STUDIES ON STORED BLOOD

IX. Further Observations on the Effects of Storage on Erythrocytes

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In an earlier communication (Crosbie and Scarborough 1941) we reported the results of investigations on the effects of storage on the erythrocyte count, the colour and volume index, and the corpuscular and mean corpuscular volumes. We also reported observations on the morphological changes occurring in erythrocytes during storage, noting especially the development of spherocytosis during the first 3-10 days. It was considered that the attainment of the spheroidal state associated with an increase in corpuscular volume denoted marked alterations in the properties of the cell envelopes which occasioned increased fragility to both osmotic and mechanical forces. The present paper deals mainly with such changes.

Blood was withdrawn from healthy female donors by the apparatus already described (Stewart 1940), the special virtue of which is that the blood is mixed with the anticoagulant solution as it leaves the vein and is never in contact with excess of anticoagulant. Sodium citrate solution (3·8 g. crystalline sodium citrate B.P. in 100 ml. freshly distilled water) was mixed with the blood in the proportion of 1 part of citrate solution to 9 parts of blood. After withdrawal the citrated blood was divided at once amongst a suitable number of tubes and stored at 2°-5° C. until required. A new tube was opened on each occasion an examination was made, its contents being gently but thoroughly mixed for two minutes. As each tube was mixed on one occasion only, the results obtained are representative of those to be found in a bottle of blood stored undisturbed for a similar period. This procedure is essential because agitation of the blood is quite sufficient to produce marked damage to the cells, the extent of the damage being greater with increasing age of the blood and with the number

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of times the blood is disturbed. Having regard to the fact that the nature and concentration (tonicity) of the anticoagulant solution and the dilution of the blood all influence the subsequent changes in the erythrocyte fragility, it is important to emphasise that the results set forth in this paper apply to blood withdrawn and stored as above described.

We have found it convenient to discuss the results under a number of separate headings.

Osmotic Fragility

The effect of storage on the behaviour of cells towards anisotonic solutions has received attention from a number of workers. Bagdassarov (1937) was the first to show that for blood stored in sodium citrate the erythrocyte fragility increased gradually from the 3rd day of storage. From the 8th-10th day of storage the increase was found to be rapid. Macdonald and Stephen (1939) using I.H.T.* as anticoagulant found very little change in the red cell osmotic fragility until the 8th-9th day when there was " an abrupt and definite increase of fragility." By the 12th day partial lysis was present even in 0·8 per cent. NaCl, and it was concluded that at the end of a month there may be complete lysis in all but isotonic solutions of salt. Lipp and Hubbard (1940) on two specimens of 500 ml. of blood received into 50 ml. of 2·5 per cent. sodium citrate found increased fragility to be apparent on the 5th day of storage and to increase gradually for 3 weeks, by which time it had become very marked. Dubash, Clegg and Vaughan (1940) found a gradual increase in the fragility of red cells stored in saline-citrate solution (1·05 per cent. sodium citrate in 0·85 per cent. sodium chloride : 180 ml. saline-citrate+ 360 ml. blood). In two samples of blood, Kolmer and Howard (1940) using 2·5 per cent. sodium citrate in the proportions of 12·6 ml. citrate solution with 90 ml. blood (final citrate concentration 0·31 per cent.) found a slight increase in erythrocyte fragility after storage for 3 days, but noted little further increase during the following 21 days. In respect of

* I.H.T. (referred to as citro-seroid in Canadian and American literature) is an anticoagulant solution of sodium chloride 7·0 g., sodium citrate 5·0 g., potassium chloride 0·2 g., magnesium sulphate 0·04 g. in 1000 ml. distilled water. It is mixed with an equal volume of blood and according to Maizels and Whittaker (1939) is isotonic with fresh human erythrocytes.

the latter observation, this report is in conflict with the results reported above and with our own.

Alterations in erythrocyte fragility have been used as the basis of methods designed to show the effect of different anti-coagulants in varying concentrations upon the storage of the cells (Kolmer and Howard 1940, Wilbrandt 1940, Dubash, Clegg and Vaughan 1940, Willenegger and Ottensooser 1940). Following the original observation of Rous and Turner (1916) all workers agree that the addition of glucose retards the changes in fragility. A final glucose concentration of 1·0-1·5 per cent. is generally considered satisfactory (see, however,

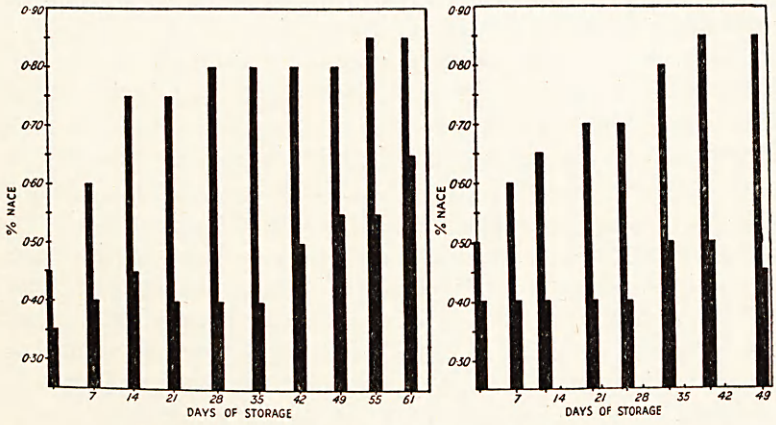

FIG. 1.—The effect of storage on osmotic fragility.

