Preservation of Blood by Freezing

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Nearly two decades have elapsed since the first successful attempts to freeze human blood following exposure to glycerol. At first glance, this seems an excessive time for the translation of an important discovery into clinical practice. Yet this was not unexpected. Although the addition of glycerol to blood was simple, the red cells could not be transfused after subsequent freezing and thawing, unless the additives first were removed. Glycerol was nontoxic and a normal component of cellular metabolism. However, in the concentrations necessary to prevent ice crystal formation, this resulted in osmotically unbalanced cells which rapidly swelled and ruptured when returned to an aqueous solution such as plasma, after thawing. This problem could be controlled only by binding the extracellular water with nonpenetrating substances such as citrate or lactate, while removing the glycerol by dialysis. To accomplish this sterilely with practical volumes of blood, it was necessary to devise elaborate new systems of blood processing which used either centrifugal cell packing or low-ionic-strength agglomeration to pass the red cells through the deglycerolizing solutions.

Detailed descriptions of these methods have been published. The mechanical system of the Cohn centrifuge has had more than ten years of testing. It has proved to be a safe, reproducible technique. The deglycerolized cells derived from it have served as a standard reference for comparison with red cells deglycerolized by subsequent methods. Yet the Cohn system has not been universally accepted because of its complexity and expense. It requires excessive time and personnel to clean, assemble and sterilize the cartridges in which the individual pints of blood are processed before freezing and again after thawing. A modified Cohn system, using the Latham bowl, is currently under development with the goal of eliminating the draw-backs of the centrifugal system, while retaining its unique advantages of high cell yield and optimal post-thaw stability. It is inexpensive, simple to operate, and can be attached directly to a commercial refrigerated centrifuge. Once this new apparatus has undergone sufficient testing to define optimal solution volumes and operating conditions, it is likely that it will be produced as a disposable plastic kit.

The agglomeration technique of Huggins was introduced in 1963, utilizing the sedimentation velocity of red cells after aggregation in media of low ionic strength. This eliminates the need for complex mechanical equipment during deglycerolization and theoretically makes possible the preparation of cells under emergent conditions when a power source is unavailable. The cell yield by this method is reported to be 80 per cent, somewhat less than by mechanical deglycerolization, and the immediate post-transfusion survival after labelling with radiochromium appears adequate. The principal drawbacks are the large freezer space required per unit of blood and the large volumes of deglycerolizing solutions, approximately twice the amount required in mechanical processing.

* Produced through Arthur D. Little, Inc., Cambridge, Massachusetts.
Whatever system is used to remove glycerol, however, there is common agreement that the final concentration after reconstitution must never be greater than 1 per cent and preferably less than 0.1 per cent in order to yield osmotically stable cells. There also is agreement that the level of free hemoglobin in the supernatant solution should be low so as not to exceed the haptoglobin binding of the recipient's plasma and that the final transfusion unit should be free of significant quantities of dead cells or cellular ghosts which might lead to circulatory sludging.

Ultra-rapid freezing without additives also has been attempted. This has the potential advantage of direct use without post-thaw processing. However, the high rate of heat exchange necessary for this technique proved impractical on any significant scale. A modified rapid freezing method was then subjected to considerable in-vitro study. This utilized extra-cellular additives which did not penetrate the cells in the manner of glycerol. This too had the theoretic advantage of direct use after thaw, but the problems of retained hemoglobin and dead cells precluded large-scale use without centrifugation.

Essentially, all of the clinical experience with frozen blood, therefore, has been with glycerolized cells which were slowly frozen to −80°C, preserved for periods from a few weeks to six years, deglycerolized by automated mechanical systems or by agglomeration and used under varying clinical conditions. This experience already has shown that the processing necessary for glycerol removal, although burdensome and slow, adds unexpected clinical flexibility. The resulting cells are thoroughly washed; they are sterile and can be resuspended in media tailored to the specific requirements of the recipient. Thus, it was reported as early as 1960 that thawed, mechanically deglycerolized cells were totally nonpyrogenic and tolerated better than ordinary ACD blood in multiple transfusions to patients with lymphoma and leukemia. Similarly, mechanically deglycerolized cells were resuspended and administered in low potassium media to patients with hyperkalemia, in citrate-free media in patients with acidosis, and as packed cells in patients with normovolemic. More recently, cells deglycerolized by the agglomeration technique have been used in an Armed Forces investigative study in Viet Nam.

