The Measurement of Sodium Concentration in Human Red Blood Cells

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Abstract Experiments are described which indicate that iodinated human serum albumin underestimates the amount of extracellular sodium trapped in the packed layer of red blood cells, when cells and plasma are separated by centrifugation. Sucrose-14C also underestimates the amount of trapped extracellular sodium, but the difference between the percentages of sucrose-14C and extracellular sodium trapped is constant and independent of mean relative centrifugal force. It is concluded that human red blood cell sodium concentration can be measured with accuracy (a) if trapped plasma sodium is estimated with radioisotopes of sodium and a correction made for entry of sodium into the cells, providing cells and plasma can be separated rapidly; (b) by the use of sucrose as a standard plasma marker to derive the amount of trapped plasma sodium; (c) by washing the cells with sodium-free solutions. Reported values for red blood cell sodium concentration in healthy adults are critically reviewed.

Many estimates of sodium concentration in red blood cells of healthy human subjects have been reported, and some of the errors associated with different methods of measurement described. RBC sodium concentration has been estimated indirectly by measuring sodium concentration in plasma and in whole blood, determining the relative volumes of plasma and cells from the hematocrit, and deriving the concentration in red blood cells by difference. This method does not allow for the presence of white blood cells. Failure to correct the apparent cell volume in the hematocrit for plasma trapped with the packed cells results in an underestimate of plasma volume and gives falsely high values for RBC sodium concentration (1). The potential error involved in deriving a small quantity (RBC sodium concentration) from the difference between two large quantities (plasma and whole blood sodium concentrations) is considerable (2) and accounts in part for the wide scatter of the results, which have been obtained with this method.
By measuring sodium concentration in red blood cells directly, after separation from plasma by centrifugation, it is possible to exclude white blood cells and to avoid the errors inherent in subtracting one large quantity from another. The problem of estimating the amount of plasma trapped in the packed cell layer, however, remains. Since sodium concentration in plasma is some twenty times greater than that in red blood cells, an error of 1% in estimating the percentage of plasma trapped with the packed red blood cells gives rise to an error of 20% in the estimate of RBC sodium concentration.

**TABLE I**

**REPORTED VALUES FOR SODIUM CONCENTRATION IN RED BLOOD CELLS, MEASURED DIRECTLY, ARE LISTED**

All values have been corrected for trapped plasma sodium. Where sodium concentration was originally given in terms of cell volume, values have been divided by 1.096 (Ponder, 19).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Plasma marker</th>
<th>Trapped plasma</th>
<th>Mean corrected RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Na meq/kg cells</td>
<td>Na meq/kg cells</td>
</tr>
<tr>
<td>Solomon (11)</td>
<td>$^{24}$Na</td>
<td>3.87</td>
<td>7.90</td>
</tr>
<tr>
<td>Love and Burch (13)</td>
<td>$^{22}$Na</td>
<td>2.44</td>
<td>7.03</td>
</tr>
<tr>
<td>Beilin et al. (14)</td>
<td>Sucrose-$^{14}$C</td>
<td>—</td>
<td>Men 7.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Women &lt; 45 yr, 6.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 45 yr, 7.53</td>
</tr>
<tr>
<td>Gold and Solomon (12)</td>
<td>$^{131}$IHSA</td>
<td>1.37</td>
<td>11.04</td>
</tr>
<tr>
<td>McCance and Widdowson (16)</td>
<td>Evans blue</td>
<td>2.73</td>
<td>13.94</td>
</tr>
<tr>
<td>Boeckelman (17)</td>
<td>$^{131}$IHSA</td>
<td>2.17</td>
<td>7.85 to 12.04</td>
</tr>
<tr>
<td>Dowben and Holley (18)</td>
<td>$^{131}$IHSA</td>
<td>1.53</td>
<td>Men 13.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Women 12.56</td>
</tr>
<tr>
<td>Czaczkes et al. (10)</td>
<td>$^{131}$IHSA</td>
<td></td>
<td>7.8</td>
</tr>
<tr>
<td>Valberg et al. (8)</td>
<td>$^{131}$IHSA</td>
<td></td>
<td>8.79</td>
</tr>
</tbody>
</table>

To disregard trapped plasma altogether gives rise to inflated estimates of RBC sodium concentration.

