ANALYTICAL REVIEW

The Preservation of Blood for Transfusion

By MAX M. STRUMIA

THE PURPOSE of this review is to present and discuss the major contributions to the solution of the problem of blood preservation, from 1947 to 1953. Primary consideration will be given to the problem of storage and preservation of red blood cells but, in addition, the essential steps in the progress of methodology of platelet collection, storage and transfusion will be mentioned briefly.

A review of progress made in blood transfusion reveals that the introduction of the use of sodium citrate solution as anticoagulant in 1914,1 the addition of dextrose to the citrate solution in 1916,2 and of the acidification of the citrate-dextrose anticoagulant in 19433 represent important steps in the improvement of erythrocyte preservation. In each instance the improvement is attributable to a better maintenance of the energy-producing mechanism and, consequently, of the integrity of the cell as a metabolic unit. This mechanism can be identified mainly with anaerobic glycolysis. When blood is stored in plain isotonic sodium citrate, dextrose reaches critically low levels by the fifth day and, at the same time, 2-3 diphosphoglycerate and adenosine triphosphate (ATP) fall rapidly.4 Erythrocytes, transfused after five days of storage in plain citrate, disappear rapidly from the blood stream.5

When the use of dextrose was reintroduced by DeGowin in 1940,6 a great improvement in viability of stored red cells was obtained: with the addition of dextrose the fall of diphosphoglycerate and ATP is slower, and blood can be transfused with a satisfactory rate of survival after storage up to about ten days.

In acidified citrate dextrose solutions, particularly with acid-citrate-dextrose (ACD), ATP is found to be much more stable, the utilization of dextrose occurs for a longer period of time and the useful storage of red cells is increased to about three weeks. The relation between cell deterioration and glycolysis has been extensively investigated by Rapoport.7

By 1947, ACD solution had generally become adopted as the standard anticoagulant and preservative. Several formulae have been proposed which offer variations in the volume of water and in the dextrose content. These two factors and the temperature control have often been arbitrarily chosen.

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PRESERVATION OF BLOOD FOR TRANSFUSION

As a consequence of reports by a number of investigators* it became a generally accepted fact that with proper technique the erythrocytes of blood collected in ACD and stored continuously at temperatures between 4 and 10 C. would be satisfactory for transfusion for as long as twenty one days. This arbitrary limit of blood storage for clinical purposes is based on another likewise arbitrary qualification: stored blood is considered satisfactory for transfusion if 70 per cent or more of the transfused cells remain in the circulation of a normal recipient for at least 48 hours. It is worth considering what these standards actually mean.

When freshly collected ACD blood is transfused, an average of 5 per cent of the cells are lost during the first 48 hours post-transfusion. The Ashby survival studies indicate that the disappearance time averages 115 days. On the basis of the customary 120 day optimal average life span, and using as the unit of measure of survival: red cell mass/time, the survival value of transfused fresh blood is about 90 per cent of the optimal.

The erythrocytes of blood collected in ACD solution and maintained at 4 to 7 C. for twenty one days, disappear from the circulation within a period of 100 days or less after transfusion. On the basis of the same standards noted above, and again using as measure of red cell survival: red cell mass/time, the value of transfused ACD blood stored for twenty one days, and which has reached the lowest limit of tolerance mentioned, amounts to about only 60 per cent of the total possible red cell survival.†

In reviewing the literature on the subject, one notes that rather than attempting to achieve a higher and more constant rate of survival within the storage period of three weeks, most efforts have been directed toward extending the period of storage, on the basis of the standards referred to above, beyond the twenty one day period. These attempts to prolong the period of useful storage with such rather low standards stem, in part at least, from the ideal of war-time transfusion service; that is, to establish a stock pile of blood for an indefinite period of time. In establishing this goal, consideration is undoubtedly given to the fact that for the treatment of a wounded patient in shock from loss of blood, and possibly in need of preparation for an emergency surgical operation, there is less concern for the survival of erythrocytes beyond a few days, than for the immediate availability of whole blood in quantity.

