A STUDY OF HUMAN RED BLOOD CELL PERMEABILITY.

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In their recent studies (20) on the water and electrolyte distribution between cells and serum, Van Slyke, Wu, and McLean assumed for their theoretical considerations that the red blood cells were impermeable to the cations sodium and potassium. They showed that when the serum anion concentration was changed in whole blood CO₂ saturation experiments, the reestablishment of electrolyte equilibrium could be entirely accounted for by the transfer of water and anions across the cell membrane. Several previous investigators (5, 6, 11, 12, 18) had reported similar findings in CO₂ saturation experiments; but Hamburger (8, 9) found that when the concentration of the cations in the serum was changed by adding sodium or potassium salts or water to whole blood, both basic and acid ions appeared to traverse the cell membrane in the electrolyte readjustment. At present, therefore, since Hamburger's latter findings stand undisputed, any assumption of the impermeability of the red blood cells to sodium or potassium seems open to doubt.

Since this question is of fundamental importance to an understanding of electrolyte equilibrium, the present writers have repeated some of Hamburger's experiments using the more refined laboratory procedures that have been recently acquired, because it was believed on theoretical grounds that the red blood cells were impermeable to cations within the limits of electrolyte change found in human disease. They found that when the electrolyte equilibrium was disturbed by the addition of salt or water to blood even beyond the extreme limits of variation recorded in human blood, the red blood cell membrane apparently remained impermeable to the cations sodium and potassium.
Red Blood Cell Permeability

It seemed of unusual interest to include in the experiments the salts of both sodium and potassium inasmuch as the extraordinary and little understood separation of potassium within the cells and sodium in the serum and tissue fluids of the body suggested that the cell membrane might show a selective permeability for potassium.

EXPERIMENTAL.

Method.

Human venous blood was collected into large Pyrex tubes and defibrinated with a glass stirring rod. From 70 to 120 cc. of blood were taken for each experiment depending upon whether the blood was divided into two or three samples. In either case, one sample was analyzed directly. In three experiments equimolar amounts of similar sodium and potassium salts were added to a second and a third sample. In the remaining experiments, a single salt or water was added to the second sample. Salts of chloride and carbonate were used because of the ease of determining their anion distribution between the cells and serum. The amounts of salt added varied from 21.9 to 100.4 milli-equivalents of base in 1000 cc. of serum. The smaller amounts of carbonate salt added represent the maximal amount of CO₂ that could be conveniently analyzed. Water was added in the proportion of 250 cc. of H₂O per 1000 cc. of blood. The dried weighed salt and the measured amount of water were added directly to the whole blood without causing noticeable hemolysis by first centrifuging down the cells and adding the salts or water to the supernatant serum, and then remixing the cells and serum. In order to prevent any organic change within the blood system during the delays between the various determinations, the blood was kept on ice until ready to use and then quickly brought to room temperature.

All specimens were saturated with 40 mm. of CO₂ in air at 38°C. by the technique previously described (1). Oxygen capacity determinations were done to assure an equal hemoglobin concentration in the duplicate or triplicate specimens. Cell volume and serum protein were determined to indicate the water shift between the cells and serum. CO₂ content and chloride concentration were determined in the whole blood and the serum to show the change.
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in their distribution between the cells and serum. The total base in the serum was determined to indicate whether there had been any exchange of base across the cell membrane. And finally, the sodium concentration of the serum was determined in five experiments to show whether in case the serum total base content remained constant there occurred an equimolar shift of sodium and potassium in opposite directions across the membrane.

Analytical Procedures.

Oxygen capacity was determined in the Van Slyke constant pressure pipette by a method devised by Lundsgaard and Neill. Blood cells from which the plasma had been removed were used for the O₂ determination as described in a previous communication (15). Where whole blood and diluted cells were compared, the agreement was fairly satisfactory.

Cell volume was determined by using a Daland hematocrit fitted to a No. 1 International Equipment Company centrifuge. Duplicate determinations were carried out on each blood sample and always agreed within 1 volume per cent.