What then are the full implications of frozen red cells in clinical medicine? What are their therapeutic indications and limitations? Is the state of research such that cell freezing should be undertaken in routine hospital blood banking? Finally, and most importantly, what evidence exists that thawed red cells have an in-vitro metabolism and stability commensurate with their post-transfusion viability?

Biochemical Characteristics of Frozen Blood

Many biochemical changes may be observed in erythrocytes during the post-thaw storage, reflecting the myriad of changes brought about during exposure to the preservative solutions and environmental conditions. However, there appears to be little effect from the glycerol per se. When red cells are incubated with glycerol in metabolic quantities, that is, 5–10 millimolar as opposed to the 5 molar quantities used to protect against freezing and thawing, the glycerol has been found to be metabolically inert. That is to say that glycerol does not affect the glycolytic capacities of human erythrocytes as manifested by glucose consumption or lactate production, nor is the oxygen uptake affected in the presence of methylene blue. Neither do erythrocytes phosphorylate glycerol to form glycerophosphoric acid. It appears then that the only significant effect of glycerol on erythrocytes is an osmotic one related to its concentration as modified by the rate of exchange across erythrocyte membranes at various temperatures.

Major Metabolic Pathways in Erythrocytes.

Biochemical comparison of preserved erythrocytes with fresh cells is a standard procedure in order to relate the metabolic or enzymatic state of the cells to their viability characteristics. Hopefully, the day is almost at hand when simple biochemical tests such as those for adenosinetriphosphate (ATP), extracellular hemoglobin, or ejection transport will predict the subsequent in-vitro survival.

PRESERVATION OF BLOOD BY FREEZING

The major chemical reactions of metabolic significance in the human erythrocyte are shown in figure 1. The scheme consists of a branch at the left indicating the phosphorylation and breakdown of hexoses to trioses with the utilization of ATP, and a branch at the right representative of pathways of nucleoside metabolism which contribute both purine and pentosephosphates. In this sequence also, hexoses and trioses in the phosphorylated forms are made. The middle branch represents the conversion of trioses to lactate with a concomitant production of high energy phosphate (ATP). Sequentially, glucose, the natural substrate of abundance in plasma, is phosphorylated at the expense of ATP and broken down to triosephosphate which is oxidized with the regeneration of ATP. The nucleoside branch for which adenosine is a useful exogenous substrate, provides erythrocytes with a source of fructose-6-phosphate and glyceraldehyde-3-phosphate. These are formed with no requirement for ATP, and since they yield ATP when oxidized in the triose-to-lactate pathway, the nucleoside branch is energetically more efficient than the glycolytic one. Adenosine, biochemically, is a source of both ribosephosphates and adenosine. The adenine is also biochemically useful to erythrocytes as it can be incorporated into nucleotides (AMP, ADP, ATP) replenishing those cells which are depleted during aging at 4° C. or when erythrocytes are frozen.