Such is not the case in civilian practice, where more than half the patients requiring transfusion of whole blood or packed red cells have depressed erythropoietic activity, and are often in need of repeated blood transfusions. Erythrocytes for transfusion into this type of patient must have as long a post-trans-

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* Practically the entire July 1947 number (Vol. XXVI) of the Journal of Clinical Investigation was devoted to a series of articles on the basic principles underlying the in vitro storage of red blood cells and their post-transfusion survival. The reader is referred to these articles, some of which will be individually cited, for a comprehensive view of much of the work on blood preservation done up to that time.

† These data are based partly on Mollison's work and partly on the work by several other groups of workers, including J. F. Ross, et al., J. Clin. Investigation 26: 687, 1947; J. G. Gibson, 2nd, et al., J. Clin. Investigation 26: 704, 715, 1947. Data from recent personal communication with C. A. Finch and J. G. Gibson, 2nd, et al., as well as work by M. M. Strumia and coworkers, have been taken into consideration in arriving at the estimates given in the text.
fusion survival as possible, to reduce the number of transfusions required and therefore minimize the risk of transmission of homologous serum hepatitis and of iron overload. For these patients there is a substantial difference between fresh or well-stored blood and blood indifferently stored for two to three weeks or more.

It may be fairly stated that in the period from 1947 to 1953 there has been no practical and substantial contribution to the problem of longer storage of blood save for the promising although still highly experimental and impractical storage of erythrocytes in the frozen state in a glycerol solution. There have been, however, contributions to the basic knowledge of the physiology of erythrocytes as well as several improvements in details of technic all intended to assure better red cell preservation for periods up to about four weeks. This period is considered as satisfactory for the proper functioning of any blood bank, except possibly those handling less than 1000 to 1500 units of blood yearly.

CONTAINERS

Since the introduction of silicone⁶,¹⁰ and arquad, plastic containers and tubing¹¹ have come into use to obtain nonwettable surfaces in the entire pathway of blood from donor to container to patient.

The use of nonwettable surfaces was readily and successfully adopted for optimal platelet survival.¹²⁻¹⁴ However, the use of plastic containers in the preservation of erythrocytes needs some comments. The obvious advantages of plastic containers are their lightness of weight, the fact that they are not breakable, and that they permit a closed gravity technic for collection and rapid administration by external pressure. These are essentially logistic advantages. Concerning the preservation of erythrocytes in plastic containers, variable results have been reported by different observers.

Sack, et al.,¹⁵ using the Fenwal plastic container, obtained results equal in every respect to those obtained with the standard glass containers. Likewise, Muether, et al.¹⁷ and Knutson¹⁸ obtained satisfactory results with the use of plastic bags manufactured by Fenwal, Abbott† and Cutter.‡ Wall, et al.,¹⁹ however, found minor in vitro differences between samples of blood stored in the plastic containers and those stored in the standard glass containers. Wall, et al.²⁰ also found that erythrocytes stored as whole blood in glass containers for fourteen, twenty-one, and twenty-four days exhibited better survival than blood stored in plastic containers. It is to be noted that in this study plastic bags and glass bottles contained different ACD formulas, NIH formulas A and B respectively. The survival studies were carried out with the nonagglutinated cell count technic (Ashby).

Results of studies made by our group over a period of about two years, using over one hundred bags prepared by the Abbott Laboratories and containing several types of ACD solutions,²¹ offer a possible explanation of the variations in the results noted by different observers. During the period from August 1951 to June 1952, the use of plastic bags for storage of red cells as whole blood or in various degrees of packing gave us good results, being equal in every way to those obtained with the conventional glass bottles containing a similar anticoagulant. But in the period between September 1952 and May 1953, with all conditions of technic remaining unchanged and with the same technical assistants, the results with plastic bags could not be duplicated. The experimental work reported by Wall, et al. was carried out at about the same period of time.

It is of interest to note that Wall received the supply of plastic containers used in his

† Abbott Laboratories, North Chicago, Ill.
‡ Cutter Laboratories, Berkeley, Calif.
§ Formula A: tri-sodium citrate 1.65 Gm.; citric acid 0.60; dextrose 1.84; distilled water to 75 ml. formula B; tri-sodium citrate 1.71 Gm.; citric acid 0.62; dextrose 2.0; distilled water to 130 ml.
experimental work in July 1952 from the Abbott Laboratories. Very likely these containers were of the same type as those received and used by us during the same period of time and found to give poor results.* We have also had some experience with the plastic containers made by the Fenwal Laboratories and have found them uniformly satisfactory for the usual storage period.