Serum proteins were determined by macro-Kjeldahl procedure using about ½ cc. samples of serum diluted with physiological saline.

CO₂ was determined by the Van Slyke method (22) in a calibrated constant volume pipette.

Chlorides were determined by the Van Slyke method (21). A few of the whole blood chlorides were determined by the Whitehorn method (23).

Total base was determined by a modification of Stadie and Ross’s adaptation of Fiske’s urine method (16).

Sodium was determined by the following modification of the Kramer and Gittleman method (14).

Solutions.—The alcohol, KOH, and Na₂S₂O₃ solutions were prepared exactly as described by Kramer and Gittleman (14). 2 per cent KI was used in place of 20 per cent. Great difficulty was experienced

1 Personal communication.

2 The serum protein results in Experiments 1, 2, and 3 are probably too low because no superoxol was added to assure complete digestion. In the remaining experiments, where superoxol was used, the results are believed to be more accurate.

3 The whole blood chlorides in the first three experiments were determined by Whitehorn’s method. It was discovered that added NaCl was not always recovered completely by this method. Hence the Van Slyke method was adopted for the last five experiments for both whole blood and serum. Inasmuch as the serum chlorides in the first three experiments were determined by the Van Slyke method which gave good checks, the cell chlorides in these experiments were recalculated on the basis of the known serum volume, serum chloride, and added chloride.
in obtaining a satisfactory potassium pyroantimonate solution. The Baker salt, suggested by Kramer and Gittleman, gave good results with only one of three lots. When the material was analyzed for its antimony content, varying amounts of Sb were found. The use of the Baker salt was therefore replaced by that of a Kahlbaum salt, pure $K_2H_2Sb_2O_7$. Made up as described by Kramer and Gittleman, this solution gave satisfactory results. Starch was found to be unnecessary in the final titration.

Analysis.—2.5 cc. of serum were ashed as described by Kramer (13). Kramer and Gittleman (14) determined sodium directly on the unashed serum. This method, however, gives lower results than when the serum is ashed; and recovery of added sodium is not quantitative. Therefore, the present writers believe, as was pointed out by Balint (2), that more complete sodium recovery is obtained by ashing the serum. The ash is dissolved in 2.5 cc. of 0.1 N HCl. 1 cc. portions are placed in 25 cc. conical Pyrex centrifuge tubes. (These tubes may not be used for other analyses and must be washed with water and brush only.) 2 drops of 10 per cent KOH (Na-free) are added and the contents of the tube thoroughly mixed by stirring with a glass rod. 5 cc. of $K_2H_2Sb_2O_7$ solution are added. 1.5 cc. of 95 per cent $C_2H_6OH$ are added with constant stirring. The tubes are allowed to stand for 30 minutes and are then centrifuged for 5 minutes. The supernatant fluid is decanted and the tube is drained on filter paper. The precipitate is washed with 5 cc. of 30 per cent $C_2H_6OH$ and the tube is again centrifuged, the fluid decanted, and drained as before. The precipitate is dissolved in 2.5 cc. of 10 N HCl and 2.5 cc. of $H_2O$. 2 cc. of 2 per cent KI are added and the solution is titrated immediately with 0.1 N $Na_2S_2O_3$ until the iodine color disappears.

The above proportions of reagents will adequately take care of 2.00 to 3.75 mg. of Na. If the presence of less Na is suspected more serum must be used. If 1 cc. of serum contains more than 3.75 mg. of Na, the ash should be dissolved in 5 cc. of 0.1 N HCl and 2 cc. portions (equivalent to 1 cc. of serum) used in the analysis. In this case double quantities of all the reagents must be used.

Using 1 cc. of HCl-containing ash and the amounts of reagents described above, the blank is 6 mg. of Na per 100 cc. of serum. Using 2 cc. of HCl-containing ash and double the reagents, the blank is 12 mg. The method yields, as Balint (2) showed, 103 per cent sodium.

Calculation.—When the equivalent of 1 cc. of serum is used:

$$\frac{(Cc. Na_2S_2O_3 \times 115) - 6}{1.03} = \text{mg. Na per 100 cc. serum.}$$

Results.

