Report on Studies on the Preservation of Blood at Low Temperatures (1964)

Pages
27

Size
8.5 x 10

ISBN
0309363713

Committee on Blood and Transfusion Problems; Division of Medical Sciences; National Research Council

REPORT ON STUDIES ON THE PRESERVATION OF BLOOD AT LOW TEMPERATURES

Prepared by

NRC [The] Committee on Blood and Transfusion Problems

September, 1964

With the Support of

The Departments of the Army, Navy, and Air Force
Contract No. DA-49-193-MD-2077
and
The National Institutes of Health, Public Health Service
Contract No. PH 43-64-44

Division of Medical Sciences
National Academy of Sciences-National Research Council

Section I

INTRODUCTION AND HISTORICAL REVIEW

During the first quarter of this century, delineation of the ABO blood group systems provided a rational scientific foundation for blood transfusion. However, general use of the procedure had to await the development of methods for the preservation of blood outside the body - the now-familiar blood banking.

The two principal conditions that had to be controlled in this remarkable development were the normal senescence of red cells and the effects on the cell of an artificial environment. In its natural milieu the red cell has an average life span of about 120 days. If this milieu could be precisely duplicated in vitro, one could expect to be able to store a unit of blood containing a linearly decreasing number of viable red cells for about 120 days outside the body. Obviously, however, at some arbitrary point during storage the blood would have to be considered to have become therapeutically ineffective, there being such a low percentage of viable cells remaining. Thus, it was to be expected that even under artificial conditions that mimicked the natural environment ideally, the practical limit of storage would be less than 4 months.

Early in the studies leading to modern methods of blood banking, it was demonstrated that the very act of removing red cells from the circulation adversely affects their viability, and that cells deteriorate rapidly during storage at 37° C. To explain these findings, it was postulated that the cells are damaged by the physical and chemical agents and conditions to which they are necessarily exposed during withdrawal from the circulation. It was also postulated that, possibly partly as a consequence of the damage inflicted during collection and of unphysiological conditions to which the cells must be exposed during storage, the metabolism of the cells is adversely affected and deleterious processes are accelerated.

A considerable amount of effort has been spent in an attempt to minimize the damage inflicted on the cells during collection, including improvements in technical procedures and the development of improved anticoagulant solutions. The recent licensing of the citrate-phosphate-dextrose (CPD) anticoagulant is one result of these efforts. Much work has also been done to find ways to minimize or eliminate the relatively rapid deterioration of the cells during storage.

In most of these latter efforts, the conceptual approach has been the following: If the metabolic activities of the cells could be depressed during storage, the deterioration of the cells might be retarded. Cold storage was chosen as the most suitable method to accomplish this end. It was shown

that if proper precautions are taken to minimize damage to the cells during collection, blood in ACD solution can be stored at 1 to 6° C. for up to 21 days if the temperature is not permitted to fluctuate more than 2° C., and that in most instances at least 70 per cent of the cells will continue to circulate 24 hours after infusion; beyond 21 days, the viability of the cells decreases rapidly. These results attest to the clinical usefulness of stored blood: the infusion of 200 to 250 ml. of red cells, of which approximately 70 to 80 per cent are viable, constitutes an addition of 7 to 10 per cent to the recipient's circulating red cell mass. Therefore, the "70 per cent in vivo survival rate at 24 hours" became the criterion of successful preservation, and the "21-day dating limitation" became a most important governing factor in blood banking and transfusion services.

The 21-day limitation on the storage period does cause problems in blood banking and transfusion services. However, it has not seriously interfered with acceptably effective and efficient operations, as evidenced by the fact that in the United States over 5 million transfusions are given annually. There have been no pressing demands, therefore, to make major efforts to improve the methods for storing blood.

It was recognized, of course, that if stored blood could be kept cold enough, it might be possible to retard further or even suspend the metabolic activities of the cells and thereby achieve greatly prolonged or even indefinite storage. It was generally agreed that freezing and thawing of red cells resulted in hemolysis, until it was shown in the 1940's that ACD blood could be solidified without hemolysis if the temperature was not below -3° C. In 1950, quite by accident, it was discovered that red cells could be protected against hemolysis caused by freezing and thawing if they were suspended in a medium containing glycerol. Thus, the possibility of practical long-term preservation of red cells was opened.