At best, it appears that plastic containers are not superior to conventional glass bottles, and that nonwettable surfaces offer no advantage insofar as preservation of erythrocytes is concerned. The great logistic advantages, however, fully justify continued effort to standardize plastic material for the manufacturing of containers for biologic purposes.

Nonwettable surfaces offer definite advantages in the preparation of platelets for transfusion.

**ION EXCHANGE RESINS**

Replacement of Ca**++** by Na**+** to prevent coagulation, in lieu of citration, was first suggested by Steinberg.24 He employed a resinous cation exchanger treated with sodium. Quick,25 Stefanini, et al.26 and deNicola and Rosti27 achieved similar results using Amberlite, a modified phenol-formaldehyde sulfonic acid type resin.

Walter28 introduced a plastic system made of polyvinyl resin in which is incorporated an ion exchange column containing Dowex 50, a sulfonated aromatic hydrocarbon polymer. Buckley, et al.,29 Buckley and Gibson,30 and Gibson31 studied the properties of Dowex 50-treated blood in vitro and its post-transfusion survival. They found this survival to be similar to that of the erythrocytes of ACD-collected blood.

Buckley32 studied the effect on erythrocytes of Dowex 50, of IRC-50, the latter a cation exchange resin in which the activity is due to the carboxylic group exclusively, and or Permutit Q, a sulfonated hydro-carbon polymer. He found that Dowex 50 was the best, transfused cells so treated surviving as well as those of ACD blood.

It is to be noted that in this study, as in the works previously referred to,28-30 the resin-collected blood was mixed with 75 ml. of ACD (NIH formula A) to obtain a suitable pH and additional dextrose.

Our experience with the ion exchange resins (Dowex 50 and Amberlite) is similar to that reported by Gibson and Buckley.

It may be stated in conclusion that post-transfusion survival of erythrocytes of blood rendered incoagulable by the use of ion exchange resins treated with Na**+** is at best comparable with that of erythrocytes of blood stored in ACD solution. The greater cost makes the use of ion exchange resin columns, as they are at present available, impractical. The possible elution of viable platelets from the ion exchange column, with the help of EDTA,32 does not appear to offset other disadvantages of this method of blood decalcification.

* Abbott Laboratories, manufacturers of the plastic containers, are not aware of any change in the composition of the plastic at any time during the 1951-53 period. They report, however, that a special detergent solution rinse was employed to wash the plastic tubing used to manufacture the bags in 1950 and 1951, but omitted later.23 Many variables may give substantial changes in the final effect of the plastic containers on blood cells. The first one is the nature of the plastic itself; the second is the nature of the material used in the extrusion; and thirdly, the choice of the plasticizer. Other factors are the washing of the plastic material and the sterilization of the bags with the contained anticoagulant.
ETHYLENE DIAMINE TETRAACETIC ACID (EDTA)

Chelating compounds, long known to industry, were introduced to bind Ca$^{++}$ and act as blood anticoagulants for blood transfusion by Proescher.$^{33}$ Dykeroff,$^{34}$ who first discovered the anticoagulant properties of this compound, states that it is 10 times more effective than citrate. Proescher used the disodium salt of ethylene diamine tetraacetic acid,* which is readily soluble in water, and found that human blood could be preserved in an 0.3 per cent solution containing 1 per cent glucose. The solution has a pH of 5.0. Blood, thus preserved, was transfused without reactions.

Chelation of Ca$^{++}$ occurs much in the same manner as with citrate; but in addition EDTA Na$_2$ inhibits the second stage of conversion of fibrinogen to fibrin$^{35}$ and also causes inhibition of some enzyme systems.$^{36}$ Dillow, et al.$^{37}$ used EDTA Na$_2$ alone for the preparation of human, dog, and guinea pig platelets. Good therapeutic effects were obtained in irradiated thrombocytopenic dogs. Stefanini, et al.$^{38}$ used EDTA Na$_2$ in combination with Tween 80 or Triton W-1399 for preparation of blood platelets for use in humans. Sprague, et al.$^{39}$ used 1.5 Gm. of EDTA Na$_2$ in 100 ml. of 5 per cent dextrose solution for preservation of 500 ml. of blood. They found good post-transfusion survival of the erythrocytes up to twenty eight days of storage at 4 to 6 C. These authors considered the EDTA Na$_2$ solution to be as good as ACD for preservation of blood.

