THE MEASUREMENT OF POST–TRANSFUSION SURVIVAL OF PRESERVED STORED HUMAN ERYTHROCYTES BY MEANS OF TWO ISOTOPES OF RADIO–ACTIVE IRON 1

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The evaluation of the efficacy of whole blood transfusion in augmenting total red cell volume has always been of interest to physicians called upon to care for patients with hemorrhage, burns, blood dyscrasias, or traumatic shock. The establishment of blood banks, resulting in an increasing use of stored blood, has focused more attention on the problem. Military requirements have created a demand for the preservation of whole blood over far longer periods than are required for civilian purposes. The urgent need for better preservative solutions and for the selection of the best conditions for overseas air transport of whole blood for the Armed Forces made it imperative that an accurate method of measuring the post-transfusion survival of stored human erythrocytes be available.

The morphological and chemical changes that take place in red cells during storage have been studied by several laboratory methods. The rate of spontaneous hemolysis, changes in cell dimension, changes in the permeability of the cell membrane, changes in osmotic resistance to hypotonic solutions of NaCl, rate of diffusion of potassium, and disturbances in carbohydrate metabolism have all been proposed as in vitro tests for the evaluation of the ability of stored red cells to survive after transfusion.

Each of these tests assuys only changes in one functional characteristic of the erythrocyte and it is for this reason that the opinion has been expressed that in vitro tests fail as a guide to the viability of stored blood (1). When several of these techniques have been applied in conjunction, they have served a useful purpose in screening suggested preservative solutions for further evaluation. But, until the changes demonstrated by in vitro studies are proved to reflect truly the degree of red cell deterioration, the final evaluation of any preservative must be based on the results of in vivo post-transfusion survival studies.

The simplest in vivo method is the measurement of the recipient's red cell count, hemoglobin, or hematocrit before and after transfusion. In uncomplicated anemia or blood loss this procedure does give some idea of the improvement in red cell volume, but in hemolytic anemias or in the presence of continuing hemorrhage the interpretation of data is difficult or impossible. It has been our experience that the hematocrit and hemoglobin level of venous blood, as well as the red cell count from finger blood, vary greatly in random samples from a given individual, depending upon the degree of venous stasis incident to venipuncture, temperature of extremities, and the general circulatory state of the subject. This variation may be as great as the net increase in hematocrit, hemoglobin, or red count expected to result from a single 500-ml. transfusion.

The increase in circulating red cell volume resulting from one or more transfusions may be measured by the dye-plasma volume hematocrit technic with a fair degree of accuracy. A transfusion of 500 ml. of whole blood contains about 200 ml. of erythrocytes. This quantity is about 10 per cent of the normal human circulating red cell volume (2). In hemorrhage or blood loss the percentage of transfused to recipient circulating red cells varies directly with the degree of cell volume...
deficit. A single transfusion will rarely represent more than 30 per cent of the pre-transfusion erythrocyte volume.

Plasma volume can be measured by the dye method within ±5 per cent. An additional error is introduced by unavoidable variations in the hematocrit determination on venous blood samples, upon which the red cell volume is calculated. Circulating red cell volume measured by the dye-plasma volume hematocrit technic is greater than the true volume (3, 4, 5), because the hematocrit of capillary blood is less than that of blood in large vessels (6, 7). This error may be minimized by correcting the calculated cell volume by the factor 0.85. Hence, the intrinsic error of the technic, which is about ±5 per cent, may be the equivalent of a large fraction of the cells transfused. It is desirable to follow the fate of stored cells at frequent intervals after transfusion, but the dye method gives only data for a single period, and repeated blood volume determinations at short intervals are not always practicable.

The occurrence of hemoglobinuria or hemoglobinemia following a compatible transfusion is, of course, evidence of massive destruction of donor or recipient cells. Hemolysis produces a rise in recipient serum bilirubin levels. Strumia has shown a progressive hyperbilirubinemia with increasing age of stored blood (8). Gilligan and Altschule (9) found large variations in the net rise in plasma hemoglobin levels following the intravenous administration of hemoglobin solutions, indicating wide individual differences in the ability to handle pigments derived from blood. Bilirubin data are misleading in patients with liver disease or hemolytic tendencies. The measurement does not permit quantitative evaluation of cell destruction.

