

THE PRESERVATION OF WHOLE BLOOD¹

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The purpose of this paper² is to present the results of experimental work on the preservation of whole blood *in vivo* and *in vitro* carried out over a period of several years. Certain standards of preservation *in vitro* (the osmotic resistance) and *in vivo* (the serum bilirubin variations) have been carried out extensively in the early as well as in the late experiments, thus affording a means of comparison.

More specifically, it will present results obtained from comparative studies of several preserving solutions employing the radioactive iron technic.

The conclusions from early experimental work (1) can be summarized as follows:

(1) The absence of spontaneous hemolysis is not a satisfactory proof of good preservation of erythrocytes of stored blood.

(2) The fragility of erythrocytes to hypotonic salt solution is a dependable measure of blood preservation and parallels the *in vivo* studies provided that the preserving solution does not contain dextrose in high concentration.

(3) Serum bilirubin determinations before and after transfusion are readily available and constitute a reliable index of the survival of transfused erythrocytes.

(4) The determination of the total bile pigment is a good means of measuring post-transfusion destruction of erythrocytes.

(5) The safe period of preservation at 4° C. of blood collected in plain sodium citrate solution is not over 5 days.

(6) Experimental work has shown that the addition of glucose to sodium citrate solution does increase the period of safe preservation of whole blood (2 to 6) but generally not enough to compensate for the disadvantage of the greater dilution of the plasma proteins.

(7) The administration of blood collected in a solution with a high volume of water and relatively high glucose content (4, 6) has been found to be followed often by an increase in serum bilirubin even when the blood is fresh. This bilirubinemia does not increase noticeably for the first few days of storage, suggesting that it probably has to do with the immediate effect of glucose on a certain type of cells, the least resistant, possibly either the youngest or the oldest.

In the present work the radioactive iron technic was applied to the study of whole blood preservation along with other means.

RADIOACTIVE IRON TECHNIC

The iron isotope 55, with a half life of 5 years, was used in these experiments. This material was synthesized into ferric ammonium citrate in neutral aqueous solutions. Two donors were selected for preparation with radioactive iron. They were both healthy young white males belonging to the "O" blood group, Rh positive and with low anti-A and anti-B titre. Each received 12 intravenous injections of 10 ml. of radioactive ferric ammonium citrate at 2- to 3-day intervals over a 4-week period. The preparation of the iron isotopes used in this work and the radioactivity of blood samples were carried out by Gibson *et al*, as a cooperative plan outlined elsewhere (7). At the end of this period, one donor (SD-1) had a unit activity of 15.35 and the second (SD-2), a unit activity of 15.8.

The interpretation of the results is based on the assumption that as long as the cell membrane remains intact there is no interchange of radioactive iron between the cells and plasma, therefore the cells become "tagged" for the duration of their life.

¹ This paper is the 19th of a series on blood, plasma, and plasma substitutes from the Laboratory of Clinical Pathology of the Bryn Mawr Hospital. The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Bryn Mawr Hospital, and with the aid of the Bryn Mawr Research Foundation.

² To avoid useless repetitions, references to previous work and to work published in this series of papers have been kept to a minimum.

At the end of the 12 intravenous injections, the radioactivity titre of the donor blood was high enough to permit bleeding and transfusing into recipients.

Technic of bleeding, division into aliquots, and storage

The donors were bled of 400 to 500 ml. into the solution to be tested. This was precooled and by mechanical means maintained in constant gentle motion during collection and for 3 minutes after completion of bleeding. The blood immediately after mixing was divided by a closed system into a number of aliquots, of approximately equal size, and these were stored at 4° C. except for 1 specimen, which was used for immediate transfusion.

Administration of blood

The aliquots from each donor were administered to different recipients. The recipients used were all hospital patients known to have a normal erythropoiesis. All were convalescing and ranged in age between 18 and 86.

Proper grouping, Rh typing, and cross matching were carried out in all instances. Blood volume measurements, hemoglobin, and hematocrit determinations were also carried out. All recipients were Rh positive.