In studies conducted by our group$^{40}$ it was found that EDTA Na$_2$ concentrations from 300 to 2100 mg./500 ml. of blood were satisfactory to maintain blood uncoagulated for at least twenty eight days. Addition of dextrose is essential for proper preservation of erythrocytes, but the optimal amount was found to be from 750 to 1500 mg./500 ml. of blood for storage at temperatures from 1 to 4 C. When less than 1200 mg. of EDTA Na$_2$/500 ml. of blood are used, citric acid must be added to obtain a pH of 7.0 to 7.1 after collection of blood.

As yet not too much is known of the toxicity of EDTA Na$_2$ in man. Dogs receiving 150, 250, and 500 mg./Kg. twice daily showed 50 per cent mortality at 40, 18.5, and 13 days respectively.$^{41}$ These doses appear to be far in excess of any likely to be needed for transfusion purposes in man. Slow infusion of EDTA Na$_2$ at the rate of 4 Gm./day for one to six days produces increased calciuria, but no severe serum calcium reduction, and no toxic manifestations have been noted.

Rubin$^{42}$ states that the acute toxic manifestations following the use of EDTA Na$_2$ are due to hypocalcemia, and are strictly time dependent. Slow infusion permits calcium to be removed from the skeletal reserves, thus maintaining an adequate level in the blood. The following dosage limits for intravenous use in the humans are recommended by this investigator: not over 0.5 Gm./30 lbs./hour; not over 1 Gm./30 lbs./24 hours; the maximal dose per week should not exceed five Gm./30 lbs. in divided doses; 7.5 Gm. is the maximum per 30 lbs. over a ten day period.

Spencer, et al.$^{43}$ found that slow intravenous administration of 4 Gm. of EDTA Na$_2$ daily for six consecutive days to patients produced calciuria, without

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* EDTA Na$_2$ is commercially available under the trade names of Sequestrene (Alrose Chemical Company, Providence, R. I.) and Versene (Bersworth Chemical Company, Framingham, Mass.).
lowering the serum calcium level, and without producing any other signs of un-
toward reaction.

The importance of slow administration to avoid toxic effects from this com-
pound is further emphasized by the studies of Gehres and Raymond44: 3 to 5 mg.
of EDTA Na₂ administered intraperitoneally in 20 Gm. mice caused death in
ten minutes when given as a single dose. The same amount administered in ten
doses at fifteen minute intervals caused no toxic effect.

So far as I am aware, only single transfusions of whole blood preserved with
EDTA Na₂ have been given to man.

It may be stated in conclusion that from evidence of studies in vitro and in
vivo, EDTA Na₂ appears to be the choice for the preparation of platelets for
transfusion.

For the preservation of erythrocytes, while EDTA Na₂ appears to be as good
as ACD solution, it offers no advantage. Studies in humans receiving multiple
rapid transfusions are needed.

**Antienzymatic Compounds**

Schales⁴ investigated a variety of inhibitors of lipases, proteases, and pepti-
dases to determine if their use is capable of retarding the deterioration of stored
erthrocytes. Osmotic fragility was used as a test of cell deterioration. The most
promising results so far have been obtained with 10-(2-dimethyl-amino-isopropyl)
phenothiazine, which was first used as an antihistaminic agent. This
compound* was found by Maral, et al.⁴⁶ to have an inhibitory effect on the
action of trypsin and pepsin.

It must be emphasized that as yet Phenergan treated blood has not been tested
in vivo, and that osmotic resistance studies are hardly sufficient to justify any
conclusion.

