A CRITICAL EVALUATION OF RED CELL AND PLASMA VOLUME TECHNIQUES IN PATIENTS WITH BURNS

BY

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From the results in 110 patients, in whom 207 red cell volume estimations were made at various times after burning using either $^{51}$Cr, $^{32}$P, or Group NN-labelled cells, and 17 plasma volume estimations on the same patients using either T-1824- or $^{131}$I-labelled albumin, it is concluded that:

(a) The $^{51}$Cr red cell volume method is the most reliable method; it gives results which are probably not in error by more than $\pm 10\%$ when the labelled cells are washed three times and so has a place in the assessment of the changes in red cell volume that follow a severe burn. In conjunction with a well type scintillation counter the radiation dosage received by the patient with $^{51}$Cr is about one-fifth of that with any other method.

(b) The $^{32}$P method is slightly less reliable, as shown by on average higher supernatant radioactivity levels and a greater scatter in the rate of disappearance of the isotope from the circulation.

(c) The Ashby (Group NN) method and the plasma volume and haematocrit method are inaccurate in patients with burns.

The changes in circulatory volume that follow a severe burn may be divided into those affecting plasma and those affecting red cells. In moderately severe burns up to about one-third of body surface, any loss of red cells, if it occurs at all, is so small as to be undetectable by the methods currently available, and a change in plasma volume is reflected by a change in the haematocrit. With large burns, however, replacement of the lost plasma by a suitable colloid using the haematocrit as a guide to the level of the plasma volume may lead to oligoemia owing to an unknown loss of red cells. It is in these cases that methods of estimating red cell volume may be of value.

The accuracy of a red cell volume estimation is mainly dependent upon complete mixing of the injected labelled cells in the circulation before sampling. However, in patients with severe burns the peculiar circulatory state with gross leakage of plasma and venoconstriction might be expected to impair mixing and hence the accuracy of blood volume estimations. An investigation was therefore carried out to show whether red cell volume estimations were likely to be accurate enough to improve the clinical management of patients with severe burns. The methods were similar to those previously described for patients with injuries other than burns (Davies and Topley, 1959).

METHODS

Two hundred and seven estimations of red cell volume using $^{32}$P- or $^{51}$Cr-labelled red cells, and 17 plasma volumes using T-1824- or $^{131}$I-labelled human albumin, have been made on 110 patients with burns involving between 10 and 90% of the body surface. About half the patients were children under 12 years old. Most of the estimations (167/224) were made within 48 hours of burning.

Red Cells Labelled with Radioactive Phosphorus [$^{32}$P]

The technique based on that of Chaplin (1954) has already been described in detail (Davies and Topley, 1959).

Red Cells Labelled with Radioactive Chromium [$^{51}$Cr]

The following slight modifications of the technique described by Davies and Topley (1959) are based on the method of Mollison and Veall (1955). The dosage of radioactivity injected into the children did not exceed 0.5 μC/kg. body weight with burns of less than 45% of the body surface and with larger burns it did not exceed 2 μC/kg. During the last six months of the five-year investigation a well type scintillation counter superseded the annular type. The increase in sensitivity of the equipment for the detection of the isotopes emitting γ rays ($^{51}$Cr and $^{131}$I) was approximately seven times, i.e., for $^{51}$Cr, 1 μC changes from 9,530 counts per minute to 65,660 counts per minute. This change made possible a fivefold reduction in the
amount of $^{51}$Cr required for a red cell volume estimation and thus also in the radiation dosage received by the patient.

As occasionally it was not possible to obtain venous blood for the sample taken after mixing was assumed to be complete, 0.5–1.0 ml of capillary blood from a free-flowing ear or toe prick was used.

**Red Cells Labelled with the Group NN Antigen**

The method as described by Mollison (1951) was used.

**Plasma Proteins labelled with T-1824**

The method described by Davies and Topley (1959) was used on 12 occasions in conjunction with the $^{32}$P red cell volume method.

**Human Albumin Labelled with Radioactive Iodine $[^{131}I]$**

The published method (Davies and Topley, 1959) was used on five occasions in conjunction with the $^{51}$Cr red cell volume method.

**Other Methods and Calculations**

The blood volume was calculated from either the plasma volume or the red cell volume and the venous and body haematocrits. The ratio between the body and venous haematocrits was taken as 0.90 (Davies and Topley, 1959).

