The estimation of red cell volume with $^{51}$Cr-labelled erythrocytes and plasma volume with radioiodinated human serum albumin

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SYNOPSIS  The validity of a simplified procedure for the direct estimation of both red cell and plasma volume, using $^{51}$Cr-labelled erythrocytes and radioiodinated human serum albumin, has been examined. It is suggested that the procedure, involving three venepunctures only, will yield accurate measurements of both compartments.

Knowledge of the red cell and plasma volumes is important in a variety of clinical conditions. In practice, either the red cell or plasma volume is measured directly, and the other compartment then calculated from the haematocrit. The body haematocrit is derived by multiplying the observed venous haematocrit by the body/venous haematocrit ratio. This ratio, however, varies with altitude (Metz, Levin, and Hart, 1962), pregnancy (Caton, Roby, Reid, Caswell, Maletskos, Fluharty, and Gibson, 1951), and disease (Rothschild, Bauman, Yalow, and Berson, 1954; Schreiber, Bauman, Yalow, and Berson, 1954). In any condition associated with fluid retention, the venous haematocrit probably does not reflect accurately the true status of the total blood volume (Fudenberg, Baldini, Mahoney, and Dameshek, 1961). For reliable blood volume studies it is therefore desirable that both red cell and plasma volumes be measured directly.

Red cell volume is measured directly with labelled erythrocytes, the $^{51}$Cr method being the most widely used. The plasma volume is usually measured directly with the dye T-1824, or with radioiodinated human serum albumin. The two methods yield comparable results but the radioiodinated serum albumin method has in practice largely replaced the dye method.

The simultaneous measurement of red cell and plasma volumes with $^{51}$Cr-labelled erythrocytes and radioiodinated serum albumin involves the counting of mixtures of $^{51}$Cr and $^{131}$I, and their separation is not easily achieved without equipment not generally available in the average hospital radioisotope laboratory. This difficulty could be obviated if the $^{51}$Cr red cell volume measurement was completed first, and the plasma volume measured with radioiodinated serum albumin immediately after drawing the last $^{51}$Cr specimen. Whole blood samples containing $^{51}$Cr only, and plasma samples with $^{131}$I only, could then be counted in a well-type scintillation counter.

This procedure has possible drawbacks. A significant amount of $^{51}$Cr in plasma would invalidate the plasma $^{131}$I measurement. Multiple venepunctures would be necessary; after the re-injection of $^{51}$Cr-labelled erythrocytes two samples are usually withdrawn, and a further three samples are required to measure the rate of clearance of the radioiodinated serum albumin. The study by Pritchard, Moir, MacIntyre, and Inkley (1955) suggests, however, that the activity of a single sample withdrawn 10 minutes after the injection of radioiodinated serum albumin, multiplied by a factor of 1.015, would give the plasma counts at zero time.

The present investigation was undertaken to test the validity of a simplified method of direct measurement of both red cell and plasma volume by studying (1) the amount of $^{51}$Cr in the plasma after the injection of $^{51}$Cr-labelled erythrocytes; (2) the significance of the difference in the radioactivity of any two whole blood specimens withdrawn between 10 and 20 minutes after the injection of $^{51}$Cr-labelled erythrocytes; and (3) the radioactivity of the plasma at 10-minute intervals after the injection of radioiodinated serum albumin.
MATERIAL AND METHODS

MATERIAL. The subjects studied were 60 healthy young white adult males.

$^{51}$CR BLOOD VOLUME Venous blood (15 to 20 ml.) was mixed with 6 ml. acid-citrate-dextrose solution in a sterile container. The blood was centrifuged and the supernatant removed. High specific activity (10 mc./mg. Cr) radioactive chromium 51 obtained from the Radiochemical Centre, Amersham, U.K., in the form of hexavalent sodium chromate, was added to the cells in a dose of 20 to 25 μc. and the mixture stood at room temperature for 30 minutes. After washing three times in isotonic saline, the cells were resuspended in approximately 25 ml. saline. Exactly 20 ml. of this volume was injected intravenously with a calibrated syringe. At 10 and 15 minutes after the injection, 10 ml. samples of blood were withdrawn and placed in heparinized containers. A 1 : 100 dilution of the residue of the injected blood was made, and 5 ml. aliquots of this standard, and of the 10- and 15-minute samples haemolysed by the addition of powdered saponin, were assayed for radioactivity in a well-type scintillation counter containing a 2-in. thallium-activated sodium iodide crystal.

\[
\text{Red cell volume} = \frac{\text{volume injected (ml.)} \times \text{counts/min.} \times \text{haematocrit}}{\text{mean of counts/min. of 10- and 15-minute samples} \times 100}
\]

\[
\text{Total blood volume} = \frac{\text{red cell volume} \times 100}{\text{venous haematocrit} \times \text{body haematocrit ratio}}
\]

\[
\text{Plasma volume} = \text{total blood volume} - \text{red cell volume}
\]

$^{131}$I ALBUMIN BLOOD VOLUME Radiiodinated human serum albumin was obtained from the Radiochemical Centre, Amersham, U.K. Carrier albumin was added to a concentration greater than 200 μg. per ml. With the 15-minute $^{51}$Cr sample, an additional sample ('pre-$^{131}$I plasma') was withdrawn to determine any radioactivity in the plasma due to $^{51}$Cr. Following this exactly 10 ml. of a solution of $^{131}$I albumin (3 μc.) was injected intravenously with a calibrated syringe, and three blood samples collected in heparinized containers 10, 20, and 30 minutes later. These samples were centrifuged and the plasma ('post-$^{131}$I plasmas') removed. Thyroid uptake of $^{131}$I was blocked by the prior administration of potassium iodide. Aliquots (5 ml.) of a 1 : 100 dilution of the $^{131}$I albumin solution injected and of the pre-$^{131}$I and post-$^{131}$I plasmas were assayed for radioactivity in the well counter.

