.

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6. Abnormal Gravity and Early Development - John Torrey

More information is needed from ground-based studies as to the effect of gravity on development before useful experiments can be planned for a satellite. In particular, studies of embryogenesis and histogenesis with clinostat and centrifuge are relevant. The relation of normal syngamy to gravity makes this process of particular interest under clinostat and low-gravity environments. Histological and microscopical analysis is important in this context and this requires recovery of the experimental plants after exposure to low gravity in spacecraft.

7. Anatomical Responses to Gravity - Frank Salisbury and Frits Went

In discussing anatomical responses to gravity, anatomy is taken to be a derivative adaption tc gravity. Moreover, there is a large background of plant research on the effect of reorientation on plant responses. Information from clinostat experiments is considered susceptible of extrapolation to low gravity conditions because the threshold period for gravitational triggering is relatively long. The above considerations are elaborated in some detail as a basis for suggesting several experiments on anatomical responses to gravity. A more extensive pursuit of positioning, clinostat, and centrifuge experiments is emphasized. Also stressed is the necessity for highly competent anatomists in the examination of material previously subjected to low gravitational environments. Here again observations are suggested at the stages of syngamy, embryo morphogenesis, and on the development of organs initiated *de novo* under low gravity.

Ecological interactions in the plant community under low gravity is a relatively novel area of concern. It also leads to concern about interactions between abnormal gravity and other environmental factors, particularly for exposures of long duration. It is suggested that the ecological aspects are sufficiently important to merit intensive expert study solely to suggest reasonable first questions to ask.

8. Growth Phenomena in Plants - Solon Gordon

Since plant growth and development are modifiable by reorientation in normal gravity, gravity may be assumed to have a determinant effect on the initiation or nature of polarization. From this he infers that the probability is high that extended exposure to low gravity will change growth and morphogenesis.

On the basis of geotropic responses of higher plants, Gordon calculates the order of dimension of the gravitational sensor, and infers that experiments on the direct effect of low gravity on bacteria, unicellular algae, and protozoa do not seem worthwhile.

Several low gravity experiments are proposed in the area of dimensional growth and in stimulus response reactions involving differential growth. Changes in orientation on the ground elicit electrical polarization which precedes cytological and histological polarization. On this basis, it is suggested that transverse and longitudinal potential gradients in differentiated and undifferentiated tissues could be monitored for correlation with subsequent changes in growth and development. If the readings were telemetered, some useful information could be obtained even if recovery of the experiment should fail.

9. Effects of Low Gravitational Force on Plants - Kenneth Thimann

A brief historical background to the idea of discrete intra-cellular georeceptors is given and the desirability of several types of ground-based investigations to add to our rudimentary knowledge of georesponse is stressed. These are the nature of the georeceptor and the morphological onset of geosensitivity, particularly where vegetative forms are insensitive. The widest field for low gravity studies is probably in the area of cell morphology and anatomy.

No experiment should be carried out in a satellite before thorough exploration on ground in a "balanced" or compensated field, i.e. the clinostat. An explicit example with the germinating grass seed is given, and then extrapolated to a low gravity condition.

The fungal sporangiophore might also be considered in this area of research.

10. Embryogenesis - Charles Metz

Embryogenesis in the animal is examined. The uniqueness of extended low gravity as a parameter is emphasized, as is the importance of trial runs before flight, particularly with respect to recording of data. The rationale of back extrapolation from centrifuge responses is discussed, with particular emphasis on the invertebrate egg. Investigation of the early histogenetic events after syngamy in low gravity appears worthwhile (see also Jensen, above). The egg of the sea urchin seems to have much to offer in this respect. The development of the amphibian egg under low gravity also merits consideration, particularly in view of the early disagreements in the results with clinostats.

Concerning experiments on higher vertebrates, the orientative dependence of morphogenetic cell movement in the chick embryo and its hydro-dynamic phenomena appear promising for investigation, particularly because preliminary information could be readily obtained in the laboratory. A comment is made about pre-parturition phenomena in the mammal that are potentially influenced by low gravity (uterine implantation and foetal position).

11. Membrance Phenomena - Karl Wilbur

The biological importance of membrane properties is noted, and the effects on them of radiation, weightlessness and high gravity fields. Wilbur does not believe that membrane components would be displaced at accelerations of less than 1 g, and that cytoplasmic displacement would be relatively unimportant except in a few cell types. Studies on the direct effect of low gravity on permeability do not seem justified. Direct effects on permeability would be manifest, if at all, under conditions of high gravity, studies that would best be carried out with centrifuges in the laboratory.

12. Embryogenesis and Regeneration - Mac V. Edds

Embryological and regenerative phenomena in the animal appear to be, by and large, not influenced by gravity. As a result, there seems no point in exploring such systems in low-gravity environments. Our understanding of tissue and organ development is still too imperfect to interpret even an apparently positive result. Even at the cellular and subcellular level we know too little to design useful and sophisticated experiments.

13. CNS and Vascular Effects of Abnormal Gravity - C. Ladd Prosser

Central nervous systems receive a continuous background input from various receptors which are modulated by reorientation to

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gravity. The possibility of changes in characteristics, reflex behavior, and ultimate loss of functional integrity when such modulation impulses become subthreshold is discussed. The primary question is whether central neurones require continual input impulse for normal excitability and capacity to respond. Do reduced proprioceptive inputs over a major fraction of early development alter brain development? The arthropod and lower vertebrate are suggested as suitable experimental material to investigate this question. The broader physiological question is raised of comparative energy requirements of an organism under low gravity.

The problem of vascular tonus is discussed, both in the reduction of continuous gravitational stress and in the venous return of statically oriented animals. Prosser broaches an area of interest hitherto untouched, namely the changes in ion flux across cell membranes under different orientations and deformations (cf. Wilbur and Pollard). Implicitly this relates to the changes in electrical polarity discussed by Gordon.

14. Discussion

In retrospect, several comments may be made concerning the above submissions. The plant scientists apparently see more biological repercussions of low gravity than do the animal biologists. This may be in part a reflection of the particular constitution of the panel. It may however reflect fundamental differences in response of biological groups. Once over critical minimum dimensions, the major effects of low gravity would be assumed to occur in those heterocellular organisms that develop in more or less fixed orientation with respect to terrestrial gravity and which respond to changes in orientation with relatively long induction periods; these are the higher plant orders. On the other extreme are the complex primates which respond rapidly, but whose multiplicity of organs and correlative mechanisms make the occurrence of malfunction and disorganization probable, but not certain. It may be suggested that the heterocellular lower plants and invertebrates will be less affected. However, there is simply insufficient information, as Edds states, to assume that even the vertebrate will be affected seriously by a low gravity environment.

Some of the panel believe in the scientific value now of low gravity biological experiments in spacecraft; Salisbury, Went, Jensen, Prosser, Gordon, Snodgrass, Metz. Others, viz. Morrison, Thimann and Torrey, would substitute laboratory experiments for most of the experiments in space, at least for the present. In their areas of concern, Wilbur and Edds consider that the probable biological return from investigations using orbiting spacecraft would be low, not justifying the expenditure of time, effort and money.

Independently, several panel members reached agreement on specific aspects of the experimental approach to the study of the biological effects of low gravity. These were:

1. Perturbations of the environment to which the experimental organism is exposed must be limited or controlled in order to reduce uncertainties in interpretation of the results. At the same time, the introduction of known perturbations may assist in isolating the effects due solely to gravity. (Cf. Metz, Snodgrass, Gordon)

2. Study of de novo differentiation and other phenomena immediately after syngamy may be of particular importance. (Salisbury, Went, Metz, Jensen)

3. The probability of finding direct effects of low gravity on single small cells is likely to be low. (Pollard, Morrison, Gordon)

4. Study of anatomical changes after exposure of the organism to low gravity is important. (Salisbury, Went, Thimann)

The Panel believes that it could not add significantly to the discussions recorded here until new experimental evidence becomes available. Several of its recommendations for desirable preliminary experiments have already been incorporated in the investigations planned for the first series of biological research satellites.

THE ELEMENTARY PHYSICS OF WEIGHTLESSNESS

Phillip Morrison - Department of Physics
Cornell University

It is very easy to make misstatements about this topic. Perhaps a few simple observations might form the context of the more difficult and detailed biological discussions.

The key point is the recognition that Newton's law of motion can be put into the form $\underline{F} = \frac{d\underline{p}}{dt}$, where $\underline{p}$ is the momentum measured in any inertial frame. If the force is due to gravitation, then $\underline{F} = m\underline{g}$ and we have:

$$F = mg = \frac{dp}{dt} \quad \text{and} \quad g = \frac{1}{m} \cdot \frac{dp}{dt}$$

In short, a gravitational field is a region in which momentum flows into every gram of matter at a fixed rate, about ten meters per second times the mass, per second, for the case of the earth's surface. This momentum flow is the quantity to seek.

Any mass whatever must pick up this momentum while it is in a gravity field. Now, if the mass is to gain momentum, as an orbiting object does, then it can be isolated except for the gravity field, and its momentum intake is exactly as expected. It is in free fall -- another word for orbit, and no additional momentum is flowing to it at all. Weight is the measure of the flow of momentum which an object takes from the supports which surround it. In orbit, no additional flow of momentum is required, and the object, any object, is said to be weightless.

Consider next a ball of solid plastic adjusted to have the mean density of water. If it floats in a pool on the earth's surface, the momentum which the field pours into it is just balanced vectorially by the momentum taken out by the integrated forces of the surrounding water. It too is overall weightless. If it were absolutely uniform and of water density the momentum flow would be a net zero in every small volume of the ball. It would be genuinely weightless, in the true sense of the word. For no additional momentum flow would be taken by it from any contacting surface. Of course the earth's field would act fully on it; weight is never to be thought of as the effect of a gravity field, for surely that cannot depend (non-relativistically) on the motion of an object. Rather, weight is the momentum flow taken by the object when required to remain in contact with its surroundings.

Next, take a more realistic case. Here the plastic ball has the mean density of water. But some of its volume is denser and some lighter than the pool. All of it is at rest. Then the overall momentum flow from the water to the ball through its surface balances the flow out of the spherical surface into the earth's field. But within the ball, the denser portions have a higher momentum intake from the field per unit volume, and their gain of momentum -- motion -- is prevented only by a matching higher momentum flow into the surrounding solid materials within the ball. Stresses must exist within the ball, and small strains to represent the distortion of the lattices which supplies the internal flows of momentum.

But if the ball is in orbit, all volumes suck up momentum at just the rate to give them all the same acceleration, and no internal stresses remain from this cause.