**RESULTS**

The factors which may cause inaccuracy will first be described. In the second section will be given results of simultaneous estimations using various pairs of methods, and the third section will show the consistency between sequential estimations before and after an operation at which there was a known blood loss and transfusion.

**Factors which May Cause Inaccuracy**

**Radioactivity in the Supernatant.**—The radioactivity in the solution in which the labelled cells were suspended (the supernatant) has been expressed as a percentage of the total quantity of radioactivity injected (Table I). The results obtained during the first 48 hours after burning have been compared with those obtained later. A comparison has also been made between different isotopes and the number of washes with saline alone or plasma saline. There were significantly more results above 1% when $^{32}$P was used (27 out of 97 or 28%) than when $^{51}$Cr was used (seven out of 92 or 8%) ($\sigma=0.689, t=3.94, P<0.001$). The data also suggest that three washes of $^{51}$Cr-labelled cells are better than two for reducing the number of observations above 1%.

<table>
<thead>
<tr>
<th>Table I</th>
<th>RADIOACTIVITY LEVEL OF PLASMA SALINE (SUPERNATANT) IN WHICH THE LABELLED CELLS WERE SUSPENDED AS A PERCENTAGE OF RADIOACTIVITY INJECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Washes</td>
<td>Isotope</td>
</tr>
<tr>
<td>Patients' cells labelled during first 48 hours after burning</td>
<td></td>
</tr>
<tr>
<td>3 $^{32}$P</td>
<td>52</td>
</tr>
<tr>
<td>3 $^{51}$Cr</td>
<td>17</td>
</tr>
<tr>
<td>2 $^{51}$Cr</td>
<td>66</td>
</tr>
<tr>
<td>Patients' cells labelled after first 48 hours after burning</td>
<td></td>
</tr>
<tr>
<td>3 $^{32}$P</td>
<td>45</td>
</tr>
<tr>
<td>3 $^{51}$Cr</td>
<td>1</td>
</tr>
<tr>
<td>2 $^{51}$Cr</td>
<td>8</td>
</tr>
<tr>
<td>Cells washed with plasma saline</td>
<td></td>
</tr>
<tr>
<td>3 $^{32}$P</td>
<td>34</td>
</tr>
<tr>
<td>3 $^{51}$Cr</td>
<td>3</td>
</tr>
<tr>
<td>2 $^{51}$Cr</td>
<td>29</td>
</tr>
<tr>
<td>Cells washed with saline alone</td>
<td></td>
</tr>
<tr>
<td>3 $^{32}$P</td>
<td>27</td>
</tr>
<tr>
<td>3 $^{51}$Cr</td>
<td>2</td>
</tr>
<tr>
<td>2 $^{51}$Cr</td>
<td>35</td>
</tr>
</tbody>
</table>

It has also been shown that washing $^{32}$P-labelled cells with plasma saline compared with saline alone results in a small number of observations above 1%.

**Adequacy of Intravascular Mixing and Survival of Labelled Materials in the Circulation.**—The changing levels of radioactivity in the circulation have been plotted for each patient, when multiple venous sampling was possible as the concentration of label per unit volume of red cells (log scale) against the time after injection (linear scale). Extrapolation of the best straight line through these points to zero time gives the theoretical initial concentration (100%), and other observations are expressed as a percentage of this value. The mean of all the percentages at each time interval after injection together with the standard deviations are plotted in Figs. 1a and 1b for each type of radioactive label. Most of the observations were made within 48 hours of burning. Thirty estimations on 25 patients have been made with $^{51}$Cr and 22 estimations on 15 patients with $^{32}$P. The best straight line has been drawn through the mean values to indicate the average rate of disappearance. Ten observations were made with T-1824. With the Ashby (Group NN) technique only one blood sample was taken at the end of the transfusion so it was not possible to show whether mixing had been adequate.

On 12 occasions venous sampling at approximately two-hour intervals was possible for up to 18 hours after the injection of $^{51}$Cr-labelled red cells. The data (Fig. 1c) have been calculated by the same means as the observations made within
Fig. 1.—The mean rates of disappearance of $^{51}$Cr- and $^{32}$P-labelled red cells. The shaded area shows the standard error.
Fig. 2.—Simultaneous estimations of red cell and plasma volume using a number of methods. The scatter around the line of equivalence is shown together with the ±10% limits.
one hour of injection. The 10 patients had burns ranging between 18 and 65% of the body surface.