The agglutination method of following the fate of transfused cells was introduced by Ashby in 1919 (10, 11). Whole blood from a group O donor is given to a group A recipient. The transfused O cells are, of course, of all different ages and represent mixed-age cells. Samples of recipient blood are taken and the A cells agglutinated with anti-A typing serum and thrown down by centrifugation. The unagglutinated O cells remaining in suspension are then counted with standard blood counting pipettes and chambers. The count in a sample drawn at the completion of the transfusion or shortly thereafter is usually taken as the quantity of O cells in circulation resulting from complete retention of all the transfused cells. Counts upon subsequent samples are referred to this "100 per cent value" for calculation of the percentage of surviving O cells. The method has been widely used in the study of red cell preservation by Mollison (12), Maizels (13), Denstedt (14), and more recently by Thalhimer (15).

Scrutiny of the results obtained by these workers shows that the non-agglutinable counts on samples taken only a few days apart may vary by as much as 20 per cent (14, 15), even when the greatest possible care is taken. This suggests a large intrinsic probable error in the method.

All workers report that a certain proportion of the recipient A cells are not agglutinated by anti-A sera, even though of very high titre. In practice this non-agglutinatable cell count is determined prior to transfusion and subtracted from subsequent counts. The assumption is that this non-agglutinatable portion of recipient cells remains a constant throughout the observation period, which in some instances has been as long as 130 days. Such an assumption may or may not be valid.

It is assumed that a complete separation of the unagglutinated O from the agglutinated A cells is effected by the addition of serum and subsequent shaking and centrifugation. It is, however, possible that numbers of the O cells may become enmeshed in the large A agglutinates and carried down therewith in centrifuging.

It has been suggested (14) that in instances in which there is an abrupt rise in cell count from the previously prevailing slope of cell disappearance, some of the transfused cells may have been temporarily withdrawn from circulation (in the spleen or liver), and later have re-entered the circulation. This hardly seems likely, since it is improbable that there are any great "depots" of red cells in the body but rather that in the normal state practically all the erythrocytes are in active circulation at all times (7).

The agglutination technic permits one to follow the presence of transfused O cells throughout their entire life span in the recipient, information that cannot be obtained by the radioactive technic.

A method of measuring post-transfusion survival of human erythrocytes by means of radioactive isotopes of iron was first described by Ross and Chapin in 1943 (16). The modifications de-
Five hundred ml. of whole blood from a radioactive donor was taken in 4 per cent sodium citrate and divided into 2 equal parts. One aliquot was transfused on the day drawn, and the other given to the same recipient after refrigerated storage for 7 days. The Ua of the recipient's red cells following the first transfusion indicates good retention of the tagged cells for 1 day and a slight loss on the second day. After the second transfusion the recipient Ua rose, but not to twice the level resulting from the first transfusion. Since both aliquots contained the same amount of radioactivity, some of the tagged cells from the second transfusion disappeared from the circulation during the administration of the blood. Only about $\frac{1}{2}$ of the 7-day old cells were viable as shown by the fall in recipient Ua through the twelfth day. The subsequent rise in Ua is due to re-utilization of iron from non-viable cells in hemoglobin synthesis.

scribed herein were developed from our studies in measuring circulating red cell volume in shock. The physical principles involved, the characteristics of the iron isotopes used, the apparatus for radioactivity detection, as well as the experimental techniques of using tagged human erythrocytes for cell volume determination have been described previously (17, 18). For the purposes of this study a brief resumé will suffice.