The initial observation concerning the protective effect of glycerol was quickly confirmed and the work was extended. Progress was such that in 1954 the Division of Medical Sciences, NAS-NRC, held a conference to review the information available on the preservation of red cells at low temperatures. In opening that meeting, Dr. Carl V. Moore, Chairman of the sponsoring NRC committee, stated the purpose of the meeting as follows:

"One of the principal interests of the Subcommittee on Blood and Related Problems is to explore methods by which red cell preservation might be improved.* Periodic conferences have been held in which investigators concerned with specific aspects of erythrocyte preservation have been brought together to discuss their efforts. These conferences have served to inform the Subcommittee members of the most recent advances in studies on preservation and to provide a common meeting ground where investigators from different fields could pool and exchange pertinent information. The previous conferences have been rewarding. It is hoped that today's discussion will

*Up to that time the "methods" referred to had been almost exclusively limited to those involving preservation of blood in the liquid state.

help the Subcommittee to determine whether low-temperature preservation of red blood cells is still in the phase where further basic experimental investigation is needed, or whether work has reached the point where concerted emphasis on practical problems might be appropriate. A minimum goal is to achieve normal survival of red cells in vivo after one year of storage."

At the conclusion of this conference, the Subcommittee approved the following statement:

"Considerable knowledge has been accumulated concerning the physical and chemical changes attending the freezing and thawing of stored red blood cells. Of particular concern at this time is the significant loss of cells which occurs during the preparation or pre-transfusion stage and during the first 24 hours following transfusion of the stored cells. The causes of these losses are not clear and are being studied.

"The Subcommittee believes that the preservation of the red cell by freezing holds great promise and should be pursued. The technique is not yet ready for application in large-scale pilot operations, but research at a scientific and developmental level should certainly be encouraged."

The armed forces, principally the Department of the Navy, promptly contributed support for research in the field, and more recently the American National Red Cross has also done so. It is estimated that the operating agencies in the National Blood Program spent approximately $3.5 million for these purposes during the period 1954 to 1964 (Appendix A).

In the studies that were undertaken, efforts were devoted principally to the development of preservation techniques. Starting with blood collected in ACD solution, studies were conducted to determine how the blood might best be processed before freezing, what additives might be necessary to protect the cells, and the optimum procedures and conditions of freezing, storing, thawing, and reconstituting the cells. The criteria of success in these studies have been the in vitro recovery of intact cells at different steps in the technique and the in vivo survival 24 hours after infusion of the final preparation of the cells.

Remarkably soon after the work began, a technique had been sufficiently developed to permit clinical investigation. It involved the partial fractionation of blood, washing of the red cell mass, freezing in the presence of glycerol, storage at -80 to -120° C., slow thawing, removal of the glycerol, and resuspension of the cells. To date, over 3000 units of blood prepared in this manner have been transfused. The proponents of the technique have reported that blood so preserved is safe and efficacious.

Other preservation techniques were also studied: one is based on rapid freezing and storage using liquid nitrogen, and others use different stabilizing additives or different methods of washing the recovered cells. Blood prepared by these techniques has also been infused into a small number of patients.

During the past 10 years, the Committee on Blood and Related Problems of the NAS-NRC has continued to monitor the progress of work in the field. It soon became evident that all the techniques being developed impose technical and procedural requirements and limitations that appear economically or operationally unacceptable, and that some yield products of doubtful clinical acceptability. About a year ago the Committee became convinced that these efforts were yielding diminishing returns and that a critical evaluation of this area of research was in order. More recently, the Chairman of the Interdepartmental Committee on National Blood Program Research requested the Chairman of the Division of Medical Sciences, NAS-NRC, to conduct such an evaluation and to advise him on the prospects of future research in the field. Within the Division, this task was assigned to the Committee on Blood and Transfusion Problems.

In its approach to the undertaking, the Committee decided to depart from its usual custom of concentrating primarily on acquiring information about new and proposed research. It decided to give primary attention to assembling data on the preservation of blood at low temperatures that had been firmly established during the past decade, and to review recent data and research efforts in less detail. The Committee agreed to accomplish these purposes chiefly by means of a conference.

In anticipation of such a conference, an ad hoc panel, consisting of one member of the Committee and one representative from each of the seven laboratories active in the field, formulated "guidelines" that established standard definitions of key terms, specified the types of data that would be essential or desirable to report, and outlined the requirements for a description of the methods used in acquiring these data. It was hoped thus to establish, by agreement among the Committee and the investigators who would be reporting the results of their work to the Committee, a common terminology and formulation of data that would facilitate the comparison of results of different workers.

