BLOOD VOLUME DETERMINATION IN THE HUMAN WITH RED CELLS CONTAINING RADIOACTIVE PHOSPHORUS (P³₂) AND WITH PURE HUMAN ALBUMIN

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The work presented in this paper is an outgrowth of a previous study made in this clinic (1) and deals with the use of a relatively simple method by which red cells containing radioactive phosphorus (P³₂) are used to measure blood volume in human subjects. Observations were also made on blood volume changes as measured by the effect of an intravenous injection of plasma and pure human albumin.

PREVIOUS OBSERVATIONS

Methods proposed for the determination of blood volume include both direct and indirect means. Complete historical reviews of these various methods have been reported by Keith, Rowntree and Geraghty (2) and by Erlanger (3). More recently Gregersen (4) and Gibson (5) summarized their results with the dye dilution method in normal and pathological conditions.

With the advent of radioactive tracer elements for use in biology and medicine, a new tool became available. Two radioactive isotopes (iron and phosphorus) have been employed to date. Radioactive iron (Fe⁵⁹) was first used by Hahn, Balfour, Ross, Bale and Whipple (6). Considerable data obtained by this method have been reported (6-14). The advantages of the radioactive iron lie in the fact that the activated red cells are stable for many weeks. The disadvantages are as follows: The need of a donor whose red cells have been synthesized with radioactive iron and whose blood has been carefully matched with the recipient's, the relatively large amount of blood needed, and the rather complicated procedure for determining radioactivity. Finally, in the determination of red cell volume before and after hemorrhage or transfusion a second injection of donor red blood cells containing a different radioactive isotope of iron is necessary (8).

Radioactive phosphorus was used initially by Hahn and Hevesy (15). Two procedures have been described. The first, like the radioactive iron method, requires a donor animal whose red cells have been activated by the administration of P³₂ as Na₂HPO₄. The second method, more recently developed by Nylin (16), uses the subject's own red blood cells which are activated and reinjected. The advantages of the second radioactive phosphorus method lie in the much greater simplicity with which P³₂ can be measured, the fact that no donor is needed, and that the volume of blood injected is small. The disadvantages lie in the short period (one hour) during which the red cells maintain constant radioactivity. Considerable data obtained by the two phosphorus methods have been reported (1, 15-26).

METHOD OF PROCEDURE

These methods will be described under two headings dealing with the use of radioactive phosphorus and of plasma albumin injections respectively.

I. The first method used, involving the radioactive red cells, was modified from that of Nylin (16). Into a clean, dry, 25-cc. pyrex tube were placed 1 to 2 cc. of a solution containing 50 microcuries of radioactive phosphorus. (Material with high activity was diluted to the

³ Radioactive phosphorus emits beta rays having a maximal energy of 1.8 and an average energy of 0.6 million electron volts. The maximum range of penetration of these rays through the body tissues is approximately 0.7 cm. The half-life of P³₂ is 14.3 days (27). The 50 microcuries or less of P³₂ used in each determination of blood volume corresponds to less than 0.01 roentgen equivalents physical per day for 100 days. The accepted limit of tolerance for man is 0.1 roentgen per day (5, 28, 29). The exposure of the investigators to radiation was negligible and no extensive protective measures were required.

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³ Aided by a grant from the Commonwealth Fund.

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same range of concentration with isotonic saline solution.) The mouth of the tube was plugged with cotton. All syringes and needles were washed thoroughly with distilled water. A 10-cc. pipette and a rubber stopper were similarly prepared. Since aseptic technic must be maintained throughout, all equipment was sterilized in an autoclave for 30 minutes at 15 lbs. of steam pressure.

From the subject, a sample of approximately 15 cc. of heparinized blood was withdrawn through the antecubital vein without stasis with a 19 gauge needle, and transferred to the test tube containing the radioactive solution. The tube was sealed with the rubber stopper and after mixing thoroughly by inversion was placed in an incubator maintained at 37° C., and agitated for two hours. A motor-driven stirrer with an eccentric shaft to which the samples were attached provided adequate mixing and prevented settling of the red cells.