The former taller column of each pair indicates the concentration of salt solution at which hæmolysis was present: the latter smaller column the concentration at which hæmolysis was complete.

the Army Manual on Resuscitation, which recommends a final glucose concentration of 0·25-0·5 per cent.). Dextrin 2·83 per cent., starch (Maizels and Whittaker 1939), and sucrose 4·12 per cent. (Wilbrandt 1940) have been used for the same purpose.

The effect of storage on the osmotic fragility of human erythrocytes is shown in Fig. 1, which represents the results obtained in two typical experiments. The effect upon the point at which hæmolysis commences is marked and occurs within the first week of storage. By the end of the second week of storage hæmolysis is produced by very slight alteration in osmotic pressure (represented by the difference between

0·85 and 0·75-0·70 per cent. NaCl). The point at which hæmolysis becomes complete, however, remains remarkably constant for the first four weeks of storage, after which progressive alterations occur, the effect of which is that hæmolysis becomes complete with higher concentrations of salt. The range of salt concentration over which hæmolysis occurs increases within the first week of storage and does not decrease again until some 30-40 days after the blood has been withdrawn. These observations may be interpreted in cytological terms as indicating that during the first week of storage the weaker cells (from an osmotic point of view) rapidly become weaker, whereas the more resistant cells maintain their resistance to osmotic changes for some 30-40 days. These results are surprising in view of earlier observations (Crosbie and Scarborough 1941) which showed that the development of spherocytosis occurred uniformly, all cells being affected at approximately equal rates. The two results taken together seem to suggest that not all spherocytes behave in the same way towards anisotonic media. Alteration in the resistance of the cellular membranes to changes in osmotic pressure may be revealed in other ways. For example, by assessing in a quantitative manner the amount of hæmolysis at each concentration of salt solution and expressing this as a percentage of the value when 100 per cent. of the cells are hæmolysed, we are able to express graphically a relation between saline concentration and the percentage of cells hæmolysed at each concentration (*cf.* Creed 1938, Dacie and Vaughan 1938). From such a graph it is possible to obtain the mean corpuscular fragility (M.C.F.), viz., that percentage of saline at which 50 per cent. of the cells hæmolyse. According to Whitby and Britton (1939) this is an index of no practical value in ordinary hæmatology. However, it may be conveniently used to demonstrate alterations in the osmotic fragility of the average cell during storage, whereas the method previously illustrated (Fig. 1) determines the effect of storage on the extremes of the cell population—the weaker cells becoming weaker rapidly, the stronger cells remaining apparently unaffected for a considerable time. Fig. 2 illustrates the uniform changes which occur in the M.C.F. of four different bloods during storage for 32-45 days. The changes are gradually progressive during the first 25-35 days of storage, at which time an alteration of osmotic pressure represented by a change

from 0·85-0·80 per cent. NaCl is sufficient to hæmolyse 50 per cent. of the cells.

A third method of investigating alteration in osmotic fragility is by observing the changes in behaviour of cells subjected to a fixed concentration of sodium chloride. For this purpose a 0·50 per cent. solution is used and the cells remaining after one hour's contact with this solution are counted, the number being used as an expression of fragility. It is clear that in this method only the more resistant cells are counted: it differs, however, from the method first described in that it includes more than the most resistant cells in the

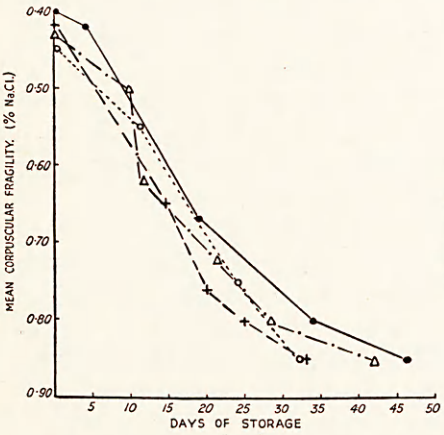

FIG. 2.—The effect of storage on mean corpuscular fragility (M.C.F.).

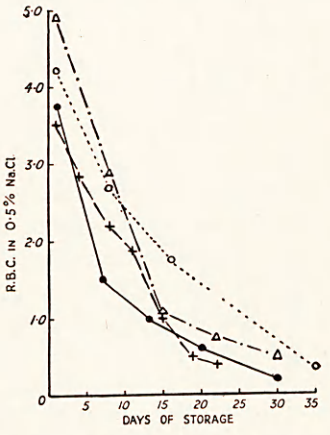

FIG. 3.—The effect of storage on erythrocyte fragility in 0·5% NaCl.

specimen. Fig. 3 illustrates the results obtained with four different bloods by this method. It is evident that an increasing fragility occurs progressively during the first four weeks of storage.

