The Measure of Erythropoiesis in Anemias. II. The Immediate and Subsequent Loss of Transfused Erythrocytes in Healthy Subjects

By Max M. Strumia, Ann Dugan and Louise S. Colwell

With the technical aid of Helen Munroe

The study of survival of transfused, compatible red cells, or of autotransfused red cells, has become of general use as a hematologic procedure since the introduction of the Cr\textsuperscript{51} tagging method.\textsuperscript{1} In the determination of red cell survival there is a tendency to ignore the immediate loss of the transfused cells, by taking as 100 per cent value the radioactivity measurements obtained at arbitrary periods of time, ranging from 15 minutes to 24 hours, following transfusion, as summarized by Aufderheide.\textsuperscript{2}

It will be shown, in accordance with findings of other investigators, that a portion of the radioactivity of the transfused cells is rapidly lost from circulation. Additionally, it will be shown that (1) the loss of radioactivity represents an actual loss of transfused cells; (2) when the transfused cells are damaged by prolonged or improper storage, the first 24 hour loss is in excess of that occurring with fresh undamaged cells, but the rate of loss in subsequent days is similar or smaller than that which occurs with normal cells.

METHODS

The procedures for evaluation of the survival of transfused red cells by the differential agglutination method (Ashby) and by Cr\textsuperscript{51} tagging have been previously described.\textsuperscript{3,4} Unless otherwise noted, blood was transfused within one hour of collection in ACD solution.

In our studies the 100 per cent survival of Cr\textsuperscript{51} tagged red cells was calculated as follows:

\[
100 \text{ per cent survival} = \frac{\text{Counts/minute in transfused cells}}{\text{Total red cell volume (ml.)}}
\]

and the apparent survival:

\[
\text{Apparent per cent survival} = \frac{\text{Counts/minute/ml. of whole blood} \times \frac{100}{\text{hmc}} \times 100}{100 \text{ per cent survival}}
\]

The red cell volume was determined with the Cr\textsuperscript{51} tagged red cell radioactivity dilution method when the transfused cells are fresh and undamaged. In all other instances the red

From The John S. Sharpe Research Foundation and the Laboratory of Clinical Pathology of The Bryn Mawr Hospital, Bryn Mawr, Pa.

This work was carried out with the aid of a grant from the National Advisory Heart Council of the National Institutes of Health, and with funds from the Army Medical Research and Development Board.

Submitted July 25, 1961; accepted for publication Oct. 17, 1961.

cell volume was determined independently by the measure of the plasma volume with the T-1824 dye and the determination of the hematocrit, with a correction factor of 0.93. It has been our experience that when the measure of the blood volume is carried out simultaneously by dilution of radioactivity of transfused Cr\textsuperscript{51} tagged red cells in healthy individuals and by the T-1824 method, the results are within 5 per cent in 98 per cent of the individuals studied and within 3 per cent in 80 per cent.\textsuperscript{5}

**RESULTS**

Thirty-eight autotransfusions of fresh blood in normal subjects were performed. The mean loss of radioactivity of the red cells, tagged with Cr\textsuperscript{51}, was found to be 6.0 per cent in the first 24 hours, with a S.D. of 2.0. The

![Radioactivity survival of Cr\textsuperscript{51} tagged fresh red cells, autotransfused in 38 normal subjects.](image-url)
daily decline of radioactivity between the second and the tenth day was found to average 2.2 per cent with a S.D. of 0.26, so that at 10 days the survival of radioactivity averages 74 per cent (fig. 1). The reasons for limiting the study of survival of transfused tagged red cells to about ten days have been previously discussed.\textsuperscript{3}

Studies were carried out on healthy subjects to determine whether a similar loss of fresh, transfused red cells occurred when the survival was determined with the differential agglutination method. In this manner it could be determined how much the first 24 hour \(\text{Cr}^{51}\) radioactivity loss can be attributed to elution and how much to actual loss of red cells.