At the end of the two-hour period of incubation and agitation, exactly 10 cc. of blood were removed from the tube with a pipette, transferred to a syringe, and injected into the subject. Blood was drawn back and forth into the syringe several times in order to insure the complete injection of all of the active material. Specimens were then removed at various intervals from the opposite arm after a five-minute period had elapsed to allow for adequate mixing. Samples of both the injected blood and that obtained subsequently were prepared for the determination of radioactivity by placing each specimen in hematocrit tubes of 8 mm. diameter and centrifuging them at 3000 r.p.m. for a period of 40 minutes. The plasma was then very carefully removed and replaced by distilled water to the original level. Resuspension of the cells in the water and subsequent hemolysis were accomplished by inverting or shaking the tube gently many times. (The originally incubated blood was obviously too active for direct counting; therefore after separation and hemolysis as described, it was diluted 1:500 and 1 cc. of this was used for counting.) One cc. of the hemolysate was placed on a pyrex watch glass and allowed to dry overnight in the absence of drafts. In order that the geometry of counting be maintained uniformly from sample to sample, the 1 cc. was confined to the area of a circle with a diameter of 25 mm. marked on the watch glasses with a china marking pencil. All the samples were counted at a uniform distance (2.5 cm.) below the counting window. All glassware was checked for contamination from previous samples. The samples were counted with a Geiger-Müller counter for 15 to 30 minutes each. Background corrections were determined before and after each counting series. All counts were at least three times the background count. When repeated determinations of blood volume were made in the same individual one day or more later, an aliquot of the initial sample of blood, i.e., the blood which was to be incubated with P2 was prepared and counted in like manner. The activity observed was subtracted from that present after incubation with P4 and from all subsequent samples following injection of the activated blood.

Inasmuch as the hemolysate was the same volume as that of the original whole blood, its activity is the same as that of the red cells in 1 cc. of whole blood. This assumes that the activity of the phosphorus injected in the plasma fraction is independent of that in the red cells. That this assumption is actually correct within the limits of error of the experiment will be shown subsequently.

**Calculation**

The value for the total circulating blood is obtained from the following calculation. Activity is expressed as counts per cc. per minute after subtraction of background count.

\[
W.B.V. = \frac{C_1}{C_2} \times V \quad \text{Formula 1}
\]

Where:

- W.B.V. is the whole blood volume
- \(C_1\) is the activity per cc. of hemolysate in the sample injected and
- \(C_2\) is the activity per cc. of hemolysate after injection
- \(V\) is the volume of blood injected.

It will be noted that in the above calculation of whole blood volume the use of the measured hematocrit has been avoided. However, Formula 1 is based upon the assumption that the cell-plasma ratio is the same throughout the body. If this is not true, then the whole blood volume will not be accurately measured. For calculation of red cell and plasma volumes the hematocrit is used in the following calculations.

\[
R.C.V. = \frac{C_1}{C_2} \times (V) \times \frac{H_2}{100} \quad \text{Formula 2}
\]

Where:

- R.C.V. is red cell volume
- \(H_2\) equals hematocrit of sample removed after injection.

After complete mixing of the injected activated red blood cells the ratio of activated to non-activated red cells is a constant throughout the vascular system. This ratio is independent of the cell-plasma ratio. Therefore the red cell volume as calculated according to Formula 2 is accurate since the use of the hematocrit value, in effect, converts the expression to one of the ratio of activated to non-activated red cells.

\[
P.V = W.B.V. - R.C.V. \quad \text{Formula 3}
\]

Where \(P.V\) = plasma volume.

Since, according to Formula 3, the plasma volume is calculated from the whole blood volume and the red cell volume, it is subject to the same possible inaccuracy as is Formula 1.

II. The second method of measuring blood volume by means of plasma and albumin injections was employed in patients with malnutrition, hypoproteinemia and edema. (Simultaneous whole blood, plasma and red cell volumes were also determined by the P4 method.) The procedure is based upon the changes in hematocrit and plasma albumin concentration following injection of a known amount of albumin, either as salt-poor human albumin (25 per cent) or as double strength plasma. Samples before and after the injection were collected and treated as already described. Fractionation was carried out by the method of Campbell and Hanna (30). Total and
fractional protein determinations were made by the colorimetric method of Weichselbaum (31).