A conference was held on 21 and 22 May 1964 and representatives from all the laboratories in the United States that are actively studying the preservation of red cells by freezing participated. The proceedings of this conference will be issued in due course as an informal NAS-NRC publication.

Section II

SUMMARY AND CRITICISM OF THE INFORMATION PRESENTED AT THE CONFERENCE ON LONG-TERM PRESERVATION OF RED BLOOD CELLS

The conference was called primarily to determine and review the firmly established data developed during the past 10 years concerning the preservation of blood at low temperatures. Accordingly, for the most part, there evolved a chronicle of essentially empirical observations on the methodology of the bulk processing and the clinical trial of preparations of reconstituted cells that had been frozen and stored under a variety of conditions well described almost a decade ago. No specific hypotheses were advanced to explain the mechanism of the damage to the cells caused by freezing, storing, and thawing. The conference, indeed, was specifically intended to provide the means to answer the technical question posed by the Chairman of the Interdepartmental Committee on National Blood Program Research rather than to formulate fundamental concepts of the reaction of red cells to low temperatures.

Techniques Employing Glycerol

The advantages and limitations of glycerol-preserved cells processed in the Cohn fractionator were succinctly reviewed by Dr. James L. Tullis. In vivo studies have revealed 24-hour survival of 75 to 80 per cent of the cells originally bled from the donor; storage periods have been as long as 7 years. It was implied that processing could probably be simplified and made less expensive by further engineering development, although the overall yield of viable cells might be reduced.

Dr. H. A. Sloviter reviewed his experience with a moderately rapid freezing method and subsequent storage of cells at -20 and -75° C. in a relatively low concentration of glycerol. After thawing, the glycerol is removed from the cells by adding hypertonic glucose and then diluting with large amounts of saline. The resuspended cells may be transfused directly, if the large volume of suspending medium containing glycerol, glucose, hemoglobin, and cell ghosts is not considered undesirable, or the cells may be separated by centrifugation before transfusion. Post-thaw recoveries and 24-hour in vivo survivals were disappointingly low for such blood stored at -20° C. for more than 3 months and for blood stored at -75° C. for more than 12 months.

In contrast to the orderly and informative clinical trials carried out at the New England Deaconess and Chelsea Naval Hospitals, some of the recent clinical trials of glycerol-stored cells reported from other parts of the country appeared to be in the hands of investigators who lacked sufficient experience to obtain definitive results in the specialized area of

evaluating the survival, function, and toxicity in man of the reconstituted red cell suspensions.

A number of peripheral investigative areas requiring amplification were brought out by the presentations and the discussions of the glycerol method of preservation. None of these was confined to this method. They are:

(1) the definition of possible nephrotoxicity of free and bound hemoglobin and the possible importance of components of red cell stroma;

(2) the nature and extent of any significant alterations in the recipients' plasma potassium levels after multiple-unit transfusions;

(3) the need to reassess methods of evaluating immediate red cell survival, regarding both blood volume measurements in ill patients and the effects of the resuspending medium on the binding of the radioactive chromium to the test cells; and

(4) the need to re-evaluate generalizations about long-term preservation based on short-term storage studies.

It was also apparent from several of the presentations that, after thawing, erythrocytes that have been frozen differ from the cells in ACD banked blood in their subsequent storage characteristics. The shortened post-thaw shelf life of cells preserved for long periods, although not strictly relevant to the problem of successful frozen storage, points to the need for intensified study of both thawed and fresh cells to gain a fuller understanding of the lesion of storage that occurs at 4° C.

Technique Being Developed by Dr. Charles Huggins

In view of the uncertain toxicological status of dimethylsulfoxide, Dr. Huggins has abandoned the use of this compound in favor of glycerol. The essential feature of his method is the use of repeated cellular agglomeration by low-ionic-strength sugar solutions with rapid sedimentation of the cells to remove the glycerol. The embodiment of this feature in an apparatus for preparing preserved blood for clinical use appears to have been successful. Red cells so processed have not been studied in many recipients, but they appear to be comparable with the products obtained by other methods employing glycerol, although losses of cells during the reconstitution cycle may be somewhat higher.

The studies Dr. Huggins presented were basically those of freeze-thaw cycles with short-term storage. The characteristics and shelf life of cells preserved and recovered by this technique will probably compare favorably with those of glycerolized cells stored under equivalent conditions by others.