The transfused blood was well mixed by gentle rotation before and during the administration. The rate of transfusion was maintained at about 5 ml. per minute, the whole procedure requiring 24 to 30 minutes. The amount of blood given to the various recipients was measured volumetrically by calibration of each bottle before and after transfusion. All the blood remaining in the tubing and filter was washed into the recipient by saline solution immediately following the blood transfusion. The amount of blood remaining in the bottle, usually amounting to 10 ml. was carefully measured after removal with measured amounts of saline solution and used for radioactivity measurements. The exact number of packed red cells given was also determined by this method.

Samples of blood were taken from each of the recipients within 1 minute of the completion of the transfusion, ½ hour and 3 hours after transfusion, and twice daily for 5 days and at the end of the seventh day. These samples were withdrawn with new syringes and needles and without stasis. The blood was drawn into a double oxalated tube, and the hematocrit was determined. The red cells were then "laked" with 30 ml. of distilled water and used for radioactivity measurements. The patient's blood volume was measured with the dye method (8 to 11) and also calculated from the unit activity of the donor's blood and the unit activity of the first sample after transfusion.

The cell survival was calculated from the unit activity of the donor's blood, the volume of cells given, the unit activity of the recipient's blood, and the volume of the recipient's blood cells.

Citrate-glucose solution (McGill No. 1)

The 2 radioactive donors were bled of about 400 ml. into 200 ml. of McGill solution No. 1. This latter solution was prepared by using 80 ml. of a 3.2 per cent solu-

tion of sodium citrate and 120 ml. of a 5.4 per cent dextrose solution. These 2 solutions were sterilized separately and mixed in the proper proportion before use in the standard donor bottles.

The results are expressed as per cent of cell survival against time (see Figure 1). The survival of erythrocytes using the fresh blood appeared to be optimal in recipient SR-1. In recipient SR-2, however, a drop of 10 per cent of the transfused blood apparently occurred in the first 24 hours with further cell loss in the first 7 days to a total of about 16 per cent. Erythrocytes from blood stored at plus 4° C. for 10 days appear to be definitely less resistant. An average of 50 per cent of the cells appear to be lost from the circulation within the first 24 hours. This confirms findings obtained by the determination of the serum bilirubin and total bile pigment. The state of preservation of erythrocytes from blood stored for 20 and 30 days appears to be extremely poor. Eighty per cent or more of the erythrocytes from 4 samples appears to be destroyed within the first 3 hours.

No untoward reactions were encountered in any of the transfusions given.

A.C.D. solution (Rapoport)

The formula suggested by Rapoport is as follows:

Sodium citrate (2H ₂ O)	1.66 grams
Citric acid (H ₂ O)	0.59 gram
Distilled water	100.00 ml.

To 90 ml. of this solution 22.5 ml. of 15 per cent dextrose were added in the collection bottles and the mixture was sterilized by autoclaving for 20 minutes at 120° C.

Four hundred and fifty ml. of the donor's blood were collected in the above A.C.D. solution according to the method described above. The blood was then divided by a closed system into 4 aliquots of approximately 150 ml. each.

The 4 aliquots from each donor were transfused into 4 human recipients at the following intervals of time: immediately, 10 days, 20 days, and 30 days in one case; and immediately, 10 days, 21 days, and 31 days in the other.

The results of this experiment are summarized in Figure 2.

With fresh blood, in the first 24 hours after transfusion, recipient SR-9 showed a drop of 10 per cent of the transfused blood and recipient SR-13 showed a drop of 6 per cent. After 10 days of storage, recipient SR-10 showed a 10 per cent drop in 24 hours and SR-14, a 9 per cent drop. With blood stored 20 and 21 days respectively, SR-11 showed a drop of 61 per cent in the first 24 hours and SR-15, a drop of 58 per cent. At the end of 30 and 31 days of storage, in recipient SR-12 there was a drop of 89 per cent in the transfused cells and in recipient SR-16, a drop of 85 per cent in the first 24 hours following transfusion.

The preservation of erythrocytes with this A.C.D. solution is good for a period of storage up to 10 or 11 days. The erythrocyte survival after 20 to 21 days' storage is very poor.