Calculation of plasma volume involved the following formulae:

\[ PV_0 = \left( \frac{\text{grams albumin injected}}{A_1} \right) \times 100 \times \left( \frac{H_0}{100 - H_0} \right) \left( \frac{100 - H_1}{H_1} \right) - A_0 \]  \quad \text{Formula 4}

Where:

- \( PV_0 \) = plasma volume before injection in cc.
- \( A_0 \) = concentration of plasma albumin before injection in grams per cent
- \( A_1 \) = concentration of plasma albumin immediately after injection in grams per cent
- \( H_0 \) = hematocrit before injection in per cent
- \( H_1 \) = hematocrit immediately after injection in per cent.

Subsequent plasma volumes were calculated according to the following:

\[ PV_1 = PV_0 \left( \frac{H_0}{100 - H_0} \right) \left( \frac{100 - H_1}{H_1} \right) \]  \quad \text{Formula 5}

Where:

- \( PV_1 \) = plasma volume after injection in cc.
- \( PV_0 \) = plasma volume before injection in cc.
- \( H_0 \) = plasma volume before injection in cc.
- \( H_1 \) = plasma volume after injection in cc.

In Table I are listed the data obtained with radioactive red cells on nine normal individuals. This table also includes values for the blood volume and its fractions in normal men and women as obtained by several investigators using various methods (2, 4, 7, 18, 21, 32–36). It is readily apparent that our results are in close agreement with those obtained by Hevesy et al. (37), by Nylin and Hedlund (21), and by Govaerts and Lambrechts (18), who employed the same method in man, as well as the data presented by Gibson et al. (7) using the radioactive iron method. In the majority of instances the results obtained with \( {\text{P}}^{32} \) are lower than those obtained by the dye and carbon monoxide methods. This has also been noted with radioactive iron in man (Gibson et al. [7], Meneely et al. [13]) and in animals (10). Our results are somewhat higher

### TABLE I

**Eleven blood volume determinations using \( {\text{P}}^{32} \) in red cells in normal human subjects compared with findings of other authors**

<table>
<thead>
<tr>
<th>Bibliographic reference of other authors</th>
<th>Method</th>
<th>Subjects</th>
<th>cc./kilo.</th>
<th>cc./kilo.</th>
<th>cc./kilo.</th>
<th>cc./sq. m.</th>
<th>cc./sq. m.</th>
<th>cc./sq. m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2) Dye</td>
<td>42 m. &amp; f.</td>
<td>85.0</td>
<td>34.62</td>
<td>43.08</td>
<td>3019</td>
<td>1399</td>
<td>1620</td>
<td>1520</td>
</tr>
<tr>
<td>(32) Dye</td>
<td>49 m.</td>
<td>77.7</td>
<td>24.6</td>
<td>41.5</td>
<td>2522</td>
<td>1002</td>
<td>1620</td>
<td>1520</td>
</tr>
<tr>
<td>(4) Dye</td>
<td>41 f.</td>
<td>85±8.9</td>
<td>45.0±4.0</td>
<td>2467</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(33) CO</td>
<td>16 m. &amp; f.</td>
<td>66.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(34) CO Dye</td>
<td>9 m. &amp; f.</td>
<td>80.2±5.5</td>
<td>34.9</td>
<td>45.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(35) CO Dye</td>
<td>20 m. &amp; f.</td>
<td>71.0±5.0</td>
<td>35.0</td>
<td>45.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(36) Dye Plasma</td>
<td>8</td>
<td></td>
<td></td>
<td>3350</td>
<td>3350</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(37) ( {\text{P}}^{32} )</td>
<td>21 m. &amp; f.</td>
<td>38.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(21) ( {\text{P}}^{32} )</td>
<td>19 m. &amp; f.</td>
<td>33.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(18) ( {\text{P}}^{32} )</td>
<td></td>
<td>74</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7) ( {\text{Fe}}^{59} )</td>
<td>40 m.</td>
<td>29.7</td>
<td>1150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Key: W.B.V. = whole blood volume.  R.C.V. = red cell volume.  P.V. = plasma volume.  m. = male.  f. = female.
than those obtained by Meneely et al. (13) in their patients.  