The apparatus designed by Dr. Huggins has an advantage in speed over other methods of deglycerolization. Fifteen units of blood may be processed per hour using a five-station machine. Cost, reliability, and personnel requirements for large-scale or general use cannot be evaluated or extrapolated from the data presented.

The principle of reversible cytoagglomeration may be adaptable to a variety of engineering applications to meet varying requirements. The apparatus designed by Dr. Huggins and built by the International Equipment Company appears to be practicable only where blood banking practice is much more sophisticated than usual.

Techniques Being Studied by the American National Red Cross

The work reported emphasized the use of an additive consisting of 14 per cent glycerol and 2.7 to 6 per cent sucrose in a custom-designed rapid-freezing unit. The procedure allows washing of the cells in the same container, with as little as 750 ml. of wash fluid, in 30 minutes. After three washes, the residual content of glycerol was 600 mg. per 100 ml. and the hemoglobin was 80 mg. per 100 ml. Recovery of the cells was 96 to 99 per cent, and 24-hour survival was "up to" 95 per cent in an unspecified small number of subjects. The blood was thawed after 24 hours of storage. No long-term storage studies have been done. The double Cr^{51} tag method was used in evaluating the in vivo survival of the cells.

The data presented are interesting but insufficient for any conclusion or recommendations other than that more work is necessary. In particular, intermediate and long-term storage studies are needed.

Techniques Being Studied by the Bryn Mawr Hospital Group

This group originally demonstrated that the addition of dextrose-lactose mixtures to whole blood exerts a protective effect on erythrocytes when rapidly frozen, stored at temperatures of about -93° C., rapidly thawed, and transfused without further modification. Blood so processed has been stored for approximately 3 years. Simple removal of the supernatant would permit transfusion of several full units of red cells without approaching the toxicity level of lactose.

More recently, the group has shown that treatment of packed red cells with lactose, followed by removal of lactose to a concentration in the final supernatant of approximately 1 per cent, followed by freezing of the red cells in the presence of low molecular weight dextran or human albumin, results in a recovery rate of red cells of approximately 98.5 per cent, including all losses. This preparation appears to be safe for transfusion with or without removal of the supernatant. The red cells have a 24-hour survival of 69 per cent when given to healthy subjects in small amounts. There is evidence that when red cells are so treated, the chromium tag is labile.

Section III

CONCLUSIONS

In assessing the information that was brought before it, the Committee took the following viewpoints:

(1) that the subject under review has its roots in fundamental biological phenomena;

(2) that research on the preservation of blood at low temperatures promises to make important contributions to the operation of both peacetime and emergency blood and transfusion services; and

(3) that research on the preservation of red cells has broad application in the whole field of biology.

The Committee was also mindful that on occasion a chance observation may provide the answer to a difficult scientific problem and that it may be possible to solve such a problem by dogged adherence to an essentially empirical research approach. However, best results are more frequently gained when, in the face of an impasse, the research approach is redirected. Redirection may be toward the re-examination of basic biological phenomena that had been thought to be well understood. In this respect, such a change of course might appear to be a retreat from a developmental accomplishment that seems to be tantalizingly close. Accordingly, redirection of a major program of study is not to be undertaken without just cause and precise definition of the new areas of investigation that should be initiated.

In consideration of these viewpoints, the Committee reached several conclusions unanimously, as set forth below.

(1) During the past 10 years, a number of significant advances in the preservation of red cells at low temperatures have been made by the investigators who participated in the conference. The large body of information obtained has established important guideposts for the direction which additional research should take, and will provide an invaluable background against which to compare new observations. Therefore, the money that the agencies supporting research in the field have provided for these studies constitutes a sound investment for the future in spite of the limited present return.

(2) All the subzero erythrocyte preservation techniques studied share the serious limitations of being too costly and cumbersome to meet the day-to-day needs for blood transfusion services or the projected needs of the armed forces and the civil defense agencies under conditions of limited war or thermonuclear disaster. In fact, because of the magnitude of current

and projected requirements for blood for transfusion, the accessibility of donors, and the relative simplicity and economy of the process of banking ACD blood, it is doubtful that preserved blood will ever be competitive with ACD blood in ordinary transfusion services. This is not to say, however, that studies of the glycerol preservation method, particularly as practiced by the Chelsea Naval Hospital and the Protein Foundation groups, have not demonstrated convincingly the feasibility of subzero preservation to meet certain special purposes. These purposes currently include stockpiling units of rare types of blood and storing blood for anticipated autotransfusion to patients with uncommon red cell antigens or known isosensitization to very common antigens. Also, the availability of an acceptable and economically feasible preservation technique would make it possible to salvage the red cells in much of the ACD blood that would otherwise become outdated.