Biological Implications

Evidently the ball of the last example is a good stand-in for the biological systems of cell size and beyond, in which there are differential densities and stresses. These stresses are gone in orbit, and out of orbit they reflect the density differences with the supporting medium. Sea-animals or cells in broth feel stresses depending only on differences in density, and can sense them only by the strains resulting, whether in muscle, vascular walls carrying fluids under pressure, special members supporting little dense loads, as in statocysts, and so on. These are all small fractions of the stresses felt by organisms whose medium is air and density like that of water, in a one-g field. Evidently the use of centrifuges on water-borne organisms is the extrapolated inverse of putting air-borne organisms into orbit. To be sure, the perfect nullification of the differential momentum flows achieved in orbit (barring small tidal effects when finite size is considered) is a very precisely defined state, while the continuum of increased (or decreased by overall flotation) momentum flows is not a single null state. How important this is for organisms is a problem for biologists. I would hazard the prejudice that some other axis will take the place of a gravity field once momentum flow vanishes completely. I would like to see plants or starfish, used to sea water, grown in centrifuges.

Molecular Phenomena

All of the above has been viewed in classical physics only, where in solids, nothing moves, and in fluids only mass flow occurs. In reality, of course, diffusion of molecular species is always possible through any phase, but with widely differing rates. This diffusion can be driven by the momentum flowing into individual molecules from a gravity field. Thus, the air separates under one-g gravity by molecular species as well as for total density. Calculation indicates that a one percent concentration difference, to take a value which might be of biological significance, is set up at equilibrium (in any phase) across a distance of about one hundred meters for a molecular weight of some thirty grams/mole (as in air). To get this gradient within say ten microns, as might be important for cells or small organisms, the diffusing substance needs a molecular weight ten million times greater. It certainly doesn't seem that molecular diffusion under gravity in equilibrium is going to be of much importance. Note that small granules, or globules, bigger than molecules might very well be important, say balls on a scale of a couple of hundred angstroms. If such structures, with a density differing by some percent from the watery surround, can influence processes by their concentration, they can be gravity sensors. In orbit, naturally, all these molecular concentration gradients will smooth out.

Finally, these equilibrium diffusion gradients should be less than powerful. But one could think of non-equilibrium phenomena of the same sort. When one starts to set up these barometer-like distributions, larger concentration gradients can occur transiently. If they are to be gravity-sensitive, though, it seems probable that the large molecular weight is needed. The kind of mechanism which tells a seed which is up might be the time difference between the state of having a substance concentrated uniformly over its coat, and later washed down to have a gradient with more material at the lower portion of the seed. If this depends on actual mass flow of fluid, it can be a low-molecular weight substance, soluble, and of course not in equilibrium except at uniform concentration with respect to diffusion unaffected by flow.

Biological Implications

It will be clear that I am rather bearish on the influence of orbit on living forms. I will freely predict that bacteria, viruses, DNA processes, and the like will not be affected by zero gravity. Here the only proviso is that gas transport be maintained by convective flows. Organisms with radial symmetry but multicellular will not be affected either. Axially symmetrical organisms may have trouble developing, but even they probably will find another gradient to guide them easily. Since it is not hard, as section 1 above shows, to replace some 90 to 98% of gravity here on earth, and since the possible information has quite well understood physical limits, I strongly favor much ground based experiment for every expensive experiment in orbit. Centrifuges and water baths and flowing streams and the like are a lot cheaper and more replicable than rocket flights. After all, I have the strongest belief that organisms cannot do anything but respond to known physical inputs. We might be overlooking something, but not much. Of course I recognize that this can be wrong. But if an astronaut is not effected, I do not see how any DNA molecular process can be.

SOME PHYSICAL ASPECTS OF LOW-G BIOLOGICAL EXPERIMENTS

Ernest Pollard - Department of Biophysics
Pennsylvania State University

1. LACK OF INTEREST IN SMALL CELLS

The fact that random thermal agitation is so powerful an agent on small molecules makes it very unlikely that gravity will have much effect on small cells. These cells almost certainly operate in terms of molecular systems which are never larger than the units of DNA which they contain. They do not contain organelles such as mitochondria, or even whole nuclei. In such cells, and I refer to <u>single</u> cells, it is most unlikely that there could be any distribution due to gravity which is not already far less than the distribution which the cell is manifestly able to sustain. Cells which definitely gain their nutrient from one side only such as those on agar, will almost certainly be able to take a variation of less than 0.1% in some substance across their whole structure.

Therefore, experiments on <u>single</u> cells which are smaller than approximately 10 microns across should be discouraged. This means that at the very smallest, work with yeast is possible, though not at all likely to give results. Cell <u>colonies</u> might show some effects. They are not meant to be excluded.

2. DISTRIBUTION OF THE ENZYME FORMING SYSTEMS

In larger cells a very important factor will be the distribution of the enzyme forming systems. These are presumably the agents which have picked up the messenger RNA and they are now endowed with ability to make enzyme and so materially alter the behavior of the cells. In a cell which is larger than 10 microns in diameter there could be conceived a distribution of these enzyme forming systems. Moreover, it is quite conceivable that in an array of cells, communication of these enzyme forming systems from one cell to another is possible. It seems unlikely that objects as large as ribosomes can travel from one cell to another. Under such circumstances there is no question that gravity effects are to be expected. Therefore, work which is done on <u>systems of cells which are undergoing development</u> is very favorable from the point of view of gravity studies and should be encouraged.

HYDROSTATIC STRESS ON MEMBRANES

The membrane of many cells seems to consist of lipoprotein elements. The evidence seems to be that these are not interconnected by chemical bonds, but instead have physical forces only between them. The result is a membrane of very low surface tension. No good estimates of this surface tension are available, and it would be very desirable to get them, but it seems to be in the neighborhood of 1 dyne per centimeter or less. With such membranes a very small hydrostatic pressure will introduce distortion. This distortion may very possibly modify the diffusion properties of the membranes from the point of view of small or intermediate size molecules. The effect is by no means a small one, and it would be expected to modity in a very material way, the transport of hormones through membranes. It is very likely that this effect operates in groups of developing cells in quite a sharp way that may very well be related to plant morphogenesis.

SUGGESTED EXPERIMENTAL WORK

It is very important that experiments designed for flight should succeed, and that only about half the experiments done at so high cost "succeed" in giving negative results. For this reason, it would be very desirable to fly those experiments which seem likely to be successful in the sense of showing a real effect in flight. Far and away the most probable effect of weightlessness lies in developing plant cells. It would make very good sense to me if all our efforts were put onto good, well-conceived, experiments studying developing plants under conditions of weightlessness. It would look as though these experiments are certain to give positive answers. Positive answers always lead to much better deductions than negative answers. They are more easy to build upon.

Therefore, from the point of view of a biophysicist, I suggest that much more priority go on the developing plant, or developing embryo experiments, and that the probably negative experiments such as zero-g-and-radiation be done, but with lower priority.

ACOUSTICO-VIBRATIONAL CONSIDERATIONS IN A LOW G ENVIRONMENT

James M. Snodgrass - Scripps Institution of Oceanography
University of California

Since we are primarily interested in determining and measuring the effects of low g on biological systems, it is extremely important that the experimental situation should be designed insofar as possible to minimize undesirable stimuli which may as a result of the experimental vehicle dominate the environment to the detriment of the prime desired measurements.

It would seem to be of utmost importance that the properties of the experimental environment be determined insofar as possible throughout the experimental period. Vibration amplitudes and frequencies, as well as noise, may well become seriously perturbing factors and must therefore be known.

There are many possible sources of noise aside from the vehicle itself. Some of these sources are, of course, related to ancillary equipment necessary to maintain the proper biological environment, such as pumps, circulators, filters, etc. In the necessarily somewhat compact assemblies, noise and vibration may easily reach unexpectedly high amplitudes. It is therefore highly desirable that adequate mechanical and acoustic isolation be provided.

Without question, it is recommended that suitable tests of the systems should be carried out in properly stimulated space environment. It would also seem to be of utmost importance to design suitable sensing and recording equipment to record both desirable and possibly undesirable parameters of the environment throughout the experimental period. Though continuous recording is desirable, it is perhaps impossible to obtain, due to space and weight limitations. However, it would seem that intermittent sampling of the environment throughout the period might, however, be properly recorded. There would seem to be need of this type of recorder in almost every biological environmental experiment. Since undoubtedly some of the parameters will tend to vary over rather wide ranges, perhaps it would be well to give consideration to designing the recorders as logarithmic recorders rather than linear.

Undoubtedly, it would be advisable to specify maximum acceptable limits for noise and vibration. However, I rather suspect that these limits would be easily subject to considerable debate. Nevertheless, at the risk of some valid objections, I would propose that we limit all components of vibration below 100 cycles to amplitudes that would result in less than 0.01 g. Above 100 cycles I am quite frankly somewhat in the dark as to the proper specifications. I am quite certain there are individuals who are able to specify some intensities for frequencies and maximum amplitudes which we should permit. (Acoustic noise as such, of course, is quite possible to generate in the bioenvironment, since undoubtedly gases at near atmospheric pressures will be maintained in the system and these may easily be excited by vibration.)

EMBRYOGENIC - HISTOGENIC EFFECTS OF GRAVITY

William Jensen - Department of Botany
University of California

I would like to address my own considerations to the question of embryogenic and histogenic effects of gravity on plants. As discussed by the panel in their meeting, there are two aspects of this type of experimentation, particularly with regard to experiments in orbiting vehicles. The first is the completely unknown developmental response that plant systems may have to a weightless condition; the second is to examine certain well-known, and as yet unresolved, features of plant development in such a condition.

With regard to the first point, it must be admitted that it is extremely intriguing to consider the question of what happens to plant tissue when it is allowed to develop in a condition of weightlessness. The simple act of allowing seeds to germinate under these conditions might be an interesting and fruitful exploratory experiment. It would be necessary to select plants about which a great deal is already understood concerning their normal development. Corn and certain of the grasses come immediately to mind, as materials of considerable interest. Germinating seeds need not be, however, the only system considered for such an experiment. Certain tissue culture strains are known to undergo specific lines of development, and these might also be used. The aim of such a primitive experiment would be to see simply what does happen under these conditions. Certainly, the possibility that no changes will be observed is perfectly possible, but it is also conceivable that some interesting morphological developments might take place in the tissue. With this in mind, it might be well to preserve some of the material when recovered, not only for standard observation but also for electron microscopy and histochemical analysis.

The other question of examining specific systems which might be believed to be influenced by gravity, and therefore, interesting to study in the absence of it, is in some ways more difficult to approach while being the much more sophisticated avenue of attack. One of the truly important questions in tissue development is that of the factors influencing the unequal division of cells.

Throughout the plant kingdom, in virtually all tissues in which specific cell differentiation takes place, there occurs early in the pattern of development a very significant event. This is an unequal division which gives rise to a small generate cell and a larger daughter cell. This latter cell normally undergoes either little differentiation or quite strikingly different differentiation than the generative cell. One prime example of this is in the development of the embryo where it is the small cell which gives rise to the embryo proper, while the large one gives rise to the suspensor only. The development of guard cells, sieve tube cells, and companion cells, cambial initials, the proto-xylem, and many other events are related to this unequal division. Virtually nothing is known concerning the basic underlying factors involved in this critical division. Extremely interesting work has been done following the course of development of these cells, particularly with regard to hair cells in roots and guard cells in leaves, but little indication of the underlying mechanism of this division has been indicated.