In Table II are listed determinations made with the radioactive phosphorus technic in five patients with hypoproteinemia showing a variety of values from which few inferences can be drawn except to say that the procedure was fairly simple and was well tolerated.

Table III summarizes four representative cases in which the findings of plasma volume as obtained with the injection of salt-poor albumin or plasma (Formula 4) were compared with those as determined by the $^{32}P$ method. The former gave values which were usually 500 to 600 cc. higher. However, the increase in plasma volume following injection of albumin or plasma as determined by $^{32}P$ was in close agreement with the change calculated from Formula 5. No significant change in red cell volume was observed by either method.

### TABLE II

**Blood volume determinations using $^{32}P$ in red cells in patients**

<table>
<thead>
<tr>
<th>Patient, age, sex</th>
<th>Diagnosis</th>
<th>W.B.V. (cc./kilo.)</th>
<th>R.C.V. (cc./kilo.)</th>
<th>P.V. (cc./kilo.)</th>
<th>W.B.V. (cc./sq. m.)</th>
<th>R.C.V. (cc./sq. m.)</th>
<th>P.V. (cc./sq. m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. S. m. 71</td>
<td>Carcinoma of pancreas, malnutrition, edema</td>
<td>50.5</td>
<td>22.9</td>
<td>27.6</td>
<td>1860</td>
<td>845</td>
<td>1015</td>
</tr>
<tr>
<td>G. S. m. 71</td>
<td>Cirrhosis of liver; no edema or ascites</td>
<td>85.2</td>
<td>32.6</td>
<td>52.6</td>
<td>2731</td>
<td>1044</td>
<td>1887</td>
</tr>
<tr>
<td>E. R. m. 75</td>
<td>Carcinoma of pancreas, malnutrition, edema</td>
<td>77.2</td>
<td>31.6</td>
<td>45.6</td>
<td>2820</td>
<td>1155</td>
<td>1665</td>
</tr>
<tr>
<td>B. P. m. 48</td>
<td>Cirrhosis of liver, ascites, anemia</td>
<td>76.9</td>
<td>23.3</td>
<td>53.6</td>
<td>2918</td>
<td>878</td>
<td>2039</td>
</tr>
<tr>
<td>G. H. f. 45</td>
<td>Non-tropical sprue, hypoproteinemia; generalized edema</td>
<td>57.8</td>
<td>24.2</td>
<td>33.6</td>
<td>2010</td>
<td>842</td>
<td>1168</td>
</tr>
</tbody>
</table>


### TABLE III

**Simultaneous plasma volume determinations by $^{32}P$ and albumin injection methods**

<table>
<thead>
<tr>
<th>Case</th>
<th>Before injection of albumin</th>
<th>After injection of albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P.V. $^{32}P$</td>
<td>P.V. albumin</td>
</tr>
<tr>
<td>1</td>
<td>1942</td>
<td>2450</td>
</tr>
<tr>
<td>2</td>
<td>2000</td>
<td>2580</td>
</tr>
<tr>
<td>3</td>
<td>2997</td>
<td>3450</td>
</tr>
<tr>
<td>4</td>
<td>3246</td>
<td>3880</td>
</tr>
</tbody>
</table>

* Key: P.V. $^{32}P$ = Plasma volume calculated from injection of red blood cells containing $^{32}P$, Formula 3.

P.V. album = Plasma volume calculated from changes in albumin concentration and hematocrit following injection of a known amount of albumin, Formulas 4 and 5.  

Note that while the initial plasma volume is 500 cc. lower, as measured with radioactive phosphorus, the increase after the injection of albumin is about the same with each method.

5 Their findings are not included in Table I because it was impossible to calculate their data in the same manner since the body weight and surface areas were not reported.

Aside from the data on blood volume, other observations were made on three of the patients in whom we injected radioactive blood, who were operated upon one, three, and five days afterwards. In them it was possible to obtain liver and rectus muscle biopsies, as well as blood samples. The findings are recorded in Table IV, which shows that the liver and muscle tissue contain considerably more $^{32}P$ than the plasma, but less than the red cells in two of the three cases. After such a time interval the $^{32}P$ would probably...
be contained in the phosphatide fraction of the red cells, plasma and liver rather than the acid soluble fractions (19).