It can be seen (Table II) that with $^{51}$Cr none of the 34 results taken between nine and 20 minutes after injection varied from the mean value by more than $\pm$10%. With $^{32}$P, two out of 29 exceeded these limits during the same period. The number of observations outside $\pm$10% of the mean increased as the time between injection and sampling increased. The observations over 18 hours after injection show a greater scatter than those over one hour. Nevertheless only three of the 54 observations exceeded $\pm$15% of the mean values.

Variations in Ratio between Body and Venous Haematocrits.—Seventeen simultaneous estimations of red cell and plasma volume using $^{32}$P or $^{51}$Cr- and T-1824- or $^{131}$I-labelled albumin enabled the body haematocrit to be calculated and hence its ratio to the venous haematocrit. Eight of the 17 observations were made within 48 hours of burning. The mean ratio was 0.931. Seven of the observations lay outside the limits of $\pm$10%, whilst only two lay outside $\pm$20% of the mean value.

Simultaneous Estimation of Red Cell and Plasma Volume.—These estimations were made by two methods in parallel on the same subjects. The labelled materials were injected from two different syringes with not more than one-minute intervals between the injections. The amount of the two labels was determined separately in the sample of blood taken after mixing was complete. The equivalence of the results is shown in Fig. 2a, b, c.

Red Cells Labelled with Both $^{32}$P and $^{51}$Cr.—The five pairs of estimations using both $^{32}$P and $^{51}$Cr (Fig. 2a) agree within a range of $\pm$7% of the line of equivalence. For three of the five experiments red cells stored in A.C.D. for transfusion purposes were labelled with $^{51}$Cr whilst the patients' own cells were labelled with $^{32}$P. In the remaining two experiments the patients' own cells were labelled with both isotopes.

Red Cells Labelled with $^{51}$Cr and the Group NN Antigen.—The 14 simultaneous estimations show a considerable scatter (Fig. 2c). Nine of the estimations lie outside $\pm$20% of the line of complete equivalence. On average the red cell volume calculated from the Group NN antigen method was higher than that estimated by $^{51}$Cr.

Simultaneous Estimations of Red Cell and Plasma Volume Using $^{32}$P- and $^{51}$Cr-labelled Red Cells and T-1824- or $^{131}$I-labelled Albumin.—The $^{32}$P-labelled red cells and T-1824 were injected within one minute of each other, whereas the $^{51}$Cr-labelled red cells and $^{131}$I-labelled albumin were usually injected with 15 to 20 minutes between them so that the red cell volume estimation was not affected by the plasma volume radioactivity. Twelve simultaneous estimations were made using $^{32}$P and T-1824 (solid circles, Fig. 2b), and five using $^{51}$Cr- and $^{131}$I-labelled albumin (open circles, Fig. 2b). The scatter of the result is considerably greater than that observed with simultaneous red cell volume estimations, only seven of the 17 lying within $\pm$10% of the line of complete equivalence. Eight of the 17 estimations were made within 48 hours of burning.

Sequential Estimations Before and After a Known Blood Loss and Transfusion

On 12 occasions a red cell volume estimation using either $^{32}$P or $^{51}$Cr was made before and another after a primary excision and grafting operation at which there was a measured blood loss by swab weighing and a known volume of blood transfused. The results (Table III) have been calculated by first converting volumes of blood lost and transfused into volumes of red cells lost and transfused using the haematocrit, and then comparing the difference between the volume of red cells lost and transfused with the

<table>
<thead>
<tr>
<th>Estimated Area of Burn (%)</th>
<th>% of Body Surface Excised</th>
<th>Error as % of Normal Red Cell Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>8</td>
<td>+10.9</td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>+0.6</td>
</tr>
<tr>
<td>13</td>
<td>11</td>
<td>+1.9</td>
</tr>
<tr>
<td>25</td>
<td>13</td>
<td>-8.5</td>
</tr>
<tr>
<td>32</td>
<td>15</td>
<td>-7.8</td>
</tr>
<tr>
<td>33</td>
<td>15</td>
<td>-3.2</td>
</tr>
<tr>
<td>24</td>
<td>15</td>
<td>+5.6</td>
</tr>
<tr>
<td>18</td>
<td>15</td>
<td>+26.7</td>
</tr>
<tr>
<td>38</td>
<td>15</td>
<td>-19.2</td>
</tr>
<tr>
<td>25</td>
<td>18</td>
<td>-5.3</td>
</tr>
<tr>
<td>30</td>
<td>21</td>
<td>-15.3</td>
</tr>
<tr>
<td>30</td>
<td>22</td>
<td>-16.1</td>
</tr>
</tbody>
</table>