When small amounts of radioactive ferric ammonium citrate (0.5 to 1.5 mgm.) are given intravenously, the salt is rapidly removed from plasma and deposited in body iron stores (18). In the process of erythrocyte formation in the marrow, some of the iron atoms that become incorporated in the hemoglobin molecule of developing cells are radioactive. Since hemoglobin does not escape from the healthy erythrocyte, it follows that as long as the tagged red cells remain intact, their presence may be detected in the blood stream. If these cells are destroyed, the released radioactive iron returns from the plasma to the iron stores, and eventually is re-utilized and resynthesized into hemoglobin, again appearing in new red cells in the blood stream. Thus the immediate retention (24 to 48 hours) of stored tagged cells can be determined, and the eventual utilization of the iron from those cells that did not survive can subsequently be measured.

Data obtained in the first experiment of this type which we carried out are shown in Figure 1. Following a transfusion of fresh radioactive whole blood (drawn in 4 per cent sodium citrate), the recipient's cell Unit Activity (Ua) remained constant for 24 hours, followed by a slight fall from

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2 Defined as counts per minute (cpm) per ml. of red cells referred to cpm of a radioactive iron standard measured at the same time, and therefore independent of the rate of radioactive decay of the active isotope and counter variability.
the initial level, indicating that about 90 per cent of the transfused cells were retained. On the eighth day a transfusion of week-old cells from the same blood donor, and equal in amount to the first, was given. There was a rise in Ua to almost, but not quite, twice that resulting from the first infusion. During the next 5 days Ua progressively dropped, leveling off at a value corresponding to a retention of about 50 per cent of the cells given in the second transfusion. Thereafter Ua rose to about 75 per cent of the original level. Since the only source of radioactive iron in the subject was the transfused red cells, it follows that about \( \frac{1}{2} \) of the iron released from destroyed cells was re-utilized by the end of the observation period.

This experiment brings out a fact of importance. The level to which Ua rose immediately following the second transfusion was less than twice the level following the first transfusion even though both contained an equal amount of radioactivity. It is, therefore, evident that the initial post-transfusion activity level following the second transfusion did not correspond to 100 per cent retention, but that some of the tagged cells were withdrawn from circulation while the blood was being administered. Thus it is essential to supplement this simple procedure with an independent method of determining the radioactivity level corresponding to 100 per cent retention of the transfused tagged red cells.

It is therefore necessary to determine the recipient's circulating cell volume at the time the transfusion is given. In some of our experiments this was determined by the dye-plasma volume hemocrit technic. The calculated cell volume was corrected by the factor 0.85 which we have found to be the average ratio of the values for the true cell volume measured by radio-iron and the cell volume calculated from the dye-plasma-hemocrit (5). Post-transfusion survivals calculated upon these values probably are accurate to within \( \pm 5 \) per cent.

In many experiments, the recipient's pre-transfusion red cell volume was more accurately measured by a small infusion of fresh group O cells tagged with Fe\(^{55}\). The preserved transfused cells were tagged with Fe\(^{59}\). Both isotopes can be measured in the same blood sample (17).

The transfused quantity of preserved stored cells tagged with Fe\(^{55}\) and the radioactivity of those cells is measured. Knowing the recipient's red cell volume the radioactivity level corresponding to 100 per cent retention of the transfused cells is calculated by the equation

\[
Ua_T = \frac{C \times UaD}{Vrr_1 + Vrr_2}
\]

where

- \( Ua_T \) is the 100 per cent value of the unit activity Ua.
- C is the quantity of cells transfused in ml.
- \( UaD \) is the unit activity of those cells, and
- \( Vrr_1 \) is the recipient's pre-transfusion red cell volume in ml. as measured by Fe\(^{59}\).
- \( Vrr_2 \) is the quantity of stored cells transfused.

The percentage of radioactive cells in the recipient's circulation at any time in terms of the quantity transfused therefore is

\[
\frac{Ua_R \times 100}{Ua_T}
\]

where \( Ua_R \) is the recipient's red cell radioactivity level.

