BLOOD EXCHANGE IN REPLACEMENT TRANSFUSIONS

II. STUDIES WITH ERYTHROCYTES TAGGED WITH RADIOACTIVE PHOSPHORUS

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THE PRECEDING paper\(^1\) was concerned with the theoretic aspects of exchange transfusions. Mathematical expressions for the instantaneous concentration of erythrocytes during a replacement transfusion under ideal conditions were developed and relevant curves were presented. The applicability of these formulae and curves to experimental results will be demonstrated in the following report.

The usual method of investigating the survival of red cells, i.e., their retention within the circulation, utilizes the well known Ashby agglutination technic.\(^2\)\(^,\)\(^3\) In this procedure, donor erythrocytes, compatible with the recipient’s blood but differing in a major or minor agglutinogen, are injected into the patient. Selective agglutination of the donor or recipient cells is then performed at intervals using high titered antiserum, the number of unagglutinated cells is estimated and the survival rate of the donor cells determined. Valid results may be obtained with this technic and valuable information concerning the destruction of normal and abnormal cells within the circulation has been reported.

Although the Ashby technic was used by us in some of the experiments, the scatter of the results, particularly in the greater dilution ranges, rendered the method unsuitable for following the changes in replacement transfusion. More satisfactory data were obtained by a sedimentation technic using standardized agglutinating sera. With this method Stats\(^4\) was able to obtain results that agreed favorably with the radioactive red cell technic described below. The radioactive red cell method, however, possesses advantages over the other two methods particularly in the simplicity of the procedure and in the reproducibility of the results.

A method for labeling red cells with radioactivity was first described by Hahn and Hevesy\(^5\) in 1940. P\(^{32}\) was injected intravenously into rabbits and hens and some of the isotope was found to be incorporated in the red cells. A measured quantity of these tagged red cells was then removed, injected into homologous animals, and the red cell volumes determined. Hevesy and Zerahn\(^6\) later simplified the procedure by demonstrating that rabbit erythrocytes take up P\(^{32}\) when incubated in vitro at 37 C. with the isotope. The P\(^{32}\) atoms penetrated into the erythrocytes and within a short time were found in both the organic and inorganic acid soluble fractions.\(^7\)\(^,\)\(^8\) Hevesy et al.\(^9\) subsequently applied this new procedure to human red cells and used it to measure the circulating red cell volume in patients. The increase in radioactivity of red cells, when incubated with radiophosphorus at 37 C., is a function of
cellular metabolism rather than diffusion, since at 7°C there is a minimal exchange of $^{32}P$ between the red cells and the plasma. The labeled phosphate ions leave the red cells at a very slow rate, and for experiments of restricted duration such as blood volume determinations, studies on cardiac output or exchange transfusions lasting a few hours, these tagged cells may be utilized for studying changes in the circulating blood.

**Methods**

(a) **Labeling of red cells with $^{32}P$**: 10 cc. of the patient's blood are withdrawn into a sterile heparinized syringe about three hours before exchange transfusion starts. Fifteen cc. are transferred into a sterile rubber-capped centrifuge tube containing about 5 millicuries of isotonic radiophosphorus; the remaining 5 cc. are introduced into a smaller tube containing about 150 micromicrocuries of $^{32}P$, which is used for the blood volume determination. Both tubes are incubated for two hours at 37°C, during which time they are occasionally inverted. The mixtures are then centrifuged at 1000 rpm for ten minutes, the plasma anduffy coat removed sterilely with a 24-inch, 20 gage needle, cold 0.9 per cent saline added to the original volume through a 1-inch, 10 gage needle previously used as the vent. The cells are mixed thoroughly, centrifuged again for ten minutes, and the supernatant discarded. The cells are resuspended and recentrifuged for the final time for fifteen minutes, made up to the original volume with cold 0.9 per cent saline, and the prepared suspensions are kept in ice water until ready for use.