In a typical experiment on two healthy young males (\(\text{Kel}, \text{Ker}\)) the red cell mass, and consequently the initial 100 per cent value, were determined by the dye plasma-hematocrit method, by the differential agglutination method and by the \(\text{Cr}^{51}\) tagged red cell dilution method. The mean loss of red cells at 24 hours posttransfusion was five and six per cent, respectively (table 1). The line of regression of the nonagglutinated cells shows extinction by extrapolation at 118 days and the loss from the second to the 118th day is .51 per cent/diem (fig. 2).

It may be assumed from these and similar observations that during the first 24 hours there is an actual loss of transfused red cells in excess of that which occurs in subsequent 24 hour periods.

It is well known that red cells damaged by extracorporeal manipulation are readily lost from circulation when transfused in a compatible recipient. Less well known is that this loss is very rapid, practically complete in 24 hours and that a relationship exists between the magnitude of the initial loss and the subsequent rate of loss of the remaining cells.

In a typical experiment in six healthy recipients, the disappearance of the transfused cells was followed with non-agglutinable cells count. Two recipients received blood less than 24 hours old (A of fig. 3); two received blood stored for 21 days (B); two received blood stored for more than 28 days (C). It will be noted that with fresh blood the survival at 24 hours posttransfusion is 92 per cent; the extrapolation of the regression line shows extinction at 122 days. Thus the daily loss of red cells between the 2nd and 122nd day is .76 per cent/100. With blood 21 days old, the average survival at 24 hours is 84 per cent and extrapolation of the regression line shows extinction at 109 days. Thus the daily loss between the 2nd and 108th day is .77 per cent/100. With blood stored in excess of 28 days, the 24 hour survival is 61.5 per cent.

<table>
<thead>
<tr>
<th>Method of Measure of Survival</th>
<th>Red Cell Volume</th>
<th>(\text{Kel})</th>
<th>(\text{Ker})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential agglutination</td>
<td>Dye plasma vol., hematocrit</td>
<td>98</td>
<td>95</td>
</tr>
<tr>
<td>Differential agglutination</td>
<td>Non-agglutinated cell count</td>
<td>92</td>
<td>96</td>
</tr>
<tr>
<td>Differential agglutination</td>
<td>(\text{Cr}^{51}) cell volume</td>
<td>93</td>
<td>91</td>
</tr>
<tr>
<td>(\text{Cr}^{51}) tagging</td>
<td>(\text{Cr}^{51}) cell volume</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>95</td>
<td>94 ± 2.3</td>
</tr>
</tbody>
</table>
Fig. 2.—Regression line of transfused fresh red cells, estimated with the differential agglutination method. Average loss at 24 hours: 5.5 per cent; daily loss 2nd to 118th day: 0.81 per cent.

and extrapolation of the regression line shows extinction at 108 days. Thus the loss of red cells between the 2nd and 108th day is only 0.57 per cent/100/diem.

When the survival of transfused red cells is measured by Cr\textsuperscript{51} tagging, a similar pattern is observed, as shown in table 2.

The rate of loss from circulation of damaged, less resistant erythrocytes is shown in figure 4. Four recipients received 10 ml. of Cr\textsuperscript{51} tagged red cells of blood which had been stored for various periods of time. It will be noted that there is a rapid initial decline, which at one hour posttransfusion averages 13 per cent. Twenty-four hour posttransfusion the loss ranges from 15 to 27 per cent, with an average of 23 per cent. The pattern of loss varies considerably.

DISCUSSION

Observations on the rate of loss of normal, fresh and stored red cells, transfused in healthy subjects, have revealed a definite pattern in which two components are easily distinguished: (1) the immediate loss; and (2) the subsequent daily loss.

Pannacciulli et al.\textsuperscript{6} found in normal subjects the disappearance of radioactivity of Cr\textsuperscript{51} tagged red cells in the first 24 hours to be 4.5 per cent; in successive days the loss is reduced to 1.7 per cent/diem.