It will be clear from the foregoing experiments that a significant proportion of the erythrocytes in blood which has been stored for more than about a week is readily susceptible to alteration in the ionic constitution of the fluid environment. It has been pointed out in this connection by Dr Maizels that erythrocyte counts on stored blood are liable to be fallacious unless special precautions are taken to ensure that the diluting fluid is isotonic, and he has accordingly recommended that their own supernatant plasma is the only safe diluent for the red cells of stored blood during their enumeration. This point

has been referred to by Dubash, Clegg and Vaughan (1940), who found that erythrocyte counts made with normal saline diluent on six bloods stored for periods of between 22 and 43 days gave significantly lower results as compared with counts made with citrated plasma as the diluent. The divergence was found to increase with increasing age of the blood. They

TABLE I

Relation between Erythrocyte Counts in Various Diluents with reference to Length of Storage of Blood

No. of Blood and Group.	Age of Blood.	3·8% Na Citrate.	Hayem's Solution.		0·9% NaCl.		Fresh Plasma.	
	Days.	Count.	Count.	% Fall.	Count.	% Fall.	Count.	% Fall.
1430 A	2	3·36	3·32	1	3·35	0	3·28	2
1404 O	8	3·51	3·41	3	3·24	8	3·07	12
1359 A	11	3·26	3·06	6	3·04	7	3·16	3
2506 O	7	4·32	—	—	3·81	11	—	—
G. 1 O	8	4·62	—	—	4·57	1	—	—
2506 O	13	4·24	—	—	4·04	5	—	—
1294 A	14	3·47	3·37	3	2·98	14	3·50	0
1299 A	14	3·09	2·88	7	1·90	38	3·07	1
G. 2 O	16	4·40	—	—	4·31	2	—	—
2411 O	18	3·63	3·28	9	3·28	9	3·43	5
2506 O	20	4·41	—	—	3·57	19	—	—
2506 O	28	4·25	—	—	3·77	11	—	—
1156 O	34	3·00	2·80	7	1·43	52	2·95	2
1161 O	34	2·14	—	—	1·54	29	1·95	9
G. 3 O	37	4·06	—	—	4·09	0	—	—
N.S. I ?	42	5·53	5·02	6	—	—	3·65	34
N.S. II B	42	4·53	4·12	9	—	—	2·09	34
2506 O	47	3·79	—	—	3·50	7	—	—
1072 A	84	3·25	3·29	0	3·19	2	2·58	21
300 O	252	3·78	3·24	14	1·40	63	2·92	23
284 O	252	5·41	5·39	0	3·14	42	4·49	17
1006 O	252	2·72	2·85	0	1·95	26	2·14	21
803 O	252	2·83	2·36	16	2·08	26	2·06	27
751 AB	280	3·73	3·05	18	1·00	73	1·20	68
733 AB	280	3·79	3·68	3	1·20	68	1·22	68

make the point that all previous data on red cell counts in stored blood must, therefore, be viewed with suspicion. Attention was directed to this point in a previous communication (Crosbie and Scarborough 1941) in which it was stated that dilution with Hayem's solution introduced no serious error in the erythrocyte count. In this paper we present evidence to support this statement. In Tables I and II will be found erythrocyte counts on 37 different bloods stored for periods

ranging between 2 and 413 days. The counts have been made on dilutions made simultaneously with 3·8 per cent. sodium citrate, Hayem's * solution, 0·9 per cent. sodium chloride, fresh

TABLE II

Relation between Erythrocyte Counts in Various Diluents with reference to Length of Storage of Blood

No. of Blood.	Age of Blood.	3·8% Na Citrate.	Hayem's Solution		0·9% NaCl.		Fresh Plasma.		Own Plasma.	
	Days.	Count.	Count.	% Fall.	Count.	% Fall.	Count.	% Fall.	Count.	% Fall.
3424	2	4·26	3·82	11	4·06	5	3·84	10	3·92	8
3406	5	3·66	3·72	0	3·29	10	3·60	2	3·47	5
1430	9	3·80	3·70	4	3·80	0	3·74	3	3·30	15
1490	12	3·48	3·23	8	2·94	16	3·47	0	3·11	11
1404	15	3·80	3·75	2	3·60	5	3·70	3	3·70	3
1359	18	3·40	3·19	7	2·39	30	3·47	0	3·34	3
1430	23	3·75	3·40	9	2·93	22	3·60	4	3·65	3
1404	29	3·97	3·92	2	2·90	27	1·35	66	3·95	1
1359	32	4·03	3·97	2	2·98	27	3·56	12	3·87	4
2460	165	2·94	2·62	11	0·43	86	2·10	29	2·16	26
1728	213	4·24	4·22	1	0·16	96	—	—	3·20	25
803	413	2·10	1·83	13	0·55	74	1·05	50	0·72	65

TABLE III

Depression of Freezing Points (Tonicity) of Solutions
[Δ *in degrees Centigrade*]

Solution.	*p*H.	Δ.
Fresh, citrated (1 in 10) human plasma .	7·40	−0·564
Sodium chloride 0·9% . . .	6·71	−0·545
Sodium citrate 2·1% . . .	7·42	−0·398
,, 2·8% . . .	7·44	−0·518
,, 3·8% . . .	7·42	−0·678
Hayem's Solution	7·46	−1·057

citrated plasma of the same group, and supernatant plasma. It will be seen that the highest counts are obtained with 3·8 per cent. sodium citrate as diluent. It is clear from Table III (see

* The Hayem's solution used in these experiments had the following composition :—

Mercuric chloride (A.R.) . . . 0·25 g.
Sodium chloride (A.R.) . . . 0·50 g.
Sodium sulphate (pure anhydrous) . 2·50 g.
Distilled water . . . . . 100 ml.

discussion later) that the tonicity of Hayem's solution exceeds that of 3·8 per cent. sodium citrate and is almost twice that of 0·9 per cent. NaCl; yet dilution with Hayem's solution introduces no appreciable error for periods up to at least 150 days, whereas dilution with 0·9 per cent. saline becomes unsatisfactory as early as the 10th-14th day of storage. It is suggested that the mercuric chloride (0·25 per cent.) in Hayem's solution may exert a fixative action on the red cells. These results evidently dispose of criticism which has been directed towards figures published in our previous communication, No. VI. (See J. W. Mollison in *Bulletin of War Medicine*, Vol. II, No. 1, Sept. 1941). Both fresh citrated plasma of the same group and their own supernatant plasma appear to be inferior as diluents to 3·8 per cent. sodium citrate and Hayem's solution.