Membrane Defects and "Lesions" of Collection, Storage and Freezing. The biochemical changes that occur in erythrocytes when frozen and thawed in glycerol may be considered to be qualitatively similar to those which take place in ACD 1 blood stored at +4° C. As a preservative medium, ACD affords benefits to the cells owing to low temperatures (+4° C.), low pH (7.0), the presence of added glucose as a metabolic fuel, and citrate as an anticoagulant. These conditions arrest glycolysis and slow down the destruction of ATP. However, as pointed out by Gibson and his colleagues,22 erythrocytes added to ACD undergo chemical and physical stresses which greatly alter osmotic fragility, cell dimensions and composition, and ultimately cell metabolism. The "lesion" of collection takes place during phlebotomy when the initial volume of blood at 37° C. enters the hypotonic, acid (pH 5.0) ACD solution. Approximately 20 per cent of the total unit is exposed to extreme conditions of pH, temperature and glucose concentrations which result in substantial osmotic pressure changes for that aliquot. This results in changes in intracellular composition and water balance as manifested by an initial cellular swelling and temporary increase of intracellular K+ and phosphate ion.22 By rendering isotonic the medium into which blood is collected with a citrate phosphate dextrose solution, and raising the pH to approximately 6.5, during the initial phase of collection, the lesion of collection can be made much less damaging to erythrocytes as determined by studies of osmotic fragility, metabolic characteristics, and in-vitro survival tests.22 Such initial stresses leading to progressive deterioration of the affected cells, and subsequent degenerative changes which occur in storage at +4° C. have been termed "the lesion of storage."23, 24 To elucidate completely, the cell damage brought about when blood is frozen after collection in ACD, a third lesion must be added, namely that of freezing and thawing. This represents the multiple effects of osmotic imbalance, irreversible change in osmotic fragility and intracellular composition, protein denaturation and disruption of cell membranes with loss of membrane components. Hemolysis occurs, ionic gradients are no longer maintained, and eventual loss of cell viability results. When glycerol is added, the water is displaced. True freezing does not occur, and the disruptive series of events characteristic of the third lesion does not ensue.

Stability of Erythrocytic Enzymes at Low Temperatures. It has been observed consistently with ACD blood that enzymes of red cells maintained at +4° C. retain activity for weeks beyond the time when the cells no longer demonstrate acceptable in-vitro survival. This applies to nearly all of the glycolytic enzymes during storage for five to six weeks at +4° C. Subsequently, several important glycolytic enzymes become reduced.25, 26, 27 In terms of per cent of original activity, these are hexokinase-(62), phospho-

1 N.I.H. formula A.
fructokinase-(4), aldolase-(14), and pyruvate kinase-(38) \( ^{26} \) (fig. 1). Although no detailed studies have been carried out on specific enzymes of ACD blood subsequently frozen in glycerol or with liquid nitrogen, it is reasonable to expect that the trauma to cells resulting from freezing does not destroy any vital enzymic activity, as shown by acceptable \textit{in-vivo} survival. If the cells are maintained in an isotonic state through the freezing process or if they are frozen and thawed extremely rapidly, apparent maximum stability of enzymes is obtained. Multiple freezing and thawing of unprotected erythrocytes, however, is a method of liberating enzyme activity from the stroma; eventually

**Fig. 1.** Major metabolic pathways in the human erythrocyte.
repeated freezing leads to inactivation of erythrocytic enzymes.

Changes in Erythrocytic Surfaces Caused by Removal of Additives. Physically, the integrity of the surface of erythrocytes is most sensitive to preservation conditions. Merely holding ACD blood for 10 days at +4°C is sufficient to permit demonstration of loss of phospholipid and cholesterol. These compounds are also lost when red cells are frozen in hypertonic (0.8 M) NaCl. The washing of erythrocytes with large volumes of sugar solutions to remove glycerol as done in cytoggelation, sufficiently decreases the ionic strength to cause residual gamma globulins to precipitate and thereby co-precipitate the cells. The surface antigens of such cells are modified so to adsorb beta and gamma globulin from plasma or serum when the blood is reconstituted, and may give false positive reactions for iso-antibodies in the Coombs test unless EDTA previously is added. On the other hand, washing erythrocytes with large volumes of low ionic strength sugar solutions has a practical advantage in that it eliminates weakly-bound blood group isoagglutinins.