Prior to each exchange transfusion, the blood volume is determined, using tagged red cells. At the completion of some of the replacement transfusions another blood volume is performed, this time using the dye Trypan Blue. In our experience, the results obtained with both methods are comparable within 6 per cent.10 Five cc. of the thoroughly mixed cell suspension in the smaller tube, now tagged with about 25 micromicrocuries of $^{32}P$, are drawn up into a modified 5 cc. Van Slyke pipet with stopcock; an 18 gage needle is adjusted to the tip; a venipuncture is then made and an exact amount is injected by positive pressure through a rubber tube attached to the end of the pipet. Ten minutes are permitted to elapse to insure complete mixing of the blood, after which three specimens of blood are withdrawn at ten-minute intervals and placed in paraffinized tubes containing a few drops of heparin. The tubes are sealed with paraffin, inverted a number of times and placed in ice water until ready for analysis. Immediately after the injection of the tagged cells a radioactive standard is prepared by pipetting exactly 1 cc. of the red cells suspension into a 1000 cc. volumetric flask and diluting to volume with water containing saponin.

(b) **Procedure for exchange transfusion**: After the blood volume has been determined, the level of radioactivity in the patient's blood is increased by injecting the contents of the larger tube containing about 2 millicuries of radioactive phosphorus. This booster dose of radioactive cells is necessary to insure a sufficiently high radioactive count toward the end of the exchange. Usually less than 10 per cent of this amount of $^{32}P$ remained in the body at the end of the replacement transfusion.

After a ten-minute mixing period, a 13 or 15 gage needle is introduced into an antecubital vein through a novocainized area. The needle is taped into place and a specimen for analysis is taken in heparinized tubes. One hundred mg. of heparin are injected through the needle, which is then attached to either an 'open' or 'closed' donor set; another similar needle is inserted through a novocainized area into an antecubital vein of the opposite arm and attached to the phlebotomy set. In the continuous type of exchange, inflow and outflow of blood proceed simultaneously and at the same rate. Variations of the continuous exchange may be performed as desired. Specimens for radioactive assay and hematocrit determinations are collected in heparinized tubes after each phlebotomy unit (usually 500–600 cc.). From time to time it is necessary to inject more heparin to prevent blood from coagulating in the needles, usually about 100 mg. of heparin are sufficient for this purpose. Ten cc. of a 10 per cent solution of calcium gluconate are administered routinely midway through, and again at the end of, the exchange. The time necessary to complete a continuous exchange of 7500 to 10,000 cc. may vary from one to four hours.

* The radioactive phosphorus was obtained on allocation from the U. S. Atomic Energy Commission, Oak Ridge, Tenn. It was neutralized with sodium hydroxide, made isotonic with sodium chloride and autoclaved. Five-tenths cc. was injected intraperitoneally into each of 2 mice which were observed for forty-eight hours before the material was used in patients.
In the discrete type of exchange transfusion, blood is withdrawn and administered alternately or vice versa. Heparin and calcium gluconate are used as above and specimens are taken as in the continuous type of exchange. Modifications of the method may be introduced as necessary.

In both the continuous and discrete types of exchange in adults, either the antecubital or ankle veins are used for inflow of blood; only the antecubital or femoral veins have been found satisfactory for rapid withdrawal. In infants, discrete transfusions have been used exclusively, with administration and withdrawal of blood through the umbilical vein.

(c) Preparation of samples: i. (for blood volume): Prior to transfusion, a quantity of pledgets is prepared as follows: 4 cm. x 6 cm. pieces of filter paper are immersed in a wetting agent (to insure subsequent even distribution of blood) and dried. These papers are superimposed upon slightly larger rectangles of thin aluminum foil and held in place at the corners with strips of Scotch tape. Immediately after the collection of each sample, 0.5 cc. of thoroughly mixed whole blood is pipetted and evenly distributed over the filter paper. The pipet is then rinsed through its upper end with small quantities of saponin water and the washings collected on the same filter paper. After the filter paper is air dried, the pledget is wrapped in a rectangle of 0.001 inch polystyrene and rolled to fit snugly around a cylindrical aluminum Geiger-Mueller tube (Victoreen tube 1B85, wall thickness 30 mg./cm.2). About $7 \times 10^3$ counts/sec./microcurie of $P^{32}$ are obtained, giving an efficiency of about 20 per cent. All counts are performed to 1 per cent precision. Another sample is taken for hematocrit determination, and the remainder of the specimen is centrifuged in the cold at 3000 rpm for forty minutes and the plasma is separated. One cc. of the packed red cells is hemolyzed in 1 cc. of saponin water and 0.5 cc. of this hemolysate is placed on a filter paper pledget as described above. Similarly, pledgets are prepared with 1 cc. of the supernatant plasma and 0.5 cc. of the red cell standard respectively.