This points up a part of the problem. Namely, that while it is conceivable that these systems would yield valuable information if studied in a weightless condition, too little is understood at the present about the development as it occurs under normal gravitational fields. This suggests that before emphasis should be placed on experiments in orbiting vehicles, considerable effort should be made in ground-based laboratories to uncover at least some of the possible mechanisms underlying this division. For one thing, it would be interesting to know in a detailed fashion the actual changes in the cell on the ultrastructural level associated with the events leading up to this unequal division. Chemical enzymatic polarization which may occur in the mother cell under various conditions must also be examined as the effect of various chemical stimulating agents on the development of the cell. On the basis of this kind of evidence, it might be well to consider experiments in orbiting vehicles using the root hair system in grasses and the guard cell system in leaves. The effect of growth regulators, in particular, both in this problem and the general problem of cell elongation in a weightless condition would also seem to be extremely interesting to approach.

One could suggest many other problems which might be interesting to approach, but which require a tremendous backlog of information which can be obtained in ground-based experiments. At present, my inclination is to believe that the first type of experiment, a general all-inclusive, simple approach, would be the most worthwhile and meaningful in terms of space and design problems. For a relatively small investment of material and preliminary experimentation it is conceivable that a good deal of information could be obtained. It is equally possible that the results would be negative, but this is, of course, the chance anyone takes with any experiment anywhere.

I believe that the availability of orbiting vehicles as experimental laboratories is an important advance in the study of many biological problems. My personal feeling is that, with the exceptions noted above, the problem of plant embryogenesis and histogenesis is still too ill-defined to make the most sophisticated use of this complicated, costly, and magnificent piece of equipment.

ABNORMAL G AND EARLY DEVELOPMENT

John Torrey - Biological Laboratories
Harvard University

The action of abnormal g, including weightlessness, on biological material and in particular on the early developmental processes which depend on the phenomena of cell division and cell differentiation is little understood and has been little studied. Yet there is reason to believe that what we describe as normal development proceeds in its course with gravity as an important and influential force of the environment. In experiments with plants rotated on clinostats, normally bilateral symmetry of floral structures may be converted to radial symmetry, reflecting presumably a changed developmental pattern in the early stages of development (cf. Kaldeway, vol. 17, Encycl. Plant Physiol., 1962). The importance of unequal divisions in setting up cellular differences leading to the differentiation of cell types also suggests the potential importance of gravity in determining normal patterns of development. High-speed centrifugation of cells clearly leads to rearrangement of cell organelles and cell structure and under appropriate circumstances can be expected to profoundly affect subsequent developmental patterns, especially in meristematic systems still capable of further cell division. But the evidence is quite fragmentary and there are many avenues of research to be followed using simple, ground-based equipment and facilities, before we can intelligently plan experiments for orbiting vehicles.. Ground-based work on the effects of abnormal g should be encouraged in studies, not only of g-detection mechanisms, which are already being studied since they have a clearly demonstrable role in the physiology of the plant, but also in the more subtle effects of abnormal g on early embryonic development and in meristematic tissues which have yet to express themselves via coordinated mitoses, cell divisions and cell differentiation processes.

An integral part of the determination of normal developmental patterns in plants depends on mechanisms which determine cellular, organ, and organism polarity. Our present state of knowledge about these mechanisms is so poor as to lead largely to speculation (cf. E. Bünning, Entwicklungsphysiologie, 1953). Unequal cell divisions based on polarized cytoplasmic distribution within cells determined by unknown forces are claimed to play a role in establishing polarized developmental systems. The distribution of correlation mediators in the form of plant hormones, moving about the plant at least partially under the influence of gravity, also is important in polarizing plant developmental systems. Here again our ignorance needs to be alleviated by careful ground-based experiments using clinostats, centrifugation and other readily available techniques.

The effect of weightlessness on the inception of pattern in developing systems is a special problem which can be approached only through a study using satellites. In earth-bound systems, polarity may always be attributable to pre-existing systems which have developed in constant response to gravity. Thus, the unfertilized egg is polar with respect to its cellular origin from a parent cell and is in turn polarized by sperm entry and fertilization under the influence of gravity. It would be interesting to know the course of developmental events in embryos fertilized and formed in a weightless state (if that is possible), and to follow the histological patterns in such systems. It is conceivable that experiments giving this information could be planned and prepared for a satellite flight. More ground-based work is needed to make this really feasible.

Ground-based studies such as those suggested above need ultimate complementation with experiments designed to test the effects of weightlessness. Our present lack of information makes difficult the proper planning of such experiments, as is clearly evident by a consideration of the proposed experiments, already submitted for consideration. Although some information may be forthcoming from telemetered experiments in non-recoverable satellites (for example, whether a seedling appears to grow and develop normally or not), in fact, the fundamental information from the interesting experiments on plant development would be found only at the tissue and cell level and would require detailed histological and microscopic analysis. Thus, experiments of this sort should be based on the assumption that recovery of the experimental material is essential.

In summary, we need considerably more work done on the effects of gravity on developing plant systems, carried out in ground-based laboratories before we are ready even to plan useful experiments for satellite study!

ANATOMICAL RESPONSES TO GRAVITY, AND ECOLOGY OF PLANTS IN THE "GRAVITY-FREE" STATE

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I. ANATOMICAL RESPONSES TO GRAVITY

A. Theoretical Background

At least three kinds of observation have indicated that the anatomical features of a plant are extensively adapted to the constant, unidirectional gravity field at the earth's surface and even that they may be modified strongly when the plant's normal relationship to this field is disturbed:

First, it is apparent that much of the land plant's anatomy is an adaptation to gravity. All supporting structures, such as wood, fiber elements, etc., may be thought of in this way, and the orientation of stems and leaves also bears an obvious relationship to the direction of the gravity field. The direction of light may also influence the orientation of plant organs, but by suitable experimental procedures it is possible to separate effects of light from those of gravity.

Second, experimenters have manipulated plant position in relation to the gravity field and observed subsequent response of the plant. Experiments of this type go back at least to those of Wiesner in 1868.

Third, an attempt has been made to stimulate the gravity-free state by growing plants on clinostats (in which the vertical axis of the plant is rotated in a horizontal position, and it is assumed that the gravity-sensing mechanism of the plant has a sufficiently slow response-time that it will act as though gravity were non-directional). Plants have been subjected to acceleration forces greater than the gravitational force at the earth's surface by growing them on centrifuges.

Anatomical responses of plants to gravity as observed in nature by changing plant position or on clinostats or centrifuges have been summarized by Edmund W. Sinnott (Plant Morphogenesis, McGraw-Hill, New York-Toronto-London, 1960 -- see particularly pages 354 to 358, but many portions of this book are devoted to description of responses to gravity). Such anatomical responses divide rather easily into two main categories: First, tropistic effects which have to do with orientation of the plant body in relation to the earth's gravity field. This is the topic of another report.

Second, formative effects which involve specific plant structures. Some of the most extensively studied of these involve modification of symmetry in leaf and flower-part arrangement. A number of zygomorphous flowers are known in which irregularities bear a direct relationship to gravity, and change of the plant's position during the development of the flower will result in a modified symmetry. The gladiolus is a classical example, and a number of very small, rapidly growing desert annuals are also known which display zygomorphy (the species of the subgenus Langloisia of Gilia). Such small annuals would be quite suitable for experimentation in a satellite. In most instances (both flowers and leaf arrangements) there is a change in pattern of leaves in response to gravity. The phenomenon has been termed anisophylly and is seen, e.g., in Acer and Aesculus .

On a clinostat cells are typically much shorter and wider than in the control plants. Thus cell elongation appears to be a response to gravity.

A number of recent experiments indicate that the formation of flowers may be strongly influenced by gravity. It was shown, for example, that a pineapple plant placed on its side will immediately produce flowers, and soybeans, in which the stems are maintained upside down by weights, flower earlier at the basal nodes. This is also the case when branches of certain woody species are held in a horizontal or other abnormal position (works of van Overbeek, Cruzado, Fisher, Wareing, Nasr, and Longman -- see review in F. Salisbury, Annual Review of Plant Physiology, 12: 293-326, 1961).

Considerable work has been done on the formation of reaction wood. If a tree is bent down towards the horizontal, it will tend to bend back towards the vertical by its growth processes. This occurs by the formation by a special kind of wood that is anatomically different from that in the vertically oriented tree trunk. In the case of the gymnosperms, reaction wood forms on the underside of a horizontal trunk. Its cells are somewhat shorter than those of normal wood, and the micellae in their walls are less steeply pitched. Such wood grows somewhat more in length than does normal wood and thus produces considerable longitudinal compression which tends to bend the trunk back to the vertical position. In angiosperms the reaction wood is normally on the upperside of a horizontally placed trunk or branch and acts by producing tension rather than compression, thus pulling the trunk or branches into place rather than pushing. The gravitational compression itself is not responsible for the formation of reaction wood, as may be shown by bending a terminal shoot into a vertical loop, in which case reaction wood is formed on the underside of both the upper and lower parts of the loop. In the former the wood is under compression but in the latter under tension. Gravity is also not directly responsible, since reaction wood will form on the uppersides of branches which are being pushed down and on the lower side of branches which are being forced up. In each case the development of reaction wood is such that it will tend to bring the plant organ back to its "normal" position in relation to the gravity field.

One may distinguish between direct responses to gravity as indicated in the experimental examples cited above, and morphological adaptions which insure a more profitable form of a plant growing in a gravitational field but being controlled by the plant's genetics rather than direct application of the gravitational field. Thus we might imagine that a plant growing in the gravity-free state would produce xylem elements, fibers, and other supporting materials (the actual cell types being genetically controlled), but the orientation of these tissues might be quite random (as often occurs in a tissue culture) or determined by some other environmental factor, such as light. If the anatomical aspects of a plant which seem to bear some relationship to the earth's gravitational field actually depend upon this field for their development, then we might imagine that a plant growing in the gravity-free condition would be unable to differentiate tissues at all and might grow into an amorphous mass of cells. This seems rather unlikely, but some workers have felt that the presence of the gravitational field might be this essential, and only experimentation in the gravity-free state can positively answer the question. We know that organisms which have essentially completed their growth can exist for a number of days in the gravity-free state (e.g. various mammals including the astronauts). We do not know for sure that a land plant can grow and differentiate in the absence of gravity. It seems extremely likely that the orientation of the organs of this plant would be upset under these conditions but less likely that the actual formation of the organs themselves would be upset.

The question of how gravity acts in tropistic responses is to be discussed in another portion of this report. Its action on formative effects may be closely related. In any case, it appears that gravity produces tensions and compressions, and these may result in redistribution of growth substances. The mechanism by which growth substances may be redistributed in response to tensions and compressions, or directly in response to gravity, have not been elucidated and constitute a valid and perhaps the most important phase of research on the gravity problem.