Effects on Cellular Metabolism

The major biochemical goal in erythrocyte preservation is to provide at the time of transfusion a unit of cells whose glycolytic capacity, ionic flux, nucleotides, phosphate esters, mean corpuscular hemoglobin, cell volume, shape, fragility and integrity are the same as in fresh cells. As yet, the precise biochemical factors on which in-cuio survival of the cells critically depends have not been delineated. Properly frozen and thawed erythrocytes have biochemical properties which are comparable in many respects to those of fresh cells. When stored in the frozen state in liquid nitrogen for as long as one year, the ability of erythrocytes to carry oxygen is almost 100 per cent retained. Even after three months of storage at -80°C, no significant change occurred in the oxyhemoglobin dissociation curve on reconstitution with autologous ACD plasma. When previously frozen erythrocytes are deglycerolized, suspended in their original ACD plasma and held at +4°C, their metabolic characteristics are very similar to those of unfrozen erythrocytes in ACD. However, many degenerative changes are accelerated, and at +4°C, the post-thaw dating period, of previ-

Abbreviations

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<thead>
<tr>
<th>Substrates</th>
<th>Enzymes</th>
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<tr>
<td>G-6-P, Glucose-6-phosphate</td>
<td>1. Glucokinase</td>
</tr>
<tr>
<td>F-6-P, Fructose-6-phosphate</td>
<td>2. Phosphohexokinase</td>
</tr>
<tr>
<td>FDP, Fructosediphosphate</td>
<td>3. Phosphofructokinase</td>
</tr>
<tr>
<td>DHAP, Dihydroxyacetone-phosphate</td>
<td>4. Aldolase</td>
</tr>
<tr>
<td>GAP, Glyceraldehyde-3-phosphate</td>
<td>5. Triosephosphate isomerase</td>
</tr>
<tr>
<td>1,3DPC, 1,3 Diphosphoglycerate</td>
<td>6. Glyceraldehyde-3-P dehydrogenase</td>
</tr>
<tr>
<td>3-PGA, 3-Phosphoglycerate</td>
<td>7. Phosphoglycerate kinase</td>
</tr>
<tr>
<td>2-PGA, 2-Phosphoglycerate</td>
<td>8. Phosphoglycerate mutase</td>
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<tr>
<td>2,3 DPG, 2,3 Diphosphoglycerate</td>
<td>9. Diphosphoglycerate mutase</td>
</tr>
<tr>
<td>PEP, Phosphoenolpyruvate</td>
<td>10. Diphosphoglycerate phosphatase</td>
</tr>
<tr>
<td>ADP, Adenosinediphosphate</td>
<td>11. Enolase</td>
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<tr>
<td>ATP, Adenosinetriphosphate</td>
<td>12. Pyruvate kinase</td>
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<tr>
<td>6-PG, 6 phosphogluconate</td>
<td>13. Lactate dehydrogenase</td>
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<tr>
<td>E-4-P, Erythrose-1-phosphate</td>
<td>14. Glucose-6-P dehydrogenase</td>
</tr>
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<td>Pi, Inorganic phosphate</td>
<td>15. Adenosine deaminase</td>
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<td>R-1-P, Ribose-1-phosphate</td>
<td>16. Nucleoside phosphorylase</td>
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<td>R-5-P, Ribose-5-phosphate</td>
<td>17. Ribose-1-P mutase</td>
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<tr>
<td>Ru-5-P, Ribulose-5-phosphate</td>
<td>18. Phosphoribosimutase</td>
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<tr>
<td>Xu-5-P, Xylulose-5-phosphate</td>
<td>19. Ru-5-P epimerase</td>
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<tr>
<td>S-7-P, Sedoheptulose-7-phosphate</td>
<td>20. Transketolase</td>
</tr>
<tr>
<td>Hx, hypoxanthine</td>
<td>21. Transaldolase</td>
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<tr>
<td>NAD+, NADH: oxidized and reduced nicotinamide adenine diphosphopyridinnucleotide.</td>
<td></td>
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<tr>
<td>NADP+, NADPH: oxidized and reduced nicotinamide adenine triphosphopyridinenucleotide.</td>
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ously frozen erythrocytes is of necessity short—about one week. In erythrocytes of ACD blood, even though the enzymes present are more than sufficient to accomodate relatively large quantities of substrates and cofactors, the intracellular intermediates consisting principally of phosphate esters, are very labile. In ACD blood at +4°C, the 2,3 diphosphoglycerate disappears from erythrocytes within two weeks while the ATP diminishes more slowly, about 50 per cent in four weeks.32