Plasma trapping is usually measured by adding to whole blood a marker substance which does not enter red blood cells, centrifuging the blood, and measuring the distribution of the substance between plasma and the packed cell layer. Substances used or suggested for use as markers have included hemoglobin, plasma proteins, Evans blue, human serum albumin-$^{131}$I ($^{131}$IHSA), inulin, lactose, $^{24}$Na, and $^{22}$Na. Some reported estimates of RBC sodium concentration, corrected for trapped plasma sodium, are listed in Table I.

The studies of Chaplin and Mollison (3), using Evans blue, clarified the effects of varying duration of centrifugation and of varying mean relative centrifugal force (MRCF) on plasma trapping. In particular they demonstrated the relationship between hematocrit and MRCF and showed that
cells in blood of low hematocrit are exposed to greater MRCF than are those in blood of high hematocrit; hence, plasma trapping is less.

Maizels and Remington (4) described the culminating experiments of a series by Maizels and his coworkers and showed that, under standard centrifugal conditions, the use of markers of high molecular weight (\textsuperscript{131}I-HSA, Evans blue-albumin, hemoglobin) gives lower estimates of plasma trapping than do smaller molecules (lactose, inulin).

The results of experiments in which the entry of sodium into human red blood cells was studied in vivo are described elsewhere (5). In these experiments, \textsuperscript{22}Na was injected intravenously and plasma and RBC specific activity estimated in blood samples obtained at varying intervals of time. Plasma trapping under standard centrifugal conditions was repeatedly estimated in each experiment using \textsuperscript{131}I-HSA. In all experiments performed, RBC specific activity was significantly greater than zero at zero time. The curves of RBC specific activity could only be simulated on an analogue computer if a compartmental model was set up which included a compartment of appreciable size, with a time constant approximating zero. Expressed in another way, one-quarter of the apparent RBC sodium was equilibrating immediately with plasma.

These results lend support to those of Maizels and Remington (4) and suggest that \textsuperscript{131}I-HSA is not suitable as a marker for the measurement of trapped plasma sodium.

This paper describes experiments which led to the choice of sucrose as a standard plasma marker for the measurement of trapped plasma sodium.

An ideal marker substance should be distributed in the same volume of extracellular water as the sodium ion; it should not enter, damage, or be bound to the surface of red blood cells; accurate estimation of concentrations one thousand times smaller than those present in plasma should be possible. Five substances, which it was thought might conform to these criteria, were selected for study. Of these \textit{d}-arabinose-\textsuperscript{14}C was found to enter red blood cells freely (RBC activity 60\% of plasma activity at 1 hr), while the samples of mannitol-\textsuperscript{14}C and sorbitol-\textsuperscript{14}C used did so to a small extent (RBC activity 2\% and 3\% of plasma activity respectively in 1 hr). The experiments to be described were, therefore, restricted to sucrose-\textsuperscript{14}C and inulin-\textsuperscript{14}C.

When cells are centrifuged from suspension in sodium-free solutions, the problem of estimating trapped extracellular sodium disappears, but this method was not considered suitable for estimating red blood cell sodium concentration in large groups of subjects, nor for studies of sodium flux involving the use of radioactive isotopes. Results obtained by others using this method are discussed later.

\textsuperscript{1} Wilbrandt (6) first reported that red blood cells appeared to be nearly impermeable to \textit{d}-arabinose.
METHODS

\(^{131}\)I-HSA, \(^{24}\)Na, and sucrose-\(^{14}\)C were obtained from the Radiochemical Center, Amersham, England, and inulin-\(^{14}\)C from the California Corporation for Biochemical Research, Los Angeles.

\(^{131}\)I-HSA was passed through an anion-exchange resin (Amberlite IRA-400 Cl) and dispensed at the appropriate concentration in isotonic saline by the Department of Physics, King's College Hospital. It was then stored at 4°C in a bottle containing free resin and used within 24 hr. These precautions were necessary to prevent degradation of the material. Sucrose-\(^{14}\)C and inulin-\(^{14}\)C were dissolved in sterile solutions of isotonic saline (5 \(\mu\)C/ml), stored frozen in ampoules, and thawed immediately before use.

Blood samples from normal subjects were taken into plastic syringes containing ammonium heparin (Evans Medical), powdered or in isotonic glucose solution, less than 5 min before each experiment.