In a personal communication Dr Maizels offers the suggestion that the superior results with 3·8 per cent. sodium citrate are due to its alkalinity (*p*H 7·42) relative to supernatant plasma (*p*H approx. 6·8), moderate alkalinity tending to shrink the red cell and prevent hæmolysis. When blood is stored for some weeks the supernatant plasma becomes faintly acid. Dilution of the remaining erythrocytes with this faintly acid plasma in the red cell pipette may well produce further swelling and hæmolysis. However, the difference between counts with 3·8 per cent. sodium citrate and fresh citrated homologous plasma cannot be explained on this basis since the *p*H of both diluents was 7·4 (by experiment). That this difference becomes progressively greater as the blood is stored is indicated in Fig. 4, which shows also that appreciable hæmolysis—that is to say, over 10 per cent. of the transfused cells hæmolysed—may be expected merely from the dilution of the stored cells with the fresh plasma of the recipient. This effect is not likely to be important with blood stored for under 30 days (Fig. 4).

The differences in erythrocyte counts obtained with 3·8 per cent. sodium citrate, 0·9 per cent. sodium chloride and Hayem's solution as diluent involve considerations as to the tonicity of these solutions with respect to the human red cell. Following the practice of Fahraeus, Westergren and others, it has been widely accepted that 3·8 per cent. sodium citrate is isotonic with human erythrocytes. In point of fact, 3·8 per cent. sodium citrate is hypertonic to fresh human erythrocytes and,

accordingly, citrate-blood mixture (1 part+9 parts) is also hypertonic. This statement is supported by the observations of Ham and Curtis (1938), Maizels and Whittaker (1940), by our own finding of a low P.C.V. which increased during storage (Crosbie and Scarborough 1941), observations on crenation quoted later in this paper, and by freezing-point determinations (see below). As the result of cell volume determinations with fresh human erythrocytes Maizels and Whittaker (1940) conclude that a concentration of sodium citrate of 2·1 per cent. (*p*H 7·8) is isotonic. In a personal

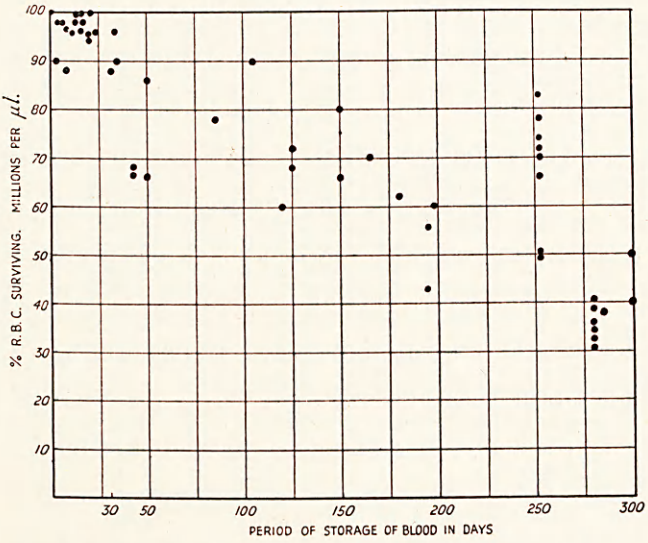

FIG. 4.—The effect of storage on percentage of erythrocytes surviving in fresh, homologous, citrated plasma diluent relative to 3·8% sodium citrate at *p*H 7·4.

communication Dr Maizels gives a more recent figure of 2·2-2·3 per cent. The question of determining what concentration of sodium citrate is isotonic with fresh human erythrocytes is a difficult one and has not yet been answered with finality. As Dr Maizels points out, the *p*H as well as the salt concentration is of importance. Criticism can be directed towards both the hæmatocrit and the freezing-point methods of investigation, neither of which is ideal. Ponder (1934) in fact draws a sharp distinction between an " isotonic " and an " isoplethecontic " solution. The latter is a solution characterised by its property of maintaining the volume of the cells suspended in it relative to their normal medium. For example,

the oxalate mixtures advocated by Heller and Paul and by Wintrobe and Landsberger for corpuscular volume determinations may be designated isoplethecontic. They are not isotonic. Finally, it may be stated that it is not yet known whether the storage of human erythrocytes in isotonic solution is advantageous.

In order to throw fresh light on the matter, and believing that freezing-point determinations afford the most accurate method of obtaining the required information, we have obtained the data set forth in Table III. The freezing-point determinations were carried out through the courtesy of Dr T. R. Bolam by Mr T. Sheddan, to whom the authors' thanks are due.

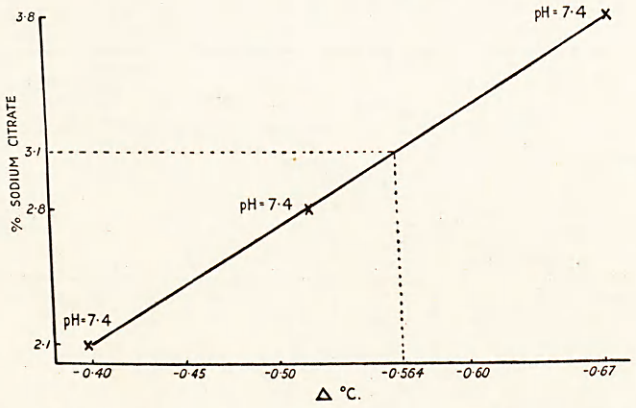

FIG. 5.—Relation between depression of freezing point (Δ) and concentration (percentage) of sodium citrate in solution.