(3) Although the adaptation of existing equipment has made it possible to meet some special needs currently, additional expenditures for engineering development may be justified in the interests of greater simplicity and efficiency and lower costs. However, there is no reason to believe that such engineering efforts will significantly diminish the serious limitations inherent in the present glycerol preservation technique, and engineering refinements should be undertaken with frank recognition of the limited application that is anticipated.

(4) The current program of studies is unlikely to lead to the successful development of a generally acceptable technique for long-term preservation of red cells. There should be frank recognition of the diminishing returns, now obvious, in the kinds of activities that have characterized the work in the field during the past few years. Reorientation of the program is therefore necessary. Greater emphasis and more support should be given to the study of fundamental problems of cell physiology and metabolism created by the exposure of the formed elements of the blood to the conditions necessary for freezing and prolonged storage at low temperatures.

(5) This reorientation should not result in the complete abandonment of the current program of studies. That would be wasteful and would eliminate a potential, if rather remote, chance of developing a more ideal technique. In addition, continuation of the current program in some form and to some extent will be essential to the development and evaluation of processes arising from more fundamentally oriented studies that might be undertaken. To achieve the intended objective of basically oriented in vitro investigations, it would be necessary to correlate the data accumulated therefrom with data from well planned and carefully controlled in vivo studies. This arrangement can best be provided by maintaining an ongoing program of clinical evaluation of preparations now being produced.

(6) The processing of preserved blood and the clinical use of reconstituted units of such blood should be limited to institutions where blood transfusion services and related research activities are of the highest quality.

(7) Before any studies on the preservation of red cells involving the use of a stabilizing additive are undertaken, it should be determined that the additive under consideration is acceptable for use in seriously ill patients who will require many blood transfusions.

Section IV

RECOMMENDATIONS

The Committee on Blood and Transfusion Problems recommends:

(1) That no large-scale program be launched, at this time, for the incorporation of any of the currently proposed techniques for long-term preservation of red cells by freezing into regular day-to-day practice in hospital or field blood transfusion services;

(2) Conversely, that current programs of study of the long-term preservation of blood by freezing continue to be developed as basic and developmental research projects;

(3) That the current program be continued, at the cost of about $500,000 annually, specifically to permit limited engineering development of improved equipment and clinical testing of preparations of preserved blood, particularly with glycerol, as appears reasonable and desirable;

(4) That greater emphasis and support, at the cost of approximately $300,000 annually, be directed to the study of the fundamental problems of cell physiology and metabolism created by the exposure of red blood cells to the conditions necessary for preservation at low temperatures (areas for such investigations are suggested in Appendix B);

(5) That the data forthcoming from in vitro studies on the fundamental biological changes that occur in the cells during low-temperature preservation be correlated with data from in vivo studies in normal recipients;

(6) That, for the present, only limited quantities of red cells frozen in the presence of glycerol be accumulated to meet emergency demands for rare types of blood, for autotransfusion of persons presenting difficult blood grouping problems, and for other such specialized and limited needs;

(7) That the clinical evaluation of red cells preserved at low temperatures, particularly those preserved with glycerol, be continued and possibly expanded modestly, to gain further experience with the blood banking aspects of the use of preserved blood and to acquire additional information about the safety and efficacy of such blood in patients;

(8) That one institution within the Department of Defense be given over-all responsibility for coordinating and monitoring the program of clinical evaluation and that the selection of other institutions for participation in the program be limited to those having high-quality blood transfusion services and in-house, or at least readily accessible, research capabilities;

(9) That Appendix C be adopted as an interim definition of the desirable properties of preparations of preserved red cells intended for use in patients;

(10) That a critical review be made of the desirability of continuing studies using PVP as a stabilizing additive to prevent hemolysis and that henceforth the possible toxicity in man of any potential additive be investigated before studies using it are begun; and

(11) That a standard protocol for the clinical evaluation of preparations of red cells preserved at low temperatures in sick and injured persons be prepared for use by all institutions undertaking such work.