The results of eight experiments are reported, three in which equimolar amounts of similar sodium and potassium salts were added to like blood samples, five in which a single salt or water was added and sodium was determined.
TABLE I.
Complete Electrolyte Determinations Expressed in Various Units
Conventionally Used.

M.-eq. = milli-equivalent, the unit for all monovalent ions, represents one-thousandth of the gram-molecular weight. The numbers following the amount of Na₂CO₃ and K₂CO₃ added, represent m.-eq. of sodium or potassium. Total base = the base-combining power of the serum expressed as m.-eq. per liter.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m.-eq. or cc. per l.</td>
<td>vols. per cent</td>
<td>vols. per cent</td>
<td>vols. per cent</td>
<td>gm. per l.</td>
<td>gm. per l.</td>
<td>m.-eq. per l.</td>
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<tr>
<td>1</td>
<td>NaCl 34.7</td>
<td>45.35</td>
<td>20.00</td>
<td>50.7</td>
<td>58.6</td>
<td>2.61</td>
<td>3.61</td>
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<td></td>
<td>KCl 34.7</td>
<td>38.73</td>
<td>20.06</td>
<td>49.4</td>
<td>59.5</td>
<td>3.66</td>
<td>5.04</td>
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<td>2</td>
<td>Na₂CO₃ 9.2</td>
<td>57.80</td>
<td>24.06</td>
<td>48.3</td>
<td>61.4</td>
<td>2.47</td>
<td>3.50</td>
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<td>K₂CO₃ 9.2</td>
<td>54.55</td>
<td>23.82</td>
<td>61.7</td>
<td>78.0</td>
<td>2.47</td>
<td>3.60</td>
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<td>3</td>
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<td>58.85</td>
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<td>61.5</td>
<td>2.52</td>
<td>3.52</td>
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<td></td>
<td>K₂CO₃ 13.9</td>
<td>54.20</td>
<td>24.10</td>
<td>67.8</td>
<td>88.6</td>
<td>2.52</td>
<td>3.61</td>
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<tr>
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<td>NaCl 50.8</td>
<td>38.45</td>
<td>16.50</td>
<td>51.1</td>
<td>60.3</td>
<td>2.76</td>
<td>3.66</td>
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<td>KCl 51.2</td>
<td>34.50</td>
<td>16.59</td>
<td>52.3</td>
<td>61.0</td>
<td>3.88</td>
<td>4.92</td>
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<td>5</td>
<td>Na₂CO₃ 13.9</td>
<td>57.20</td>
<td>15.54</td>
<td>54.0</td>
<td>63.4</td>
<td>2.87</td>
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<td>32.30</td>
<td>15.56</td>
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<td>4.02</td>
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<td>45.30</td>
<td>20.27</td>
<td>47.1</td>
<td>55.8</td>
<td>2.76</td>
<td>3.66</td>
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<td></td>
<td>K₂CO₃ 23.6</td>
<td>42.60</td>
<td>20.39</td>
<td>65.0</td>
<td>80.5</td>
<td>3.72</td>
<td>5.85</td>
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<td>7*</td>
<td>H₂O 250</td>
<td>42.20</td>
<td>18.06</td>
<td>45.8</td>
<td>53.6</td>
<td>2.71</td>
<td>3.63</td>
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<td></td>
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<td>40.50</td>
<td>13.96</td>
<td>38.1</td>
<td>45.2</td>
<td>2.21</td>
<td>2.87</td>
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<td>H₂O 250</td>
<td>43.80</td>
<td>18.98</td>
<td>42.2</td>
<td>50.0</td>
<td>2.75</td>
<td>3.68</td>
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<td></td>
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<td>41.60</td>
<td>15.20</td>
<td>36.7</td>
<td>41.8</td>
<td>2.29</td>
<td>2.85</td>
</tr>
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</table>

*In Experiment 7, due to a technical error in the delivery of the blood from sampling bulb before it was properly mixed, the hematocrit and whole blood CO₂ and Cl were inaccurate. These determinations were, therefore, corrected on the basis of the serum protein change and the serum CO₂ and Cl.
TABLE II.
Concentration of Bicarbonate, Chloride, Sodium, and Total Base in Cells and Serum before and after Addition of Salt.