ii. (for exchange transfusions): During the exchange transfusion whole blood samples of each specimen are treated as described above, except that in cases where initial samples are very radioactive, 1 cc. of whole blood is diluted to 10 cc. with saponin water and 0.5 cc. of this hemolysate used. The remainder of the original sample is centrifuged in the cold for ten minutes at 2000 rpm and the plasma and buffy coat removed. One cc. of the plasma is assayed for radioactivity. The cells are washed twice with cold 0.9 per cent saline and finally centrifuged for forty minutes at 3000 rpm. One cc. of these packed cells is diluted to 10 cc. with saponin water and 0.5 cc. of this hemolysate placed on the pledges preparatory to radioactive assay. All specimens for radioactive assay and hematocrits are taken in duplicate.

Selection of cases: Only patients with leukemia or metastatic carcinomatosis were investigated in this study. Thirteen exchange transfusions were performed, with only ten yielding sufficient data for analysis. The 3 cases not included were done early in the study and some pertinent information necessary for the computation was found to be lacking. The group studied was made up as follows: 1 cases of chronic lymphatic leukemia (Cases 6 and 9), 4 cases of acute myeloblastic leukemia (Cases 1, 5, 7, 8). One case of multiple myeloma (Case 4), one case of congenital myeloblastic leukemia (Case 10), and two cases of pulmonary carcinomatosis (Cases 2 and 3).

Results

The uptake and retention of radioactive phosphorus by the erythrocytes was determined in vitro under conditions similar to those used in the experiments. This was necessary in order to estimate the total amount of tagged red cells to be used in both the blood volume determination and the exchange transfusion, and to determine the time interval during which such red cells might give an accurate picture of the circulating blood. Whole blood was therefore incubated with an isotonic solution of radioactive phosphorus and after periodic intervals, a specimen of blood was removed, the plasma separated and the red cells washed three times with 0.9 per cent saline. After the first washing, less than 1 per cent $P^{32}$ was found in the suspending medium. The radioactivity of both the red cells and plasma was determined as described above. As is seen in figure 1, during the period studied, the increase in the ratio of red cell to plasma radioactivity at 37 C. was approximately
exponential with about 50 per cent of the total added radioactivity in the red cells at two hours. At 4°C. there was no appreciable uptake of P³² by the red cells, indicating the metabolic nature of the incorporation of radiophosphorus by the erythrocytes. Blood was therefore incubated for a two-hour period, and for these experiments it was assumed that approximately half of the added P³² was metabolized by the red cells. Since no exchange was manifest at 4°C., all specimens were kept at this temperature for a period not over one hour before being prepared for analysis, and in addition, were centrifuged in the cold.