**Disaccharides**

The addition to whole blood or resuspended erythrocytes of nonelectrolyte,
nondiffusible compounds produces a shrinkage of the red cells and diminution
of the osmotic fragility of these cells. Disaccharides have been used with particu-
larly constant results. Lactose, although producing in the same concentration less
shrinkage of the red cells than sucrose, provides a better preservation. With either
sucrose or lactose it is necessary to add glucose for prolonged storage of red cells
since the disaccharides are probably not metabolized. Red cells have been pre-
served with lactose in a concentration of 3 to 7 per cent for periods of over three
months without deterioration, as determined by hemolysis or changes in the
osmotic fragility.⁴⁷ This method of preservation has been found very useful for
maintenance of red cell samples for determination studies of blood groups and
types over long periods of time.

The removal of plasma from blood in ACD solution and replacement with a
solution of 4 per cent globin prolongs the in vitro survival of erythrocytes to about
four months. Cohen and Pondman,⁴⁸ using 4 per cent globin solution in a 5 per
cent lactose solution, preserved the antigenic structure of red cells for a period of

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* Available commercially under the name of Phenergan, Wyeth Chemical Laboratories, Philadelphia, Pa.
four months. These authors used this method of storage to maintain a blood group panel.

In vivo survival of cells preserved with the aid of lactose, with and without globin, has given very variable results. Some of the units of red cells resuspended in a globin-lactose solution survived well when transfused in vivo. Others did very poorly. Factors responsible for these variations are not clear, but this method for preservation of red cells for the purpose of transfusion is not considered practical. It is, however, recommended for the preservation of red cells for in vitro studies, such as reference cells for grouping, etc.

**Temperatures Below Freezing**

It has been generally accepted that freezing or solidification of erythrocytes at temperatures below 0 C., followed by thawing, results in hemolysis. However, hemolysis of red cells under these conditions has been avoided in a variety of ways. Luyet and his coworkers49, 50 have pioneered in the study of ultra-rapid freezing of living cells. Death of living cells in the process of freezing and thawing is attributed to the intracellular formation of ice crystals. Cooling at rates of several hundred degrees Centigrade per second permits the vitrification of water without formation of ice crystals. The dangerous zone in freezing, or range of crystallization temperatures, is considered by Luyet as extending from the freezing point of the solution to some tens of degrees below it. Crystallization must also be avoided in the process of thawing. Rewarming, therefore, through the range of crystallization temperature, must be as rapid as the cooling through the same range. With the use of liquid nitrogen Luyet succeeded in freezing and thawing oxalated ox blood in minute quantities51 with preservation of the morphologic integrity of 72 per cent of the cells.

Polge, Smith, and Parkes first reported the effect of glycerol on freezing of spermatozoa at temperatures around -79 C. Equal parts of 40 per cent glycerol solution in Ringer's and fowl semen were used in this experiment. Smith52 applied the technic of freezing at -79 C. with the aid of glycerol to the erythrocytes of guinea pig, rabbit, and man. Heparinized rabbit blood, diluted with equal quantities of mixtures of glycerol in Ringer's solution or glycerol in saline, was employed with final concentrations of glycerol of 10 to 15 per cent. With rapid freezing and storage at -79 C., followed by thawing at +40 C., good morphologic preservation of erythrocytes was obtained for periods of up to three months. In a single experiment with human red cells, similar results were obtained. An ingenious device by Smith and coworkers54 enabled the observers to study and record photographically crystal formations in blood cooled to -79 C., with and without glycerol, and its effect on the living cells. Sloviter55 succeeded in removing glycerol from the red blood cells by dialysis. This procedure is essential for the utilization of glycerol-treated cells for transfusion purposes because glycerol penetrates the red blood cells and these hemolyze rapidly when placed in plasma or any other isotonic medium, not containing glycerol.

Using this method of glycerol removal Sloviter56 succeeded in transfusing rabbit red blood cells frozen in the presence of glycerol and stored at -79 C. with survival of some of the transfused cells for at least a few days.

Mollison and Sloviter57 demonstrated that storage of human red cells treated
with glycerol at \(-79\) C. for 2 or 3 hours has no appreciable effect upon their post-transfusion survival. The same authors later demonstrated that human erythrocytes, stored in a 15 per cent solution of glycerol at \(-79\) C. for six months, were capable of normal survival after transfusion.