In incubation experiments, the isotope was added to heparinized blood in a plastic flask and gently shaken on a water bath at 37°C with 95% \(\text{O}_2\)–5% \(\text{CO}_2\), samples being withdrawn serially and centrifuged. In other experiments isotope was added to blood in plastic tubes which were capped, mixed, and centrifuged as soon as possible. The delay between addition of isotope and start of centrifugation was usually less than 2 min when low centrifuge speeds were used, and between 2 and 3 min when high speeds were used.

In experiments in which centrifugation was carried out at low speeds, 5 or 9 g samples of blood were weighed into cellulose nitrate tubes (internal diameter 11 mm) to which 0.1 to 0.5 ml of isotope solution was added. Tubes were then centrifuged in an MSE hematocrit centrifuge governed at 3000 ± 30 RPM (RCF at base of tube 1460 g) for 60 min. In experiments in which centrifugation was carried out at high speeds, 3.6 g samples of blood were weighed into polypropylene tubes (internal diameter 11 mm). After addition of isotope and mixing the tubes were centrifuged in a MSE Superspeed 40 fitted with a three bucket swing-out head. Centrifuge speed and times varied in different experiments.

After centrifugation, weighed samples of plasma containing \(^{24}\)Na or \(^{131}\)I-HSA were counted in a Panax scintillation counter with well-type crystal and pulse height analyzer. The centrifuge tubes containing the packed cells and a layer of plasma were frozen in an ethanol-\(\text{CO}_2\) mixture and sectioned horizontally approximately 0.5 to 1 mm below the buffy coat–RBC interface by an electric saw in apparatus specially constructed by the Department of Physics. For studies with \(^{24}\)Na and \(^{131}\)I-HSA, the lower section of the centrifuge tube containing frozen red blood cells was inserted in a beaker, weighed, and counted. The weight of red blood cells counted was obtained by reweighing the beaker and cut centrifuge tube, after washing clean and drying in an oven. All counts were corrected for dead time and background, and RBC counts were additionally corrected for geometrical factors introduced by the presence of the centrifuge tube. Counting errors were less than 1%.

\(^{14}\)C was counted in a Nuclear-Chicago gas-flow counter, on steel-ribbed planchets. Sample composition was standardized by adding to plasma samples an appropriate volume of a nonradioactive solution of hemolyzed red cells. A concentrated solution of nonradioactive sucrose was added to all samples to ensure even drying of the material.
Sample thickness was standardized by adding the same weight of sample to all planchets. After drying, variation in sample weights was less than 10%. To minimize the effect of variations in sample thickness on counting efficiency, weights were selected to provide relatively thick samples, and sufficient radioactivity was always added to overcome the resulting reduction in counting efficiency. Counts were corrected from a standard curve relating sample weight to counting efficiency, and also for dead time and background. Counting error was less than 1% for plasma samples and less than 3% for RBC samples.

The sodium concentration of samples was determined, after appropriate dilution, with an EEL flame photometer.

**TABLE II**

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>MRCF 1300 g 50 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation time, min</td>
<td>3.5</td>
</tr>
<tr>
<td>Percentage trapped</td>
<td>Mean 3.120</td>
</tr>
<tr>
<td></td>
<td>SD 0.027</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 2</th>
<th>MRCF 170,000 g 6 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation time, min</td>
<td>12</td>
</tr>
<tr>
<td>Percentage trapped</td>
<td>Mean 1.519</td>
</tr>
<tr>
<td></td>
<td>SD 0.114</td>
</tr>
</tbody>
</table>

*The between-times effect is not significant.

The sodium concentration of RBC samples was corrected for trapped plasma sodium as follows:

$$\text{Corrected RBC [Na]} = \frac{\text{Observed RBC [Na]} - T \times \text{plasma [Na]}}{1 - T}$$

where $T$ is the fraction of plasma trapped in the packed cell column.

**RESULTS**

Table II lists the results of experiments in which sucrose-$^{14}$C was incubated with blood in vitro. These demonstrated that sucrose does not enter red blood cells in significant amount for periods between 4 and 60 min. (The problem of radiation decomposition of $^{14}$C-labeled samples and the effect this may have on estimates of plasma trapping are considered later.)

