WHOLE BLOOD PRESERVATION; A PROBLEM IN GENERAL PHYSIOLOGY.[1] AN *IN VITRO* ANALYSIS OF THE PROBLEM OF BLOOD STORAGE

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A study of the storage of whole blood presents numerous problems in the field of cellular physiology. An understanding and solution of these problems can be of great aid in prolonging the viability of red cells of stored blood.

A major part of the problem centers around experimental studies on the influence of a number of environmental factors on survival. The more important factors are: temperature, range and optimum; degree of dilution; tonicity of the diluent; composition of the diluent; ion balance, maintenance of the colloidal osmotic pressure; pH range and optimum; buffer systems; glucose and rate of glycolysis; acid metabolite formation.

There have been many studies of the effect of some of these environmental factors on blood storage. In most cases, however, they have been studied as isolated, incomplete events by methods that frequently did not allow proper judgment of the state of preservation of the red cells. More recently, several excellent studies along these lines have appeared (Loutit [1]).

It is essential that an *in vitro* study of the blood preservation be accomplished by a thorough examination of a number of carefully controlled experimental criteria. We have found that the correlation obtained from the following *in vitro* methods permits one to arrive at a reasonable judgment of the state of preservation of the stored red cells and to predict the *in vivo* survival of such cells. Such predictions have been confirmed by *in vivo* tests (Gibson, *et al* [2]).

IN VITRO METHODS EMPLOYED IN THESE STUDIES

1. *The degree of spontaneous hemolysis,* obtained spectrophotometrically on the plasma after thorough re-suspension of the cells and subsequent separation in an air turbine (Chase, *et al* [3]).

2. *The mean and spread of the osmotic resistance,* determined by an experimental analysis of the full curve of osmotic resistance, in buffered salt solution at fixed pH and temperature (Parpart, *et al* [4]).

3. *Rate of loss of potassium from the red cells,* obtained by potassium analysis on red cells and plasma (Weichselbaum, Somogyi, and Rusk [5]).

4. *The rate of glycolysis,* as determined by the rate of disappearance of glucose from the stored blood (Hoffman [6]).

5. *Rate and amount of lactic acid formation* (Barker and Summerson [7]).

6. *Changes in hydrogen ion concentration of the plasma,* by glass electrode measurements.

7. *Hematocrit and cell volume changes,* determined by an accurate (standard deviation of 0.86 per cent) air turbine method (Parpart and Ballentine [8]).

8. *Initial total hemoglobin, methemoglobin formation and cell counts,* methods of Chase, *et al* (3) and Parpart (9).

9. *Rate of exchange of anions across the membrane of the red cells,* measured photoelectrically (Parpart [10]).

10. *Rate of passage of non-toxic organic molecules into the red cells,* measured photoelectrically (Parpart [10]).

All of the data reported in this paper were obtained on blood samples collected by venipuncture from healthy human males (18 to 40 years of age).

RESULTS

Appropriate correlations between these criteria have served as a satisfactory index of the state of preservation obtained in the various procedures we have applied to stored blood. The results of these studies will be outlined under separate headings, though it is important to realize that this separation is purely for convenience. Only full cognizance of the interaction of the various variables studied will allow for proper comprehension of the factors involved in blood storage.

1. Optimal temperature for preservation.

Studies have been made on whole blood samples stored at different temperatures under a variety of
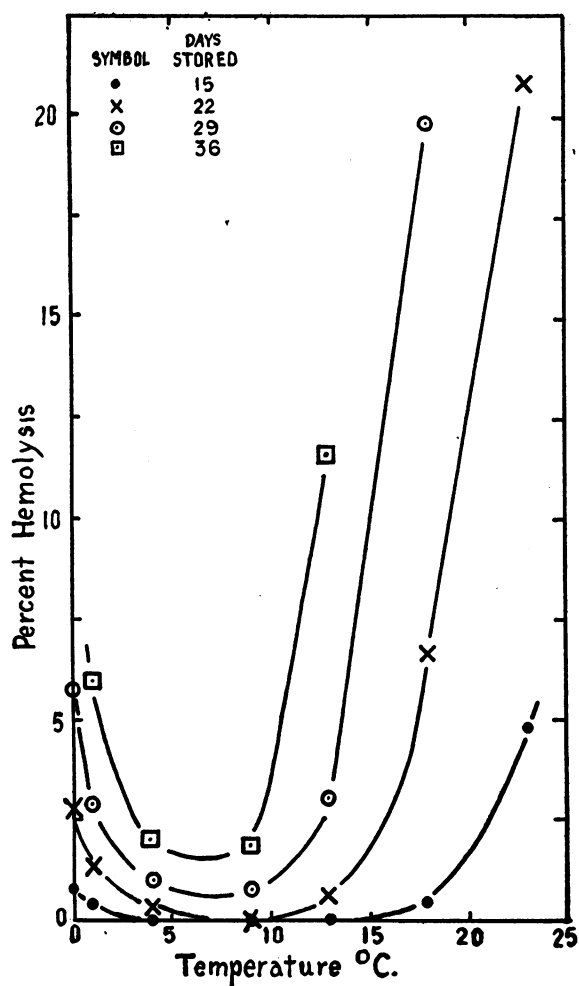
FIG. 1. EFFECT OF TEMPERATURE ON THE SPONTANEOUS
HEMOLYSIS OF STORED BLOOD

ability of the red cells to retain their normal potassium content. Four complete repetitions of these experiments gave the same results.

It may be seen from Figures 1, 2, and 3, that the optimum temperatures for survival of the human red cell lie between 4° and 9° C.; with an optimal at about 7° C. ± 1°. This has been confirmed by the *in vivo* studies of Gibson, *et al* (2).

These experimental conditions do not represent the best we have found for human red cell survival, but they do illustrate the optimal temperature conditions for such survival.

Figure 4 presents data on survival of red cells

experimental conditions (*e.g.* diluted, defibrinated, citrated, normal, and added glucose).

Figures 1, 2, and 3 illustrate the typical influence of temperature on the survival of stored human blood. These data were obtained on 270 ml. of blood collected in a bottle containing 30 ml. of 3.25 per cent $Na_3C_6H_5O_7 \cdot 2 H_2O$ (0.11 M). This blood was then diluted to 70 per cent of normal whole blood by the addition of an isosmotic solution (5.4 per cent) of glucose and within 30 minutes of collection stored at the temperature indicated in the figures.