**DISCUSSION**

The behavior of the phosphorus under the conditions of our study is obviously important. Much light has been shed on the subject by many investigations. The uptake of inorganic phosphorus by the red blood cells has been studied in vitro and in vivo in man and in animals by the use of both quantitative and tracer methods. In 1940 Hahn and Hevesy (15) demonstrated that $^32P$ injected subcutaneously into rabbits entered the acid soluble fraction (hexose monophosphates and adenine triphosphate), and the phosphatides of the red blood cells. In short term experiments, the uptake of phosphorus into the carbohydrate cycle is much more significant than that into the phosphatides (19). Factors concerned with the uptake of phosphorus into the carbohydrate cycle of the red blood cells are:

1. **Time**: Lawazek (38) demonstrated that it required two to three hours for the glucose of red blood cells to be broken down to lactic acid.

2. **pH**: Alkalinization favors the synthesis, and acidification the breakdown, of phosphate esters [Halpern (39), Rapoport and Guest (40), and more recently Tulin, Danowski, Hald and Peters (41)].

3. **Temperature**: Halpern (39), Eisenman, Ott, Smith and Winkler (42), and Hahn and Hevesy (19) have shown that there is little or no uptake of phosphorus by the red blood cells at $0^\circ$ C., whereas the uptake at $37^\circ$ is considerable.

We have found that, following incubation of whole blood with $^32P$ for two hours at $37^\circ$, the activity of $^32P$ in the red blood cells is greater than that of the plasma. This difference in the distribution of $^32P$ was the same in the control subjects and patients with various diseases studied (see Table V).

Hahn and Hevesy (19) offer further evidence that the uptake of phosphorus by the red blood cells is actually due to metabolic activity by demonstrating that the addition of KCN to the system reduces markedly the formation of organic phosphorus compounds; that the uptake of phosphorus is independent of concentration of phosphorus in the plasma; and that hemolysate at $37^\circ$ takes up $^32P$, though at a slower rate than intact red blood cells.

In general, then, it may be said that the phosphorus enters the red blood cells in a large part by a process of organic synthesis (i.e., metabolic activity) rather than simple diffusion according to concentration gradients and that the metabolic process primarily concerned is glycolysis during which organic phosphorus esters are synthesized. That the phosphorus taken up by the red blood cells during incubation is held within the red blood cells at a constant level for sufficient time to allow for mixing of the injected blood and subsequent sampling is demonstrated below. The reason the $^32P$ that has entered the red blood cells does not begin to leave the red blood cells immediately after injection is not known.

The method used herein of measuring blood volume by means of $^32P$ is subject to the following four sources of error, each of which is discussed in detail. (1) Error of hematocrit, i.e., plasma adherence to packed red blood cells. (2)
Loss of \( P^{32} \) from the red blood cells within the time of determination of the blood volume. (3) Intrusion of \( P^{32} \) from the plasma into the red blood cells after injection (since activated whole blood is injected). (4) Hemolysis of the injected blood cells.

1. The error of the hematocrit due to adherence of plasma to the packed red blood cells has been studied and is found to be about 2 to 3 per cent (16, 19, 20, 43). Now if the plasma adherent to the red blood cells in the aliquot of the injected blood has the same activity as the red blood cells, there is no error in determination of \( C_1 \) in Formula 1. After injection and mixing time have passed, however, there will be an error in the determination of blood volume in the positive direction, i.e., the counts per cc. of sample after mixing (\( C_2 \) in Formula 1) will be lower than if no plasma were adherent because the activity of the plasma has fallen to about 20 per cent of the activity at zero time. As time passes this error will increase.

2. The error due to loss of \( P^{32} \) from the cells to the plasma is in the order of 1 to 2 per cent (1, 17, 19, 20, 37). This will also render the error in determination of blood volume positive because the counts per cc. (\( C_2 \)) of blood after mixing will be lower than at zero time. It has been shown in vitro by Hahn and Hevesy et al. (19, 20, 37) and by Brown, Hempelman and

![Graph](image)

**Fig. 1.** Radioactivity of Red Cells and Plasma after Injection

Each point represents a single determination of the radioactivity present in the red cells and in the plasma of 1 cc. of whole blood at various time intervals following the injection of the activated sample. The activity is expressed as a percentage of the theoretical activity at zero time which is set at 100 per cent. In the case of the red cells this zero value was obtained by extrapolation from the values at five, ten and 15 minutes. In the case of plasma this zero value was calculated from the plasma volume as subsequently measured together with the known amount of activity present in the injected plasma.