The effect of different centrifugal forces applied for constant time on the percentage trapping of $^{3}$HIHSA and sucrose-$^{14}$C, and on total sodium in the
packed cell layer was studied. The results for $^{131}$I HSA and sucrose-$^{14}$C are illustrated in Fig. 1. It can be seen that the percentages of both substances trapped in the packed cell layer change in a similar manner with increasing centrifugal force, but that for all forces sucrose-$^{14}$C trapping is greater than $^{131}$I HSA trapping, and is approximately four times as great between 50,000 and 150,000 g. When trapped plasma sodium, calculated directly from the observed percentage of sucrose-$^{14}$C trapped at each centrifugal force, was subtracted from total sodium in the cell layer, the resulting values for cell 

![Figure 1. The percentages of sucrose-$^{14}$C and $^{131}$I HSA trapped at different MRCF's are compared. Means and standard deviations of samples in triplicate are illustrated.](https://example.com/figure1.png)

sodium concentration were constant, and independent of g force. When similar calculations were made, using the observed percentage of trapped $^{131}$I HSA to estimate trapped plasma sodium, values for cell sodium concentration were not constant and were influenced by centrifugal force. The results, which are given in Table III, indicate that the relationship between trapped extracellular sodium and sucrose is constant and independent of applied g force.

They also illustrate the quantitative importance of small changes in estimates of plasma trapping. Sucrose trapping was approximately 1.5% greater than albumin trapping, but the calculated RBC sodium concentration was reduced by approximately 25%.

A comparison of sucrose-$^{14}$C and $^{24}$Na trapping under identical conditions was made, to determine whether sucrose and extracellular sodium are dis-
tributed in a similar volume of extracellular water in the trapped cell layer. It is necessary to apply corrections to the results to allow first for sodium binding to plasma proteins and to heparin and second for $^{24}\text{Na}$ entry into the red blood cells.

Salminen (7) investigated the sodium-binding properties of normal human serum and showed that the mean percentage of nonultrafiltrable sodium was 7.1% at filtration pressures of 110 cm Hg, and 5.1 to 5.6% at 18 cm Hg. It has been assumed for these calculations that 6% of plasma sodium is bound to protein, and after centrifugation is trapped to the same extent as $^{131}\text{IHSA}$.

Salminen (7) also investigated the effect of heparin on the ultrafiltration of sodium and found that, at heparin concentrations of 120 units per ml plasma and pressures of 110 cm Hg, 10.1% of plasma sodium was not ultrafiltrable.

The results of an experiment, in which the effect of increasing heparin concentration on percentage trapping of $^{24}\text{Na}$ was studied, are listed in Table IV. The results show that $^{24}\text{Na}$ trapping was increasingly depressed relative to sucrose trapping, as heparin concentration was increased, and indicate that the distribution of sodium bound to heparin in the packed cell layer was restricted relative to sucrose. Because hematocrit and the percentage of sucrose trapped were also affected by heparin concentration, it was not possible to draw any quantitative conclusions from this experiment about the amount

### Table III

<table>
<thead>
<tr>
<th>MRCF From percentage sucrose-$^{14}\text{C}$ trapped</th>
<th>From percentage $^{131}\text{IHSA}$ trapped</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t \times 10^8$</td>
<td>meq/kg cells</td>
</tr>
<tr>
<td>-----</td>
<td>-----------</td>
</tr>
<tr>
<td>2</td>
<td>6.60</td>
</tr>
<tr>
<td>6</td>
<td>6.67</td>
</tr>
<tr>
<td>25</td>
<td>6.90</td>
</tr>
<tr>
<td>69</td>
<td>6.90</td>
</tr>
<tr>
<td>176</td>
<td>6.66</td>
</tr>
</tbody>
</table>

Analysis of variance of RBC sodium concentration from estimates of trapped plasma sodium

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>$F$</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G$ force</td>
<td>4</td>
<td>0.0552</td>
<td>0.0138</td>
<td>&lt;1*</td>
<td>0.7771</td>
<td>0.1943</td>
<td>7.476‡</td>
</tr>
<tr>
<td>Residual</td>
<td>10</td>
<td>0.2896</td>
<td>0.02896</td>
<td></td>
<td>0.2599</td>
<td>0.02599</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>0.3450</td>
<td>0.02450</td>
<td></td>
<td>1.0370</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Not significant.
‡ (0.01 > $P > 0.001$).
of sodium bound to heparin. Instead, it has been assumed for these calculations that at a concentration of 30 units/ml plasma 1% of plasma sodium is bound to heparin. It has also been assumed that heparin (mol wt, 57,000) and albumin (mol wt 65,000) are distributed in a similar manner in the trapped cell layer.