B. Experimental Approach

It should be clear from the above summary that gravity effects on plant morphology and anatomy constitute an interesting field for investigation. Ground based experimentation and observation might well continue to follow the pattern set nearly a century ago (positioning, clinostat and centrifuge). Satellite experimentation provides a new tool which conceivably might open doors which have until now remained closed to the experimental morphologist. The basic approach would, at the beginning at least, be quite simple. Plants would be allowed to grow in the gravity-free state, and their morphology would subsequently be observed both on the gross and the micro levels. Such an approach must be an important phase of any plant satellite experiment. Regardless of the purpose of the experiment, if higher plants are utilized, a well trained and imaginative plant anatomist must be part of the team. Observations might be made during development of the plant in the gravity-free state (using recoverable photographs or television), and careful analysis of the material should be made upon recovery of the satellite.

Anatomical responses might be observed on at least three levels. First, fertilization may be allowed to occur in the gravity-free state, and development of polarity in the zygote would be observed. Second, morphogenesis of the embryo, during which plant organs first make their appearance, should be allowed to occur in the gravity-free state. Both sets of observations could be made on peas since they are self pollizators. Third, development of preformed organs should be allowed to occur in the absence of gravity. Such an experiment might involve plants such as *Geum turbinatum* which grow in the alpine and which form rather well-developed leaf and flower primordia before going into dormancy. These plants may develop mature leaves and flowers from preformed primorida within five to seven days after they break dormancy (e.g. some hours following launch).

We might imagine that formation of polarity in the zygote would be most affected by lack of gravity, formation of organs less so, and development of preformed organs even less, but only actual experimentation can bear out or refute these expectations.

II. ECOLOGY OF PLANTS IN THE "GRAVITY-FREE" STATE

A. Theoretical Background

The term ecology, implying all relationships between all plants and their environment (including each other), is so broad that it might encompass all of the topics here being discussed. Yet current ecological investigations seem to follow two broad approaches. The first considers the entire plant community with its interactions and its relationships to the over-all physical environment. We might imagine that gravity has played a very important role in evolution of plant communities. The sum of all gravitational effects upon plant anatomy and morphology might be considered in the ecological context. Probably in the present fragmentary state of our knowledge, the effects of gravity on a plant community cannot be underestimated.

The second approach being followed in contemporary ecology involves the study of the response of individual plants to specific environmental factors such as light, temperature, soil nutrients, soil moisture, etc. The response to gravity might be considered in this ecological sense. Of course, direct responses of plants to gravity are discussed in other parts of this report, but from the ecological standpoint, one might consider possible interactions between gravity and the other environmental factors. A test of such possible interactions cannot be carried out in a completely satisfactory way in any environment except the gravity-free one. Interactions between light and various gravitational forces, for example, cannot be satisfactorily reproduced in earth based laboratories. We have little theoretical grounds for expecting any important interactions to become apparent, but they should always be carefully watched for in satellite experiments. An example of a plant response which might be influenced by gravity could be the endogenous rhythms of leaf movement, metabolic activity, etc., which have been studied so intensively in recent years. Perhaps our reason for mentioning this specific example lies in our ignorance of the mechanisms underlying these rhythms, yet exactly because these mechanisms have defied the conventional approaches, it would be well to search for them under conditions in which new parameters (e.g. gravity) are controlled.

B. Experimental Approach

Perhaps one of the most important contributions that could be made in the field of ecological responses to gravity would be a more thorough theoretical consideration by a number of specialists throughout the world, of the problems involved. This might be said of virtually any aspect being reported upon here, but the ecological phase seems particularly suited to further theoretical treatment. The importance of gravity as an ecological parameter has probably been greatly overlooked and certainly should be emphasized in future studies.

It is difficult, without a more detailed theoretical background, to propose specific,gravity-free experiments aimed at furthering our ecological understandings. Yet in any gravity-free experiments the ecological implications should not be ignored. This applies particularly to interactions between gravity and other environmental parameters. Such interactions must always be looked for in evaluation of the experimental results obtained in satellite experiments.

GROWTH PHENOMENA IN PLANTS

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Growth and morphogenesis are defined by the constituents of an organism, its structure, and by various gradients across that structure. Both structure and gradient are in turn a consequence of polar differences induced sequentially at cell, tissue, and organ levels of organization. There is accumulating evidence that such polarity is modifiable by reorientation with respect to gravity. Hence gravity may be assumed to have a determinative effect on either the initiation or nature of the polarization. If the preceding assumption is correct, it may be inferred that development in an abnormal or sub-threshold field would result in alterations in growth rate, growth phenomena such as tropisms, and in morphogenesis.

On this basis, three lines of research on responses of the plant to abnormal g may be suggested: growth in the sense of changes in dimension and mass, stimulus-response reactions involving asymmetric growth, and morphogenesis in the sense of initiation and development of tissues and organs. Within these lines I will try to ask several relatively simple questions, of relatively simple plant systems, whose answers might provide useful guides for more extended, sophisticated experiments.

By plant I have in mind a heterocellular organism of minimally millimeter order of dimension, normally oriented with respect to gravity, in which both steady-state function and reaction to stimuli are correlative phenomena. I would exclude most lower plant forms (unicellular or homogenous multicellular bacteria algae and fungi) as desirable material for initial investigations of low-g effects *per se*, recognizing that bioengineering research on these forms for maintenance function may be highly important. Is the exclusion of the small organism, even though of fixed orientation, justified? Rigorously, I'm not sure. I think so on the basis of the following argument.

Assume that response to reorientation with respect to earth g is a consequence of positional displacement of a material, stable, intracellular sensor. Assume also that the sensor is near spherical. What is the order of dimensional magnitude that would be minimal for such a sensor for it to be displaced within the times and dimensions known for responsive plant tissues?

Georeceptive cells, which are approximately isodiametric and unvacuolated, are from 10-20μ in diameter. The minimal presentation time, as determined by Larsen for Artemisia roots, is about 10 seconds. We can therefore begin with an upper limit of sensor velocity of about 10^{-4} cm · sec^{-1}. On the basis of Brownian displacement in situ, it has been calculated that the viscosity of cytoplasm is about 5 centipoise. The density of the sensor may be approximated as lying between 0.9 (natural oils) and 2.2 (calcium oxalate; starch grains are about 1.5).

Upon shift of orientation of a tissue, the initial acceleration of the sensor should be: $a_s = (\rho_s - \rho_c) \cdot \rho_s^{-1} \cdot g$, where ρ_s and ρ_c are respectively the densities of the sensor and cytoplasm, and g is the gravitational acceleration. The velocity so generated would subject the statolith to a force definable by Stokes Law till a terminal velocity is reached where the acceleration is zero, i.e., the velocity at which the gravitational force is equal to the retarding force:

$$4/3\pi r^3(\rho_s - \rho_c)\, g = 6\pi \eta r v$$

$$r^2 = \frac{9}{2} \times \frac{\eta v}{g(\rho_s - \rho_c)}$$

The minimal sensor diameters required for 3 density differences at a ten-fold range of sensor velocities are:

	Velocity (cm · sec^{-1})					
	0.5 x 10^{-4}			0.5 x 10^{-5}		
$\rho_s - \rho_c$	1.1	0.1	0.02	1.1	0.1	0.02
Diameter (μ)	2	7	15	0.6	2	5

The two velocities represent a traverse by the sensor of 50 and 5% of the diameter of the georeceptive cell in the minimal presentation time. Traverses of less than several percent are probably within noise level. If the difference in densities is .02, a 15μ sensor is suggested by the velocity required for 1/2 cell traverse, and 5μ for a 1/20 movement. For a density difference of 1.1, the sensor diameters are, respectively, 2 and 0.6μ. It is therefore unlikely that organelles or inclusions smaller than mitochondria are functioning as sensors in higher plants. It also seems improbable that 1 g will directly cause stratification within cells or of cell components smaller than the above range of dimension. Hence, a direct effect of low g on similarly dimensioned organisms would not be anticipated.

GROWTH

In the higher plant, growth may be defined as an irreversible increase in volume. Cellular replication may, though not necessarily need to, accompany the increase. What the observer measures as dimensional change in plants is largely a product of cell enlargement. An excellent experimental organ in which such enlargement occurs, uncomplicated by cell division, is the young grass shoot. Its extension is chiefly along the longitudinal axis. I would pose the simple question: How is cell enlargement affected by a low g environment? The experimental period required would be 2 to 4 days. The experiment would be: measure periodically the length of a germinating grass shoot.

What about tissues in which cell division occurs continuously along with cell enlargement? Undifferentiated plant tissues can be grown in a medium designed so that there is continuous cell replication and enlargement without differentiation. What would be the effect of an abnormal g environment on this growth by incremental replication of a single cell type? A shadowgraph periodically taken along two coordinates would be a simple indication of an increase in tissue volume .

However, it is entirely possible that cells in a tissue culture mass under abnormal g conditions will alter in behavior so that at first they will enlarge excessively without dividing or increasing appreciably in dry weight. In other words, the tissue mass can grow for some time essentially by an enhanced absorption of water, without elaborating new cell structure. It may be suggested that both total volume and the acquisition of new cell material can be followed quantitatively by radiation absorption techniques. For example, shadow area could be used as an index of volume change and shadow density as the index of the deposition of dry weight. This technique would lend itself to the use of visible light. Where photomorphogenic effects are to be excluded, infrared radiation with no component in the visible spectrum, at a band where water as a low extinction, appears feasible. I suspect that the resolution obtained by using radio frequencies would be inadequate for the purpose.

TROPISMS

Tropisms, the movement of plant organs in the plane of a stimulus gradient, are caused by differences in the growth rates of tissues in that plane. It seems to me that alterations in tropic responsivity would provide a useful index of fundamental changes in the physiology of the organism subjected to abnormal g.

The above differences in growth rate are caused by differences in concentration of the growth hormone auxin in the plane of the stimulus gradient. These auxin inequalities are generated in reactive cells and are transmitted to tissues where the growth actually takes place. Thus, alterations in tropic responsivity would be an indication of an alteration in either the sensitivity of the sensor, the metabolism or transport of the growth hormone auxin, or in the reactivity of cells to auxin. Any of such changes will result eventually in alterations of both growth and development of the plant.

Therefore, a useful experiment would be the evaluation of the tropic reactivity of an organism allowed to develop in an abnormal field for an appreciable part of its ontogeny. Experimentally, for example, do phototropic and geotropic sensitivities change? More specifically, germinate a grass seed in orbit and challenge its shoot by unilateral phototropic and geo-(centrifugal) stimuli. We now have as a basis for evaluation of such responsivities a considerable data on the phototropism and geotropism of the plant at earth g. This could be readily supplemented by related data on the responsivity of the seedling allowed to develop in the clinostat.