Although little data concerning frozen erythrocytes have been published, reconstituted glycerolized erythrocytes frozen at −79°C. show a glycolysis similar to that of fresh cells.33 The intracellular phosphate esters of blood frozen for as long as two years at −80°C. were found to be very similar to those of freshly collected blood,34 and the sodium and potassium fluxes of red cells reconstituted after preservation in glycerol at −80°C. were similar to those of fresh cells collected in heparin or ACD.35 Some potassium leakage occurred during storage at −80°C. Care is necessary during deglycerolization, because substantial potassium losses may ensue when cells are excessively conditioned. However, in the study noted above, potassium losses during post-thaw storage at +4°C. in low potassium media, were less than those observed in ACD blood kept at +4°C. throughout storage. Upon resumption of glycolysis (incubation of thawed cells at 37°C.), an active transport of potassium took place with reconstitution of the potassium content of the cells. Cation flux measurements thus reflect the overall metabolism and are useful indices of the metabolic state and integrity of the membrane of erythrocytes. In a study of glycerol treated red cells frozen rapidly with liquid nitrogen and deglycerolized by washing the cells with sorbitol, Dutch workers demonstrated that metabolic properties, osmotic fragility, and post-transfusion survival were satisfactory.36 These cells were normal or nearly normal with respect to glycolytic capacity, ATP, and plasma potassium concentrations.

**Nucleotides and Frozen Erythrocytes.** The nucleotides, adenosinetriphosphate (ATP) and related mono- and diphosphates (AMP and ADP), function as “energy currency” in erythrocytes, hence they change in composition in response to chemical and physical stresses imposed upon them. Erythrocytes with normal ATP content have a normal or nearly normal life span in the circulation while those with low ATP content survive poorly.37, 38 In addition to the relation to the overall metabolism of erythrocytes, ATP provides energy to support the ionic gradient of cations between the plasma and the cells. Adenosine triphosphatases (ATPases) in erythrocyte membranes cause hydrolysis of ATP and provide the energy required for the ejection of sodium ions from the cells.39 Such “pumps” exercise a profound influence on the biconcave shape of erythrocytes. Cells which cease to participate in metabolism lose their ATP; active transport of ions then ceases, water enters and the cells become spherical and hemolyze.

In frozen blood, high energy phosphate is a good indicator of the shape distortion and membrane damage imposed on erythrocytes in the various stages. Low temperature storage causes a decrease in intracellular and membrane-bound ATP.40 On the other hand, the acid stable phosphate (2,3 diphosphoglycerate principally) and other nucleotides are little changed in comparison to fresh cells (when the pH and inorganic phosphate content are comparable).

To elucidate the cellular mechanisms responsible for preservation of ATP,40 adenine nucleotides were studied in post-thawed erythrocytes previously collected in ACD, frozen in glycerol and deglycerolized by means of either the Cohn-ADL fractionator (centrifugation) or with the Huggins cytovagglomaterator (agglomeration). One of us (F.J.L.) found that the major losses of nucleotides took place during deglycerolization. With either method the nucleotide composition was not changed appreciably during glycerolization and slow freezing. Cells which underwent extensive losses of ATP during deglycerolization also lost substantial quantities of hemoglobin and exhibited varying degrees of hemolysis. The nucleotides of post-thawed deglycerolized erythrocytes were in several respects similar to those of ACD blood maintained at +4°C. Both ATP and total nucleotides (the sum
ATP + ADP + AMP) decayed in storage at the same rate over a three-week interval. Only small compensating increases in ADP or AMP were observed. When erythrocytes were deglycerolized optimally by either centrifugation or agglomeration, the same relation was observed; namely, a parallel rate of decay was found for ATP and total nucleotides with slopes the same for both, as in unfrozen ACD whole blood. The nucleotide composition of agglomerated erythrocytes was markedly dependent upon the conditions under which glycerol was removed, in particular the extent of crenation of the cells and the time they were allowed to remain in hypertonic glucose.