The second correction necessary is to allow for entry of $^{24}$Na into red blood cells. There is an unavoidable delay of nearly 3 min between additions of isotope to the blood and starting the centrifuge (due to sealing and loading the tubes, and closing and evacuating the machine). In addition there is a delay of unknown duration before plasma and red blood cells are effectively separated. With MRCF of 69,000 g (reached in approximately 2 min), this additional delay is unlikely to be less than 1 or more than 3 min. The total delay is thus 4 to 6 min, during which time sodium is entering the cells. Assuming a sodium flux between plasma and red blood cells of the order of 2.75 meq Na/kg cells/hr (12) and plasma concentration of 140 meq Na per liter, 0.164% of the plasma sodium will enter the red blood cells in a period of 5 min.

Thus when sucrose-$^{14}$C and $^{24}$Na trapping were compared, the following corrections were applied:

$$S_e = S_o - 0.07(S_o - A_o)$$
$$Na_e = Na_o - 0.164$$

where $S_e$ and $Na_e$ are the corrected percentages of sucrose-$^{14}$C and $^{24}$Na trapped, and $S_o$, $A_o$, and $Na_o$ are the observed percentages of sucrose-$^{14}$C, $^{11}$HSA, and $^{24}$Na trapped.

If sucrose trapping provides a true measure of sodium trapping, $S_e$ should equal $Na_e$.

In Fig. 2 are shown the results of an experiment in which the percentages of sucrose and of $^{24}$Na trapped at different centrifugal forces were compared.
It can be noted that sucrose trapping was slightly but consistently greater than $^{24}$Na trapping at all forces; this finding remained after application of the corrections mentioned.

No explanation for this rather surprising result was apparent at the time, for these experiments were performed shortly after the in vitro incubation experiments, which showed that no significant entry of the material into red blood cells was occurring between 4 and 60 min. Repeated estimation of sucrose-$^{14}$C trapping under standard conditions, however, using the same material over a period of 8 months, showed a slow but significant rise in percentage trapping. The results fitted the equation, $y = 1.62 + 0.0020 \times$, where $y$ was the percentage sucrose-$^{14}$C trapped and $x$ was the number of days since recrystallization of sucrose. When fresh material was used within a week of recrystallization (by Dr. Bayly, Radiochemical Center, Amersham), the observed trapping under identical conditions (MRCF 69,000 g: 20 min) was 1.598%. It was concluded, therefore, that self-destruction of the material was the cause of the anomalous result, and that the decomposition products equilibrated rapidly with red blood cells. The general conclusions of the experiments described remain valid, except that values for percentage of sucrose-$^{14}$C trapped are spuriously elevated by a constant but unknown amount.

When recrystallized sucrose-$^{14}$C trapped was 1.598% (± 0.098%), $^{1}$HSA trapped was 0.406% (± 0.076%), and $^{24}$Na trapped was 2.088% (± 0.063%).

Figure 2. The trapping percentages of sucrose-$^{14}$C and $^{24}$Na are compared at different MRCF's. Means and standard deviations of samples in triplicate are illustrated.
Applying the corrections given in equations 1 and 2, \( S_e = 1.515\% \) and \( N_a = 1.924\% \). Thus sucrose trapping is less than sodium trapping. The difference between observed sucrose trapping and corrected sodium trapping is 0.326\%.

Two conclusions may be drawn from these results. If sodium and sucrose are distributed in the same volume of extracellular water in the packed cell column, some sodium must additionally be associated with the surface of the red blood cells (approximately 0.5 to 0.6 meq/kg cells). Alternatively, if sodium distribution in plasma water in the packed cell column is uniform, approximately 3 ml plasma water/kg RBC must be inaccessible to the sucrose molecule. Whichever mechanism or combination of mechanisms is the cause of the discrepancy between sucrose-\(^{14}\)C-trapping and the calculated value for trapped plasma sodium, the difference between the two appears to be constant and independent of applied centrifugal force.