Figure 1 gives the rate of spontaneous hemolysis with respect to temperature and length of storage; Figure 2 presents the data on the mean osmotic resistance and Figure 3 illustrates the effect on the
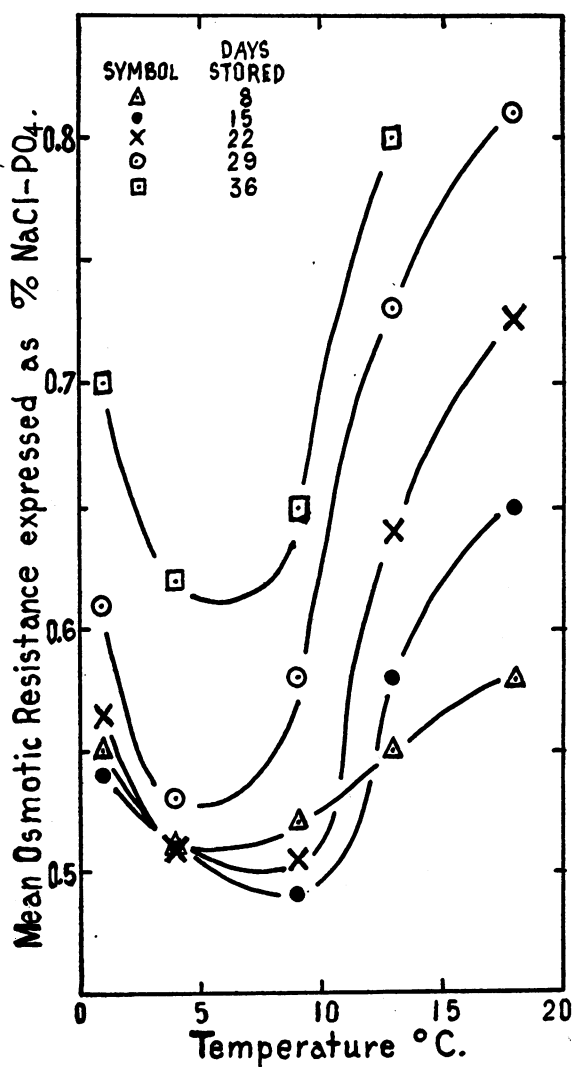

FIG. 2. EFFECT OF TEMPERATURE ON THE MEAN OSMOTIC
RESISTANCE

under a variety of storage conditions at 2 different temperatures. One of these temperatures is in the optimal range (5° C.) while the other is well outside this range (23°). Figure 4 quite clearly shows that while temperature is a variable in the survival of stored red cells (one of prime importance as shown in Figures 1, 2, 3), and one whose effect is consistently in the same direction irrespective of large variations in the composition of the environment of stored red cells, nevertheless the effect of temperature may be overshadowed by the environmental composition. Curves a and b (Figure 4) contrast the rate of spontaneous hemolysis in the case of 12 collections of carefully defibrinated blood stored at 23° (a) and 6 collections stored at 5° (b) respectively. These blood samples had nothing else done to them except defibrination. It may be observed that the effect of temperature is present but its action is slight.

Compare these curves a and b with curves c and d (Figure 4). The latter 2 curves were obtained in 5 collections of blood according to the method of De Gowin, et al. (11) and stored at 23° and 5° respectively. In this case the effect
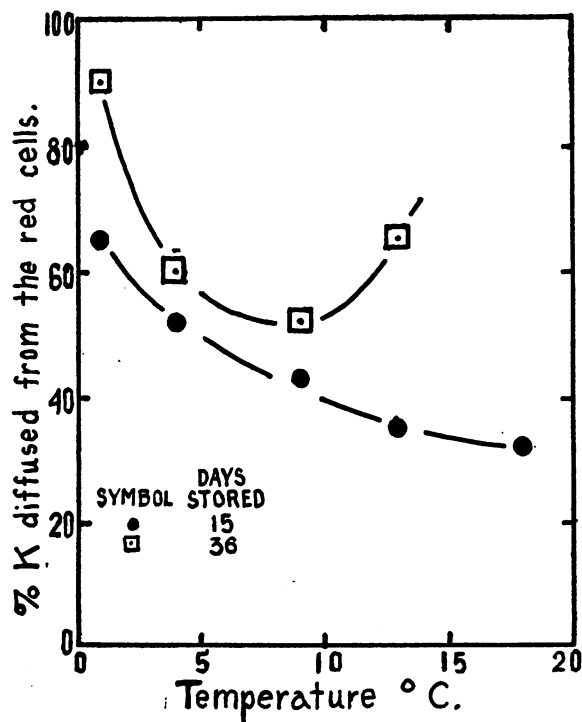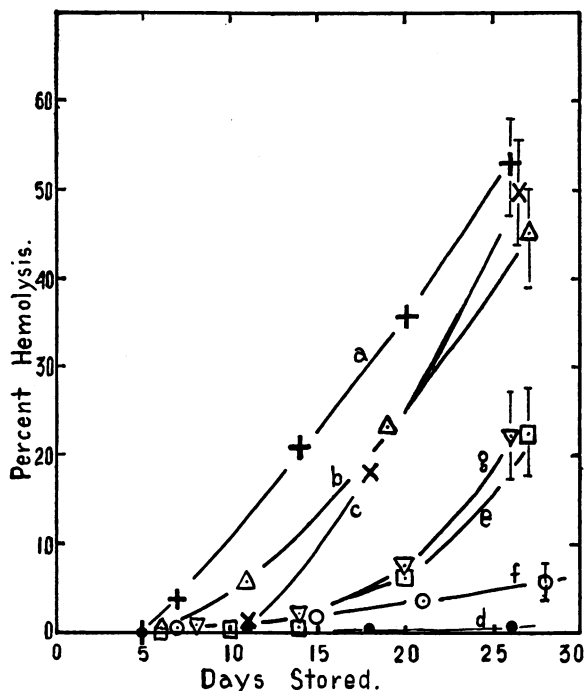

FIG. 4. SPONTANEOUS HEMOLYSIS AT 2 TEMPERATURES IN A VARIETY OF SOLUTIONS

of temperature is exceedingly striking and could have been deduced from the effect of temperature on the rate of penetration of glucose into human red cells, since the citrated bloods stored by De Gowin's method have been diluted to 40 per cent of their original concentration with an isosmotic solution of a compound that penetrates the red cells (5.4 per cent glucose). Such penetration of glucose leads to an abnormal increase in volume of the red cells (Figure 8).

One may also contrast curves e and f with a and b (Figure 4). The only difference other than temperature between the stored bloods represented in these 4 curves is the addition of sterile, solid glucose to give a final concentration of 0.5 per cent added glucose to the blood samples of curves e and f. These latter curves are the average for 6 and 9 individual blood collections. Comparison of curve e with f shows a very marked effect of temperature, over and above the strikingly better survival of the red cells to which glucose has been added.

Spontaneous hemolysis with time of storage for 90 parts of blood collected into 10 parts of an isosmotic $Na_3C_6H_5O_7 \cdot 2 H_2O$ (0.11 M) solution

FIG. 3. INFLUENCE OF TEMPERATURE ON THE LOSS OF POTASSIUM FROM THE RED CELLS

is shown in curve g (Figure 4). The better, though still poor, preservation under these conditions as compared with defibrinated blood stored at 5° C. (curve b) is primarily associated with the dilution of the blood and not with any effect of the added citrate. This point will be taken up later under the following section on the effect of dilution.

Figure 4 is intended solely to illustrate further effects of temperature on spontaneous hemolysis. It is not intended, however, to indicate that any of the methods of preservation illustrated in it is better than any other when considered from the point of view of in vivo survival. The other in vitro criteria that have been used on all of the samples of stored blood in Figure 4 indicate that none of these methods of preservation would give satisfactory in vivo survival expectancy.