The difference is shown as a percentage of the patient's normal red cell volume. The estimated area of the burn and the area excised are also shown.
difference between the pre- and post-operation red cell volume estimations. These observed differences have been expressed as a percentage of the patients' expected red cell volume for height. It can be seen that with burns up to 38% of the body surface, and when up to 22% of the body surface was excised, the error between expected and found values only exceeded ±20% once and only on five occasions did it exceed ±10%. Larger excisions on larger burns were excluded because of obvious and unmeasured post-operative haemorrhage.

**DISCUSSION**

This study has revealed variations in the amount of radioactivity in the suspending saline, variations in the mean rate of disappearance of the labelled materials from the circulation, and differences between two methods in parallel. These variations will be compared with findings in patients with other injuries, and discussed in terms of their probable effect on the accuracy of red cell volume measurement after burns.

**Factors Causing Variation**

**Level of Radioactivity in the Supernatant.**—This indicates that red cells from patients with burns are less satisfactorily labelled with \( ^{32} \)P compared with red cells from patients with injuries as there were two to three times as many observations above 1% (Davies and Topley, 1959). The data suggest that \( ^{51} \)Cr-labelled red cells washed three times is the preferred method even in the absence of plasma saline. This is in contrast to the evidence in normal individuals (Mollison, 1958), where only two or sometimes one wash is necessary.

**The Adequacy of Intravascular Mixing and the Survival of Labelled Materials in the Circulation.**—The concentration of the labelled material per unit volume of blood at various times up to 40 minutes after injection was not significantly different from that found in normal individuals or for patients with injuries other than burns (Davies and Topley, 1959). These data, together with the observations up to 18 hours after injection, suggest that mixing was complete by nine to 20 minutes after injection and that no preferential disappearance of labelled cells occurred in this time. A single sample during this time is therefore probably a fair sample for a red cell volume estimation. The chances of a single observation exceeding ±10% of the mean value are shown in Table II.

**The Ratio Between the Body and Venous Haematocrits.**—Patients with burns showed a range of ratios similar to those found in patients with other injuries (Davies and Topley, 1959), and thus, as in normal individuals, errors of ±10–20% could at times be produced in a red cell volume when calculated from a plasma volume and the haematocrit.

**Simultaneous Estimations by Different Methods**

The simultaneous red cell volume estimations using \( ^{32} \)P and \( ^{51} \)Cr show good agreement similar to that found in patients with injuries other than burns and in normal individuals (Davies and Topley, 1959) whereas the Ashby technique using Group NN cells has often proved inaccurate.

The simultaneous red cell and plasma volume estimations have shown a greater scatter than that observed in patients with injuries other than burns. About one-third of the results (in six out of 17 patients) exceeded ±20% of the line of equivalence compared with only one-sixth in patients with injuries other than burns (Davies and Topley, 1959). The results suggest that the plasma volume and haematocrit method is not a suitable way of estimating red cell volume in patients with burns, probably because of the changes in capillary permeability which may lead to a leakage of the dye or labelled albumin from the circulation.

**Radiation Dosage**

The considerations of radiation dosage due to \( ^{51} \)Cr, \( ^{32} \)P, and \( ^{131} \)I already described (Davies and Topley, 1959) also apply to patients with burns. In general, however, smaller doses of the isotopes were used, as in the children the dosage of \( ^{51} \)Cr never exceeded 0.5 \( \mu \)C/kg with burns of less than 45% of the body surface and 2.0 \( \mu \)C/kg when the burned area exceeded this value.

In adult patients 25 \( \mu \)C was usually given and gave a radiation dosage of 0.025 r.e.p. on the assumption that the isotope was evenly distributed throughout the body of a 70 kg. patient. A similar radiation dosage was received from the 2 \( \mu \)C of \( ^{32} \)P required for a red cell volume estimation using this isotope. The reduction in the amount of \( ^{51} \)Cr required from 25 \( \mu \)C to 5 \( \mu \)C by use of the well type scintillation counter reduced the radiation dosage to 0.005 r.e.p. in the more recently burned patients.

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