In these experiments, dialysis of glycerol-treated blood was carried out with cellulose sausage casing. After dialysis, the red cells are washed three times with saline solution. This operation eliminates most damaged cells, and the loss of red cells during this operation, when the blood has been stored for five months, amounts to 40 to 50 per cent of the blood originally frozen. Of the cells which escape destruction by hemolysis, during the process of thawing and dialysis, 10 to 30 per cent disappear from circulation 48 hours after transfusion. In these figures allowance is made for an additional eleven days of storage at \(+4\) C. following the dialysis of the blood. In determining the survival of transfused cells preserved by freezing with glycerol, Mollison and Sloviter\(^5\) employ contemporaneous transfusion of \(O\) cells from blood collected in ACD solution and kept only a few hours, and of frozen \(AN\) cells to \(AM\) recipients. The results are expressed as a ratio, the 100 per cent value being obtained thus:

\[
\frac{\text{Test cells (frozen AN cells) ml. transfused} \times \text{RBC count millions/cu. mm.}}{\text{Control cells (fresh O cells) transfused} \times \text{RBC count millions/cu mm.}} = 100 \text{ per cent.}
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The assumption that the control fresh \(O\) cells survive at a rate of 100 per cent is not entirely tenable. The method has the advantage of testing certain variables in the recipient such as changes in the plasma volume. The advantages appear to be too small to justify this cumbersome method. At any rate it may be stated that at 48 hours post-transfusion the number of cells remaining amounts to 35 to 45 per cent of the total blood frozen and stored for five months.

Lovelock\(^5\) suggested the use of concentrated sodium citrate solution for the removal of glycerol, in place of dialysis. The removal of glycerol here is due to increased external osmotic pressure, and to obtain this, enough sodium citrate is used to raise the concentration to 0.33 M. After 5 minutes the cells are separated by centrifugation, and may be resuspended directly in saline solution or in plasma. As yet, the effect of this method of removal of glycerol has not been studied by in vivo survival studies.

Originally it was supposed that the mechanism of the effect of glycerol lay in the modification of the character of crystallization of the medium and of the internal cell environment. It is evident, however, from the study of freezing of large quantities of blood, that this explanation does not apply. Parkes\(^6\) now considers that the presence of glycerol alone does not prevent or essentially modify the formation of crystals. During the relatively slow freezing of large quantities of blood, water is progressively removed from the medium so that the residual solution becomes progressively more hypertonic. In addition, glycerol in the concentration used is in itself a strong dehydrating agent. Dehydration of the red cells therefore occurs, and in the method used by Mollison and Sloviter dehydration may be the effective mechanism in protecting the cells against low temperatures. However, it may also be the cause of progressive deterioration of
the red cells frozen at low temperature with the aid of glycerol. Red cells undoubtedly would be subjected to increasingly higher concentrations of glycerol from progressive freezing out of water. This process affects the red cells, reducing progressively the post-transfusion survival of frozen, transfused cells. If this assumption is correct, there is little likelihood of storage of frozen red cells in glycerol at low temperatures for undetermined periods of time.

In conclusion, this method of red cell preservation offers as yet no practical solution to the problem of prolonged storage without considerable loss. It offers, however, a challenge for continued investigative work.

Hemolysis due to freezing because of low temperature of storage may be prevented by maintaining the blood under considerably increased pressure, thus lowering the freezing point. Walter suggested this method of storing blood in the liquid state at temperatures below 0 C.64 Laurant, et al.65 likewise studied the effect of pressures of 680 to 1530 atmospheres on blood in the liquid state at temperatures between +4 C. and -13 C. This procedure was accompanied by considerable hemolysis, and the observers do not consider this a promising procedure for blood preservation. The same investigators used ethanol in a final concentration of 21 per cent with storage of blood in the liquid state at -12 C. Under these conditions immediate hemolysis is avoided, and at four months of storage the hemolysis was found to be equal or slightly less than the hemolysis of ACD blood maintained at 4 C. without ethanol.

Strumia and coworkers,63 however, found that red cells preserved with the aid of ethanol, either in the liquid or frozen state and at temperatures from -3 to -17 C., undergo severe degenerative changes, so that although no hemolysis occurs, evidence from osmotic fragility studies indicates that the cells are profoundly altered, and surely unfit for transfusion.