2. The degree of dilution and preservation.

A review of the extensive literature on blood preservation reveals that a great variety of dilutions of the whole blood have been used. This has varied from 10 per cent whole blood through a great many intermediate degrees of dilution up to undiluted blood. In most cases in the literature, the use of a particular dilution, whether 10, 40, 50, 80, 90 per cent of whole blood or whole blood itself, has been purely arbitrary with no supporting data for a particular dilution. Further, the tonicity and glucose content of the dilution medium have varied along with the degree of dilution. The work described below was an attempt to correlate the survival of red cells with the degree of dilution of the stored blood.

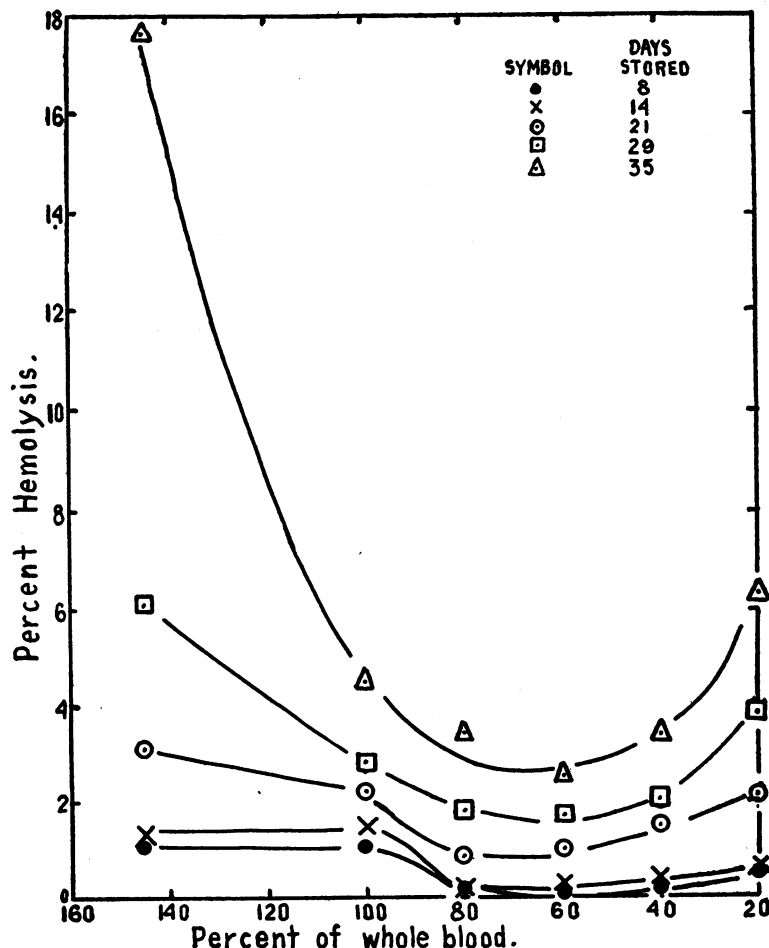In addition to the immediate problem of the

FIG. 5. DILUTION OF WHOLE BLOOD WITH PLASMA AND THE SPONTANEOUS HEMOLYSIS DURING STORAGE

degree of dilution of stored blood on the survival of the red cells, there is a practical aspect of this problem in relation to transfusion. If it is necessary to increase the available hemoglobin of a patient, minimum dilution of the blood would be advisable. On the other hand the transfusion may also be used to combat dehydration, to supply glucose or salts, or plasma proteins, in which event high dilution of the red cells might be desirable. The answer to this must hinge upon a knowledge of what dilution is best for the survival of stored red cells.

An obvious perfect diluent might be plasma. We have run 5 experiments in which the donor's blood was diluted by varying amounts with its own plasma. A typical set of such data is given in Figure 5. The blood used in this experiment was collected by venipuncture into a bottle containing enough solid $Na_3C_6O_7 \cdot 2\ H_2O$ to give a final citrate content of 400 mgm. per 100 ml. of blood. A portion of the blood sample was transferred to sterile centrifuge tubes and citrated plasma obtained from it. This citrated plasma was then used to dilute the original blood by the amount indicated on the abscissa of Figure 5. Each of these diluted blood samples had 500 mgm. of glucose added to them and they were stored at the optimum temperature, 7° C. One of these blood samples was concentrated centrifugally and enough plasma removed to give a final concentration of 145 per cent relative to the whole blood concentration which was designated as 100 per cent. The blood of 3 other individuals had been stored and examined periodically in this way. Exactly similar results were obtained with only minor fluctuations in the absolute values.

The data contained in Figure 5 show quite clearly that the optimum dilution range for red cell preservation, with these conditions, lies between 80 per cent and 60 per cent of the whole blood concentration. It may also be noted that the optimum shifts from close to 80 per cent toward 60 per cent as the time of storage increases. On the average, blood which has been diluted to 70 per cent of its original concentration might be expected to give the best preservations of its red cells.

The question arises as to whether other types of diluents have an analogous effect. The data in Figure 6 illustrate this. Curve a (Figure 6) is
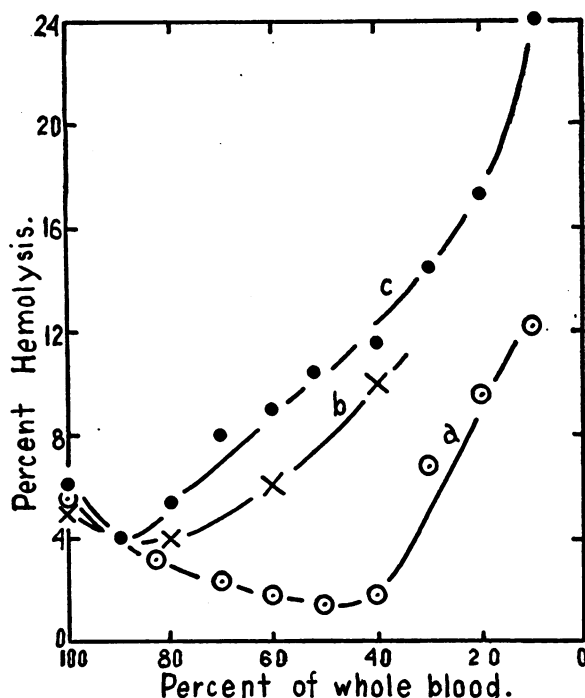

FIG. 6. EFFECT OF SEVERAL DILUENTS ON THE HEMOLYSIS RATE OF STORED BLOOD

a plot of the degree of spontaneous hemolysis in blood stored for 48 days, at 7° C., under the following conditions. Five parts of blood were collected into 1 part of a 3.2 per cent solution of $Na_3C_6H_5O_7 \cdot 2\ H_2O$, cold (5° C.). A portion of this blood had 500 mgm. per cent (mgm. per 100 ml.) of glucose added, was stored at 7° C. and was examined at weekly intervals. This appears as 83.3 per cent of whole blood in curve a. Aliquots of the rest of this citrated blood were diluted with an isosmotic solution of glucose (5.4 per cent) to give the percentage concentrations of curve a. All were stored at 7° C. and examined at weekly intervals. The data in curve a (Figure 6) are those for the forty-eighth day of storage. They show that the optimum dilution lies between 60 and 40 per cent of the whole blood concentration and that a dilution which gives 40 per cent of the whole blood concentration is close to the borderline which may lead to rapid hemolysis. The latter is the preservative solution used by De Gowin, et al (11). The fact that survival of red cells in this solution is very poor when judged from the other *in vitro* criteria we have used has been previously mentioned under Figure 4, curve d.