Any counts in the pre-$^{131}$I plasmas were subtracted from those of the post-$^{131}$I plasmas and the latter then plotted on semi-log. paper and extrapolated to zero time.

\[
\frac{\text{volume injected (ml.)} \times \text{counts/min.}}{\text{ml. standard} \times \text{dilution of standard}}
\]

\[
\text{Plasma volume} = \frac{\text{counts/min./ml. at zero time}}{\text{plasma volume} \times 100}
\]

\[
\text{Total blood volume} = \frac{100 - \text{(venous haematocrit \times body haematocrit ratio)}}{\text{red cell volume} \times \text{body haematocrit ratio}}
\]

Red cell volume = total blood volume - plasma volume

The venous haematocrit was estimated from venous samples collected into dried sequestrine, and centrifuged at 12,000 g. in duplicate capillary tubes in an International microhaematocrit centrifuge.

The body haematocrit was calculated as follows:

\[
\text{Body haematocrit} = \frac{\text{red cell volume} \times (^{51}\text{Cr})}{\text{red cell volume} \times (^{51}\text{Cr}) + \text{plasma volume} \times (^{131}\text{I albumin})}
\]

RESULTS

The results are shown in Tables I to IV. The mean number of counts in the plasma due to $^{51}$Cr, expressed as a percentage of the counts due to $^{131}$I at zero time, is $0.93 \pm 0.17\%$. The difference in the mean red cell volumes calculated from the two $^{51}$Cr samples.

### TABLE I

<table>
<thead>
<tr>
<th>No.</th>
<th>(1) $^{51}$Cr in Plasma (counts/min.)</th>
<th>(2) $^{131}$I in Plasma at Zero Time (counts/min.)</th>
<th>(1)/(2) × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>60</td>
<td>27.5 ± 4.7</td>
<td>0-143</td>
<td>3438.8 ± 273.9</td>
</tr>
</tbody>
</table>

### TABLE II

<table>
<thead>
<tr>
<th>No.</th>
<th>Red Cell Volume (ml./kg.)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specimen 1</td>
<td>Specimen 2</td>
</tr>
<tr>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>60</td>
<td>33·28 ± 0·66</td>
<td>23·8-51·9</td>
</tr>
</tbody>
</table>

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The estimation of red cell volume and plasma volume

The estimation of red cell volume and plasma volume

TABLE III

<table>
<thead>
<tr>
<th>No.</th>
<th>Plasma Volume (ml./kg.)</th>
<th>% Difference</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extrapolation to Zero Time</td>
<td>10-minute Sample × 1-015</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
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</tr>
<tr>
<td>48</td>
<td>44-12 ± 0-74</td>
<td>34-6-56-4</td>
<td>44-47 ± 0-78</td>
<td>36-4-55-9</td>
<td>1-85 ± 0-26</td>
<td>0-7-0</td>
</tr>
</tbody>
</table>

TABLE IV

<table>
<thead>
<tr>
<th>No.</th>
<th>Red Cell Volume (ml./kg.)</th>
<th>Plasma Volume (ml./kg.)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>^51Cr Erythrocytes</td>
<td>Radioiodinated Serum Albumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>33-34 ± 0-69</td>
<td>32-59 ± 0-58</td>
<td>45-16 ± 0-46</td>
<td>45-70 ± 0-99</td>
</tr>
</tbody>
</table>

^The differences are not statistically significant.

is 0-13 ml./kg. and is not statistically significant (p = >0-05). In comparing the plasma volume calculated by correcting the 10-minute ^131I sample with that obtained by extrapolation to zero time, only curves where the three values fell on one straight line were considered; this was the case in 48 out of 60 subjects studied. The difference in the means of 0-35 ml./kg. is not statistically significant (p = >0-05).

DISCUSSION

The results confirm the validity of a simplified method of measuring both red cell volume and plasma volume directly. Following the injection of labelled erythrocytes, only a single sample of blood need be taken for the calculation of red cell volume. Although the number of counts in the plasma due to ^51Cr is generally insignificant, it is advisable to draw an extra sample at this time as a blank for the radioiodinated serum albumin plasma volume. After the withdrawal of this specimen, the radioiodinated serum albumin solution can be injected through the same needle, and 10 minutes later a single sample drawn from another vein. The radioactivity of this sample, when multiplied by a factor of 1-015, does not yield a significantly different figure from that obtained by drawing multiple samples and extrapolating to zero time. This procedure, however, would be invalid in conditions where intravascular mixing is delayed, or where there is abnormally rapid plasma clearance of albumin as in nephrosis or protein-losing gastroenteropathy.

We wish to thank the Director, the South African Institute for Medical Research, for facilities to carry out this study, and Dr. N. W. Levin for his help.

REFERENCES