POLARITY

Earlier I stated, more or less explicitly, that growth and morphogenesis in an organism were controlled by polarization at various levels of organization. Temporally, initial polarizations are almost certainly an interrelation of molecular concentration and electrical charge. It is interesting though not particularly pertinent at the moment as to which of these phenomena cause the other; probably the two phenomena are in intimate feedback relationship. The important point is this: It would be difficult at the present time to measure micro- or semi-micro changes in molecular concentration in organisms, or across organisms, maintained in orbit. Measurement of such changes in material subsequently recovered is of little interest insofar as these changes are involved as morphogenic determinants. However, it is feasible to measure alterations in electrical polarity, and to record and transmit such changes.

An example of the pertinency of such determination is the work of Hertz on geoelectric potentials. Hertz has solved the problem of measuring potential gradients across tissues without direct two-electrode contact by recognizing the extremely high impedance of tissues. Thus, by using the plant organ as one electrode of a vibrating reed in a capacitive field, he has been able to measure quantitatively microvolt changes occurring across organs. Pertinently, he has shown that reorientation of the grass shoot with respect to earth g causes a potential gradient to develop across the organ in the plant of the gravitational field. A potential difference appears within 10-20 minutes after reorientation, and rises to magnitudes of 60-80 millivolts. This electrical polarization is a rapid and sensitive index of the subsequent physiological and tropic responses. Although I am unaware of experimental investigations on this topic, it is likely that bioelectric gradients are induced or are altered an appreciable time before visible differentiation as well.

I would suggest the following types of experiments. The young grass shoot, when growing normally under earth-g conditions, has no potential gradient transverse to its longitudinal axis, but possesses a longitudinal gradient. What would happen to longitudinal and transverse potentials when the shoot is grown under conditions of abnormal g? What would be the potential gradients across undifferentiated tissue masses before and after differentiation under earth-g conditions? In orbit? With either type of biological material, what would be the bioelectric changes induced by tropic stimulation? I believe that this area of biological research is worth pursuit under both earth as well as orbit conditions. At the least, it would enhance our understanding of the phenomena that precede morphogenesis.

In the discussion so far I have assumed that the package would not be recovered after orbit. Where a package is recovered, there are several useful and potentially significant questions that can be asked of the organism, particularly if asked quickly after free-fall. In materials such as the young grass shoot, a dicot stem segment, or a stem segment to which a leaf is attached, what is the polarity of auxin transport? What are the velocities and rates of auxin transport? Since growth and development are in part determined by these polar behaviors of auxin, such information would yield a basis for guess as to what might happen on flights of longer duration.

EFFECTS OF LOW GRAVITATIONAL FORCE ON PLANTS

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The action of gravity on organisms has never enjoyed a very prominent place in biology, either with plants or with animals. Since the elucidation of the otolith mechanisms for the static detection of gravity in the mammalian ear and the Cristae mechanism for detection of acceleration therein, most of the later work has been devoted to the interactions between these primary detecting systems and the body-wall muscles or the eyes, and to the controlling role of the brain. It is these secondary but important influences which, according to Adrian, differ widely from one genus to another among vertebrates, while the labyrinth of the ear remains much more constant in function, and thus has called for much less continued study.

A parallel but simpler mechanism for the static detection of gravity in plants was brought to light by Nemec, Haberlandt and others early in this century. It was shown that specific organelles, the "statoliths", fall down through the cell under the influence of gravity, and it became widely assumed that these constitute the georeceptors for higher plants. It will be noted that the system differs from that of vertebrates in two respects: 1) instead of being contained in a single specialized organ it is found in many locations in the plant, 2) instead of depending on an organ (i.e. a multicellular body) it makes use of organelles (subcellular bodies). The latter difference is of course in line with the general direction of evolution.

The first point to be made is that the actual role of these statoliths is far from proven. They are found in such geo-perceptive organs as the few millimeters of tissue just behind the extreme tip of most roots, or the apical parts of the seedling shoot of numerous dicotyledons, as well as along the whole length of the coleoptiles of oats and wheat. In only extremely few cases has it been shown that the threshold time of exposure to gravity (i.e. the time a plant must be held horizontal to give a minimal geotropic response) agrees with the time that the statoliths take to fall. But even these are only correlations; critical experiments are largely lacking. The statoliths, being subcellular, cannot be excised as can the otoliths of animals, and no physiological procedure for causing them to disappear from the cells has even been successful (though many have been tried).

A major need, in work on the effect of gravity on plants, is thus to elucidate more fully the nature of the georeceptors. This will not, of course, depend on studies with low or zero gravitational force but will require only microscopes, centrifuges, and that scarce component -- experimental skill.

It should be noted that many plants, including liverworts, filamentous algae, mycelial fungi, etc., appear insensitive to gravity. However, the fact that their fructifications often grow upright seems to show that the sensitivity is latent and georeceptors can be produced when desired. A study of this onset of geosensitivity might well prove most illuminating.

The second major point to be made is that the limits of the effects of gravity are unknown. The most obvious limit is simply the re-dressing of the root or shoot axis with respect to the perpendicular. But the variety of angles to the perpendicular assumed by laterally growing organs (e.g. pine branches or the lateral roots of the lupin) shows that even orientation to gravity is not a simple matter. When we further note that the lower side of the pine branch develops a peculiar type of cellular structure known as compression wood or "Rotholz", and the upper side of birch and other tree branches develops another type called tension wood, it is clear that the effects of gravity are not limited to orientation, but may show themselves in the details of cellular morphology. To what degree of subcellular refinement they extend is not known. Furthermore, for every one of the very few such cases sufficiently obvious to have come to the attention of sharp-eyed botanists many more must be still awaiting discovery. It is here that the widest field for the investigation of low-g or zero-g effects doubtless lies, namely at the level of cell morphology and anatomy and fine structure. Since we do not know which among the effects that we record may be due to gravity it is quite possible that it will take the use of low-g to find out.

It will be essential, however, to preface experiments on low-g with adequate studies of the effects of balanced 1-g at the earth's surface. In the last few years the author has been advising Prof. C. J. Lyon of Dartmouth on his experiments on epinasty. This phenomenon is a curvature of lateral organs in which the effect of gravity is modified by forces inherent in the integrity of the plant. The necessary accompanying survey of the literature brought out the surprising result that extremely few observations of the growth of whole plants on clinostats have been made since the days of Pfeffer and Jost, 30 or

40 years ago. Such curious neglect of a field is hard to explain, but in any case it means that more than the usual amount of background work with balanced 1-g, using plants from several groups and clinostats of various types, will be essential prolegomena to any low-g observations.

Work of this kind should be started at once. Comparisons between the growth and the morphology of normally oriented plants and plants subject to balanced 1-g should bring out the points to be looked for in a study of low-g. They may even determine whether it is worthwhile to embark on a low-g program at all.

It should be noted that in many of the proposals for experiments in satellites, the authors are evidently quite unaware of the value, or even the possibility, of work on earth with balanced 1-g. It is not going too far to say that not a single experiment should ever be carried out in a satellite which has not been thoroughly explored on the ground in a balanced gravitational field.

The actual design of low-g experiments is limited by the size of the plants and the short duration of the flight. Since the plants should not have been exposed beforehand to 1-g, let alone to the multiple-g values of the launching pad, one is essentially limited to dry seeds or spores. The experiment is simply to bring them into the appropriate gravitational field, with their orientation fixed, then soak them in water or other germination-inducing fluid, photograph or sample them during growth, and at the end of the experiment drop them into fixative for cytological examination. At start the most effective classes would be:

1. Seeds in dark, oriented for vertical shoots and roots, exposed to 1-g.
2. Seeds in dark, oriented for horizontally emerging shoots and roots, exposed to 1-g.
3. As 1 but on clinostat, ∴ balanced g.
4. As 2 but with orienting light vertically above.
5. As 3 but with orienting light, fixed to clinostat, vertically above seed.

Comparison between 1 and 2 gives the normal geotropic response, and comparison with 5 will show the modifying effect, if any, of the orienting light. Comparison between both 1 and 2 and 3 will give the general effect of 1-g on growth and morphology, and any differences from 3 observed in 5 must be taken into account in evaluating 4. With a good understanding of the growth behavior and the morphology of these plants, which may require many repeats and modifications, we should be in a position to repeat 3 and 5 under truly low-g conditions. Needless to say, this last will call for elaborate mechanization and timing; no water should reach the seeds until they are in the required field, and fixation must occur before they leave it.

As to material, prehusked Victory oat seeds (a widely used strain) reach 25 mm in height, with few roots, in 72 hours at 25°. Choice of a dicotyledonous seed calls for a survey, since most of them require over 24 hours for mere germination, -- some lettuce varieties begin in 14 hours at 25°, however, and mustard perhaps even sooner. High percentage germination and uniformly rapid seedling development are both essential; the latter normally implies a genetically pure line.

The exigencies of time would make it most desirable to explore the behavior of a fungus with vertically-growing sporangiophores; perhaps some strains of Rhizopus will form these in 72 hours. It is not certain, however that the orientation of these is mainly due to the gravitational field, and the interaction of light and gravity on them needs to be worked out. It seems certain that one or more fungi will yield sporangiophores that prove useful in these studies.

In summary, a certain number of experiments on the effects of low g on plants are desirable and practical, but they need careful exploration on earth with balanced-g first. Given a flight time of not less than 72 hours, useful studies with both higher and lower plants can be carried out. A minimum experimental plan is proposed.

EMBRYOGENESIS

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I. General

The known factors of special biological interest in the space environment appear to be weightlessness and complex mixtures of radiations. With these factors in mind space flight experiments can be designed with one of two objectives, namely, exploration of space using biological systems as indicators, and utilization of the space environment as a tool to study biological problems. The second of these objectives seems to be uppermost in the minds of most investigators.

Since most radiations can be duplicated in earthbound laboratories, under controlled conditions, most radiation experiments should be done in such laboratories. The only known, unique, parameter in the space environment is weightlessness. This cannot be duplicated in earthbound laboratories except in very short term "free-fall" experiments. Such free fall experiments do not provide a practical means for studying the effect of weightlessness on biological systems because most biological phenomena of interest involve slow changes and usually require relatively long exposure to this type of altered environment to obtain a measurable result. In view of the uniqueness of the weightless environment, space flight experiments should properly emphasize studies on the effects of zero gravity alone and in combination with other variables (e.g. radiations) to examine for possible synergistic effects.

The argument for an effect of weightlessness on biological systems in general is based on the proposition that the most constant, invariable environmental parameter throughout the time of organic evolution is the one g force of the earth's gravitational field. This view is supported by the fact that the majority of organisms have morphological, physiological and behavioral orientation with respect to the one g environment. Many animals have evolved special sense organs to obtain directional information relative to their position in the one g field.