A typical experiment in which recipient cell volume was measured by radio-iron is shown in Figure 2. The subject was a normal 22-year-old male, blood group A, who had been bled 1 day prior to transfusion. On the morning of the experiment plasma volume was determined by the dye method. At the same time, radio-iron red cell volume was determined, the subject receiving 44.8 ml. of cells drawn as whole citrated blood from a group O donor who had been built up with Fe\(^{59}\). Three venous blood samples were drawn at 10-minute intervals after the injection of dye and tagged cells for measurement of plasma dye concentration and red cell radioactivity. Four hours later the subject received 160 ml. of cells tagged with Fe\(^{55}\), drawn as whole blood in Alsever's solution and stored 19 days.

Venous blood samples were then drawn at 20 minutes and approximately 1, 4, and 8 hours after the end of the transfusion of stored blood. Two blood samples were taken on both the first and second day, and 1 sample on the third, fourth, and fifth day after transfusion, and a final sample was taken on the twenty-eighth day. All of these samples were analyzed for both Fe\(^{59}\) and Fe\(^{55}\). Data obtained are given in Protocol GR–78 and are plotted in Figure 2.
Fig. 2. Method of Measuring Post-transfusion Survival of Stored Human Erythrocytes by Means of 2 Isotopes of Radioactive Iron

The recipient's pre-transfusion circulating red cell volume was determined by giving 44.8 ml. of freshly drawn group O cells tagged with Fe\(^{59}\) and found to be 2,140 ml. Then 160 ml. of group O cells tagged with Fe\(^{59}\) and stored 19 days were given. The resultant expected red cell volume was 2,300 ml. The theoretical recipient red cell Ua corresponding to 100 per cent retention of the Fe\(^{59}\) cells was 0.497. The lowest recipient Fe\(^{59}\) Ua value was 0.129, and survival was computed at 26 per cent.

Recipient's pre-transfusion red cell volume computed from the quantity of Fe\(^{59}\) cells given, the Ua thereof, and the average Fe\(^{59}\) Ua value of the first 4 recipient's blood samples, is

\[
\frac{44.8 \times \text{Ua Fe}^{59} (1.58)}{0.0332} = 2,140 \text{ ml.}
\]

This agrees well with the calculated corrected dye-plasmina-hematocrit red cell volume of 2,110 ml.

The total quantity of recipient circulating red cells is equal to the red cell volume plus the amount of cells transfused, or 2,140 ml. + 160 ml. = 2,300 ml.

The recipient's 100 per cent retention value Fe\(^{59}\) Ua then becomes

\[
\frac{160 \times \text{Ua Fe}^{59} (7.1)}{2,300} = 0.497.
\]

This value, 0.497, is higher than that of the first post-transfusion sample, 0.418, indicating a loss of about 16 per cent of the transfused cells in the 50-minute interval between starting the transfusion and drawing the first sample.

The lowest recipient Fe\(^{59}\) red cell Ua \(^{3}\) during the first post-transfusion day is used for calculating percentage survival, which is

\[
\frac{0.129}{0.497} = 26 \text{ per cent.}
\]

It will be noted that the Fe\(^{59}\) Ua values of the

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\(^{3}\) No correction for blood loss in sampling was made since the total whole blood drawn, after the transfusion of stored cells, was about 150 ml. or about 3 per cent of the recipient's total blood volume, an average of less than 1 per cent per day.
RED CELL SURVIVAL in vivo: RADIO-IRON METHOD

Protocol Exp. No. GR-78

December 7, 1944

POST-TRANSFUSION SURVIVAL OF WHOLE BLOOD IN ALSEVER'S SOLUTION TRANSFUSED 19 DAYS AFTER DRAWING

Subject: J. G.  
Age: 28.  
Sex: male.  
Height: 183 cm.  
Weight: 77.2 kgm.

Plasma volume: 3,430 ml.  
Hct.: 42.1 per cent.  
Total blood vol.: 5,920 ml.  
Red cell vol.: 2,490 ml.  
× 0.85 = 2,110 ml.