The sodium and total base "calculated" represent their expected concentrations in the serum if there is no transfer of base across the cell membrane.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Salt added to blood</th>
<th>Serum volume.</th>
<th>HCO₃⁻</th>
<th>Cl</th>
<th>Na serum.</th>
<th>Total base serum.</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>m.-eq. per l.</td>
<td>Serum.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NaCl 34.7</td>
<td>54.65</td>
<td>17.3</td>
<td>24.9</td>
<td>39.5</td>
<td>101.8</td>
</tr>
<tr>
<td></td>
<td>KCl 34.7</td>
<td>61.15</td>
<td>14.5</td>
<td>25.3</td>
<td>40.7</td>
<td>142.3</td>
</tr>
<tr>
<td>2</td>
<td>Na₂CO₃ 9.2</td>
<td>42.20</td>
<td>16.2</td>
<td>26.2</td>
<td>49.3</td>
<td>99.0</td>
</tr>
<tr>
<td></td>
<td>K₂CO₃ 9.2</td>
<td>45.75</td>
<td>20.0</td>
<td>33.2</td>
<td>43.6</td>
<td>101.3</td>
</tr>
<tr>
<td>3</td>
<td>Na₂CO₃ 13.9</td>
<td>41.15</td>
<td>16.8</td>
<td>26.2</td>
<td>50.8</td>
<td>99.4</td>
</tr>
<tr>
<td></td>
<td>K₂CO₃ 13.9</td>
<td>46.80</td>
<td>21.8</td>
<td>37.5</td>
<td>44.5</td>
<td>102.0</td>
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<tr>
<td>4</td>
<td>NaCl 50.8</td>
<td>61.55</td>
<td>15.2</td>
<td>25.6</td>
<td>37.3</td>
<td>103.1</td>
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<td>KCl 51.2</td>
<td>65.50</td>
<td>15.0</td>
<td>26.0</td>
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<td>5</td>
<td>Na₂CO₃ 23.6</td>
<td>54.70</td>
<td>15.2</td>
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<td>46.6</td>
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<td>57.40</td>
<td>18.6</td>
<td>34.6</td>
<td>41.1</td>
<td>105.0</td>
</tr>
</tbody>
</table>

Sodium and Total Base Calculated.

\[ B_2 = \frac{B_1 V_1 + B_a}{V_2} \]

Where \( B_1 = \) Sodium or total base found in 1000 cc. of untreated serum.
\( B_a = \) M.-eq. base added per liter.
\( B_2 = \) Sodium or total base calculated in 1000 cc. of treated serum.
\( V_1 = \) Serum volume per cent in untreated blood.
\( V_2 = \) " " " " treated blood.

Table I contains the complete electrolyte determinations.

Tables II and III represent changes in serum volume, cell and serum bicarbonate and chloride concentration, and sodium and total base concentration produced by the addition of salt or water.
TABLE III.

Concentration of Bicarbonate, Chloride, Sodium, and Total Base in Cells and Serum before and after Addition of Water.

The cell volume is expressed in relation to 125 cc. of blood in the specimens to which water has been added, instead of 100 cc. as it is in the untreated specimens. The figures in parentheses represent the concentration of BHCO₃ and Cl calculated on the assumption that there is no transfer across the cell membrane.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Water added to blood.</th>
<th>HCO₃</th>
<th>Cl</th>
<th>Na serum.</th>
<th>Total base serum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>42.20</td>
<td>14.6</td>
<td>22.6</td>
<td>38.5</td>
<td>102.4</td>
</tr>
<tr>
<td>H₂O 250</td>
<td>50.60</td>
<td>11.3</td>
<td>18.9</td>
<td>34.6</td>
<td>80.9</td>
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<td>8</td>
<td>43.83</td>
<td>13.0</td>
<td>21.3</td>
<td>43.6</td>
<td>103.6</td>
</tr>
<tr>
<td>H₂O 250</td>
<td>52.00</td>
<td>12.1</td>
<td>17.4</td>
<td>41.9</td>
<td>80.4</td>
</tr>
</tbody>
</table>

Sodium and Total Base Calculated.

\[ B_2 = \frac{B_1 V_1}{V_2} \]

Where \( V_1 \) = Serum volume in 125 cc. of treated blood.