Curve b (Figure 6) contains data for blood collected into solid citrate (see page 645) while curve c gives data for defibrinated blood. These blood samples were diluted with a sterile isotonic salt solution of the following composition: NaCl, 0.90 per cent; KCl, 0.042 per cent; $NaHCO_3$, 0.024 per cent; NaH_2PO_4, 0.05 per cent. They had 500 and·1,000 mgm. per cent of glucose added respectively and were stored at 7° C. The curves represent the degree of spontaneous hemolysis occurring at 35 and 21 days of storage respectively. The shapes of these curves and of curve a did not alter appreciably with time of storage, i.e. after the first week, though their position on the ordinate altered. It will be shown in section 5 that the difference in glucose concentration of these 2 samples does not account for the survival differences.

Curves b and c also show an optimum dilution and this occurs at about 80 and 90 per cent of whole blood. The effect is slight until blood concentrations of 60 per cent or lower are examined. It is obvious that dilutions of 60 per cent or greater are definitely deleterious when a balanced salt solution is used as the diluent. It is again noteworthy that defibrinated blood does not survive as well as citrated blood.

Figures 5 and 6 are convincing evidence of the fact that earlier experiments in the literature on blood preservation which failed to take account of the effect of dilution on survival of the red cells must be considered with respect to the actual dilution that was used. The foregoing experiments, combined with an analysis of data from a number of others, have led us to the conclusion that the survival time of stored red cells is increased when the blood is diluted. The data in Figures 5 and 6 would further indicate that for a variety of diluents a dilution consisting of 70 parts of blood and 30 parts of diluent (70 per cent whole blood) might be expected to give optimum survival.

Even this value of 70 per cent must be employed with caution, as the data of Figure 7 will show. In this case blood was collected into a minimum amount of citrate and diluted with a 6 per cent gelatine solution prepared in 0.9 per cent NaCl at pH 7.2.[2] The days of storage are indicated on

[2] This diluent was supplied through the courtesy of Dr. D. Tourtellotte, and is a product of Knox Gelatine Protein Products, Inc.
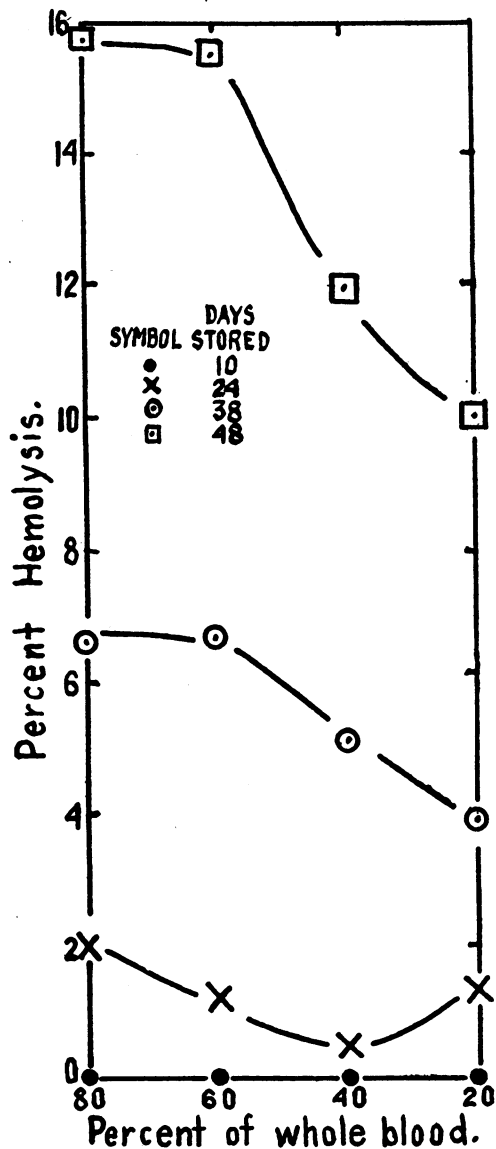

FIG. 7. DILUTION WITH GELATIN

the figure. Through 24 days of storage the survival was good at all dilutions and excellent at 40 per cent. At later periods of storage the survival was poor, but it is evident that the greater dilutions (40 and 20 per cent of whole blood concentration) gave better protection.

3. Tonicity, chemical composition and colloidal osmotic pressure of the diluent.

The diluent of historical interest is an isosmotic solution (5.4 per cent) of glucose (Rous and Turner [12]). They diluted whole blood as fol-

lows: 30 parts blood, 20 parts of 3.8 per cent $Na_3C_6H_5O_7 \cdot 5\ H_2O$ and 50 parts of a 5.4 per cent glucose solution. This has been used more recently at a 40 per cent concentration by De Gowin and his coworkers (11).

Those who have used this isosmotic glucose solution as a diluent for human red cells have ignored the fundamental fact that the human red cell is readily permeable to glucose. Thus, human blood collected by the methods of Rous and Turner or of De Gowin would be expected to show a marked increase in the volume of its red cells. That this is the case is shown in Figure 8, curve a, which expresses the relation between the red cell volume and the degree of dilution of the whole blood, under conditions where whole citrated blood was diluted with isosmotic glucose solution. The measurements given are for the seventh and forty-eighth days of storage at 7° C.

Contrasted with the very marked increase in volume of curve a are the much smaller red cell volume changes which occur during the storage of citrated blood which has been diluted with Ringer-Locke solution (calcium-free). The latter are

represented in Figure 8, curves b and c, and are the data for the first and forty-ninth days of storage at 7° C. These diluted blood samples had 500 mgm. of added glucose.

The pronounced swelling of red cells diluted under the conditions of De Gowin or of Rous-Turner is the result of the penetration of the glucose. These red cells would continue to swell to the hemolytic volume were it not for the fact that they are being subjected to a shrinking effect brought on in part by the loss of chloride ions from the cells (Jacobs and Parpart [13]). An additional and more important factor in checking the swelling of human red cells diluted with isosmotic glucose solution is the rapid loss of potassium from these cells. Our data on potassium ion loss from the red cells stored under these conditions indicate that 50 per cent of the diffusion equilibrium is attained between the fifth and eighth days of storage, while the outward diffusion of potassium is complete after 10 to 20 days.

The balance between these several factors acting on the cell volume is a delicate one and needs only a slight change in environmental conditions (*e.g.* temperature rise) to cause a rapid rate of spontaneous hemolysis by the red cells of blood thus diluted, see curve c (Figure 4). Human blood stored under conditions where it is diluted to 40 or 30 per cent of its original concentration with an isosmotic glucose solution would be expected to show very poor *in vivo* survival of its red cells (Gibson [2]).