It is evident from Table III that 0·9 per cent. NaCl was found to give a slightly lower depression of freezing point than plasma. 2·1 per cent. sodium citrate was found to be significantly hypotonic and 3·8 per cent. sodium citrate definitely hypertonic. The latter solutions were at *p*H 7·4, the *p*H of fresh, normal, citrated plasma. If the values for these citrate solutions (at *p*H 7·4) be plotted in the form of a graph against the salt concentration (Fig. 5) the result is a straight line, from which it may be found that the concentration of sodium citrate which will give the same depression of freezing point as fresh citrated normal plasma is 3·1 per cent. (at *p*H 7·4). This concentration of citrate is, therefore, on the basis of this method, isotonic with plasma and, consequently, with fresh human erythrocytes. Hayem's solution is evidently grossly

hypertonic at approximately the same *p*H. As has already been mentioned, it is considered that it may exert a fixative action on the red cells.

Mechanical Fragility

A property of the erythrocytes at least as important as their ability to withstand alterations in the osmotic pressure of their fluid environment is the power of maintaining their integrity when subjected to the mechanical influence of shaking. This property may be referred to as their mechanical fragility. It may be estimated in the following way: 2 ml. of blood are pipetted into a tightly stoppered test tube having a capacity of 2·5 ml. The tube is then shaken in its long axis on

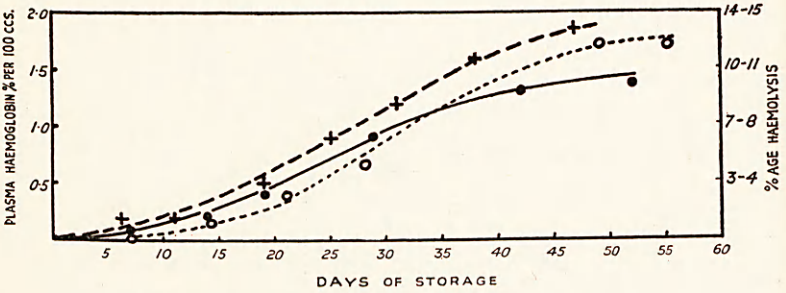

FIG. 6.—The effect of storage on mechanical fragility.

a mechanical shaker at a rate of 70 per min. for 2 min. At the end of this time the tube is centrifuged and the amount of hæmoglobin in the supernatant plasma is determined by the method of Newcomer. The results of such an investigation on three bloods are illustrated in Fig. 6, which shows the relationship between the period of storage and both the concentration of hæmoglobin in the plasma (on the left) and the percentage hæmolysis corresponding to this concentration (on the right). It is evident that on or about the 20th day of storage some 3-4 per cent. of the total hæmoglobin is liberated into the plasma by the experimental procedure; on or about the 30th day the percentage has risen to 7-8 per cent. There is remarkable uniformity between the three bloods examined: in our experience variations in mechanical fragility are more uniform than those of any other property of the cells. Comparison of these results with those previously described under the heading of osmotic fragility suggest that the erythrocytes

are considerably more susceptible to alterations in the ionic concentration of the fluid medium surrounding them than to the more mechanical influence of shaking.

Spontaneous Hæmolysis

When citrated blood is stored undisturbed at 4° C. the cell and plasma layers remain more or less distinct until hæmoglobin gradually diffuses upwards from the cellular layer into the plasma. Whether this phenomenon be due to the actual rupture of erythrocytes or to the diffusion of hæmoglobin from the cells is not at present known. It is referred to here as spontaneous hæmolysis and may be assessed in terms of the height of the column of extracellular hæmoglobin. Fig. 7

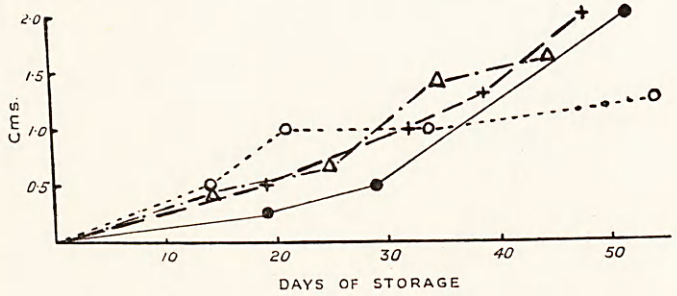

Fig. 7.—The effect of storage on spontaneous hæmolysis.

shows the development of typical changes in four different bloods. Spontaneous hæmolysis develops gradually within the first 10 days of storage and is appreciable by the 20th day. Thereafter further gradual increase occurs. The presence of spontaneous hæmolysis has been considered by some to preclude the specimens being used for transfusion. We have, however, used blood having 0·5-1·0 cm. of spontaneous hæmolysis with an effect which was apparently entirely beneficial.

Crenation

It is generally supposed that crenation is evidence of a decrease in volume of the erythrocytes and occurs in the presence of a hypertonic solution outside the cell. Ponder (1934), however, finds very little evidence to support this view. According to him crenation " is better regarded as a failure

on the part of the cell to maintain its special shape." Miller (1925) distinguishes two forms of crenation, one which is characterised by short coarse processes broad at the base, is found in hypertonic solutions : and a second, where the processes are much finer and where there is no distortion of the general form of the cell, which he regards as a prelude to the attainment of the spherical form.