Mollison and Veall\textsuperscript{7} and Mollison\textsuperscript{8} found an excessive loss of radioactivity of Cr\textsuperscript{51} tagged transfused red cells in the first day posttransfusion. These authors
favor the hypothesis that the loss is only apparent, being due to elution of 
Cr\textsuperscript{51} from the cells and not to destruction.

Immediate loss occurs very rapidly and at one hour is approximately 50 per 
cent of the total loss at 24 hours. It is customary, for convenience, to express 
as immediate loss the value obtained 24 hours posttransfusion.

The first 24 hour posttransfusion loss of radioactivity of Cr\textsuperscript{51} tagged auto-
transfused fresh red cells in normal subjects in a steady state averages 6 per 
cent. This loss is interpreted as being due to:

1. Daily loss of red cells due to senescence, 0.83 per cent.
2. Daily loss by "elution" of Cr\textsuperscript{51}, estimated at 1.35 per cent.
3. Loss of tagged cells due to sampling, varying from .4 to .6 per cent. This 
   loss is significant only during the first 24 hours, because of multiple 
sampling, and is related to the method employed.
4. Excess loss of red cells during the first 24 hours posttransfusion: 3 per cent.

The true excess loss of red cells during the first 24 hours posttransfusion by 
the Cr\textsuperscript{51} method is thus approximately 3 per cent more than that occurring
Table 2.—Effect of Extracorporeal Damage on the Immediate and Subsequent Loss of Transfused Cr\textsuperscript{51} Tagged Erythrocytes

<table>
<thead>
<tr>
<th>Days of Storage</th>
<th>No. of Observations</th>
<th>Radioactivity Survival 24 hrs. Posttransf.</th>
<th>Mean % Radioactivity Loss/Diem*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1</td>
<td>38</td>
<td>94</td>
<td>2.20</td>
</tr>
<tr>
<td>A 20–22</td>
<td>9</td>
<td>81</td>
<td>1.74</td>
</tr>
<tr>
<td>B 20–21</td>
<td>10</td>
<td>73</td>
<td>1.47</td>
</tr>
<tr>
<td>C 21–22</td>
<td>9</td>
<td>71</td>
<td>1.75</td>
</tr>
<tr>
<td>D 34–36</td>
<td>3</td>
<td>50</td>
<td>1.17</td>
</tr>
<tr>
<td>D 41–43</td>
<td>6</td>
<td>47</td>
<td>1.13</td>
</tr>
</tbody>
</table>

A, B, C and D represent different conditions of blood collection and storage: containers, temperature, anti-coagulant, etc.

*Second to 10th day.

in subsequent 24 hour periods. When the differential agglutination method is used to measure the first 24 hour posttransfusion loss of red cells, only the daily loss of red cells due to senescence and the loss due to sampling need be taken into consideration. Thus, with an average loss, as reported, of 5.3 per cent in the first 24 hours, the net excess loss of red cells in the first 24 hours by the non-agglutinable method is 4.1 per cent.

Extracorporeal damage to red cells produces an increase in the first 24
hour posttransfusion loss. This loss is proportionate to the severity of the damage. When the damage is not excessive, the loss of cells after the first 24 hour period is similar to that noted for undamaged or minimally damaged cells. However, when the immediate loss is excessive, paradoxically the cells remaining in circulation will be lost in successive days at a rate lower than noted with undamaged red cells. The slope of the regression line in such cases yields incomplete and often misleading information.

These findings suggest the existence of a cell population more susceptible to extracorporeal factors, and that these cells disappear more readily from circulation, leaving a more resistant cell population.

It is also apparent that when the transfused red cells are damaged, extracorporeally, as by prolonged or improper storage, the measure of the red cell volume based on the dilution of radioactivity of the Cr$^{51}$ tagged cells results in erroneously large values. If the red cell volume thus obtained is used to determine the per cent survival of transfused cells, the values will be incorrectly high. An independent measure of the red cell volume is necessary in these cases.