It seems clear that some well selected and properly controlled empirical experiments should be performed to examine for possible zero g effects however remote the possibility that effects will be found. However, the main effort should be with systems where an effect of weightlessness can be expected from the results of observation and experiment in earthbound laboratories. It is of great importance to recognize that flight experiments have drawbacks relative to earthbound laboratory experiments. These drawbacks are (1) that flight experiments are generally far more expensive than laboratory experiments and (2) that precise and exact controls and recording of data are at present more difficult to obtain than in earth laboratories. In view of this second factor the reliability and validity of data from flight experiments is likely to be lower than that from earth laboratories.

In view of these factors it is important that all possible data bearing upon the effects of gravity be obtained in earth laboratories for the particular experiment before the system is examined at zero g in space vehicles. Two means are available for such studies on embryological systems. Indeed they have been employed for over 60 years in a variety of experiments. These are the centrifuge to obtain forces in excess of one g and the clinostat to produce a scrambling effect - e.g. continuous change in the direction of applied gravitational force.

The rationale of the centrifugation studies in the present context is to obtain a curve for effects of one g and larger forces which can be extrapolated back to zero g. Although valid in principle, it should be noted that significant effects of centrifugation are only obtained in most embryological systems at high g forces, e.g. in the hundred or thousand x g range. A large literature exists with respect to the effect of centrifugation on embryological systems. However, in most experiments the high forces used were applied for a relatively short time (usually a few minutes) and the effects, if any, do not necessarily reflect the effect of continuous centrifugation during the course of development.

With respect to the "scrambling" type of analysis simple observation should lead to first order predictions with respect to a possible zero g effect. For example, it is readily seen that the small, holoblastically cleaving eggs of many invertebrates pass through the early stages of development with their primary developmental axes oriented at random with respect to gravity.

Furthermore these (e.g. sea urchin blastulae) frequently develop cilia before major biochemical and morphological differentiation begin. Such ciliated embryos rotate and move apparently at random with respect to gravity. It seems likely that such material will develop normally in the zero g environment.

On the other hand, the eggs of a variety of species orient specifically to and develop in relation to gravity. In general, these are larger, yolky eggs of which the avian egg is the most striking case. Other interesting examples include cephalopods, some fish and amphibian eggs. Such orientation in some cases may be the fortuitous result of an asymmetry with respect to the egg's center of gravity and without developmental significance. Nevertheless, the possibility that such orientation has developmental significance is compelling in all such cases and can only be evaluated by appropriate experimentati n.

Theoretically important effects of weightlessness that could be of developmental significance.

Physical effects of zero g as opposed to an environment of a significant gravitational field relate to the differential localization of formed elements, cytoplasmic regions, etc. in single cells (eggs) which may have developmental significance; morphogenetic cell movements in multicellular systems (e.g. regeneration, epiboly, gastrulation, specific cell migration) which may have gravity orientation; and the absence of convection in fluid systems (both intra and extracellular) under zero g conditions. The latter could critically influence localized pH, and dispersion of nutrients and metabolic products. Systems that are highly sensitive to such factors (for example polarity of the Fucus egg) could be critically influenced by the zero g environment.

Suggested Experiments

The following are a few selected experiments in the embryological area that appear to this writer to be especially suitable for investigation.

1) Sea urchin fertilization. Attempts have been made to test for an effect of weightlessness on fertilization of sea urchin eggs. This is an empirical experiment. There is no a priori reason to expect that fertilization of the sea urchin egg will be affected by zero g. The experiment is recommended because it is a precise experiment in cellular interaction, a great deal of basic information is available on the mechanics, morphology and biochemistry of sea urchin fertilization and finally because the results of the experiment can be examined with great precision both qualitatively and quantitatively. The fertilization of this egg preceeds very rapidly and to a considerable extent gives an "all-or-none" result. It is probably the best system for use in a direct, empirical biological experiment on weightlessness.

2) Cell division. Experiments to test for effects of zero g and/or other space environmental factors on cell division should be performed on a variety of materials such as microorganisms, tissue culture cells and embryonic systems. Invertebrate eggs at earliest developmental stages are especially favorable for such cell division experiments because in such material cell division is rapid, uncomplicated by an extended G1 (protein synthesis) phase, and can be evaluated in terms of specific cell number and cell orientation.

3) Development of the amphibian egg. The fertilized amphibian (frog) egg orients to gravity. Numerous experiments have shown that gravity has important morphogenetic effects since alteration of the direction of gravity can result in shifts (even reversal) of the egg's polarity and plains of symmetry. Clinostat experiments are reported in the early literature with some disagreement over results, but it appears likely that clinostat eggs develop normally. It is important to confirm and extend this type of analysis under weightless conditions. Normal development at zero g would strongly indicate a primary polarity in this egg comparable to that of other eggs and tend to show that gravitational effects are secondary. Extension of this line of investigation could lead to a significant understanding of organic polarity in this material.

4) Experiments on higher verbebrates.

a. Cell movements in morphogenesis. The chick embryo presents unusually favorable material for studies of cell movements in morphogenesis. The embryo in the egg and the morphogenetic cell movements are oriented with respect to gravity and could be gravity influenced. One g preliminary laboratory experiments on the effects of gravity on morphogenesis could be performed easily using standard explanation -- culture techniques.

The development of the vascular system presents possible problems of interest with respect to gravity. As the chick yolk sac develops a hydrodynamic problem in relation to gravity appears. Although this factor (venous return of blood against gravity) is probably small as compared to fluid friction in the capillaries, etc., it might nevertheless, influence the distribution of blood vessels, capillaries and the development of the embryonic heart.

b) Mammalian development. The internal fertilization and development in mammals present special problems some of which might be related to gravity to some degree. These include implantation in the uterus, spacing of embryos, various aspects of morphogenesis, and orientation of the fetus at birth.

MEMBRANE PHENOMENA

Karl Wilbur - Department of Zoology
Duke University

The term membrane embraces biological structures varying in complexity from those of less than 100 Å in thickness to those consisting of several cell layers. Membranes may be intracellular or at the cell surface. Intracellular membranes have important metabolic functions, as in mitochondria and endoplasmic reticulum. The membrane of the cell surface functions to aid the maintenance of equilibrium between the cell and its environment by limiting free diffusion of certain materials both inward and outward and by facilitating the movement of particular substances through special energy-requiring mechanisms. In nerve cells membrane phenomena are important in transmission of impulses. At the level of the whole organism the state of the membranes of the capillaries will govern the normal fluid balance. Membranes consisting of a single cell layer are important in intestinal absorption and kidney function. In view of these several functions of membranes, factors affecting their properties will understandably affect the normal functioning of the cell and the organism.

We may inquire briefly as to the relative importance of studies of membrane phenomena in relation to space biology. Three factors, at least, come into consideration: Ionizing radiation, weightlessness, and high gravitational fields.

Radiation. That ionizing radiation affects the permeability of single cells and alters fluid distribution in the organism is well known. From knowledge of the doses required to produce clear-cut changes in cell permeability, it is to be anticipated that other cellular functions (e.g., cell division) will usually be more sensitive. Thus during space flight, radiation effects other than permeability changes will probably be of first concern. On this basis, studies of permeability would not merit a high priority in rockets and orbiting vehicles.

As our understanding of permeability mechanisms in the cell membrane advances, it is probable that radiation effects on these mechanisms may be of special interest. Such studies should obviously find their development in ground-based laboratories.

The action of ionizing radiation on intracellular membranes is an area of experimentation of considerable interest. It has been suggested that the primary effect of radiation leading to cell death is a change in intracellular membranes resulting in a release of enzymes. The expansion of these studies on intracellular membranes is to be encouraged -- but in ground-based laboratories rather than in space vehicles.

Weightlessness. In animal cells a gravitational component in membrane permeability is not to be expected at accelerations of 1 g. or less in that membrane components would not be displaced. Moreover, there would be no cytoplasmic displacement except in a few cell types. Accordingly studies of the effects of the weightless state on permeability do not seem justified.

High gravitational fields. In high gravitational fields, changes in permeability of single cells would not be surprising as the cells become distorted or forced against a supporting surface. On the other hand, in cells cushioned against other cells as in multicellular organisms, there may well be no permeability alterations at accelerations of a few g. We may mention that the abnormal distribution of fluid seen in organisms in centrifugal fields need not imply a primary change in permeability of capillary membranes. However, their permeability may change secondarily as a result of reduced oxygen supply to the cells of the membranes. Although abnormal distribution of fluid is of major concern with marked changes in acceleration, the influence of gravity on the permeability of cell membranes or capillaries would seem to have a limited interest at present. In any case, studies of gravitation effects on permeability are best carried out in centrifuges rather than in space vehicles.

EMBRYOGENESIS AND REGENERATION

Mac V. Edds - Division of Medical Sciences
Brown University

I have reflected at some length about experiments in embryogenesis and regeneration that might be feasible and meaningful to include in the near future in space vehicles.

I have come to the conclusion that there is no scientific justification for undertaking a program of this sort at this time. The essential reasons for my view are as follows:

By its nature the Space Program permits experiments to be carried out in an environment which differs from the terrestrial in two respects: first, the absence of gravitational forces; second, the presence of diverse radiations. The availability of the latter in terrestrial laboratories has long permitted investigations of the effects of radiation on developing objects. A few such experiments have also been performed in connection with free balloon flights but little of additional biological interest has emerged, nor indeed was it expected. The absence of gravitational forces in space vehicles has led to numerous suggestions for experiments on biological objects already demonstrated to be sensitive to gravity. However, so far as we now understand embryological and regenerative phenomena at the level of tissues, organs and whole organisms, there are extremely few events which seem to be influenced by gravity. Those that clearly are so influenced are complex phenomena representing the net outcome of several incompletely understood events.

I can see no profit in exploring such systems in the proposed way merely because the absence of gravitational forces happens to be an available condition. My reasoning leads me to the same conclusion when I think of other objects that have been used previously to analyze developmental events at the level of tissues and organisms. We still understand these events too imperfectly to design experiments bearing on possible gravity influences. The prediction would be that, even with an apparently positive result, no meaningful interpretations could be placed upon the findings.

Finally, as regards investigation of developmental biology at the cellular, subcellular and molecular levels, I would take the position that this is an area of research which has only recently opened up in terrestrial environments, that we still know precious little about it, and that much laboratory-based research must still be done before we will be in a position to decide upon a useful and sophisticated experiment.

I regret the negative position I have had to take here. If it is in error, I hope somebody will convey the appropriate counter-arguments. But in my own reflections and in my conversations with other biologists over the past year or two, no other conclusion has seemed reasonable to me.