When blood is mixed with 3·8 per cent. citrate in the proportion of 1 part of citrate solution to 9 of blood

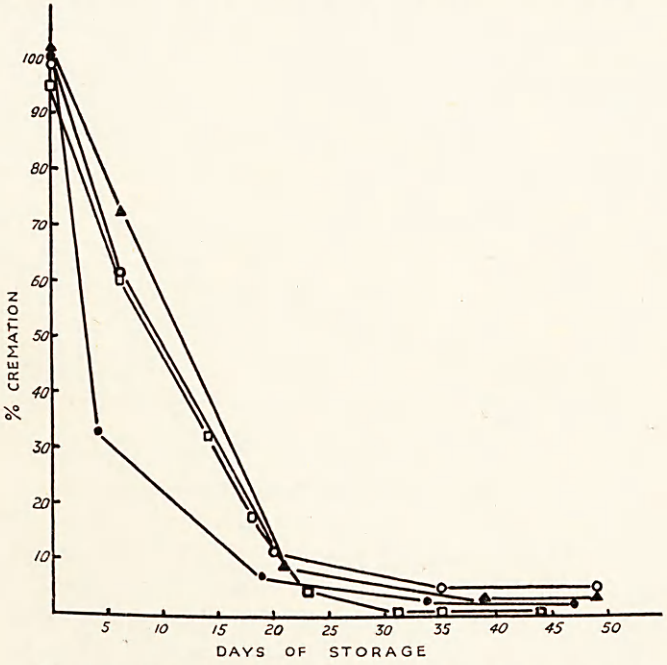

FIG. 8.—The effect of storage on crenation (Miller's first type).

crenation of the red cells very frequently, but not invariably, occurs. This crenation affects some 90-100 per cent. of the erythrocytes and gradually diminishes during the subsequent storage of the blood until about the 30th day it involves from 0-10 per cent. of the cells (Fig. 8). The appearance of the cells conforms to Miller's first type of crenation and the change is associated with a low cell volume which gradually increases during the first 20-30 days of storage (Fig. 4). (Crosbie and Scarborough 1941.) It is probable, therefore, that it may be taken as evidence of a hypertonic solution surrounding

the cells. Its gradual disappearance together with a gradually increasing cell volume we ascribe to alteration in the properties of the cell membranes consequent upon storage.

Sedimentation Rate

A retardation of the erythrocyte sedimentation rate during storage has previously been observed by other workers, for example, Dubash, Clegg and Vaughan (1940). Our own

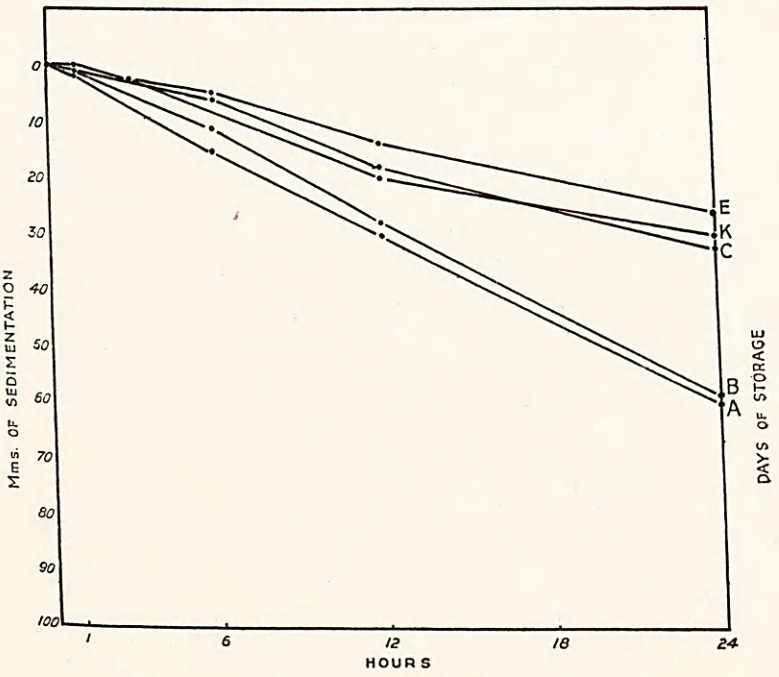

FIG. 9.—The effect of storage on erythrocyte sedimentation.

observations confirm these results (Fig. 9). That the slowing of the sedimentation rate of the erythrocytes is a property conferred upon them by the plasma in which they are suspended is demonstrated in Fig. 10, which shows the effect produced by the addition of old citrated plasma of the same group to fresh erythrocytes. In these experiments citrated blood was obtained and divided at once into two equal parts, both of which were centrifuged for 2 minutes. The supernatant plasma was removed as completely as possible from both specimens and measured. Four times this volume of fresh

citrated plasma obtained from the same subject immediately beforehand was added to one tube and an equal amount of citrated homologous plasma previously stored for 16 weeks to the other. The cells were then re-suspended in the added plasma and the sedimentation rate of the two cell suspensions was determined during the subsequent 24 hours. It is clear from Fig. 10 that the sedimentation rate of the cells suspended in stored plasma is markedly retarded in all three specimens.