The efficacy of using radiophosphorus-tagged red cells for determining the blood volume of a patient has been reported by others¹¹⁻¹³ and confirmed by us.¹⁰ Figure 2 shows typical curves of whole blood, of washed red cells and of plasma radioactivity during a ninety-minute period following the intravenous injection of P³²-labeled erythrocytes. Five other similar experiments have been performed with specimens taken at intervals up to eight hours. From the data obtained from these
<table>
<thead>
<tr>
<th>Case</th>
<th>Type Exch.</th>
<th>$V_s$</th>
<th>$V_1$</th>
<th>$V$</th>
<th>$ar{V}$</th>
<th>$V_f$</th>
<th>$T$</th>
<th>$E$</th>
<th>$R$</th>
<th>$C^R_E$</th>
<th>Hematocrit x 0.915</th>
</tr>
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</tr>
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<td>0</td>
<td>3400</td>
<td>545</td>
<td>3600</td>
<td>8195</td>
<td>2.4</td>
<td>1.8</td>
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<td>1000</td>
<td>5000</td>
<td>650</td>
<td>6000</td>
<td>8195</td>
<td>1.7</td>
<td>1.1</td>
<td>12.2</td>
<td>15.5</td>
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<td>3. I. R. (m)</td>
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<td>0</td>
<td>4300</td>
<td>500</td>
<td>6000</td>
<td>8195</td>
<td>0.7</td>
<td>1.0</td>
<td>49.3</td>
<td>47.0</td>
</tr>
<tr>
<td>4. H. B. (f)</td>
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<td>600</td>
<td>5500</td>
<td>700</td>
<td>6150</td>
<td>8195</td>
<td>1.6</td>
<td>1.5</td>
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<td>8.1</td>
</tr>
<tr>
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<td>Cont.</td>
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<td>7000</td>
<td>5100</td>
<td>500</td>
<td>8400</td>
<td>8195</td>
<td>1.1</td>
<td>3.1</td>
<td>12.2</td>
<td>12.5</td>
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<td>9280</td>
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<td>1.7</td>
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<td>6200</td>
<td>6200</td>
<td>620</td>
<td>9250</td>
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<td>8.4</td>
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<td>4650</td>
<td>620</td>
<td>4780</td>
<td>9300</td>
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<td>*9. A. C. (m)</td>
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<td>5850</td>
<td>6300</td>
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<td>38.0</td>
<td>-0.5</td>
<td>0.99</td>
<td>1.00</td>
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<tr>
<td>10. S. (inf.)</td>
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<td>1000</td>
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<td>1.0</td>
<td>0.08</td>
<td>0.08</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Each horizontal line contains the following pertinent data for the particular case:

- $V_s =$ initial blood volume of the patient.
- $V_1 =$ blood volume removed prior to, and added at the end of, the replacement transfusion.
- $V =$ volume during the exchange transfusion.
- $ar{V} =$ average increment of blood administered and withdrawn in the case of the discrete replacement transfusions. In a continuous exchange, $ar{V}$ represents the average volume administered and withdrawn between two consecutive samples.
- $V_f =$ blood volume at the end of the exchange.
- $T =$ total volume of donor blood used in the exchange (where a known volume was added at the end of the exchange it was included in $T$).
- $E =$ volume of donor blood used in the exchange.
- $R =$ patient initial hematocrit.
- $C^R_E =$ volume of original red cells remaining.
- $C^R_v =$ total volume of circulating red cells.

The observed and calculated $C^R_v$, their difference and ratio have been tabulated for each case, as well as the observed and calculated final hematocrit ratio. The results are well within the experimental error.

* Where a volume $V_1$ was withdrawn prior to the continuous exchange and a similar volume replaced at the end (Cases 2 and 4), $C^R_v$ stands for $C^R_v + V_1$ (see preceding paper).

† Both calculated and observed $C^R_v$ refer to per cent concentration of whole blood remaining.
experiments, the half time of disappearance, i.e., the time that it takes for half of the radioactivity to disappear from the red cells, has been found to be twelve to eighteen hours (fig. 3), and during a one-hour interval the loss of P\(^{32}\) from the cells is about 4 per cent. In addition to these in vivo studies, radiophosphorus-tagged cells suspended in saline were incubated at 37 C. and agitated frequently to simulate conditions within the circulating blood. Specimens were withdrawn at regular

![Graph](image1)

**Fig. 2.**—Radioactivity curves of three times washed red cells, of plasma and of whole blood during a one and one-half-hour period, following the intravenous injection of P\(^{32}\) tagged cells. Note the constancy of the red cell radioactivity (about 4 per cent decay after one hour). A single specimen taken at any interval following the initial mixing phase of a few minutes will give an accurate blood volume.