These considerations do not apply to the result of survival of red cells expressed as $T_{1/2}$. In such cases the immediate loss is ignored, and the choice of the 100 per cent, established by extrapolation to 0 time, is purely arbitrary and has no effect on the value of $T_{1/2}$. It is obvious, however, that the method of estimation of survival of red cells expressed as the $T_{1/2}$ gives an incomplete view of the behavior of the transfused red cells.

**Summary**

1. Fresh and stored red cells, transfused in healthy subjects, disappear from the circulation during the first 24 hours at a rate in excess of that prevailing in subsequent 24 hour periods. When fresh red cells are transfused the excess first day loss is approximately 3 per cent.

2. Comparative survival studies using the nonagglutinable red cell method of Ashby have indicated that this excess immediate loss is not due to elution of Cr$^{51}$, but to actual loss of red cells.

3. When red cells are stored improperly or for an extended period of time, the excess loss occurs very rapidly, and at one hour is approximately 50 per cent of the total loss at 24 hours. The limitations to the use of the dilution data of transfused stored cells to establish the 100 per cent value for the measure of the survival of the transfused red cells are implicit.

4. When the first 24 hour loss of transfused red cells is approximately 30 per cent or more, paradoxically the rate of loss in subsequent days is less than when fresh, undamaged red cells are transfused. This observation raises the question of interpretation of the measure of the red cell survival expressed as $T_{1/2}$.

**Summary in Interlingua**

1. Erythrocytos fresc e immagasinate que es transfundite in subjectos normal dispare in le curso del prime 24 horas a un rhythmo plus rapide que in le curso del subsequente periodos de 24 horas. In le caso de cellulas fresc, le
122

STRUMIA, DUGAN AND COLWELL

excesso de perdita in le curso del prime periodo de 24 horas in comparation con le subsequente periodos de 24 horas es aproximativamente 3 pro cento.

2. Comparative studios de superviventia, utilissante le metodo del nonag-glutinabile erythrocytos de Ashby, ha demonstrate que le mentionate excesso de perdita immediate non es causate per un elution de Cr51 sed representa un ver perdita de erythrocytos.

3. Quando le erythrocytos es magasinate inadequatamente o durante pro-longate periodos de tempore, le excesso del mentionate perdita occurre rapidissimente. Post un hora illo amonta a approximativamente 50 pro cento del perdita total de 24 horas. Es evidente le resultante limitation del utilite de datos de dilution del transfundite cellulas immagasinate in le establimento del valor de 100 pro cento pro le mensuramento del superviventia de transfundite erythrocytos.

4. Paradoxemente, quan do le perdita de transfundite erythrocytos durante le prime 24 horas es approximativamente 30 pro cento o plus, le perdita in subsequente periodos de 24 horas es minus que quando le transfusionate erythrocytos es fresc e non-lesionate. Iste observation subleva le problema del interpretation del mesura del superviventia erythrocytic exprimite como T ½.

REFERENCES


Max M. Strumia, M.D., Sc.D., Director of Research, The John S. Sharpe Foundation of Bryn Mawr Hospital; Director, Laboratory of Clinical Pathology, Bryn Mawr Hospital, Bryn Mawr, Pa.; Professor of Clinical Pathology, Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pa.

Ann Dugan, B.S., Research Assistant, Radioisotope Laboratory, Bryn Mawr Hospital, Bryn Mawr, Pa.

Louise S. Colwell, A.B., Research Assistant, Alison Research Laboratory, The John S. Sharpe Research Foundation of Bryn Mawr Hospital, Bryn Mawr, Pa.
The Measure of Erythropoiesis in Anemias. II. The Immediate and Subsequent Loss of Transfused Erythrocytes in Healthy Subjects

MAX M. STRUMIA, ANN DUGAN, LOUISE S. COLWELL and Helen Munroe