SOME CONSIDERATIONS ON CNS AND VASCULAR EFFECTS OF ABNORMAL G

C. Ladd Prosser - Department of Physiology and Biophysics
University of Illinois

Animals have evolved under the influence of 1 g and many adaptions, especially of reflex functions and of blood distribution, are responses to the normal gravitational environment. Aquatic animals are subject to much lower gravitational forces and some marine forms with specific gravity approximating that of sea water live in virtual absence of gravity stimulation. Most animals, including aquatic ones, have specific sense organs which signal deviations from the normal orientation of their dorso-ventral axis. There are two principal types of receptors -- dyanmic receptors of acceleration or deceleration and static receptors of orientation. Recent studies on both types of equilibrium receptors show that usually there is a continuous background of "spontaneous" activity and that stimulation causes a modulation -- either positive or negative -- of this activity. In addition, virtually all animals, especially those with appendages containing muscles, have proprioceptors which signal position. The net effect is that central nervous systems in general have a continuous input from various receptors associated with gravity. It is impossible to eliminate all such input by surgical means and most studies have been on effects of increased rather than zero stimulation. An important question to be answered by prolonged space flights is whether loss of functional capacity in central nervous systems would occur in the absence of all of the normal input from gravity receptors. Orientation can be maintained by visual cues but if these are also eliminated in an orbiting animal, it is difficult to predict reflex effects. A variety of tests of nervous function should be performed during and after prolonged flight. The basic question is whether central neurones require some continued input for maintenance of normal excitability and response capacity. These measurements should be conducted on both anthropods and terrestrial vertebrates. A related question is whether the energy requirement of an animal in a zero gravity field would be appreciably lower than at 1 g. This information should be available from measurements of metabolism under orbiting conditions.

A question of very long range might be asked concerning the effects of gravity on development of nervous systems in growing animals. It would be of real interest to know whether deprivation of this input in addition to inputs from eyes and other better-known sense organs might alter brain development.

A second basic question relating to gravity in animal physiology concerns the distribution of body fluids. There is a lack of knowledge as to how much of vascular tone in birds and mammals results from that component of flow resistance due to gravity. Observations on humans show some reduction in vascular capacity after prolonged bedrest. The mechanisms by which tone is maintained in the smooth muscle of the smaller blood vessels are very poorly understood. Venous return of blood may be importantly influenced by gravity. Prolonged exposure to negligible gravity in flight might give useful information concerning the role of gravity in normal vascular physiology. Measurements of cardiovascular functions should be made not only in mammals but in birds, such as chickens, which normally stand.

Related to the central nervous and vascular effects of prolonged exposure to zero gravity are practical considerations such as possible muscular atrophy and loss of bone calcium. These effects are well-known in bed-ridden patients. The cellular mechanisms leading to such results when muscles and skeleton are not used are virtually unknown.

Both of the preceding suggestions -- central nervous and vascular -- have applied as well as fundamental importance.

The mode of stimulation of specialized cells, either plant or animal receptors of gravity stimuli, is poorly known. In animals, recent measurements on muscle and mechanoreceptor membranes show that mechanical deformation of cell surfaces can alter the ion conductance in such a way as to cause excitation. Presumably equilibrium receptors are stimulated by similar deformation of sensitive cell processes by statoliths of different specific gravity from the fluid in which they move. In plant cells, stimulation is said to result when cytoplasmic organelles move within the cell. The molecular events associated with mechano-stimulation need to be elucidated. An abvious approach would be to measure with intracellular electrodes possible changes in ion fluxes in plant cells under different orientations. This sort of problem in cellular physiology is appropriate for support by NASA.

APPENDIX A

National Academy of Sciences
National Research Council
2101 Constitution Avenue
Washington 25, D. C.

SPACE SCIENCE BOARD

Panel on Gravity

Members:

Solon Gordon, Argonne National Laboratory - **Chairman**
Lawrence Bogorad, University of Chicago
Mac Edds, Jr., Brown University
William Jensen, University of California
Charles Metz, Florida State University
Phillip Morrison, Cornell University
Ernest Pollard, Pennsylvania State University
C. Ladd Prosser, University of Illinois
Frank Salisbury, Colorado State University
James Snodgrass, Scripps Institution of Oceanography
Kenneth Thimann, Harvard University
John Torrey, Harvard University
Frits Went, Missouri Botanical Garden
Karl Wilbur, Duke University

Liaison:

Dale Jenkins, National Aeronautics & Space Administration
Allan Brown, Space Science Board

Secretariat:

J. P. T. Pearman

Committee on Environmental Biology

Members:

Colin Pittendrigh, Princeton University - **Chairman**
W. Ross Adey, University of California
Kimball C. Atwood, University of Illinois
Allan Brown, University of Minnesota
Solon Gordon, Argonne National Laboratory
Christian J. Lambertsen, University of Pennsylvania
Ernest Pollard, Pennsylvania State University
Sheldon Wolff, Oak Ridge National Laboratory

Liaison:

Sidney Galler, Office of Naval Research
Benjamin Strickland, Air Force Systems Command

Secretariat:

G. A. Derbyshire

NATIONAL ACADEMY OF SCIENCES
National Research Council
2101 Constitution Avenue, N.W.
Washington 25, D. C.

APPENDIX B.

S P A C E   S C I E N C E   B O A R D

PANEL ON GRAVITY
Committee on Environmental Biology

MINUTES
Meeting of November 30, 1962
Washington, D. C.

Panel Members

Present: Solon Gordon, Argonne National Laboratory - **Chairman**
Lawrence Bogorad, University of Chicago
William Jensen, University of California
Ernest Pollard, Pennsylvania State University
C. Ladd Prosser, University of Illinois
James M. Snodgrass, Scripps Institution of Oceanography
Kenneth Thimann, Harvard University
John Torrey, Harvard University
Frits Went, Missouri Botanical Garden

Absent: Barry Commoner, Washington University
Mac Edds, Jr., Brown University
Charles Metz, Florida State University
Phillip Morrison, Cornell University
Frank Salisbury, Colorado State University
Karl Wilbur, Duke University

Space Science Board

Allan H. Brown, University of Minnesota

Liaison

Dale Jenkins, Office of Space Sciences, NASA

Secretariat:

J. P. T. Pearman

In opening remarks, Gordon drew attention to the magnitude of the space program, and emphasized the significance of sound judgments and advice on its scientific content. The facts known about extraterrestrial biology are so few that the fundamental problem is to decide what investigations of a biological nature should be made in space. It is the function of the Space Science Board of the National Academy to provide the Government with advice of this sort, and he asked Brown, as a member of that Board, to discuss its role.

Brown referred to the beginnings of the space program, noting that the early work during the IGY period had contained little or nothing of biological interest. At that time the immediate problems were primarily of an engineering nature. The Space Science Board came to consideration of biological problems when programs for manned space flight were initiated. Its initial concerns in this area were chiefly with exobiology.

The interest and involvement of the Board in other biological problems developed more or less in step with the evolution of the space program as a whole. During the early period, the biological activities of the Board had been in the hands of Hartline, Lederberg, Lambertsen and Pittendrigh. Three committees: Exobiology, Man in Space, and Environmental Biology, had been formed to discuss the relevant problems. While the functions of the first two of these committees were clear enough, the Committee on Environmental Biology was, in a sense, a repository for problems which would not fit conveniently under the other two headings. It was primarily concerned with the fundamental aspects of biology in space, particularly with the way space research might contribute to an improved understanding of basic biology.

Brown noted that the ability to conduct experiments in space is, in effect, a new experimental tool. The problem before this Panel and the Committee on Environmental Biology to which it belongs is to decide how this tool may best be utilized. He contrasted this interest with that of a related Working Group on Weightlessness of the Man in Space Committee. That group is concerned principally with the characteristics and functioning of man in flight.

Brown went on to mention the condensation within the Space Science Board of the three biological committees into one (Life Sciences). It is hoped that this move will integrate the biological work of the Board. To facilitate planning, all of the outstanding biological working groups and panels have been asked to report by about the end of the year. Representing the interests of biologists, Brown stated that he would depend heavily on the advice of groups such as the present panel. He noted also that the Board's primary function is advisory. The reports of its committees would not be subject to review by the Board but would be forwarded, as appropriate, to the government agencies concerned.

Since most of the panel members have had little to do with other activities of the Board, Pearman reviewed them briefly. He emphasized again the advisory function of the Board and its close relations with the Office of Space Sciences in NASA. Though the Board had, in its earliest days, been involved in reviewing scientific research proposals, this was an activity which should no longer be necessary. Existing machinery within NASA was quite capable of carrying out such reviews. Nevertheless, it might be advisable to exercise this function to some extent for newly developing biological programs.

Brown referred to the organizational structure within NASA having to do with biology. Until recently the principal focus for biological problems had been in the Office of Manned Space Flight; there the interests were almost exclusively on applied problems. The staffing of the Office of Space Sciences to deal with problems of fundamental biological interest is a very recent event. The Office of Advanced Research and Technology is responsible for investigations which may assist in the much more ambitious space flight programs of the future -- that is, beyond those involved in the Apollo program. Bearing in mind that NASA is primarily an operating agency, the advice of the Panel on Gravity and others like it, is most likely to be of immediate concern to the Office of Space Sciences -- and perhaps also to the Office of Advanced Research and Technology. The Ames Research Center can be expected to be given the task of arranging for the practical realization of any experimental biological programs which the Panel may recommend.

In discussion, reference was made to the misgivings which had been expressed at the Space Science Summer Study and elsewhere concerning the quality of the research at Ames. This panel could well encourage the participation of first-rate biologists in NASA's program. Commensurate with the magnitude of the undertaking, not enough capable biologists are concerned. Other steps, for example, the organization of symposia, should also be considered. One of the paramount requirements is the improvement in quality of the biological work supported by NASA.

In this connection, Brown observed that the facilities for conducting experiments in space are limited and costly. He felt that it was a duty to society in general to help to bring about the efficient use of the opportunities which may be available. One of the ways in which the panel can contribute in this sense is to identify fields of investigation and experiments which would be worth undertaking.

Gordon moved to the specific concern of this group -- biogravitics (defining it as the study of the action of gravity on the properties and behavior of biological matter). He reiterated the obligation of the Panel: to produce a technically explicit report for the Space Science Board as a research guide.

In discussing an outline for the Panel's report, it was evident that information about the characteristics of the proposed spacecraft would be useful. The Chairman therefore asked Jenkins to comment on characteristics of the environment within which experimental investigations would be conducted. The present program, as outlined by Jenkins, involves the use of a recoverable spacecraft, launched by means of an Atlas-Agena rocket system. The satellite portion consists of the Agena-D upper stage and a recoverable capsule derived from an Air Force design. The recoverable portion contains a volume of about 125 cubic feet and weighs about 2000 pounds. It is essentially cylindrical, 6 feet in diameter and stabilized <u>in orbit</u> so that centrifugal forces are no more than 10^{-5}g. Three flights are planned, the first to occur in the latter half of 1964 with the others following at six-month intervals. A circular polar orbit with altitude 150 to 200 miles will probably be selected, with launch from the Pacific Missile Range. The accelerations at launch are 12 to 15g, perhaps higher on re-entry. Orbital durations anticipated are 2 weeks. The spacecraft contains apparatus for maintaining a portion of the volume at constant temperature (between 70° and 80° F.) and supplying an atmosphere of oxygen and nitrogen in the ratio ~1:4 (+½% CO_2) at one atmosphere pressure. Some parts of the "life support cell" can be maintained at different temperatures. There will be television, telemetry and data storage facilities. Jenkins promised to compile for the group a summary of the stresses and environments on the prospective vehicles. (see below*)

In discussion of the facilities which the contemplated vehicle would provide, questions were raised as to the reasons for selecting such an elaborate system. One of the reasons given was the desire to be able to carry out investigations in mammalian physiology using small monkeys. Other spacecraft which could be used, for example, the BIOS payload, the Mercury capsule and the Discoverer payload suffered from various drawbacks which tended to limit their utility for mammalian work. The small size of the BIOS payload would make it extremely difficult to maintain a terrestrial atmosphere within it; it is spin stabilized at launch and re-entry, and this might involve difficulties in experiments involving weightlessness. The Mercury capsule is designed for a single gas atmospheric system (oxygen at 1/3 atmosphere) and has a very high leakage rate. Adaptation of the existing types of Mercury capsule would be possible in principle but difficult and expensive in practice.