![Graph](image2)

**Fig. 3.**—A typical curve showing the disappearance of radioactivity from the circulating red cells over an eight-hour period. Note that the P\(^{32}\) leaves the red cells in an exponential manner during this time, and that the half time of disappearance is about eighteen hours.

intervals and the washed red cells and plasma assayed for radioactivity. The results obtained mirrored the in vivo studies. It is obvious that for experiments lasting under one hour, no correction need be made for the loss of P\(^{32}\) from the red cells. For experiments lasting over one hour, a correction must be made for this decay. This correction was applied to our experiments wherever it was indicated.

Results of the ten replacement transfusions performed are seen in table 1 and the
hematologic data pertaining to these cases before and after the exchange are shown in Table 2.

The concentration of original red cells in per cent of total circulating cells remaining in the blood stream during and at the end of the exchange transfusion ($C^e_{ob}$ observed) was determined by assaying the particular samples of blood for residual radioactivity. These were compared with the theoretic values obtained from the mathematical formulae derived in the preceding paper ($C^e_{cal}$ calculated).

From Table 1 it can be seen that for the cases studied, the absolute difference between the observed and the calculated per cent concentration of original cells remaining in the circulation ($C^e_{ob}$) varies from 0 per cent to 2.3 per cent with an average deviation of 0.6 per cent. The corresponding ratios ($C^e_{cal}$) vary from 0.86 to 1.18.

The ratio of the final observed to calculated hematocrit varies from 0.89 to 1.00 with an average deviation from 1.00 of 0.06.

In general, the experimental results for $C^e_{ob}$ and hematocrit are in excellent agreement with the theoretic values.

In the case of a continuous exchange with the blood administered and withdrawn simultaneously and at the same rate, the per cent concentration of patient's original red cells in the total circulating red cell volume ($C^R_{ob}$) is given by the following formula:

$$C^R_{ob} = 100 \frac{e^{-R}}{R - (R - 1)e^{-R}} = 100 \frac{1}{R(e^{-R} - 1) + 1}.$$
where the symbols have the following meaning: 

\[ R = \frac{\text{donor hematocrit}}{\text{initial hematocrit of patient}} \]

\[ E = \frac{\text{volume of donor blood used}}{\text{blood volume of the patient during the exchange}} \]

\[ e = \text{base of natural logarithm} = 2.718 \ldots \]

The above formula with \( R = 1 \) gives the per cent concentration of patient's original whole blood in the total circulating blood (\( V \)).

For any fixed hematocrit ratio \( R \), the per cent concentration of the original cells (\( C^R_E \)) can be plotted as a function of \( E \). Such curves are shown in figure 3 of the preceding paper for different values of \( R \) ranging from 0.5 to 4.0. In this paper such curves are plotted for \( R = 1 \) and for \( R = 1.7 \) (corresponding to the donor-patient hematocrit ratio of the actual case studied), and compared with the experimental curves obtained by the method of P32-tagged red cells (fig. 4, Case 8).

In table 3 (Cases 1, 4, 5) the theoretic \( C^R_E \) values are tabulated for a series of values of \( E \), at equal intervals of one unit of blood transfused and withdrawn and compared with the experimental data obtained using radiophosphorus-tagged cells. The results show excellent agreement with the expected values.

In the discrete exchange transfusions, two distinct situations are considered. If a certain volume of blood is first withdrawn from the patient and subsequently administered, then the mathematical expression for \( F \), which is the fraction of the original whole blood of the patient remaining in the circulation after such a single discrete exchange, is given by

\[ F = \frac{V_0 - v}{V_0} \]
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Here \( V_0 \) stands for the initial total blood volume of the patient and \( v \) for the blood volume used in a single exchange. If a unit of blood is first administered to the patient and subsequently withdrawn, the above formula becomes:

\[
F = \frac{V_0}{V_0 + v}
\]

When the hematocrit of the patient is equal to that of the donor, i.e., \( R = 1 \), the per cent concentration of the original erythrocytes in the total red cell volume of the patient after a single discrete exchange is given by 100 \( F \), and after \( n \) such exchanges by

\[
C_n = 100F^n
\]

where the symbols \( R \) and \( F \) are defined as above, and where \( E \) can be written as:

\[
E = \frac{\text{vol. donor's blood used in a single exchange} \times \text{number of exchanges}}{\text{initial blood volume of the patient}}
\]

In this formula the concentration of the red cells \( (C_n) \) is seen to depend upon \( F \) which in turn depends upon \( v \) and \( V_0 \). In figure 1 of the preceding paper, the \( C_n \)

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**Table 3.** Comparison of the Theoretical and Observed Values for the per cent Concentration of Original Cells in the Total Cell Volume after \( "n" \) Units of Blood Have Been Administered and Withdrawn.