The question has been frequently raised as to whether it is important to maintain the normal colloidal osmotic pressure of the fluid surrounding the stored red cells. In an earlier section of this paper we have demonstrated that dilution of whole blood to 70 per cent of normal affords a better environment for the survival of the red cells. This was true irrespective of whether the diluent was (1) plasma, (2) a 6 per cent gelatine in isotonic NaCl solution, (3) a balanced salt solution, (4) an isosmotic glucose solution, or (5) an isotonic phosphate solution which when added to the blood established an initial pH of 7.1 (Figures 5, 7, 6, and 9).

Diluents (1) and (2) above had normal colloidal osmotic pressure while the other 3 diluents reduced the colloidal osmotic pressure of the plasma of the stored blood to 70 per cent of normal. The
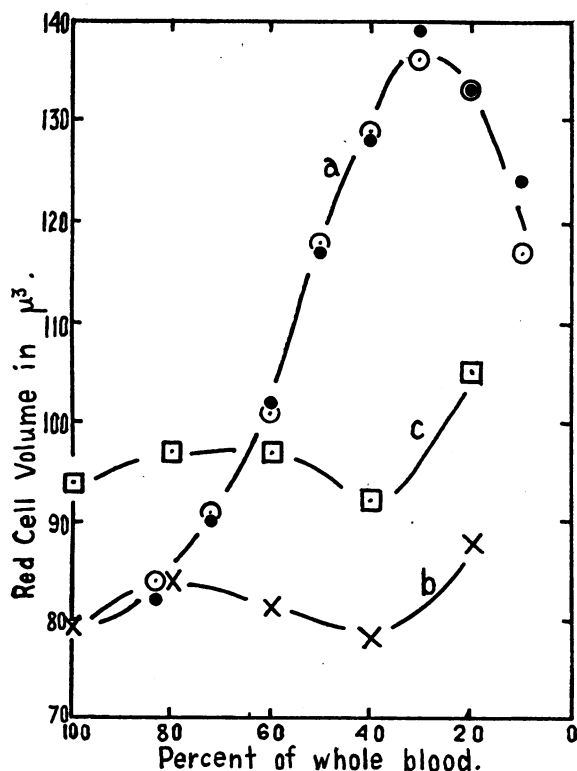

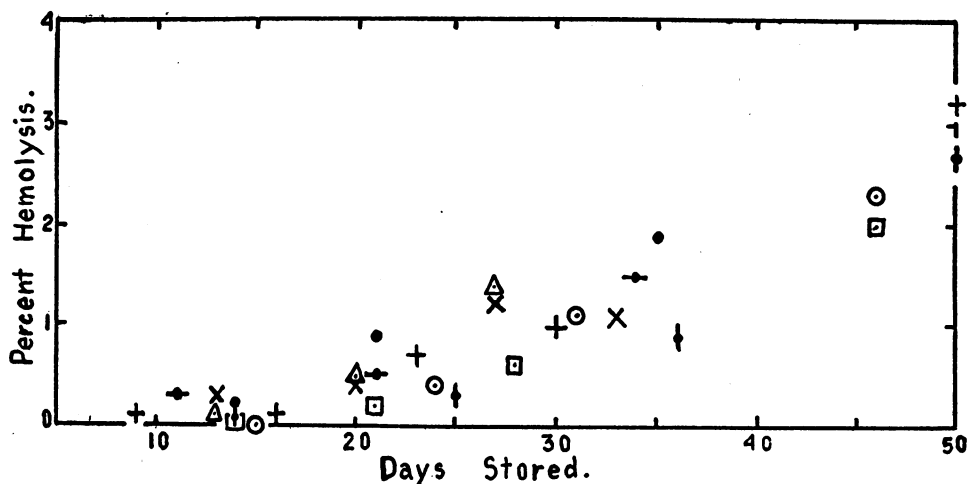
FIG. 8. DILUTION AND RED CELL VOLUME

FIG. 9. COMPARISON OF DATA ON PHOSPHATE DILUTED BLOODS

rate of spontaneous hemolysis in these various diluents, however, did not differ greatly and what differences there are certainly cannot be ascribed to this factor. In fact, the survival of the red cells in stored blood diluted to 70 per cent of whole blood concentration with the phosphate solution (Figure 9) is much better than that in plasma or gelatine solution judged from our *in vitro* data and the *in vivo* data of Gibson (2).

It should be pointed out that the differences that do exist are outside the limits of variation of individual blood samples. This is also illustrated by Figure 9, where the degree of spontaneous hemolysis is plotted against the days of storage. The 8 individual blood samples represented in Figure 9 were from data taken at random over a period of 1½ years on citrated human blood samples stored at 7° C. and diluted as follows: 70 parts of whole citrated blood (4 mgm. $Na_3C_6H_5O_7 \cdot$ 2 H_2O per ml. of blood) and 30 parts of a solution containing 60 volumes of 0.11 M Na_2 HPO_4 and 40 volumes of 0.11 M NaH_2PO_4. The initial pH of blood thus diluted is 7.1 and during 50 days of storage drops to pH 6.7.

Another series of tests on the influence of the colloidal osmotic pressure of the plasma on red cell survival were run on 4 different samples of human blood. The blood samples were collected into citrate (in amount indicated above), divided into 2 equal volumes, transferred to sterile centrifuge tubes, spun, and the major portion of the plasma removed aseptically. The plasma of 1

aliquot of cells was exactly replaced by a solution recommended by Gibson, viz.:

$Na_3C_6H_5O_7 \cdot 2H_2O$	480 mgm.
Glucose	1,080 mgm.
NaCl	590 mgm.
Conc. HCl (Sp. G. 1.19)	0.25 ml.

made up to 100 ml. with pyrogen-free water.

The other aliquot had its plasma replaced by the same solution except that 3 grams of plasma protein- lipid Fraction IV–3, 4 Run S301, Cohn (14), were added to the 100 ml. of solution before replacing the plasma.

In the case of all 4 blood samples thus treated there was good survival over 45 days storage, but the difference slightly favored the solution without the added Fraction IV. In brief, the colloidal osmotic pressure of the medium surrounding human red cells does not appear to be a factor in their *in vitro* survival.

4. pH range and optimum, buffering.

The influence of pH on the survival of stored red cells has not been consistently investigated. Recently Loutit (1) has gathered together data on a number of samples of blood stored in acid citrate-dextrose that indicate that blood diluted with this solution and having an initial pH of 7.1 to 7.3 shows a better survival of its red cells.