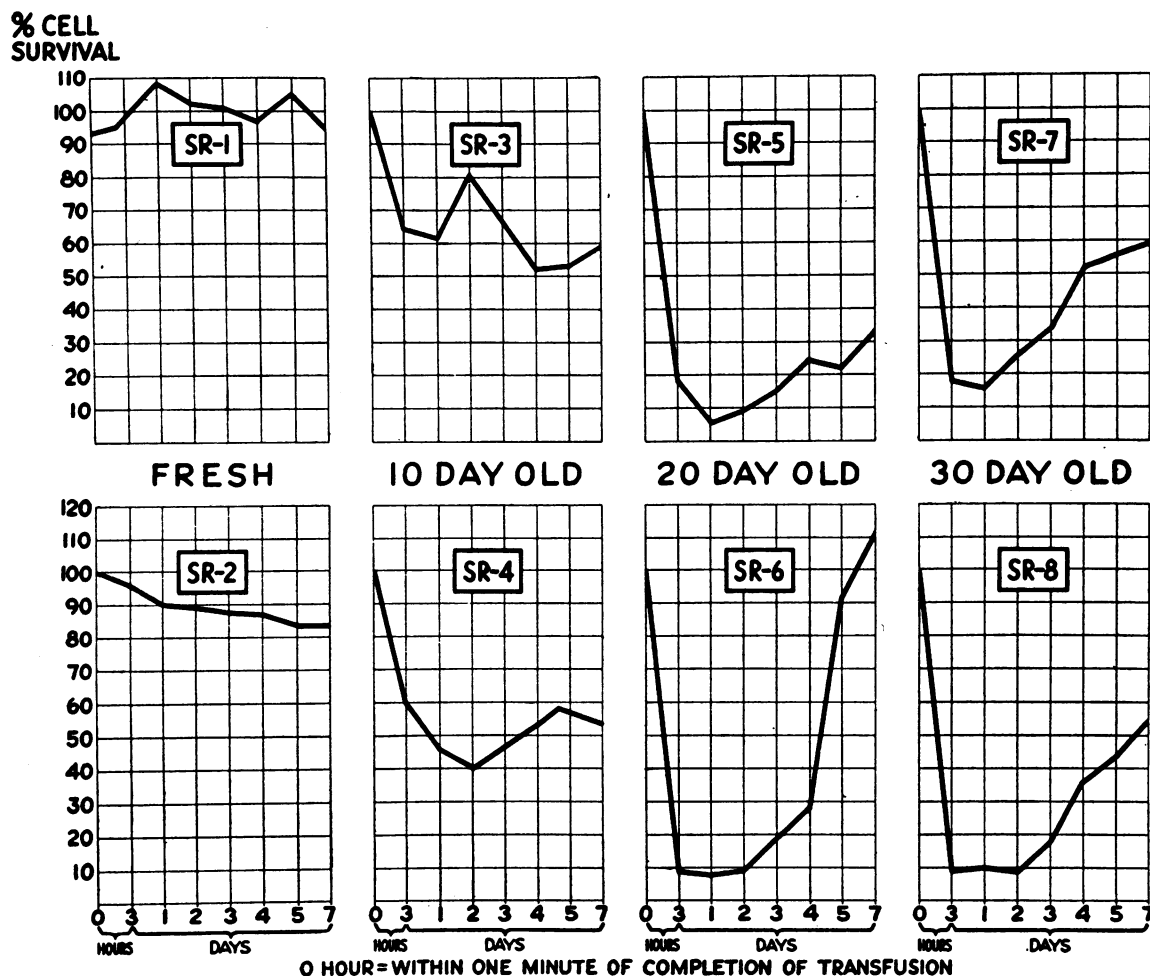


FIG. 1. POST-TRANSFUSION SURVIVAL OF ERYTHROCYTES DETERMINED BY RADIOACTIVE IRON. WHOLE BLOOD PRESERVED IN CITRATE-GLUCOSE SOL. WITHOUT BUFFER (MCGILL SOLUTION NO. 1)

Citrate-glucose solution (Baxter-Alsever)

The citrate-glucose solution (Baxter-Alsever) was furnished by the Baxter Company ready for use. The formula is as follows:

Trisodium citrate	0.8 per cent
Dextrose	2.05 per cent
Sodium chloride	0.42 per cent
Water	500 ml.

pH 6.0

Four hundred and forty-three ml. of the donor's blood were placed in 500 ml. of this solution. The blood was then stored at plus 4° C. for 13 days. At the expiration of this period of time the blood was thoroughly mixed and divided into 2 aliquots of approximately equal size. The blood was then administered immediately to 2 recipients. After 13 days of preservation, the survival of the erythrocytes transfused was 70 per cent and 64 per cent respectively. In both recipients hyperbilirubinemia occurred.