Although the freezing point of blood collected in ACD solution is approximately -0.455 C., actually blood may be super-cooled to -3 C. and maintained indefinitely at this temperature without freezing. However, blood may be obtained in the frozen state at -3 C. by cooling it to this temperature and then touching the side of the container with CO2 ice, to initiate crystallization. This method was used to study the effect of temperatures of -3 C. on blood maintained in the liquid and in the solid state.64 Under these conditions it was found that whole ACD blood maintained at -3 C. in the liquid state showed less deterioration than similar blood maintained at -3 C. in the frozen state. The reason for this difference in behavior is probably to be found in the observation of Luyet.65 Luyet points out that when the temperature is lowered to the freezing point, a fraction of the water of the plasma crystallizes so that the remaining solution becomes more concentrated and its freezing point is lowered. Blood cells thus find themselves in a hypertonic solution, lose water by osmosis, and therefore become more resistant to freezing. Under the experimental conditions one would not expect ice to form on the blood cells at -3 C. The study of red cell behavior at temperatures just below freezing had one distinct advantage: namely, that of removing the fear of erythrocyte destruction by temperatures approximating 0 C. or slightly below. Removal of this fear suggested studies of red cell preservation at temperatures just above freezing in the range of +1 C.
TEMPERATURES JUST ABOVE FREEZING AND LOW DEXTROSE CONCENTRATION

The essential factors in the preservation of erythrocytes outside of the body appear to be the reduction of metabolic activities of the cells to a minimum. This is based on the reasonable assumption that enzyme complexes responsible for maintenance of the normal metabolic activities of the red cells are probably depleted as time progresses. Ideally, complete suppression of enzymatic activity would afford best means for red cell preservation. This temperature is probably somewhere below \(-15\,\text{C}\). It has been already shown, however, that the maintenance of red cells at temperatures substantially below zero is not a practical method of red cell preservation. This consideration led Strumia and coworkers to investigate the temperature immediately above freezing for the preservation of blood. Parpart, et al.\(^6\) established the optimal temperature range for preservation of erythrocytes at between 4 and 9 C., with an optimal at about 7 C. \(\pm 1\,\text{C}\). Gibson, et al.\(^7\) confirmed this range of temperature by in vivo survival studies. However, studies on whole blood have shown that between \(+7\,\text{C.}\) and \(+1\,\text{C.}\) glycolysis is reduced to about half. It would appear from this observation alone that it is desirable to reduce the temperature of storage to a point as near as possible to 0 C. Reduction of the rate of glycolysis permits the reduction of the amount of dextrose originally added to the preserving fluid, thus reducing the amount of swelling due to dextrose penetration into the red cells. It has been shown\(^8\) that the glycolysis is also directly proportional to the concentration of dextrose; therefore further control of glycolysis is made possible by reduction of the original dextrose concentration in the preserving fluid. Strumia has also emphasized the necessity for rapid cooling of collected blood. The procedure of placing the bottle of blood in a refrigerator at \(+4\) to 9 C., especially if many units of blood are collected and stored at the same time, is hardly compatible with rapid cooling of blood. Plunging of bottles in ice-water reduces the cooling time to near optimal value.

In practice this method is easily applied, permits routine storage of red cells in whole blood for a period of twenty eight days, and of packed red cells, without addition of resuspension media, for periods up to twenty one days.

BIOMECHANICAL APPARATUS

The basis for the separation of the formed elements of blood from their native medium, plasma, prior to storage, is based essentially on the observation that removal of the red cells from the circulating blood, regardless of the means employed for collection and storage at refrigerator temperatures, radically changes some of their fundamental requirements. Thus, while we may accept that plasma is the ideal medium for red cells in the circulation, we know equally well now that plasma is not the best medium for preservation of red cells outside of the body under all conditions.\(^9\)

Separation of red cells from single units of blood immediately after phlebotomy under rigidly controlled temperature conditions has been experimentally used in our laboratory for several years. We have employed an ice-water mixture for rapid cooling of blood in the conventional glass bottles or in plastic containers and centrifugation in the cold room at 1 C. Fabrication of special portable apparatus
for processing of blood into stable components, including both the cellular elements and plasma components, has been the goal of years of painstaking efforts of Cohn and his co-workers. This method intends to obtain more rapid separation of the formed elements of blood, with rapid cooling, by the use of multichambered stainless steel rotating bowls. The design of these bowls varies with the purpose for which they are intended.