In a report, dated October, 1943, to the Blood Substitutes Committee of the Committee on Medical Research we showed the very marked influence of pH on the survival of the red cells of stored human blood. Our studies of the influence of pH
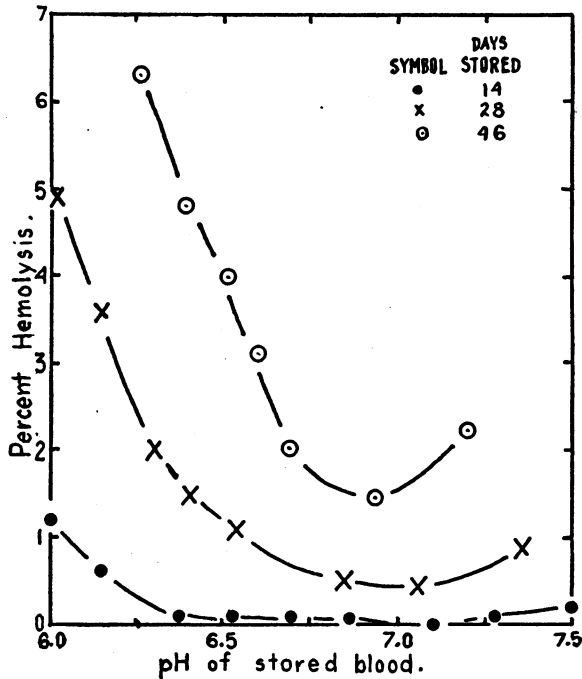
FIG. 10. INFLUENCE OF PH ON HEMOLYSIS RATE OF STORED BLOOD

on *in vitro* survival have been carried out under conditions where a minimum shift in the pH of stored blood was attained during long periods of storage. The citrate buffer system has no buffer capacity over the pH range for maximum survival of stored red cells (Figure 10). The rate of glycolysis of blood and the rapid formation of lactic acid, even at the optimum temperature (7° C.), is so high that over long storage periods blood samples will show marked decreases in pH unless adequately buffered.

We, therefore, turned to the phosphate buffer system and studied survival of the red cells at various hydrogen ion concentrations. These blood samples were followed over long time periods under conditions where a minimum decrease in pH with time of storage occurred. Citrated blood was diluted with isotonic (0.11 M) phosphate buffer solutions in the proportion of 30 parts of buffer to 70 parts of whole blood. In all cases these stored bloods had 0.5 per cent added glucose and storage was at 7° C. Thus, a number of blood samples having an initial pH between 6.0 and 7.5 were prepared and red cell survival determined at frequent intervals. Seven individual blood samples were run in this manner; the typical result of the

effect of pH is shown in Figure 10 where the per cent of spontaneous hemolysis is plotted against the pH of the blood samples at the fourteenth, twenty-eighth, and forty-sixth days of storage.

Figure 10 demonstrates that the optimum pH for prolonged storage of human blood lies between pH 6.7 and 7.0. It may also be noted that during the first 2 weeks of storage there is slight effect of pH over the range pH 6.5 to 7.5. Longer storage periods, however, show a marked influence of pH. Thus storage for 4 and 7 weeks clearly requires a pH between 6.7 and 7.0 if optimum preservation of the red cells is to be attained.

Another striking action of pH on the survival of the red cells of stored blood is on the ability of the red cells to retain their normally high potassium content. This effect is illustrated in Figure 11, where the per cent of the original amount of potassium in the red cell which has diffused out of the cell at various times of storage is plotted against the pH of storage. In this experiment the blood was buffered by phosphate solutions as described above and 0.5 per cent glucose added and stored at 7° C.

Figure 11 illustrates that human red cells stored at a pH between 6.7 and 7.0 will lose their potas-
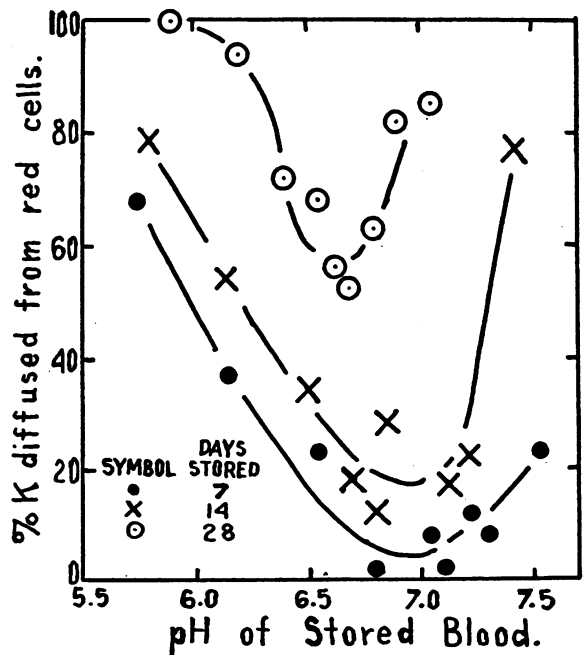

FIG. 11. EFFECT OF PH ON THE RATE OF LOSS OF POTASSIUM

sium at a much less rapid rate than at a pH higher or lower than this optimum range. Even after 4 weeks of storage at pH 6.8 the potassium of the red cells has diffused to only 60 per cent of equilibrium.

The studies thus far dealt with have employed the phosphate system. The results outlined are due primarily to the pH and not to specific action of the phosphate itself. This has been established by experiments involving storage of human blood in maleic, in maleic-succinate, and in glycyl-glycine buffer systems, under conditions otherwise identical for the phosphate system. The data obtained for these 3 systems as concern the optimum pH range for red cell survival are the same as that for the phosphate system, though the absolute magnitude at the optimum pH for cell survival does vary with the buffer used (Table I).

TABLE I

Comparison of the per cent of spontaneous hemolysis in human blood after 4 weeks' storage at 7° C. in the presence of different buffer systems

Each blood sample was composed of a mixture of 30 parts isotonic buffer solution and 70 parts whole (citrated) blood. The initial pH of each was 7.10 to 7.20 and the pH on the fourth week of storage is given. Each blood sample had 0.5 per cent glucose added.

Buffer system	pH after 4 weeks' storage	Sponta- neous hemolysis after 4 weeks	Original glucose utilized by fourth week
		per cent	*per cent*
NaH₂PO₄/Na₂HPO₄	6.82	0.5	20
Glycyl-glycine/Na glycyl- glycine	6.75	0.7	18
Maleic-succinic acids/Na maleate-Na succinate	6.95	7.0	13
Maleic acids/Na maleate	6.82	15.0	0
None	6.94	1.8	36

In this respect, Table I shows that the phosphate system was better than the other buffers. It is thus certain that in addition to the pH effect on survival the phosphate itself markedly increases survival. The glycyl-glycine buffer is also exceptionally effective in prolonging red cell survival of stored blood (Table I).

Table I also shows that red cell survival in the maleic and maleic-succinate systems is poor even at the optimum pH. This effect, however, is probably associated with the low or completely inhibited glycolytic rate in the presence of these latter buffer systems (Table I).

Citrated blood, diluted to 70 per cent of whole blood with its own plasma and with 0.5 per cent added glucose, stored 4 weeks at 7° C., but with no buffer system added is included in Table I. It may be seen that while the degree of spontaneous hemolysis is low at this time, it is, however, considerably greater than in the blood with phosphate or glycyl-glycine buffer system (see also Figure 5).

From the foregoing it would appear that the phosphate buffer solutions which have on mixing with blood an initial pH between 7.1 and 7.3 and a pH after 4 weeks of storage at about 6.9 (with 0.5 per cent added glucose and storage at 7° C.) are exceptionally fine for increasing the survival period of the red cells of stored blood of man. The question remains as to what might be the minimal concentration of phosphate buffer to add to blood for optimal or good preservation of the red cells.