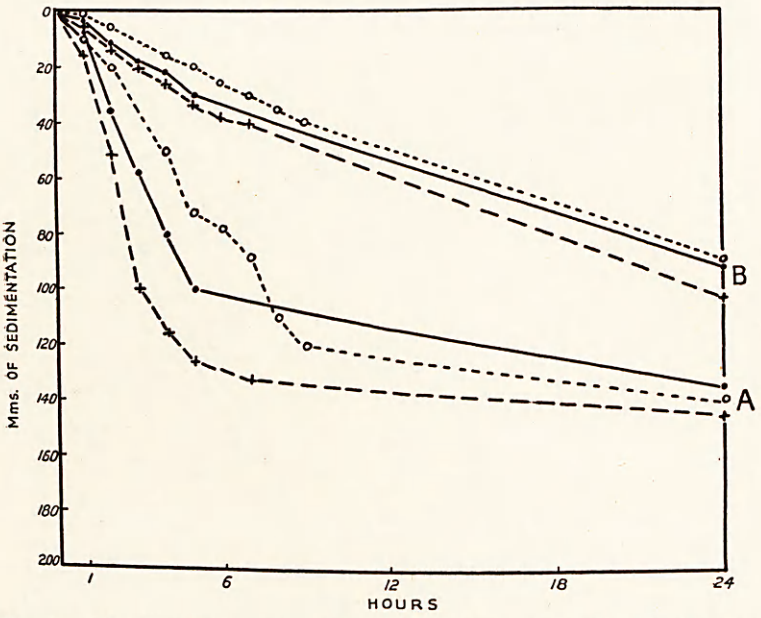

FIG. 10.—Showing the retarding effect on the sedimentation of freshly withdrawn erythrocytes of the addition to them of stored plasma.

The re-suspension of fresh cells typically discoidal in appearance in plasma which has been stored for any length of time produces within half an hour in these cells the appearance of spherocytosis and it is probably as a result of this change in form that the suspension stability of the specimen is increased. This change is not reversible. Ham and Curtis (1938) have shown that with discoidal cells, those with the larger mean corpuscular volume sediment more rapidly than smaller cells when suspended in samples of the same plasma or serum to similar cell concentrations, but Damashek (1939) has shown that spheroidal cells do not form rouleaux and such cells would, therefore, presumably not sediment so rapidly as

normal cells whatever their volume. Ponder (1934), however, who attributes rouleaux formation to the presence of a specific substance in the plasma, states that fresh cells added to plasma "several days old form rouleaux much as in fresh plasma." The alteration in the shape of fresh erythrocytes which takes place within half an hour after their admixture with old plasma is demonstrated in Figs. 11 and 12, which are three-dimensional photographs of blood films made from fresh human erythrocytes suspended in their own citrated plasma (Fig. 11) and

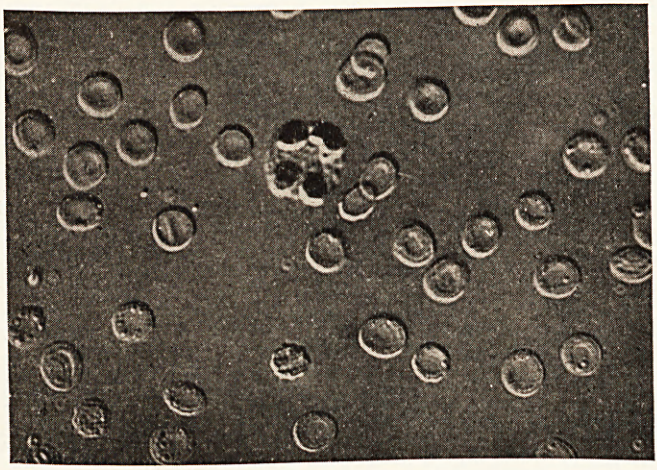

FIG. 11.—Three-dimensional photomicrograph (×1000) of stained film made from erythrocytes re-suspended in ×4 vols. of their own citrated plasma.

half an hour after the suspension of the same cells in plasma stored for 16 weeks (Fig. 12).

These facts are important in connection with the cause of the maintenance of the normal discoidal shape. During 30 mins. in homologous plasma which has been stored for 16 weeks, the component, which is responsible for the discoidal shape, gives way and the cells rapidly and uniformly assume the form which presents minimum surface for volume—the sphere. A suggestion which evidently arises is that fresh plasma contains a substance which is necessary for the proper maintenance by the red cells of their physiologically advantageous discoidal form, which substance disappears on storage. Alternatively, it is possible to attribute both changes to differences in the colloidal state and stability of the plasma. It is

of interest to consider this suggestion in relation to the statement of Damashek (1939) made relative to the controversy as to whether the spherocytes characteristic of certain naturally occurring blood disorders arise *de novo* from the bone-marrow or are produced in the peripheral blood by the action of unknown substances on the cell envelopes : " Further studies now in progress indicate that this tendency of red cells to become small, thick and spherical is dependent on the action of various hemolytic agents on mature erythrocytes, and not on an abnormal formation of red cells in the bone-marrow."