### Procedure

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Procedure</th>
<th>Unit activity</th>
<th>Ratio</th>
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<tbody>
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<td>12/7/44</td>
<td>9:45 a.m.</td>
<td>10 mgm. Evans Blue i.v.</td>
<td>Fe55</td>
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<tr>
<td></td>
<td>9:46-</td>
<td>108 ml. whole blood</td>
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<td></td>
<td>9:48</td>
<td>(44.8 ml. cells) i.v.</td>
<td>a</td>
<td>1.580</td>
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<td></td>
<td>9:59</td>
<td>Venous sample</td>
<td>b</td>
<td>0.0340</td>
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<tr>
<td></td>
<td>10:10</td>
<td>Venous sample</td>
<td>c</td>
<td>0.0320</td>
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<td></td>
<td>10:19</td>
<td>Venous sample</td>
<td>d</td>
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<td>1:28 p.m.</td>
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<td></td>
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<tr>
<td></td>
<td>1:28-</td>
<td>575 ml. blood at hct. 28 per cent =</td>
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<td>7.10</td>
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<td></td>
<td>1:56</td>
<td>160 ml. cells i.v.</td>
<td>f</td>
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<td></td>
<td>2:18</td>
<td>Venous sample</td>
<td>g</td>
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<td></td>
<td>3:25</td>
<td>Venous sample</td>
<td>h</td>
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<td>Venous sample</td>
<td>j</td>
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<td>Venous sample</td>
<td>l</td>
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<td></td>
<td>0.0318**</td>
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<td></td>
<td>12/12/44</td>
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</tr>
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</table>

* Part of sample lost in processing.  
** Ua Fe55 is average of values for samples e-o, incl., omitting sample j.

Recipient red cells remained constant, within the limit of error of the technic, throughout the first 5 days of the experiment, indicating virtually complete retention of these group O tagged cells. The slight fall in Ua samples d and e is to be explained by the additional dilution of the Fe59 tagged cells by the transfused Fe55 cells.

The ratio of Ua Fe55 to Ua Fe59 falls progressively during the first 24-hour post-transfusion period and thereafter rises. Since this ratio is an expression of the proportion of Fe55 to Fe59 tagged cells in the circulation and since virtually all of the Fe59 tagged cells were retained, this ratio can also be used for computing percentage of survival.

Thus, \( \frac{4.1}{15.6} = 26.2 \) per cent.

The rise of the ratio after the first post-transfusion day is due to the presence of radio-iron derived from non-viable transfused cells tagged with Fe55.

Figure 3 shows the course of the radioactive red cell level of a subject receiving "tagged" whole blood in McGill II solution, stored under refrigeration for 29 days prior to transfusion. Unit activity data are expressed in terms of percentage of total cells transfused Ua/Ua70. A large proportion of the cells had deteriorated during storage and their removal from the blood stream was reflected by a sharp decline in recipient red cell radioactivity in the first few hours after transfusion, falling to about \( \frac{1}{2} \) of the calculated 100 per cent retention value. During the following day this level was unchanged, but thereafter successive samples showed a rise in radioactivity, at first rapid, but leveling off at about 70 per cent of the 100 per cent value.

The question arises as to whether or not those cells which were not rapidly removed from circulation were normally functioning cells with normal life expectancy and remained in circulation throughout the observation period. Denstedt (14) and more recently Thalhimer (15) have shown that when the post-transfusion course of stored cells is followed by the agglutination technic there may be an initial abrupt fall in the recipient's group O cell count, followed by a decreased rate of disappearance, the slope of which closely ap-
proximates that obtained for transfused fresh group O cells, somewhere in the neighborhood of 1 per cent per day. This conclusion was confirmed by 2 experiments in which the fate of group O cells tagged with radioactive iron (Fe$^{55}$) transfused into group A recipients was followed by both the agglutination and radio-iron techniques.

Two such experiments, in which equal aliquots of blood drawn from the same donor into De Gowin’s solution were transfused into individual recipients, one on the day of collecting, and the other after storage for 24 days, are illustrated in Figure 4.