Similar experiments were performed with inulin-\(^{14}\)C. In incubation experiments no significant entry into red blood cells occurred between 3 and 30 min and the difference between the percentages of sucrose-\(^{14}\)C and inulin-\(^{14}\)C trapped was constant and independent of centrifugal force. As with sucrose, evidence of slow self-destruction was obtained, but since supplies of recrystallized material were not readily obtainable, these studies were not pursued.

It is concluded that when facilities are available for separating plasma and red blood cells effectively within 5 min, the simplest and most accurate method of measuring trapped plasma sodium is to measure the trapping of radioisotopes of sodium and to correct the results for sodium entry into the cells. In some experiments facilities for such rapid separation are not available. Thus, studies of in vivo \(^{24}\)Na flux (5) require the use of larger blood samples than can be satisfactorily separated on high speed centrifuges with swing-out heads. Larger blood samples are necessary to ensure counting accuracy, when only limited amounts of isotope can be injected with safety. Furthermore, fast centrifuges cannot easily be moved and in many studies it is more convenient to use a portable and slower machine. In such instances, sucrose-\(^{14}\)C can be used as a standard marker substance, since plasma sodium trapping is 0.326\% greater than the observed percentage of sucrose-\(^{14}\)C trapped, and this figure is independent of applied centrifugal force.

Sucrose-\(^{14}\)C has been used thus as a plasma sodium marker in experiments with the MSE hematocrit centrifuge (RCF at base of tube 1460 g).

Chaplin and Mollison (3), using a similar machine, showed that the rate of change of plasma trapping was still considerable after 30 min, but was much less after 50 min centrifugation. To minimize timing errors all studies were, therefore, carried out with 60 min centrifugation. It was assumed that centrifuge tubes are of constant diameter; constant volumes of red blood cells should, therefore, provide constant MRCF and identical packing of the cells.
For speed, and because many blood samples were radioactive, whole blood was added to centrifuge tubes by weight and not by volume. Three potential errors arise in estimating MRCF and therefore trapped extracellular sodium, (a) those attributable to variation in weight of whole blood added, (b) those attributable to difference in hematocrit between individuals, and (c) those attributable to variation in the distance from the buffy coat-red cell interface to the level of cutting the centrifuge tube. A fourth variable is introduced if plasma trapping, at constant MRCF, differs in blood from different subjects. That this is so when \(^{31}\)I-IHSA is used as a plasma marker is suggested by Valberg et al. (8).

Experiments were performed in which the percentage of sucrose-\(^{14}\)C trapped was estimated in three different weights of blood from different subjects. In two experiments, each with four subjects, in whom duplicated samples at each weight were taken, sucrose-\(^{14}\)C, which had not recently been recrystallized, was used. In a third experiment with nine further subjects, recently recrystallized sucrose-\(^{14}\)C was used and single samples at each weight were taken.

The equation for the regression was \(y = a + bx\), where \(y\) was the percentage of sucrose trapped, and \(x\), the weight in grams of red blood cells sampled. In the first two experiments no significant differences between subjects were present. The values for \(b\) in the three experiments were 0.2398, 0.2438, and 0.2383 respectively and as these values did not differ significantly, the results were pooled to give a figure of 0.2411. The value of \(a\) was, however, significantly lower in the third experiment using recrystallized material (2.4938) than in the pooled results of the first two experiments (3.0467). When \(a\) (2.4938) is corrected for the constant percentage by which sucrose-\(^{14}\)C underestimates trapped plasma sodium (0.326%), the regression equation becomes \(y = 2.8198 + 0.2411x\). A slightly different equation based on early calculations \(y = 2.8214 + 0.2398x\) was used in experiments to determine sodium flux into red blood cells in vivo (5); because of its close similarity to the final equation, the results were not recomputed. In the three subjects studied flux rates of 2.62, 2.39, and 2.53 meq/kg RBC/hr were obtained, slightly less than the value assumed in these calculations. The error involved in assuming that 0.164% of \(^{24}\)Na enters red blood cells in 5 min is not, however, great. With flux rate of 3 meq Na/kg cells/hr and a 6 min delay, \(^{24}\)Na entry into red blood cells equals 0.214% of plasma sodium; with flux rate of 2.3 meq/hr and a delay of 4 min, \(^{24}\)Na entry into cells equals 0.110% of plasma sodium. The use of the arbitrarily assigned figure of 0.164% may therefore cause an error in estimated plasma trapping and hence an error in RBC Na concentration of \(\pm 0.05\) plasma Na concentration or approximately 0.07 meq Na/kg cells.
DISCUSSION