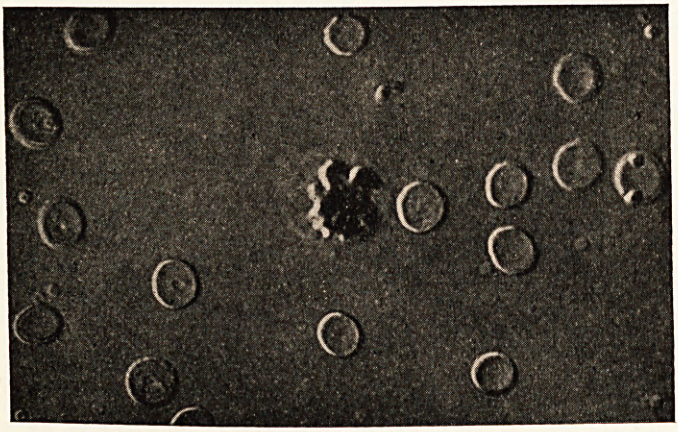

FIG. 12.—Three-dimensional photomicrograph (×1000) of stained film made from erythrocytes of same blood as Fig. 11, but taken ½-hour after their resuspension in ×4 vols. of homologous citrated plasma previously stored for 16 weeks. The cells in this film do not show the central depression nor the " granular " appearance at the edges. They are spherocytes.

(See also Damashek, Schwartz and Singer, 1939.) An alternative possible explanation for these results is that they are due to changes in *p*H in the stored plasma. This possibility has not been entirely excluded. Unfortunately, the *p*H of the plasma actually used in the above experiments was not determined. We have, however, not been able to find a change in the *p*H of stored citrated (unfiltered) plasma of sufficient magnitude to account by itself for the phenomenon. From a practical point of view it might be suggested that these results throw an element of doubt on the wisdom of transfusing large quantities of plasma rapidly. It is at any rate theoretically possible that such procedures might induce changes in the properties of the recipient's erythrocytes as a result of which

they become unduly fragile and actually rupture. We have, however, no knowledge of any such phenomenon occurring *in vivo*. It should be pointed out that the spherocytes which are characteristically found in stored blood are dissimilar to those associated with various hæmolytic syndromes because in the latter conditions the cell volume remains constant as the diameter decreases and the thickness increases (Damashek 1939), whereas in stored blood these changes are associated with an actual increase in volume (Crosbie and Scarborough 1941).

Since writing this paper we have discovered some observations of Ponder (1927) which record the development of the spheroidal state " in cells in plasma after some hours." According to Ponder, the change was not associated with increase in volume; it was considered to be irreversible and to be accompanied by a breaking apart of the cells so that no rouleaux were present. The latter statement appears to be inconsistent with that already quoted (Ponder 1934).

Finally, it may be of interest to record that the alterations in shape in fresh erythrocytes consequent upon their suspension in old serum were known to Hewson in 1773. " . . . on mixing serum (that had been kept three days in a warm place, and smelt putrid) with fresh-drawn human blood; the vesicle assumed this globular and mulberry-like appearance. In these experiments on human blood beginning to putrefy I have likewise observed some of these vesicles break into pieces, without becoming spherical." (Hewson 1773.) In connection with his observations on this phenomenon, Hewson asks the following question—" Whence is it that the serum has the property of preserving them (*i.e.* the ' vesicles ' or red cells) in that form which seems so necessary ? " and he answers this question by saying " It is principally by the salts of the serum that this effect is produced."

Summary

Further observations on the effect of storage on the erythrocytes of blood mixed with 3·8 per cent. sodium citrate in the properties of 9 parts of blood to 1 part of citrate have given the following results:

1. There is a gradual and fairly uniform increase in erythrocyte fragility during storage. The tonicity at which hæmolysis

commences rapidly decreases during the first week, but the tonicity at which hæmolysis becomes complete does not alter until after four weeks of storage. Mean corpuscular fragility and fragility in 0·5 per cent. saline are complementary (not additional) methods of investigation but show similar changes. After 25 days of storage the cells are so fragile that a change of tonicity from 0·85-0·80 per cent. NaCl is sufficient to hæmolyse 50 per cent. of the cells.

2. Both 3·8 per cent. sodium citrate and Hayem's solution are satisfactory diluents for the red cell count. There are objections to the use of plasma, whether supernatent or fresh.

3. Transfusion of stored blood will lead to hæmolysis of some of the cells as a result of their admixture with the recipient's plasma. This effect will not be important with blood stored for periods under 30 days.

4. On the basis of freezing-point determinations 3·1 per cent. sodium citrate is isotonic with fresh human citrated plasma and, therefore, with freshly withdrawn erythrocytes.

5. Alterations in the "mechanical fragility" of stored erythrocytes is gradual and remarkably uniform. On or about the 20th day of storage some 3-4 per cent. of hæmolysis is produced by the experimental procedure—fairly vigorous shaking for 2 mins.

6. Spontaneous hæmolysis becomes evident as early as the 10th day of storage and is always appreciable by the 20th day. Its presence is no contraindication to the use of blood for transfusion.

7. Crenation of the red cells up to 90-100 per cent. is found in blood obtained by the method used, but this almost disappears within the first 10-30 days of storage, during which time the corpuscular volume is increasing.

8. The erythrocyte sedimentation rate becomes progressively slower during storage. This change is believed to be due to the development of spherocytosis, which is, however, different from that form associated with certain naturally occurring hæmolytic processes in that the former is accompanied by an increased volume of the cells.

9. The development of spherocytosis and a retarded sedimentation rate can be noted within half an hour of the suspension of fresh erythrocytes in stored homologous plasma.

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CORRIGENDA

Fig. 1.—" % NACE " should read " % NaCl."

Fig. 6.—At left of figure should read—
" Plasma hæmoglobin grammes per 100 c.c."

Fig. 8.—Read " % crenation."

Fig. 9.—A = 1 day of storage.
B = 7 days of storage.
C = 14 days of storage.
E = 28 days of storage.
K = 87 days of storage.

FIG. 10.—A refers to fresh plasma.
B to stored plasma.