Following the transfusion of fresh blood, a prompt initial loss of about 10 per cent of the cells was demonstrated by both methods. Thereafter the number of radioactive cells in circulation increased, reaching the 100 per cent value on the thirtieth post-transfusion day, whereas the count of non-agglutinable cells progressively fell until, the sixtieth day, somewhat less than 40 per cent were detectable in the blood stream. A much greater initial loss occurred in the subject receiving the 24-day-old blood. The immediate (24 to 48 hour) survival was about 40 per cent by the radio-iron and about 70 per cent by the agglutination technic. This discrepancy is probably to be accounted for by a considerable destruction of cells during the interval between the beginning of the transfusion and the taking of the first blood sample for non-agglutinable count. Radioactivity data revealed that 60 per cent of cells had disappeared from the circulation in the first 6 hours after transfusion started. Since the initial post-transfusion count was used as the denominator in calculating percentage of transfused cells remaining in circulation, the computed immediate survival was falsely high.

After the initial loss, a continuous rise in the recipient’s red cell radioactivity occurred, reaching a level of about 90 per cent of the 100 per cent value on the fortieth post-transfusion day. During this period the percentage of non-agglutinable cells fell at a rather constant rate.

In the case of both the fresh and the deteriorated blood, however, after the initial drop, the non-agglutinable cells disappeared at a rate of about one per cent per day, or at a mortality rate equal to that of normal cells.

The increase in radioactivity of recipient’s cells after the initial decline is due to the presence of tagged cells newly developed since the transfusion. About 80 per cent of the transfused cells were destroyed and their contained iron may be considered as available for re-utilization. The curve of regeneration flattens out at about 50 per cent of the quantity of radioactive cells destroyed during the first day, and hence it is concluded that 5/6 of the total radio-iron from destroyed cells was eventually re-utilized and the remainder either excreted or stored.

The assumption that the retained cells accounted for an almost constant portion of the total recipient cell radioactivity appears to be warranted in this particular experiment, because only young red
Radioactivity levels clearly show the initial loss of cells and subsequent re-utilization of hemoglobin-derived iron. Non-agglutinable cell counts show an initial loss of non-viable group 0 cells, and thereafter a continued loss, the rate of which closely approximates the normal red cell mortality rate.

It is important to ascertain whether the behavior of all donor cells, ranging in age from newborn to senescent, was similar during storage. The work of Denstedt (21) and Thalhimer (15), in whose studies the survival of cells representing the entire age population of donor erythrocytes was measured, indicates that the percentage of non-viable cells is roughly proportional to the number of days elapsing between drawing and transfusion. It was therefore necessary to distinguish, radioactively, between young cells and cells that were known to be of mixed age. The known fact of re-utilization of hemoglobin-derived iron was used to advantage to tag differentially young and mixed-age cells.

For example, one donor received the first injection of 5-year iron at 133 days and the last injection 95 days prior to bleeding. He had also received 47-day iron, the first injection 28 days and the last injection 5 days prior to bleeding. Thus, all of the donor's cells tagged with Fe$^{59}$ were young cells, whereas the cells tagged with Fe$^{55}$ were representative of the entire cell population.

Blood from another donor prepared in a similar manner was drawn into acid-citrate-dextrose, and equal aliquots were transfused into 3 recipients after storage for 15, 29, and 41 days, respectively.
Blood from this donor was drawn into ACD, the plasma was removed and the cells resuspended in an acid-citrate-dextrose medium. Aliquots were transfused into 4 recipients after 3, 10, 16, and 30 days' storage at 4°C.

Red cell volume for determination of the 100 per cent retention value was calculated from plasma volume and hematocrit, and percentages of survival were calculated from both the Fe\textsuperscript{55} and Fe\textsuperscript{59} recipient blood levels. In both experiments, as shown in Table I, the survival of the young (Fe\textsuperscript{59})