Appendix A

CONTRIBUTIONS MADE BY OPERATING AGENCIES IN THE NATIONAL BLOOD PROGRAM IN SUPPORT OF STUDIES ON THE PRESERVATION OF BLOOD AT LOW TEMPERATURES

The financial data in the following tables were obtained from reports made to the Chairman of the Interdepartmental Committee on National Blood Program Research by the organizations listed. In these reports, expenditures were reported by both calendar and fiscal year and in some instances the information is incomplete. In order to approximate the support provided to date and the support projected, some arbitrary decisions were made as to times of expenditures. As a result, some duplications may have been introduced; the expenditures for 15 June 1950 through 31 December 1964 may be smaller than shown, and the amounts proposed for expenditure from 1 January 1965 through 30 June 1966 may be larger than shown.

Estimates have not been included of the amounts or sources of financial support provided by other organizations, such as universities, associations, and private institutions. Accordingly, these tabulations are to be considered as conservative approximations of expenditures, both made and proposed, rather than precise recordings. These data were compiled by the NRC staff in this form to acquaint the NRC Committee with the extent of the support that operating agencies in the National Blood Program have contributed to research in the area.

Total Expenditures by Organization

Organization	Expended 15 June 1950-31 Dec. 1964	Proposed 1 Jan. 1965-30 June 1966
Department of the Army	$ 369,000	$120,000
Department of the Navy:		
Office of Naval Research	2,445,000	436,000
Bureau of Medicine & Surgery	322,000	156,000
National Institutes of Health	20,000	---
American National Red Cross	296,000*	No estimate
	$3,452,000	$712,000

*Includes $70,000 obtained from NIH through research grants

Total Expenditures by Type of Research

Type of Research	Expended to Date	Proposed	Total
Glycerol Processes	$1,160,000	$128,000	$1,288,000
Liquid Nitrogen Process	1,435,000	336,000	1,771,000
Cell Agglomeration Process	----	75,000	75,000
Basic Technological Studies	561,000	173,000	733,000
Evaluation Program of the American National Red Cross	296,000	not reported	296,000
	$3,452,000	$712,000	$4,163,000

Details of Expenditures by Research Area and by Organization

I. Department of the Army

Type of Research	Expended 15 June 1950-31 Dec. 1964	Expended 1958-1961	Proposed 1 Jan. 1965-30 June 1966
Basic Technological[1]	$329,000	---	$ 45,000
Glycerol Processes[2]	---	$39,000	---
Cell Agglomeration Process[3]	---	---	75,000
	$329,000	$39,000	$120,000

(1) Bryn Mawr Hospital and John S. Sharpe Research Foundation

(2) Protein Foundation

(3) Massachusetts General Hospital

II. Department of the Navy

A. Office of Naval Research

Type of Research	Expended 1956-June 1963	Proposed 1 July 1963-30 June 1966
Liquid Nitrogen Process[1]	$1,435,000	$336,000
Glycerol Processes[2]	953,000	50,000
Basic Technological[3]	57,000	50,000
	$2,445,000	$436,000

(1) Until fiscal year 1963, the total support went to the laboratories of the Linde Division, Union Carbide Corporation. In 1963, contracts were let with the following laboratories: Presbyterian Medical Center, San Francisco (clinical evaluation - $41,000); Yale University (effects of PVP on renal function - $22,000); and Bjorksten Research Foundation, Madison, Wisconsin (effects on proteins of the red cell - $25,000). These three studies are to be continued at approximately the same level of funding through FY 1965. In addition, the Office of Naval Research, Department of the Navy; the British Army Medical Service; the French Center for Transfusion and Reanimation; and the Netherlands Red Cross are cooperating in a joint effort. The combined expenditures of the three foreign organizations are reported to be about $200,000 annually.

(2) Protein Foundation. Includes $216,000 provided to the A. D. Little Company in fiscal years 1961, 1962, and 1963 for development of equipment. This task is completed.

(3) Includes support to the Bryn Mawr Hospital for studies on effects of macromolecular agents on recovery and survival of red cells ($25,000) and the University of Pennsylvania for research on hemoglobin and other hemin chromoproteins ($32,000).

B. Bureau of Medicine and Surgery

Type of Research	Expended 1 July 1959-30 June 1963	Proposed 1 July 1963-30 June 1966
Glycerol Process (Chelsea Naval Hospital)	$148,000	$ 78,000
Basic Technological (Naval Medical Research Institute, Bethesda)	174,000	78,000
	$322,000	$156,000

III. National Institutes of Health

In 1954, the NIH did a study to determine the dating period of glycerolized red cells stored at -45° C. No studies have been done since.