Recipient SR-17 was a male, aged 54, convalescing from a subtotal gastrectomy, weight 60 kgm., height 170 cm. This patient received 132.8 ml. of packed erythrocytes and the survival measured with the radioactive iron technic showed within 3 hours of transfusion a drop of 21 per cent, indicating the destruction of 27.8 ml. of packed erythrocytes. The serum bilirubin before transfusion was 0.7 mgm. per cent, 3 hours after transfusion it rose to 1.0 mgm. per cent, and after 7 hours to 1.8 mgm. per cent; 24 hours after transfusion it had returned to normal, 0.7 mgm. per cent (see Figure 3).

Recipient SR-18 was a female, aged 47, convalescing from skin grafting, weight 71.8 kgm., height 162.5 cm. This patient received 107 ml. of packed erythrocytes and the survival measured with the radioactive iron technic showed a loss of 19 per cent of the transfused erythrocytes at the end of 3 hours, indicating a loss of 20.3 ml. of packed red cells. The patient's serum bilirubin before transfusion was 0.5 mgm. per cent; 3 hours after, it was 1.3 mgm. per cent; 7 hours after, it was 0.7 mgm. per

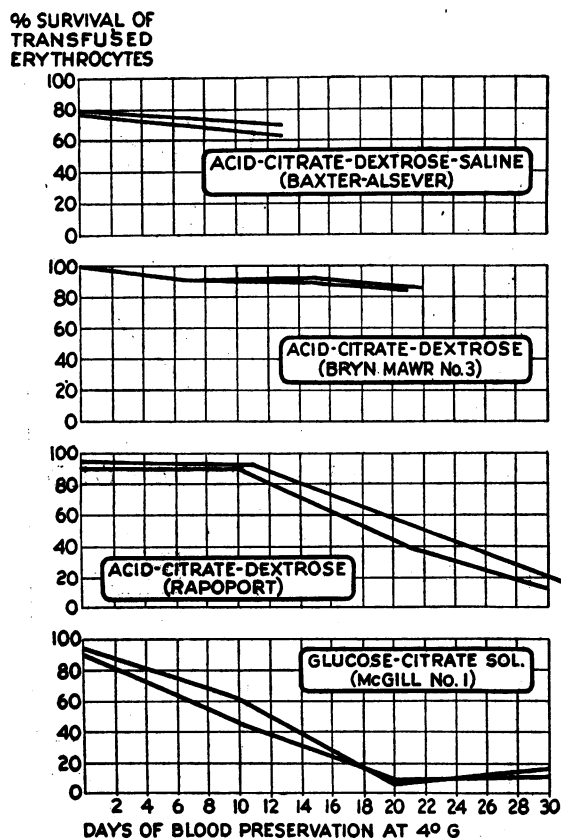


FIG. 2

cent; and 24 hours after, it was 0.8 mgm. per cent. As control, a third recipient SR-20 received a comparable amount of blood preserved in B.M. A.C.D. No. 3 for the same period of time. The radioactive iron study showed a good preservation with a destruction within 3 hours of transfusion of less than 5 ml. of packed erythrocytes. There was no significant change in this patient's serum bilirubin level.

It can be said in conclusion that with the Baxter-Elsever solution optimal preservation of whole blood is less than 13 days, probably not more than 1 week.

A.C.D. solution (Bryn Mawr No. 3)

The formula is as follows:

Sodium citrate (trisodium dihydric)	2.1 grams
Citric acid (monohydric)	0.66 gram
Dextrose (anhydrous)	2.0 grams
Water	100.0 ml.

75 ml. of this mixture contains:

Sodium citrate	1.6 grams
Citric acid	0.50 gram
Dextrose	1.5 grams

Five hundred ml. of blood from the same 2 donors were collected in 75 ml. of this solution according to the above

technic. Each sample was divided into 4 equal aliquots by a closed system and stored at plus 4° C. until ready for use.