\( HCO_3 \) and Cl Calculated.

\[ A_2 = \frac{A_1 V_1}{V_2} \]

Where \( A_1 \) = HCO₃ or Cl found in 1000 cc. of untreated serum.\( A_2 \) = ““““ calculated in 1000 cc. of treated serum.

\( HCO_3 \) (Tables II and III).

Bohr's solubility coefficients (3) for cells and serum are used throughout these experiments though it is realized there is a slight inaccuracy according to Hastings' (10) recent paper. \( HCO_3 = CO_2 \) content - \( H_2CO_3 \).

\[ H_2CO_3 = \frac{\text{Solubility coefficient} \times pCO_2}{760} \]

Where \( pCO_2 = CO_2 \) tension.

Solubility coefficient of \( CO_2 \) in serum at 38°C. = 0.555 X 0.975.

““““““ cells ““ ““ 38°C“ = 0.555 X 0.81.

Where 0.555 = solubility coefficient of \( CO_2 \) in \( H_2O \) at 38°C.
The effect of adding salt or water to whole blood will be noted in the following changes: There is an increase in serum volume or shift of water from cells to serum in all except the two water experiments where cell volume increases. There is an exchange of Cl and HCO₃ across the cell membrane when salts of chloride or carbonate are added to the blood. When chloride is added, Cl penetrates the cells and HCO₃ escapes to the serum; and similarly, on adding carbonate, HCO₃ enters the cells while Cl passes into the serum. When water is added to the blood, there is a shift of both HCO₃ and Cl from cells to serum.

The sodium and total base found in the serum agree in all the experiments within 3.5 m.-eq. per liter with that calculated on the assumption that no base traverses the cell membrane. The variation is not constantly above or below the calculated and in most of the experiments is less than 2 m.-eq. In three experiments where equal concentrations of sodium and potassium salt were added, the changes in the distribution of water, CO₂, and chloride produced by the two salts are practically identical.

DISCUSSION.

There can be little doubt that within the limits of the above experiments the red blood cell membrane has consistently remained impermeable to both sodium and potassium. Inasmuch as the most extreme changes in total base concentration found in health or disease are well within the limits of the changes produced in the present experiments (see Table IV) it is fair to assume that in the human body the red blood cell membrane remains impermeable to the cations sodium and potassium throughout all the equilibrium readjustments of the blood.

The assumption is further supported, as was mentioned in the introduction, by nearly all those workers who have made observations on the permeability of the red blood cells after exposing blood to varying tensions of CO₂. Gürber (6), in 1895, was the first to point out the apparent impermeability of red blood cells to cations. He reported that though the serum appeared to become more alkaline when blood was saturated with CO₂, he was able to demonstrate by ash analyses and estimation of serum volume changes that there was no actual increase in serum base content, but that sufficient Cl had passed into the cells to account for the
increase in BHCO₃. 2 years later, Koepppe (12) tried the effect of passing CO₂ through a mixture of washed cells in isotonic glucose solution, and reported that he was unable to recover any titratable alkali from the glucose solution. Hamburger in his splendid monograph (7) (1902), in which he reported a great many red blood cell permeability experiments with a variety of salts, apparently believed at that time that the cells were impermeable to cations. More recently, Henderson, McLean, and Murray (11) and Doisy and Eaton (5) have reported from data obtained in CO₂ saturation experiments that the electrolyte readjustments can be accounted for almost entirely by transfer of water and anions across the cell membrane. In 1917, Van Slyke and Cullen (18) stated that some base probably crossed the cell membrane when blood was saturated with CO₂; but in 1921, Van Slyke (19) was of the opinion that the

table shift must at most be slight compared with the anion transfer. Collip (4), using the same method of equilibrating blood at various CO₂ tensions, concluded that both acid and base could cross the cell membranes, but that the permeability to base only became noticeable when using CO₂ tensions considerably beyond the physiological range. The evidence that is derived from the CO₂ saturation experiments, in which the changes in CO₂ tension do not exceed the limits found in the human body, is overwhelmingly in favor of the impermeability of the red blood cells to cations.