IV. American National Red Cross

Type of Research	Expended 1960-1964	Proposed
Evaluation of Several Freezing Processes and Additives	$296,000	No estimate

V. The following agencies reported no expenditures in the field: Military Blood Program Agency, Department of the Air Force, and Atomic Energy Commission.

Number of Personnel in the Program by Type of Research, Fiscal Year 1963

Type of Research	Number of Persons*
Liquid Nitrogen Process	46
Glycerol Processes	20
Red Cross Evaluations	6
Basic Technological	11
Cell Agglomeration Process	No estimate
	83

*These estimates are very rough because of the incomplete information that was reported. However, as with the estimates of financial support, they are considered to be conservative because they do not include persons known to be working in the area in other laboratories in this country or the foreign investigators in the joint program with the Office of Naval Research, nor do they include overhead and personnel costs for several of the programs.

Appendix B

SUGGESTED AREAS OF INVESTIGATION CONCERNING THE BASIC CHARACTERISTICS AND PROPERTIES OF RED CELLS PRESERVED AT LOW TEMPERATURES

The ultimate criteria of practical usefulness of any type of preparation of red cells must remain the satisfactory in vivo survival of the transfused cells and their normal function in gas exchange. However, the measurement of these parameters provides no information concerning the cause of the failure of the cells to survive normally or to transport oxygen and carbon dioxide if, in fact, they fail to do so. Accordingly, the search for such causes, and their possible avoidance or correction, must be directed along other lines of investigation, primarily by in vitro methods, and the findings from these investigations must be correlated with the results of carefully designed and controlled in vivo studies. Also, if such combined investigations are to yield meaningful data, there must be convincing evidence that the methods for measuring in vivo survival and gas exchange are adequately sensitive and reproducible. In this respect, additional work on the measurement of in vivo survival is necessary to develop a practical method based on the differential agglutination method (Ashby) to check the results of the standard Cr^{51} technique. It is also essential to conduct additional studies to reassess the validity of measurements of the blood volume in healthy young subjects and in ill patients. Recent evidence indicates that the indirect determination of the red cell mass, using a measurement of plasma volume and a corrected hematocrit, is not applicable to all patients.

It may be anticipated that any technique for prolonged preservation of red cells will result in changes in some of the characteristics and properties of the cells as measured by both in vitro and in vivo methods. The mere demonstration of such changes does not, of course, foredoom a proposed preservation technique. It must be determined to what extent changes noted by in vitro methods are reversible after thawing and after transfusion of the cells. Also, the specific cause or causes of the changes noted by in vivo methods must be determined and corrective measures sought.

The tests outlined herein might be conducted to investigate the lesions of the cells caused by freezing, storing, and thawing which may lead to alteration of the red cell as demonstrated by in vitro methods, and excessive removal of the cells from circulation or loss through hemolysis after transfusion. These tests, and particularly those used to evaluate metabolic stability, may also be done to investigate the changes that take place in such cells during post-thaw storage.

The Red Cell and Its Membrane

The membrane determines many of the principal physical and chemical properties of the cell. The appearance of the cell, its chemical composition,

permeability, elasticity, and intrinsic enzymatic and metabolic properties require maximum definition in the fresh normal cell, assessment after whatever processing steps are necessary before freezing, and frequent reassessment at intervals during storage and after whatever processing is done before transfusion. Among the characteristics to be considered are:

- microscopic appearance and cell dimensions (by light, phase, and electron microscopy)
- antigenic properties
- membrane surface "carrier" properties
- membrane iron content
- surface charge
- viscosity
- elasticity (osmotic and mechanical fragility, ability to pass through millipore filters, colloidal protection against colloid osmotic hemolysis of the freeze-injured membrane)
- ability to withstand thermal shock
- lipid, protein, and ionic constituents
- membrane heme pigment content
- the mechanism of the protection afforded by nonpenetrating substances, such as PVP and some sugars, to the extent that such substances provide it
- permeability characteristics for:
  - electrolytes
  - sugars
  - larger molecular solutes
  - gas exchange
- intrinsic membrane enzymes
- intrinsic energy metabolism (ATP content, phosphate partition)

Intracellular Contents

Most important here are the energy metabolism systems, the electrolyte pump(s), and the heme pigments. Among the conditions to be considered are:

- intracellular pH
- intracellular fluid volume
- intrinsic viscosity
- integrity of the oxidative and anaerobic glycolytic pathways:
  - content and functional integrity of enzymes and co-enzymes involved therein
  - content of substrates
  - content of phosphorylated intermediates and inorganic phosphate
  - content of DPN, TPN, and ATP
- electrolyte composition and response to electrolyte gradients
- concentration and integrity of hemoglobin and its various oxidation and reduction products
- integrity of mechanisms for methemoglobin reduction
- hemoglobin O_2 and CO_2 association and dissociation

In the light of current knowledge, it is most difficult to define precisely which of the foregoing are most crucial and therefore deserve the most attention. However, it would appear that the primary problem to be investigated is the determination of the nature and cause of the injury to the cell membrane. Emphasis might be given initially, therefore, to study of the effects of additives and freezing, storing, and thawing of the cells on the membrane and on the energy systems that work the electrolyte pumps.