The 4 aliquots from each donor were transfused according to the technic described into 4 human recipients at varying intervals of time: immediately, 7 to 8 days, 14 to 15 days, and 21 to 22 days.

The recipients selected for use were similar to those used above, and their ages varied from 37 to 79.

The results are summarized in Figure 2.

With the injection of fresh blood there appears to be no immediate loss of cells. After 7 to 8 days, the loss is 10 per cent; after 14 to 15 days of preservation, the loss remains at 10 per cent; and after 21 to 22 days, it is only 16 per cent.

DISCUSSION

The radioactive iron technic as applied to the study of whole blood preservation has given results comparable to those previously obtained from the study of the serum bilirubin level before and after transfusion and from the measure of the total bile pigment output.

The data show that with the addition of dextrose to sodium citrate and with variable amounts of water to obtain a final dextrose concentration in the plasma of 1.45 per cent (McGill No. 1) to 1.28 (Baxter-Elsever), the period of preservation is increased over that obtained with plain citrate solution. The increased period of preservation is in any case relatively small. If the standard of optimal preservation of 90 per cent and 70 per

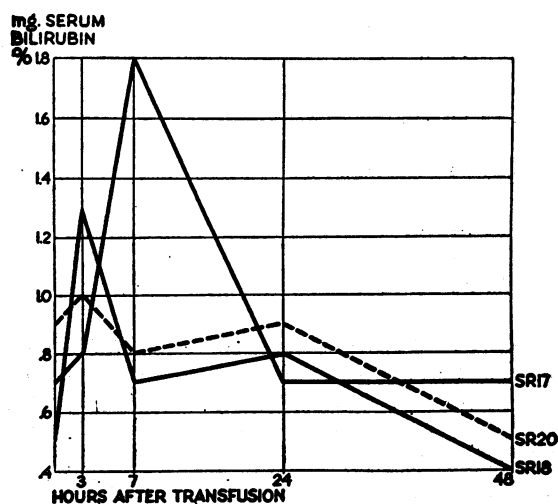


FIG. 3. HYPERBILIRUBINEMIA OCCURRING IN PATIENTS RECEIVING BLOOD PRESERVED FOR THIRTEEN DAYS IN A CITRATE-GLUCOSE SOLUTION (BAXTER-ELSEVER)

cent minimal is adopted, McGill solution No. 1 is good only for about 5 days and the Baxter-Elsever for approximately 12 to 14 days.

The acid citrate-dextrose solutions tested gave a far better preservation. The solution with less glucose (.4 per cent Bryn Mawr No. 3) gave a distinctly better preservation than the solution with more glucose (.88 per cent Rapoport).

In comparing the maximal length of satisfactory erythrocyte preservation with various solutions obtained by the different observers using a comparable technic, it will be noted that the results obtained by our group at Bryn Mawr Hospital are generally indicative of a shorter survival of the erythrocytes after transfusion. This is most likely due to the fact that at the Bryn Mawr Hospital all recipients were convalescent patients, whereas in most other studies the recipients were normal adult males. The implication of a more rapid erythrocyte destruction is borne out by other observations, particularly the higher total bile pigment output in diseases.

In addition to the means of study mentioned, we have repeatedly attempted to follow the fate of transfused red cells by means of the Ashby technic (12) as modified by Denstedt (13). The results which we have obtained are very difficult to interpret. The major difficulty, encountered by other experimenters, is due to the fact that the count of non-agglutinable cells varies a great deal. We have noted that non-agglutinable cells seem to disappear from the circulation suddenly and for irregular periods of time; their reappearance is usually at an unpredictable level. Under these circumstances, to draw a line and to interpret the slope of such line as meaning progressive loss of the transfused cells appears to us to be somewhat arbitrary. We have not been able to correlate the findings obtained with the technic of Dr. Denstedt with the results obtained with the studies of the bilirubin level following transfusion and with the results obtained from the study of the total bile pigment output in the days immediately following the transfusion nor with the results obtained with the radioactive iron technic. We are, therefore, not reporting in this paper the results of the

studies on cell preservation using the non-agglutinable cell technic.

CONCLUSIONS

A.C.D. solutions are a better blood preservative than citrate or glucose-citrate solutions. With the best formulas optimal preservation can be secured for at least 22 days.

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