Manipulation of the conditions for agglomeration is an expedient method to seek an explanation for ATP losses when cells are excessively crenated. With the agglomeration technique, erythrocytes normally are sedimented in large volumes (approximately 8 liters of 8 per cent glucose and 1 per cent fructose containing EDTA). In this medium of low ionic strength, erythrocytes sediment rapidly and the supernatant solution is displaced, carrying glycerol with it. Under optimal conditions (rapid removal of hypertonic glucose, and subsequent large volume dilution), the erythrocytes exhibit good survival characteristics. However, if cells deglycerolized by agglomeration are subsequently left in glucose for 20 to 30 minutes, they lose water, shrink excessively, and may accumulate intracellularly as much as ten times more glucose than normal (1 g./100 ml. whole blood). If such cells are not permitted to swell to normal size by means of dilution in buffers, they become extremely unstable, undergo extensive hemolysis, and may lose more than 50 per cent of their nucleotides and ATP. When EDTA is omitted from the wash solutions, ATP is also much more labile in the post-thaw state. If the cells are deliberately crenated excessively in the deglycerolization step, large quantities of ATP are broken down in the absence of EDTA, and free hypoxanthine is liberated as a breakdown product. These observations suggest that crenation of the cells causes intracellular ATP to encounter the erythrocyte membrane. With the membrane adenine deaminases, apyrases and adenine-triphosphatases are present. Excessive changes in cell dimensions as the result of exposure to hypertonic (or hypotonic) solutions may permit transport of intracellular ATP through channels thereby opened in the erythrocytic membrane. The "activated" surface (principally enzymes) would then cause irreversible decomposition of ATP through losses of phosphates and ammonia. It is of interest that EDTA which is known to form complexes of divalent cations, and particularly the magnesium required to activate adenine-triphosphatases and deaminases, protects erythrocytic ATP from much of this breakdown. Figure 2 represents our concept of the enzymatic
changes which are presumed to take place in the membrane. These enzymes may be thought to be insulated normally from the intracellular ATP, because comparatively little ATP breakdown occurs intracellularly. Although normal nucleotide composition is not the sole factor on which good survival characteristics of erythrocytes depends, conditions for deglyceralization should be sought by which nucleotides are maintained just as well in the post-thaw state at +4° C. as in unfrozen blood.

Clinical Indications for Use of Frozen Blood

No matter what the technique of freezing used, the clinical experiences to date clearly suggest the areas in which frozen blood is likely to achieve principal use.

(1) Rare Blood Banking. Under the joint sponsorship of Protein Foundation, Boston, the U. S. Naval Hospital, Chelsea, Massachusetts, and the American Association of Blood Banks, a program of rare blood banking was begun in 1961. Already this has made possible the long-term preservation of over 300 rare blood units. The types which have been stored include "Bombay," Kp*-negative, U-negative, Lu*-negative, K-negative, Le-negative, JK*-negative, Js*-negative, Vel-negative, Y*, I-negative, Ge-negative, D/D+, and --/-. Thus far, 75 of these units have been deglyceralized and furnished to approximately 45 recipients who were undergoing surgery, parturition or trauma sufficient to require transfusion but for whom satisfactory donor cross-match could not be found in local panels. The unique stability of mechanically deglyceralized cells for subsequent short-term storage at −20° C. or +4° C. makes it possible to ship the cells to diverse hospitals throughout the world from the central processing laboratory, without maintaining the −80° C. temperature in which the cells have been stored.