Appendix C

PROPERTIES OF PREPARATIONS OF RED BLOOD CELLS PRESERVED AT LOW TEMPERATURES

One of the functions of the Committee on Blood and Transfusion Problems is to evaluate the status and accomplishments of research and development studies on the preservation of blood at low temperatures, and to advise the operating agencies in the National Blood Program thereon. The Committee agrees, however, that in this area it is not possible to form critical judgments without some definition of the properties and characteristics of preparations of preserved blood that are essential or desirable from the clinical and logistical viewpoints. Because none of the available definitions was considered adequate for its purposes, the Committee undertook to formulate its own.

In this undertaking, the Committee did not intend to establish arbitrary, rigid standards for preparations of preserved blood or for methods of preservation. In fact, in consideration of the status of developments in the field, it is premature to attempt to do so. On the other hand, if the ultimate purpose of the research in the area is to develop a useful preparation for transfusion, it is logical to consider this proposition in the light of the practical realities of blood transfusion in the civilian and military practice of medicine that influence the acceptance of such a preparation. It is also logical to define standards that might be useful as guides for research and as criteria for evaluating the practical results of such research. In developing the statements that follow, the Committee employed this approach.

Definition of Properties

(1) When preserved red cells are intended to meet requirements for transfusion in circumstances for which ACD blood is usually given: If preserved red cells are to be used in large quantities as a substitute for the red cells of ACD blood, the preservation technique should not be technically very complex and should not involve prohibitive expense. The loss of cells incidental to the procedures involved should not be excessive. If, however, preserved red cells are to be used as a substitute for the red cells of ACD blood simply to augment the supplies of the latter under special circumstances, the comparative expense would not be so important. In such circumstances, it is doubtful that monetary costs would be important if the preparation met the medical need.

Apart from the considerations of operational efforts and expense, however, if preserved red cells are to be used as a substitute for ACD blood on equal clinical terms, the preserved product should meet the following biological requirements:

(a) Total losses of red cells 24 hours after transfusion should not be greater than those involved in the transfusion of ACD blood as specified by NIH regulations. In addition, the decline of the red cell population surviving the first 24 hours after transfusion should be similar to that of normal red cells.

(b) Preserved blood should not contain any additive that is toxic in man, that is not rapidly excreted or metabolized within a short period, or that is likely to sensitize the patient by reason of an antigenic component.

(c) The preserved cells should be stable at low temperatures for at least 1 year, as measured by standard red cell survival studies.

(d) Immediately and for at least 72 hours after thawing, red cells that have been stored in the frozen state should possess, as a minimum, physiological and bacteriological attributes similar to those of 21-day-old ACD blood stored under regular blood bank conditions.

(e) The immediate and long-term condition of the recipient's health should not be prejudiced by any untoward secondary effect from a single or large multiple transfusion of preserved blood.

(f) If possible, red cells should be packaged so as to permit the use of the same container for collecting, freezing, storing, thawing, and transfusing. This is particularly important to ensure maintenance of donor identification. In any event, however, the container used during the transfusion should permit direct visual inspection of the blood.

(2) <u>When preserved red cells are intended for use in meeting the limited requirements for blood transfusion in special circumstances</u>: Preparations of preserved red cells may have unique applications that are not possible with unprocessed, banked whole blood or red cells, and some experience with such applications has been gained already. Preparations of preserved cells of rare types have been used and recipients have "banked" their own blood for autologous transfusion. Preserved blood has also been used in surgical procedures requiring large quantities of blood.

Therefore, preparations of preserved red cells may be considered as a new and unique biological product that offers advantages over banked blood preparations in special circumstances. For this reason, and under these circumstances, the comparison of costs with ACD blood is not appropriate. However, the biological requirements for preserved blood used in such special circumstances are the same as for preserved blood used for any other purpose (1-a through f in the foregoing).