Note the consistency with which the activity in the red cells is maintained for a period of one hour with but little loss at two hours. By contrast note the almost immediate disappearance of the activity in the plasma which within 20 minutes had fallen to a maximum of 20 per cent.
Elman (1), that if red blood cells activated either by shaking in a thermostat at 37° or by repeated injection into a donor animal are mixed immediately with inactive NaCl, plasma or whole blood, there is little loss of activity over the period of one hour. Nylin (16, 21) has shown that in “normal” men there is no loss of activity of the red blood cells from 60 seconds to one hour after injection. We have found that there is no significant loss of activity from five minutes to one hour, and only a small loss in the second hour (Figure 1).

3. The error due to intrusion of P32 from the plasma into the red cells after injection would render the error in the determination of blood volume negative because the counts per cc. of red blood cells after mixing (i.e., C2) would be higher than at zero time. This error is thought to be in the magnitude of 3 to 4 per cent by Hahn and Hevesy et al. (19, 20, 37). Now if the activity of the red blood cells and plasma injected is equal and the ratio of P32 : P31 atoms in the plasma is ten times the ratio in the red blood cells (the acid soluble phosphorus concentration in red blood cells being ten times as great as in the plasma) the probability that P32 will pass into the cells rather than out is present until the activity of the plasma falls to 10 per cent of that at zero time. We have found that this point is reached in 20 minutes when the activity of the plasma has fallen to about 10 per cent of its activity at zero time. At this time the probability that P32 will flow into the red blood cells is equal to the probability that it will flow from the red blood cells to the plasma (the ratio of P32 : P31 in the plasma and red blood cells is now equal). From this point on, then, the error in determination of blood volume is that discussed above under Section 2, i.e., the error due to loss of P32 from the red blood cells after injection. Nylin (16), however, found no significant change in activity of the red blood cells of normal men up to 60 minutes after the injection of either activated whole blood or activated red blood cells which had been washed and resuspended in inactive plasma.

4. The error due to hemolysis of the injected red blood cells has not been studied; however, the same principles apply as discussed under Section 2, i.e., the loss of P32 from the red blood cells into the plasma. That errors 1, 2 and 3 almost cancel out for a single determination is shown by the following table taken from Hevesy (37).

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Per cent error due to adherence of plasma to corpuscles</th>
<th>Per cent error due to intrusion of P32 from plasma into RBC</th>
<th>Per cent error due to loss of P32 by corpuscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>+5</td>
<td>-5</td>
<td>+1.5</td>
</tr>
</tbody>
</table>

Nylin (16, 21) has found no significant loss of activity within one hour and we have corroborated these results, indicating that this method may be accurately applied in man for both routine determinations and accurate estimations of changes in blood volume occurring within one hour.

It should be emphasized that the primary method of calculation herein described, Formula 1, gives a value for whole blood volume without using the hematocrit. It is, however, based upon the assumption that the cell-plasma ratio is the same throughout the vascular system. Nevertheless, when the hematocrit is used for measuring red cell volume (Formula 2) the results obtained are in close agreement with those of other investigators (7, 21, 37). Moreover, the following data (Table VI) concerning the measurement of red cell volume before and after phlebotomy demonstrate the accuracy of the red cell volume calculation. These data were obtained in four normal subjects bled 500 cc. The whole blood volume was measured, according to our modification of the radioactive phosphorus technic, as described above, before, immediately after, and one-half hour after the bleeding. The red cell volume and plasma volume were calculated from the hematocrit at these times. We found that the red cell volume determined after hemorrhage was within 5 per