(2) Autologous Blood Banking. A significant amount of blood administered under ordinary conditions of liquid storage is for elective surgery, obstetrics, preoperative and postoperative anemia, and medical disorders accompanied by a reduced red cell mass. Estimates on the frequency of such non-emergent blood use run as high as 40 per cent. Thus, in the United States it appears likely that over 1 million transfusions each year are planned far enough in advance so that frozen blood could easily be processed in time to meet the clinical need. If each adolescent were to store annually one pint of frozen blood during his young, healthy years, the great majority of nonemergent transfusion needs could be fulfilled by autologous transfusion in later life. The benefits of such a program are patent. In addition to assuring an adequate supply of blood, all recipient reactions would be wholly abolished. There would be no iso-sensitization, serum hepatitis, or other transfusion disseminated disease. The patient would receive only those diseases, immune globulins, haptoglobulins, hemoglobin and other group-specific materials which already were uniquely his. The feasibility of autologous transfusion was demonstrated as early as 1962 when a patient of unusual blood type underwent cardiac surgery utilizing entirely his own blood for the pumprime and subsequent transfusions. Large-scale application of this important advance awaits only the morw widespread availability of the processing equipment.

(3) Pump-Oxyenator Surgery. Extensive logistic requirements are necessary to supply fresh donors for use in extracorporeal surgery. Cross-matching and typing must be done in advance. The phlebotomies must begin early on the morning of operation. Then, after all preparations are complete, the operation may sometimes be cancelled. Present methods of deglyceralization are insufficiently rapid so that the entire process from deep freeze locker to patient can be accomplished within one hour. This is about as rapid as cross-matching can be completed. Moreover, the latter can proceed simultaneously with samples taken from the plastic pilot tube of the blood bag. A sufficiently large frozen blood bank with

§ Processing was carried out by Commander Mary T. Sproul, U. S. Navy (Ret.). Current address: Cytology Laboratories, Protein Foundation, 110 Francis Street, Boston, Massachusetts 02215.

proper technical help would be able to keep pace with the blood needs of an active cardiopulmonary team. The capacity of thawed cells to transport oxygen has already been shown. Indeed, deglycerolized cells after long-term storage are able to give up oxygen to the tissues as readily as freshly collected ACD blood and without the shift to the left in oxyhemoglobin dissociation reported by Valtis and Kennedy noted after a week of ordinary blood storage at +4° C.

(4) Nonhemolytic Transfusion Reactions. Red cells processed through mechanical systems can be electively resuspended in their original plasma, if whole blood transfusions are desired, or in albumin or simple saline solution if only the red cell mass is required. During the early transfusion studies with cells derived from the Cohn fractionator, it was observed that pyrogenic and urticarial transfusion reactions did not occur. Accordingly, patients were sought who previously had undergone multiple transfusion. Deglycerolized cells suspended in albumin did not give rise to reactions in these patients despite a history of previous chills and malaise after standard ACD whole blood. A double-blind study was then carried out in which patients in 3 hospitals of the Boston area were observed for incidence of nonhemolytic transfusion reactions. Subsequent blood needs of proven reactors were then met during the same hospital admission by random allocation from group-specific pools containing coded units of standard ACD blood and thawed deglycerolized cells in albumin solution. Neither the transfusionists nor the recipients were aware of the source of the unit administered. Sixty-seven units of deglycerolized red cells in albumin were administered to 12 recipients without pyrogenic reactions. Seventy-six units of standard ACD blood given to the same group of 12 recipients produced 37 pyrogenic reactions.