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* The procurement of experimental satellites based on the Agena system outlined above has now been cancelled. Proposals for a new series of spacecraft are being formulated; information about their important characteristics will be supplied by the Office of Space Sciences as soon as they are known.

Thimann pointed out that while the launch and re-entry conditions were important for mammals, this may be of no concern for some types of botanical work. In any event, it is important to be able to design and conduct biological experiments as a series, and to take advantage at each stage of the results of earlier work. For this reason a number of relatively small payloads might be more useful (biologically) than a few very large and elaborate ones.

Snodgrass noted also that peripheral apparatus, which maintains the capsule environment in the proposed biological satellite, might interfere with the experiments and might in fact delay the performing of certain tasks. This refers to a point made earlier concerning the possible interactions of other parameters as the absolute magnitude of the gravitational forces is reduced.

Pollard noted also that it may not be necessary to insist on maintaining the terrestrial environment for all types of experiments. Concerning penetrating radiation, Jenkins stated that the expected dose would be very small in the circular orbits which are proposed, and promised to provide more detailed information on this point. He also said in reply to a question by Prosser that some use of the trapped radiation in an experimental sense was contemplated, in which case the orbit would be made eccentric.*

In reply to a question by Went as to the amount of experimental space in this program which has been spoken for, Jenkins stated that about 60 experiments had been proposed, 32 of these bearing on the problems of weightlessness. R. Schiffman of the Ames Research Center has been visiting all of the would-be experimenters. The Biosciences Subcommittee of the Space Sciences Steering Committee reviews the proposals at its meeting of December 13 and 14, after which it is expected that the Space Science Board will be consulted. The final selection of experiments for flight will be approved, as usual, by the Space Sciences Steering Committee. Jenkins emphasized that all of the experiments proposed for inclusion in this program will go through the same review procedure.

Brown stated that the role of the Space Science Board in connection with the selection of these experiments consisted principally in consulting with the experimenters themselves. It was not intended that the Board would conduct a full dress review and selection, but by relatively informal discussion with the experimenters it was hoped that the quality of the work might be improved.

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* D. Jenkins reports that with the abandonment of the satellite design discussed above, it has also been decided to forego the use of eccentric orbits reaching into the trapped radiation. The radiation incident on the spacecraft is that which prevails at an altitude of 100-200 miles above the Earth's surface.

Gordon noted that a list of titles of the experiments proposed had been circulated to the panel, with no useful information about the experiments themselves. Jenkins agreed to supply a set of abstracts describing the experiments which are proposed.

Thimann asked how the list of proposed experiments had been obtained and Jenkins stated that the process had been rather haphazard. Some were related to a conference which had been held in 1958 and an announcement in Science. Otherwise the information that NASA was interested in such proposals had been spread by word of mouth. Jenkins also noted that the recommendations of the Space Science Board's Panel on Radiation Biology had been taken into account in connection with proposals for the study of the biological effects of radiation in the weightless state.

Brown noted that the Panel might feel that a criticism of the procedures for soliciting experimental proposals might be in order. Jenkins described the methods which NASA followed for the review of proposals and selection of experiments. All relevant proposals are reviewed by the Biosciences Subcommittee of the Space Sciences Steering Committee and a set of experiments is recommended. The final selection and approval is then made by the Steering Committee. In discussion, some misgivings were expressed concerning the amount of effort involved in the application of these selection procedures.

Pollard expressed the view that, because of the expense which biological experiments of this sort would involve, it might be preferable to abandon such efforts and allocate the resources instead to a strengthening of the investigations of the nearby planets -- e.g., Mars. Gordon noted that this was a point certainly moot: one of the reasons for the formation of panels, indeed the SSB itself, was to enable such evaluative judgments. Pollard reiterated the point that it is necessary to decide whether any biological experiments are worth serious consideration in view of the other ways in which NASA's effort could be expended. Bogorad noted that it would not be wise to perform experiments simply because apparatus is available. Thimann expressed the opinion that the weightless state as such is not particularly interesting from a biological point of view but rather the main point is to discover how living organisms -- plants, for example -- detect the gravitational forces. In reply, Gordon said that, while information on the nature of the sensor was of no small importance, this was one potential return to the plant biologist that should not be overlooked: that plant development in the low-gravity state might yield information on the determinants of the polarizations prerequisite to differentiation.

Referring again to the reasons for selecting a large spacecraft for biological investigations, Jenkins suggested that the justification lay in the ability to make exploratory investigations from many different viewpoints at the same time, and that the results would then be useful in refining the design of more definitive experiments to be performed later. The problem of interference between experiments was mentioned and the need for dummy runs to explore this problem. It was stated also that smaller spacecraft launched by a system such as the Scout could be used for single experiments.

It appears that none of the biological experiments performed to date, either by this country or Russia, has yielded any valid data. Torrey noted that this indicates the desirability of adhering to a simple approach to experimentation in the preliminary stages. Brown mentioned also that the proposed biological satellites are recoverable and that it should therefore be possible to study also the behavior of experimental organisms when terrestrial gravity is restored. In further discussions it was noted that experiments might be divided into two categories: (1) Specific and (2) Exploratory. In this connection, Went observed that while there were so many preconceived ideas about the effects of gravitational forces it was most important to obtain at least a few reliable observations. Gordon added that a comparison between the results of work with clinostats and zero gravity would be most desirable.

In response to a question by Gordon as to what the NASA would most like to see in the way of results from the panel's function, Jenkins suggested the following points:

1) Is the biological effect of weightlessness a valid subject for experimental study in space?
2) What types of experiments are recommended?
3) What suggestions can be made concerning the general approach to the planning of such investigations?
4) What importance should be attached to the effects of:
    a. Synergism
    b. Rhythmic phenomena
    c. Radiation, etc.?
5) What new experiments can be suggested?

Brown suggested that the Panel might consider the relative importance of studies in exobiology and weightlessness and perhaps formulate a statement on this matter. It is arguable that a landing on Mars might be preferable to any investigations of the biological effects of weightlessness (v.s.).

Went suggested that polarity was a topic which would be worth investigation -- in particular, to discover the stage when polarity is established in the developing organism. It is difficult to study this problem in the prevailing terrestrial gravitational field and perhaps critical experiments on it could be performed in the weightless state. He suggested that Jaffe might be consulted on this problem.

Gordon proposed that the panel members comment on these propositions in the light of fuller information concerning the experiments which have already been proposed.

Thimann mentioned that he was also interested in the onset of gravitational sensitivity in development, and suggested this as a topic of investigation.

Pollard advised against working with small single cells ($<10\mu$) in space. Systematic work with centrifuges on the effects of increasing g would be worth support. He added that he would expect weightlessness to have effects on multicellular structures and on single cells large enough to show stratification or streaming. Gordon noted that experiments by Fox on composition changes in microspheres as a function of gravity are relevant.

Thimann posed the following as desirable ground-based preliminary studies:

1. Are there any truly ageotropic plants?
2. Are different parts of plants different in sensitivity to g?
3. What is the mechanism of georeception in aquatic plants?
4. How does geotropism begin developmentally?

Gordon asked whether an experiment on the minimum force threshold would help understand the geotropic mechanism. He noted that vibrating wire experiments indicated that the threshold for plant shoots is about 10^{-4} to 10^{-5}g but Pollard expressed great skepticism about these values.

Torrey suggested that early embryological studies of the initiation of organization might be useful but that more preliminary laboratory work is needed. Jensen suggested that many such studies could be done on seed germination, and Thimann mentioned the possibility of using the difference between horizontally and vertically growing roots. There may be great practical difficulties in growing roots horizontally, however. The study of egg development in a suitable suspension was also suggested by several. It was stated that differentiation occurs in 3-4 days and, in discussion, the reason for choice of a 14-day life for the proposed biological satellites was questioned. Jenkins replied that the main consideration had been the time required for significant decalcification and loss of muscle tone in mammals (10-14 days). He solicited comments on this point and the 10^{-5}g threshold.

Snodgrass suggested that a series of modular sizes should be specified for the satellite experiments and that each experimenter should specify the environment required. The mutual interference problem should also be considered. In this context, Brown mentioned the discussion of the role of large ("streetcar") and small satellites which had occurred during the Summer Study. Is there a need for both types? Attention should be given first to the current program but he noted that NASA is not irrevocably committed to it. Pick-a-back experiments in the lunar program are feasible in principle, but experience indicates that little science is likely to be done in Gemini and Apollo. Biological experiments are believed to have been done in the Discoverer program (USAF) but no useful results are known to have been obtained.

Bogorad felt that the panel should consider how best to interest good biologists in the space science program with a view to taking some constructive action. Preparation of an appropriate article for publication was suggested. Prosser reviewed a number of problems in animal physiology which might be suitable for experimental study. Examples are: energy requirements of movement in 0g; circulatory and respiratory physiology; vascular tone; venous return; gravity-pressure relationships (ref. work of Nello Pace); animal behavior; sensory deprivation; CNS action; proprioceptors; biological rhythms.

Pollard remarked that there seemed to be general support for more laboratory work as a prelude to experiments in space, but that he would like to see a good seed experiment performed soon. Gordon thought that, since germinating seed development involved growth on pre-existing structures, he would not expect early morphological changes at 0g. He suggested also that the threshold of 10^{-5}g may be too high on the basis of 2-axes compensation experiments. Pollard reiterated the importance of good laboratory studies -- these are essential whether or not any space experiments are indicated.

The Chairman requested each panel member to consider the questions raised by Jenkins (page 7) in their contributions to the panel report. As to the nature of that report, Thimann submitted a tentative outline based on areas of known competence of the members.* Brown asked that the members comment on the NASA's methods for selecting experiments and experimenters.

It was agreed that the need for another meeting should be decided on the basis of submissions received.

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* Although unable to attend, Morrison contributed a written outline of the physics of weightlessness which was distributed at the meeting.