To answer this question, whole citrated human blood was diluted with varying amounts of phosphate buffer (Table II). The degree of dilution

TABLE II

Blood sample	Citrated blood	Phos- phate buffer mixture	Bal- anced salt solution	Added glucose	pH 0 days storage	pH 50 days storage
no.	*ml.*	*ml.*	*ml.*	*mgm.*		
397	70	30	0	500	7.29	6.74
398	70	20	10	500	7.35	6.78
399	70	10	20	500	7.48	6.86
400	70	3	27	500	7.54	6.90
401	70	0	30	500	7.59	7.03

(70 per cent whole blood) was maintained constant (Table II), by using a calcium and phosphate-free Ringer-Dale solution. The glucose added to each blood sample was 0.5 per cent and all bloods were stored at 7° C. The data in Table II are expressed in terms of 100 ml. of blood plus diluent. The phosphate buffer mixture used consisted of 60 parts of 0.11 M Na₂HPO₄ and 40 parts of 0.11 M NaH₂PO₄.

The degree of spontaneous hemolysis which occurred in these stored samples after 14, 36, and 50 days of storage is illustrated in Figure 12. The "mean osmotic resistance" initially and at the same storage times is given in Figure 13.

As noted before, the presence or absence of the phosphate buffer has little or no effect during the first 2 weeks of storage (Figure 12). However,
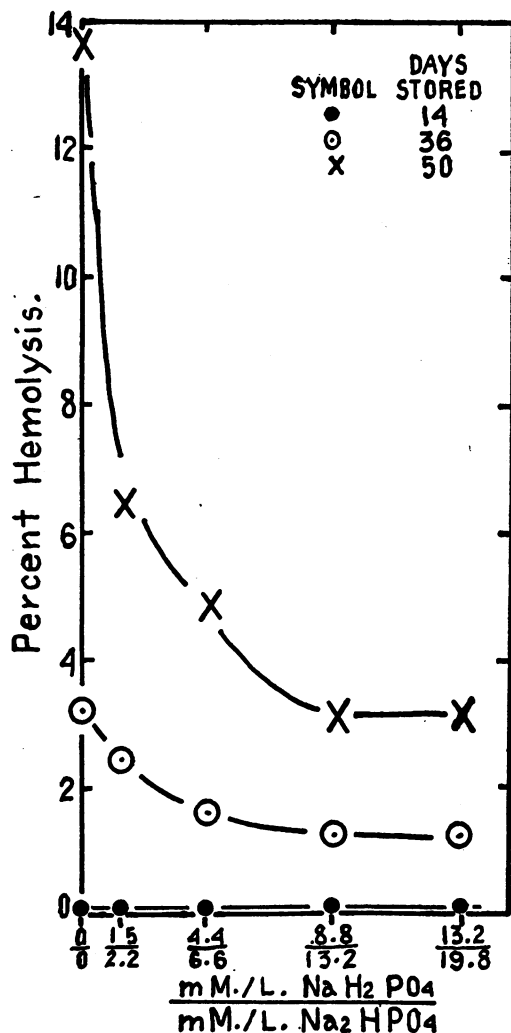
FIG. 12. EFFECT OF PHOSPHATE CONCENTRATION ON
SPONTANEOUS HEMOLYSIS

5. Optimal Glucose Content.

Glucose added to stored blood increases the red cell survival. This is a fact upon which all experiments on blood preservation have concurred. No other sugar has been observed to have a comparable effect.

Surprisingly no one has investigated the effect of varying the glucose concentration while keeping other possible variables as constant as possible. We therefore performed a series of experiments on the effect of glucose concentration on red cell survival of stored human blood. Several series compared the survival in whole blood to which varying amounts of glucose were added. Blood was collected directly into solid citrate in the proportion of 400 mgm. $Na_3C_5H_7O_6 \cdot 2$ H_2O to each 100 ml. of blood. To aliquots of this citrated blood, amounts of glucose varying from 0 to 6 per cent were added and the survival followed over a 7-week period. The glucose was added on the basis
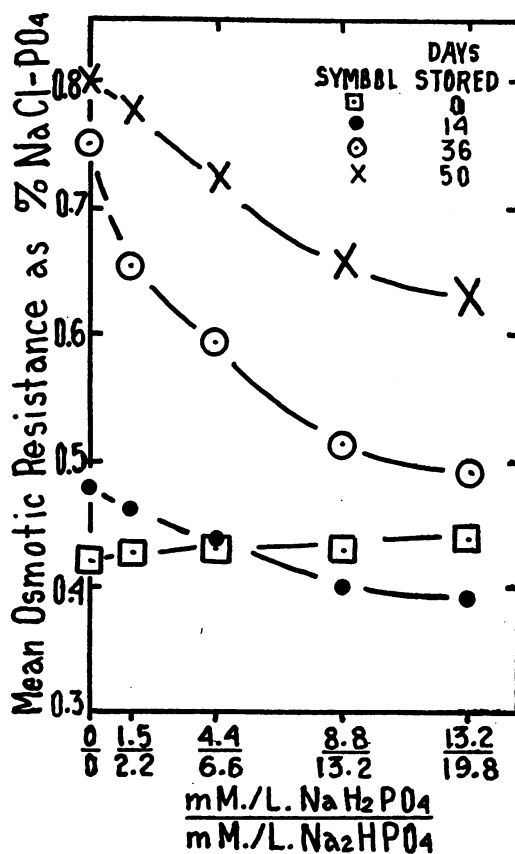
when one examines the mean osmotic resistance curves (Figure 13) one observes that even after 2 weeks of storage the bloods stored with no, or a small amount of, phosphate buffer would be poor risks in transfusion and this becomes more striking on the fifth and seventh weeks of storage.

Blood stored with 0.0044 M NaH_2PO_4 and 0.0066 M Na_2HPO_4 per liter of blood and with 0.5 per cent added glucose, 30 per cent dilution and at 7° C., has its red cells well preserved over 5 weeks of storage. Blood stored under these conditions and injected in 500 ml. amounts would increase the normal plasma phosphate level by only 40 per cent.

FIG. 13. EFFECT OF PHOSPHATE CONCENTRATION ON
MEAN OSMOTIC RESISTANCE

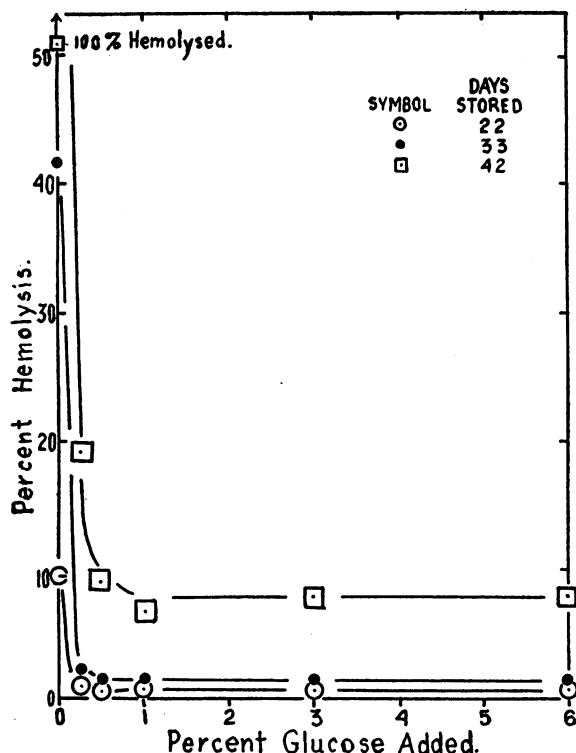| SYMBOL	DAYS STORED
⊙	22
●	33
▫	42

FIG. 14. OPTIMAL GLUCOSE CONCENTRATION FOR
STORAGE

of grams of glucose per 100 ml. of stored blood, *e.g.* 1 per cent means 1 gram per 100 ml. blood. All bloods were stored at 7° C. The results of such experiments are illustrated in Figures 14, 15, and 16.