When studies of $^{24}\text{Na}$ flux between plasma and red blood cells in man were repeated, using the percentage trapping of sucrose-$^{14}\text{C}$, corrected in the manner described, to estimate trapped plasma sodium, RBC specific activity at zero time was zero (5). The immediately exchangeable fraction of RBC sodium found when trapped extracellular sodium was estimated with $^{131}\text{IHSA}$ had been eliminated. This fraction is analogous to the "easily exchangeable" fraction described by Maizels and Remington (9) when red blood cells were washed with solutions of differing sodium concentration. These authors found that the size of the fraction varied directly with the sodium concentration in the wash solution and suggested four hypotheses which might explain its existence. First, they suggested that red blood cells may be heterogeneous, as regards permeability to the sodium ion. Beilin et al. (5) have shown that such heterogeneity, as regards the "easily" or "immediately" exchangeable fraction of sodium, is not a function of cell age, and no support was offered for this hypothesis. The other hypotheses put forward to account for the easily exchangeable fraction were, first, that sodium is adsorbed on the surface of red blood cells; second, that sodium freely enters some superficial zone on the surface of red blood cells; third, that the sodium concerned is extracellular.

The difference between these hypotheses is, to a certain extent, semantic. Quantitatively the size of the immediately exchangeable fraction found in the in vivo flux studies was reduced from approximately 2 to 0.5 meq/kg cells, when sucrose replaced albumin as the marker molecule. The experiments described do not distinguish between these hypotheses, but since the fraction is a function of extracellular sodium concentration (9), for practical purposes it can be regarded as plasma sodium.

Certain conclusions can be drawn from inspection of the results listed in Table I. When isotopes of sodium have been used to measure trapped plasma sodium, lower concentrations of RBC sodium have been found, than in studies with $^{131}\text{IHSA}$ or Evans blue as the marker substance. An exception is provided by the results reported by Czaczkes et al. (10).

These authors measured the percentage of $^{131}\text{IHSA}$ trapped in samples of blood from each subject studied, and found not only considerable variation from subject to subject (1.2 to 4.0%), but also in different samples of blood from the same subject (<1.47%). Such variation has not been encountered in this laboratory when rigid precautions were taken to prevent decomposition of $^{131}\text{IHSA}$, and when no differences in MRCF were present.

The results reported by Solomon (11) and by Gold and Solomon (12) are of some interest. In the first study $^{24}\text{Na}$ was used as plasma marker, and trapped plasma sodium amounted to 4.15 meq/liter cells; in the second study, when $^{131}\text{IHSA}$ was used as marker, trapped plasma sodium fell to 1.5 meq/liter.
cells. Gold and Solomon concluded that the earlier estimate of RBC sodium concentration was erroneous because the upper layer of cells had been excluded and because $^{24}$Na had entered the cells. Neither of these factors was sufficient to account for the discrepancy between the results which is, however, consistent with the finding that $^{131}$I-HSA is a poor marker for measuring trapped extracellular sodium. The results of Love and Burch (13), who used $^{24}$Na as a marker for trapped plasma sodium, correcting for $^{24}$Na entry into the cells, closely resemble those obtained by Beilin et al. (14), using a plasma-trapping correction obtained with sucrose-$^{14}$C and described in this paper.

Valberg et al. (8) obtained a mean figure for RBC sodium concentration of 8.84 meq/kg cells, using $^{131}$I-HSA as plasma marker. They also studied the effect on RBC sodium concentration of washing cells with choline-Tris and with magnesium chloride buffers. When the mean value of 8.84 meq Na/kg cells is corrected for the sodium reduction observed on washing, corrected values of 7.22 and 7.37 meq/kg cells are obtained for the respective buffers. These values are very close to that obtained by Beilin et al. (14).

Maizels (15) expressed a dislike, on theoretical grounds, for the use of washed cells. Nevertheless, because it is concluded that the easily exchangeable fraction of RBC sodium found when cells are washed (9) is in fact extracellular, the results obtained by this method are in general more accurate than those obtained with unwashed cells when $^{131}$I-HSA has been used to measure trapped plasma sodium.

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