The operations at present claimed for this apparatus vary from the simple operation of removing plasma from outdated whole blood, to more complete ones, such as separation of the plasma proteins by zinc interaction to yield immune globulins, and stable plasma protein solution (S.P.P.S.) for transfusion, for the reconstitution of lost plasma volume, or for nutritional purposes.

The development of such an apparatus obviously presents great mechanical difficulties. Current studies on the development of the biomechanical equipment are devoted to the problem of maintenance of sterility in the separation of red cells from plasma and for the complete separation and preservation of platelets.

It is hoped to achieve in the future better separation of platelets using a modified falling film principle for subsequent storage and use in vivo.

Further modification of the bowl assembly is under study for application to requirements for freezing of blood with the aid of glycerol, and for the removal of glycerol from previously frozen and thawed red cells. It is hoped that this apparatus will be rendered sufficiently simple to be suitable for routine use in addition to its primary use as a research tool.

THE USE OF PACKED RED CELLS

The use of red cells left after separation of plasma from freshly collected ACD blood has not received the wide applications warranted by the satisfactory results achieved. The findings of a number of investigators have shown that such cells have a good post-transfusion survival after storage up to twenty one days. In general, a good number of patients with various forms of anemia require, essentially, red cells. These two facts suggest that efforts should be made to develop a practical procedure for the salvaging of red cells left over from separation of plasma from blood collected under the National Blood Program.

It is generally not necessary to add any resuspension fluid; at most a small amount of additional dextrose may be required to make available, in the residual plasma, about 500 mg.

CONCLUSIONS

Notwithstanding a great deal of work done in several centers of investigation, it may be stated that none of the methods proposed since 1947 has contributed a practical method to achieve a preservation of erythrocytes considerably beyond the three weeks' period.

Basic studies on the biophysical and biochemical requirements of the red cell in vitro, especially on the enzymatic structure and the related energy producing mechanism, offer hope for a solution of the problem in the future. At this time preservation at temperatures substantially below freezing, sufficient to arrest or greatly diminish enzymatic activity, appears to offer the best field of exper-
mental study. However, emphasis is needed on better, rather than longer, preservation of erythrocytes. For the time being, improvement of preservation of ACD blood is best obtained by reducing the dextrose concentration, by rapid cooling of blood, and by lowering the temperature of storage to just above freezing. Under these conditions good preservation of blood for the purpose of transfusion can be achieved for periods of four weeks.

Procedures for the separation, storage, and transfusion of platelets are now available, which will permit better clinical evaluation of this method of therapy.

**SUMMARIO IN INTERLINGUA**

Es presentate e discutite le major contributiones que inter 1947 e 1953 esseva facite al solution del problema del preservation de sanguine immagazinate. Es considerate primarimente le problema del immagazinage e preservation de erythrocytos, sed etiam le phases essential del progresso methodologic in collectionar, immagazinar, e transfunder plachettas es mentionate brevemente.

In despecto de extemise recercas complite in plure centros de investigation, on debe constatar que nulle del methodos proponite ab 1947 representa un medio practic pro extender le preservation de erythrocytos considerabilemente ultra le limite de tres septimanas.

Studios fundamental in re le requerimentos biophysic e biochimic del cellula—specialmente in re le structura enzymatic e le correlate mechanismo de production de energia—justifica le spero de un solution futur del problema. Al tempore presente il pare que le plus promittente campo de studios experimental es illo del immagazinage a temperaturas sufficientemente sub 0 C. pro arrestar o grandemente diminuer le activitate enzymatic. Tamen, plus attention debe esser prestate a un melior in loco de solo un plus longe preservation del erythrocytos. Al presente tempore le plus promittente melioration del preservation de sanguine in ACD (acido-citrate-dextrosa) es obtenite per reduce le concentration del dextrosa, per rapidemente frigorificar le sanguine, e per reduce le temperatura de immagazinage a pauco supra 0 C. Sub iste conditiones il es possibile preservar sanguine in stato satisfactori pro transfusiones durante usque a 4 septimanas.

Pro separar, immagazinar, e transfunder plachettas, procedimentos es nunc disponible que va permitter un melior evaluatation clinic de iste methodo de therapia.

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