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Days of storage & Day since donor received radio-iron & Percentage survival & Relative retention of mixed-age to young cells\textsuperscript{*} \\
& Fe\textsuperscript{55} & Fe\textsuperscript{59} & Mixed-age (Fe\textsuperscript{55}) & Young (Fe\textsuperscript{59}) \\
& First & Last & First & Last & \\
\hline
Whole blood in acid-citrate-dextrose & & & & & \\
0 & 179 & 153 & 30 & 6 & \\
15 & 192 & 168 & 45 & 87 & 100 & 0.87 \\
29 & 208 & 182 & 59 & 35 & 52 & 59 & 0.88 \\
41 & 230 & 194 & 71 & 47 & 37 & 42 & 0.88 \\
\hline
Cells resuspended in acid-citrate-dextrose & & & & & \\
0 & 133 & 95 & 28 & 5 & \\
3 & 136 & 98 & 31 & 8 & 87 & 100 & 0.87 \\
10 & 143 & 105 & 38 & 15 & 81 & 93 & 0.87 \\
16 & 149 & 111 & 44 & 21 & 78 & 83 & 0.94 \\
30 & 163 & 125 & 58 & 35 & 43 & 46 & 0.93 \\
\hline
\end{tabular}
\caption{The comparative survival of young and mixed-age red cells tagged with radio-iron}
\end{table}

The use of 2 tracers also permits, under selected conditions, of measurements of changes in the recipient's own cells. In the experiment described in Figure 5, the recipient, blood group A, received 560 ml. of group O blood (220 ml. of cells) of high "anti-A" titre (1/400). Fe\textsuperscript{55} tagged group O cells for cell volume determination were given shortly before, and the value obtained agreed very closely with that calculated from the Fe\textsuperscript{55} recipient levels. During the next 7 days the recipient Fe\textsuperscript{55} and Fe\textsuperscript{59} levels both rose progressively and to about the same degree. At the same time the venous hematocrit progressively fell. It is clear that the proportion of the recipient's cells that were radioactive became greater. Since both lots of group O cells were well retained, as evidenced by the constancy of the ratio of the 2 isotopes, it follows that the rise in radioactivity was due only to destruction of the recipient's own A cells. Calculations based on the original cell volume and the changes in Fe\textsuperscript{55} levels showed a loss of about 700 ml. of A cells. (Calculations based on hematocrit changes showed a loss of only 500 ml.) During the remainder of the observation period the hematocrit rose, and both radioactivity levels declined to almost the original levels, but the ratio of the two isotopes again remained constant. These later changes reflect the regeneration of recipient A cells.

We have applied the method described to the study of a large number of solutions, recommended both for the preservation of erythrocytes as whole blood and as red cell resuspensions after removal of plasma. Results will be reported in subsequent communications.

The method has also been placed at the disposal of several collaborators working in the field of blood preservation. They have built up their own donors with radio-iron prepared in the M.I.T. cyclotron, and donor and recipient blood samples, forwarded by mail, have been chemically prepared and analyzed for radioactivity in our laboratories. Since the cells are destroyed in wet-ashing, the packed cells can be packed prior to shipping. Time in transit or temperature changes do not affect the accuracy of radioactivity measurements.

A standard system of mnemonic symbols for samples of donors' stored and recipients' bloods was developed to facilitate reporting of data to
collaborators. Because all workers used a common technic, differences in method were reduced to a minimum, and results obtained have been truly comparable.

**CONCLUSIONS**

(1) A method of measuring the post-transfusion survival of preserved stored human erythrocytes by means of 2 radioactive isotopes of iron is described.

(2) The method is specific in that only intact circulating cells containing radioactive iron bound in the hemoglobin molecule are detected.

(3) The determination of the recipient's pre-transfusion circulating red cell volume by means of fresh group O cells tagged with Fe⁵⁵ permits of accurate calculation of recipient post-transfusion red cell radioactivity level corresponding to 100 per cent retention of transfused stored cells tagged with Fe⁵⁵. This is important in the study of deteriorated bloods in which red cells may be withdrawn from circulation in the interval between starting the transfusion and obtaining the first post-transfusion blood sample.

(4) The extent to which iron derived from non-viable transfused stored cells is re-utilized in the synthesis of new hemoglobin can be measured.

(5) The destruction of the homologous cells of patients of group A and B by high anti-A or anti-B titre donor bloods of group O is discussed.
BIBLIOGRAPHY