Transfusions to Recipients with Deranged Metabolism. The extracellular phase of blood during ordinary (liquid) storage contains an excess of free potassium. At standard refrigerator temperatures, there is insufficient metabolic activity to maintain the high intracellular gradient, with a resulting leakage of K⁺ leakage into the surrounding medium. This averages 12 mEq./liter after a week of storage, 17 mEq./liter after two weeks and more than 20 mEq./liter after three weeks at +4° C. When the cells are rewarmed to body temperature, most of this potassium is pumped back inside the cells; but the extracellular concentration is still sufficient to constitute a hazard to patients. This is especially important in uremia, renal shutdown, extensive burns, and other conditions in which the degree of hyperkalemia may be so severe that it precludes much needed transfusion therapy. Unpublished data of Haynes show that if red cells are washed centrifugally in potassium-free media at the time of freezing, and subsequently resuspended in a low potassium medium at the time of reconstitution, it is possible to create a "potassium sponge." Rapid transfusion of four consecutive units of such cells into a uremic patient with an elevated potassium not only failed to elevate the serum K⁺ further but also resulted in a fall from 7.82 to 6.82 mEq./liter and concurrent electrocardiographic improvement. Owing to the anemia which constitutes such a common complication of renal disease, this observation should be able to be exploited in the future.

The excess load of citrate in ACD blood has been postulated to constitute a severe metabolic load in patients with acidosis, or in the multiply-transfused surgical patient undergoing prolonged anesthesia or suffering from anoxia. Each pint of ordinary ACD blood contains an excess of 27 millimoles of fixed acid in the form of citric acid. This ordinarily is metabolized in the liver hence does not constitute a hazard of circulatory anticoagulant effect through chelation of calcium. However, when multiple units are transfused in the presence of circulatory stasis or diminished hepatic function, hypokalemia and metabolic acidosis can supervene. Deglycerolized cells when resuspended in albumin or saline are transfused free of anticoagulant, hence do not constitute an added metabolic load in the presence of pre-existing acidosis.

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**Haynes, L. L., Capt. U. S. Navy (Ret.). Present address: Lahey Clinic Foundation, Boston, Massachusetts.**
Miscellaneous Uses of Deglycerolized Cells.

CARDIAC DECOMPENSATION: Several years ago, Goodale\textsuperscript{42} studied the pulmonary vasoconstrictive effects of citrated plasma and postulated that the cardiac decompensation which sometimes follows the transfusion of blood in anemic cardiac patients is the result of vasopressor agents in the plasma rather than to the volume of material infused. Studies were instituted recently to test the effect of rapid transfusion of deglycerolized cells resuspended in albumin, on circulatory dynamics of the recipients. Preliminary findings suggested that Goodale's original hypothesis was indeed correct and that the volume of material infused into a decompensated patient is less important than the nature of the material infused.\textsuperscript{42}

HOMOLOGOUS SERUM HEPATITIS. As yet, no valid data exist to show that the incidence of serum hepatitis is reduced in recipients of red cells that have been subjected to the large wash volumes of deglycerolization. However, it was noted early in work with frozen cells processed in the Cohn fractionator that no known cases of icteric hepatitis occurred in 2,000 recipients of washed cells resuspended in albumin, whereas, two cases occurred in the first 300 recipients of similarly processed cells resuspended in their original plasma. Attempts to subject this observation to statistical evaluation were at first impractical because of the very large pool of carefully followed recipients needed. However, more recently, separate investigators\textsuperscript{44,45} have shown that the incidence of anticteric serum hepatitis may be as much as a hundred-fold greater than the icteric variety. This has made possible a current double-blind study which is designed to compare plasma enzyme changes, chiefly SGPT and SGOT, in recipients of the two differently processed kinds of cells.

No preliminary conclusions can be drawn from this study. However, if the incidence of hepatitis in the deglycerolized, albumin resuspended cells is significantly reduced, it may well indicate the eventual desirability of "washing" even fresh cells and administering them in some type of hepatitis-free medium such as albumin.

Conclusions

It is too early to anticipate fully the impact of freezing techniques on blood banking practices; but it would appear reasonable to predict they may be of wider therapeutic significance than any single contribution since the institution of blood grouping.

References