*All blood administered was group 0 with added A and B substance. All the exchange transfusions were of the continuous type.*

<table>
<thead>
<tr>
<th>Units blood (c.c.)</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
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<tr>
<td>calc.</td>
<td>observ.</td>
<td>calc.</td>
<td>observ.</td>
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<tr>
<td>1</td>
<td>78</td>
<td>81</td>
<td>65*</td>
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<tr>
<td>2</td>
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<td>9.3</td>
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<td>16.0</td>
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<td>17</td>
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</table>

Case 1: Female, 27 years, acute myeloblastic leukemia; blood group A.
Case 2: Female, 60 years, multiple myeloma; blood group AB.
Case 3: Male, 59 years, spent polycythemia with acute myeloblastic leukemia; blood group A.
* After the initial withdrawal of 600 cc.
† After the final addition of 600 cc.
curves are plotted for discrete exchanges with withdrawal followed by the administration of blood and for values of \( v \) (volume used in a single exchange) equal to \( V_0/20 \), \( V_0/10 \), and \( V_0/5 \) respectively. In this paper, such a curve is plotted in figure 5 and compares favorably with the experimental data derived by the radiophosphorus cell method (Case 10, \( R = 1 \)).

When the donor-patient hematocrit ratio is not equal to one, the expression for the per cent concentration of the original cells becomes somewhat more complicated. In such cases:

\[
C^R_E = 100 \times \frac{\text{volume of the original red cells remaining}}{\text{final total red cell volume}} = 100 \times F^n \left( \frac{R_n}{R} \right)
\]

where \( E \) and \( F \) are defined as before, and where

\[
R = \frac{\text{donor hematocrit}}{\text{patient initial hematocrit}}
\]

\[
R_n = \frac{\text{donor hematocrit}}{\text{patient hematocrit after the } n\text{th discrete exchange}}
\]

In order to determine \( R_n \), it is necessary to calculate all the preceding hematocrit ratios, i.e., \( R_1, R_2, \ldots, R_{n-1} \). Such curves for \( R = 0.5, 1, 2 \) and 4 are plotted in figure 2 of the preceding paper. In this paper a theoretic curve for a discrete exchange transfusion with administration of blood preceding its withdrawal, and with the initial donor-patient hematocrit ratio not equal to one, can be seen in figure 6, where it is compared with the experimental results obtained in Case 9 (\( R = 2.2 \)). This patient, a male, age 45, with chronic lymphatic leukemia, was...
the only adult on whom a discrete exchange transfusion was performed, and was of such duration that only 10 units \( (E = 1.1) \) could be replaced. Samples were prepared as in the other cases but unfortunately the final red cell preparations were lost and the results could not be plotted. However, whole blood radioactive levels agreed with the theoretic values. A discrete exchange transfusion is not to be recommended for adults since it is time consuming and the patient becomes irritable.

**Summary**

Red cells labeled with radioactive phosphorus have been used by us to measure the extent and the rate of the replacement of blood in exchange transfusions. The experimental results confirmed the theoretic formulations developed in the preceding paper. On the basis of the above data, it seems reasonable to introduce these curves and formulae whenever exchange transfusions are to be performed.

**Conclusions**

1. Radiophosphorus-tagged red cells have been used for blood volume determinations and for measuring the exchange of red cells in replacement transfusions.
2. The experimental data obtained agreed with the theoretic formulations previously derived.
3. The tagged red cell method is valuable for following changes in the circulating blood.

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BLOOD EXCHANGE IN REPLACEMENT TRANSFUSIONS: II. STUDIES WITH ERYTHROCYTES TAGGED WITH RADIOACTIVE PHOSPHORUS

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