Data on the effect of added glucose on the degree of spontaneous hemolysis occurring during storage are given in Figure 14. The data in this figure show quite clearly that even a low total concentration (0.25 per cent) of added glucose aids in the red cell survival for 33 days of storage when compared with the cells of blood stored with no added glucose. From 33 to 42 days of storage, however, this low concentration becomes insufficient. It may also be noted in Figure 14 that amounts of glucose, including 0.5 per cent and greater, increase to a considerable degree the red cell survival although there is no cumulative effect of the greater amounts of glucose. It must be emphasized that these experiments were designed to vary only 1 factor, namely, glucose concentration. This, of course, precluded the possibility of studying survival under the optimal conditions of dilu-

tion and buffering against pH change. The results as far as the minimal effective amount of glucose to add to stored blood, viz. 0.5 per cent, are quite conclusive. Glucose penetrates the human red cell and, hence, its osmotic effects may be neglected under the conditions of these experiments.

Completely analogous results have been obtained on citrated blood diluted to 70 per cent with phosphate buffer at the optimal pH and stored with and without added glucose. After 42 days of storage these blood samples showed 8 per cent spontaneous hemolysis when no glucose was added and 1.8 per cent in the presence of 0.5 per cent added glucose. These data contrast only in the magnitude of the hemolysis with those for 42 days' storage in Figure 14 but are parallel as to the action of glucose. They show again the importance of dilution and buffering. In fact, blood stored under these latter conditions is in better state for

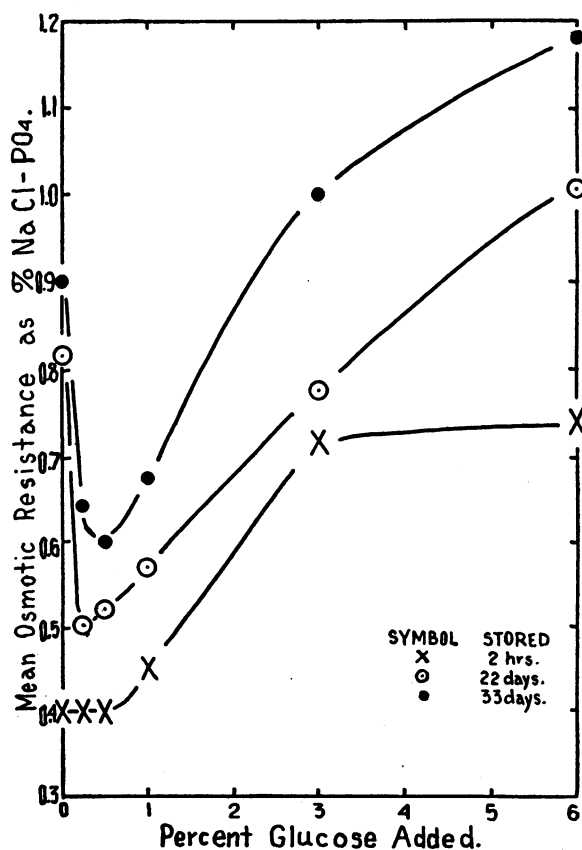| SYMBOL	STORED
X	2 hrs.
⊙	22 days.
●	33days.

FIG. 15. EFFECT OF ADDED GLUCOSE CONCENTRATION ON
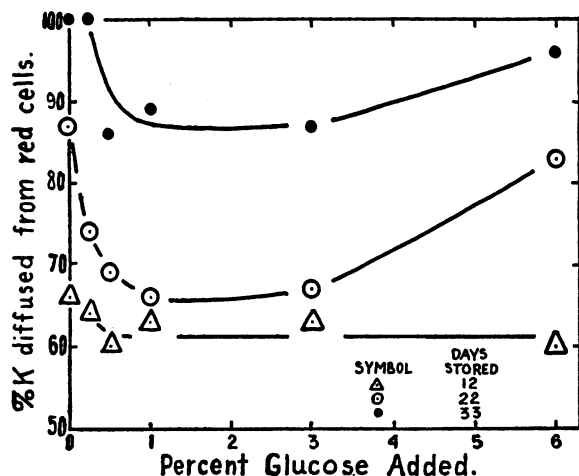THE MEAN OSMOTIC RESISTANCE

FIG. 16. EFFECT OF ADDED GLUCOSE ON THE LOSS OF
POTASSIUM

transfusion after 42 days of storage than undiluted, unbuffered blood was at 22 days, regardless of added glucose.

Figure 15 brings out the noteworthy fact that high concentrations of added glucose, 1 per cent and higher, lead to a rapid decrease in the osmotic resistance of the stored red cells. These experimental observations are obviously the resultant of the effect of the increased concentration of glucose in the cells stored with this high amount of added glucose and the consequent swelling this would produce in the hypotonic saline solution in which the osmotic resistance was measured. They do give point to the fact, however, that such red cells when transfused would undergo rapid osmotic hemolysis, thus rendering them useless for such purposes. This danger point lies between 1 and 3 per cent added glucose (Figure 15).

The slight, but noticeable effect of added glucose on the retention of potassium by the stored red cells is illustrated in Figure 16 and may be contrasted with the good retention of potassium when the blood is diluted and buffered to the optimum pH (Figure 11).

We have studied the preservative action of a number of sugars in comparison with glucose. In all of these studies the particular sugar was compared with identical conditions for survival with added glucose. The sugars used were xylose, lactose, fructose, maltose, sucrose. None of these sugars gave as good survival of the red cells of stored blood as compared with cells stored with added glucose. In all cases except xylose the red cell survival was better than under parallel experiments where no sugar was added. Xylose gave no better survival than control blood with no added sugar.

SUMMARY

An analysis has been made of a number of factors concerned in prolonging the life of the red cells of stored human blood.

Blood collected and stored under the following conditions has been found to give excellent survival of its red cells over a period of at least 6 weeks: Seventy parts of blood collected into 10 parts of 4 per cent $Na_3C_6H_5O_7 \cdot 2 H_2O$ and 20 parts of a phosphate buffer solution (composed of 60 volumes 0.11 M Na_2HPO_4 and 40 volumes of 0.11 M NaH_2PO_4); 500 mgm. per cent of glucose is added and the blood is stored at 7° C.

Blood thus stored will be under the optimal conditions of (1) pH (initially 7.2 and 6.8 after 6 weeks), (2) good buffering, (3) dilution (70 per cent), (4) glucose concentration (500 mgm. per cent), (5) temperature (7° C.).

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