<table>
<thead>
<tr>
<th>Subject</th>
<th>Initial red cell volume</th>
<th>Red cells removed</th>
<th>Expected red cell volume</th>
<th>Red cell volume determined</th>
<th>Per cent error</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. S</td>
<td>3010</td>
<td>252</td>
<td>2758</td>
<td>2900</td>
<td>4.98</td>
</tr>
<tr>
<td>R. K</td>
<td>2220</td>
<td>227</td>
<td>1993</td>
<td>2555</td>
<td>3.1</td>
</tr>
<tr>
<td>F. K</td>
<td>2535</td>
<td>258</td>
<td>2277</td>
<td>2215</td>
<td>2.73</td>
</tr>
<tr>
<td>C. R</td>
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cent of the expected red cell volume, in each case, and within 3 per cent in three of the three cases. The differences between the data obtained immediately after phlebotomy and one-half hour later were less than 2 per cent in all instances. Similar findings were reported by Gibson et al. (8), who employed radioactive iron in dogs and in one patient with secondary polycythemia, and found the red cell volume before hemorrhage equal to red cell volume after hemorrhage plus the red cell volume removed (plus or minus 3 per cent). They also observed that the red cell volume before transfusion was equal to the red cell volume after transfusion minus red cell volume infused (plus or minus 2 per cent).

Much has been written about the inaccuracy of the hematocrit (other than the error incurred because of the adherence of plasma to the packed cells). The pivotal question in the literature is whether or not the hematocrit as drawn from one portion of the body represents the true cell plasma ratio of the entire circulating blood. Smith et al. (35), Hahn et al. (10), Stead and Ebert (43), Gibson (5), Hevesy et al. (37), and Gibson et al. (9) all state that the hematocrit is not a true indication of the cell plasma ratio of the circulating blood. The opposite is expressed by Hopper et al. (34, 44) who produced evidence that the hematocrit is essentially accurate.

The experimental data presented by most workers to disprove the validity of using the hematocrit consist of determinations of the red cell volume from the dye plasma volume and the hematocrit before and after hemorrhage. Invariably they found that the red cell volume before hemorrhage is greater than that after hemorrhage plus the volume of the cells removed. They conclude that the hematocrit is therefore responsible for this discrepancy on the assumption that the dye method gives the correct figure for the plasma volume. This assumption may not be justified.

Methods employing the injection of plasma for the measurement of blood volume have been used for many decades. One of the more recent studies is that of Hopper (34, 44) who reported that the calculation of plasma volume in dogs from formulae similar to those used herein gave results which not only failed to check the figures for the dye or carbon monoxide methods, but often varied in the opposite direction. On the other hand, plasma transfusions were used in a similar way in human subjects by Phillips et al. (36), who calculated plasma volume from the changes in specific gravity of the blood as well as from the changes in hematocrit or in the hemoglobin concentration and obtained similar results by both calculations; their results were in close agreement with simultaneous dye plasma volume methods.

In spite of these possible sources of error, it is believed that the method herein described is as accurate as any other now available and it is far more convenient for routine use than the other available refined methods. Therefore, it may prove of considerable clinical value in the study of patients suffering significant alteration in the blood volume or its constituents. It has proved useful in our experience in following changes in the total circulating red cells and plasma proteins in contrast to simple measurements of their respective concentrations. This has provided a three dimensional picture of the blood changes in various blood deficiencies, particularly following various types of intravenous therapy. A summary of its use in evaluating the respective effects of plasma and pure albumin injections in patients with chronic hypoproteinemia is described in another report from this laboratory.

**SUMMARY**

1. Blood volume was determined in normal human subjects and in patients with chronic hypoproteinemia, using red blood cells labeled with radioactive phosphorus (P³²). The method consisted of incubating a small sample of the subject’s blood with an isotonic solution containing approximately 50 microcuries of radioactive phosphorus and reinjecting a portion thereof. With this technic a direct value for the whole blood volume is obtained. The plasma and red cell volumes are then calculated by means of the hematocrit.

2. Blood volume was also measured in five patients using values obtained from changes in the hematocrit and plasma albumin concentration following injection of a known amount of pure salt-poor albumin. When compared with the simultaneous determinations made with radioactive red cells, the former method gave higher results for initial plasma volume, but subsequent changes checked well.
3. Both methods proved relatively simple and would seem to be well adapted to clinical investigation.

4. The findings were in close agreement with those obtained by other investigators using similar and other methods.

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BIBLIOGRAPHY


38. Lawaczek, Quoted from 43.