But Hamburger and Bubonovic (1911) (8), and Hamburger (1916) (9) reported that the red blood cell membrane was permeable to both sodium and potassium when they added small amounts of sodium or potassium chloride or water to blood. They used an amount of salt equivalent to 0.2 gm. per 100 cc. of serum and an amount of water equivalent to 10 per cent of the serum

### TABLE IV.
Comparison of Limits of Concentration Change Produced in Experiments with These Found in Human Disease in This Clinic.

<table>
<thead>
<tr>
<th></th>
<th>Serum CO₂</th>
<th>Serum Cl</th>
<th>Total base</th>
</tr>
</thead>
<tbody>
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<td>Experiments</td>
<td>m.-eq.</td>
<td>m.-eq.</td>
<td>m.-eq.</td>
</tr>
<tr>
<td></td>
<td>per l.</td>
<td>per l.</td>
<td>per l.</td>
</tr>
<tr>
<td></td>
<td>17.4</td>
<td>37.5</td>
<td>80.4</td>
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<tr>
<td>Human disease</td>
<td>2.4</td>
<td>54.0</td>
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<td></td>
<td>116.8</td>
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</table>
volume. It will be noted that considerably larger amounts of salt and water are added in the present experiments and that in spite of the consequent greater disturbance of electrolyte equilibrium, no cations appear to traverse the cell membrane.

In trying to explain these discordant findings, the methods used by Hamburger and Bubonovic were carefully reviewed. In both papers, permeability to base was ascertained by sodium and potassium estimations in cells and serum. Potassium was determined either as cobalti-nitrite or chloroplatinate and sodium was calculated by difference after the gravimetric determination of the total KCl + NaCl content. In the earlier paper, 900 cc. of beef blood were used for each experiment and the chloroplatinate method was used. After a preliminary saturation of the blood with 5 volumes per cent of CO₂ very little precaution seems to have been taken against loss of CO₂ between the time cell volume was determined and the cells and serum were separated. Further, the salt or water was added to serum that had been decanted from the sedimented cells into a second vessel. This treated serum was then poured back into the original container without, apparently, taking any care against loss of CO₂ or serum in the transfer. In the second paper, the potassium was determined by a volumetric estimation of the potassium cobalti-nitrite precipitate. Although individual determinations show excellent duplicate agreement, it is conceivable, since potassium cobalti-nitrite is known (17) to be an unstable compound, that some of the variation in potassium found might be accounted for by changes in the serum in the different experiments. It is worth mentioning, also, that in all the experiments the sodium and potassium are reported to have crossed the membrane in opposite directions. Since sodium was determined by difference, any analytical error in the potassium would cause an opposite error in the estimation of sodium. The actual shift of base across the membrane is reported as being tremendous. In both series of experiments, when the serum NaCl content was increased by 25 per cent, approximately 20 to 30 m.-eq. per liter of sodium entered the cells while the serum potassium increased about 2 m.-eq. When an amount of water equal to 10 per cent of the serum volume was added, there was again a loss of about 15 m.-eq. per liter of sodium from the serum with an increase of 1.5 m.-eq. per liter of potassium. In the experiments,
therefore, where the NaCl was added, over 80 per cent of this sodium is reported to have entered the cells. In spite of this, the cells are shown to have shrunk to about 88 per cent of their original volume. These findings seem quite unreasonable. Some of the incongruities may be accounted for by the changes in cell volume attendant upon an unguarded loss of CO₂. Finally, it is quite inconceivable how these workers were able to obtain accurate CO₂ equilibrations while using such large quantities of blood.

The disadvantage of using such small quantities of blood as were taken for the present experiments appears to be more than compensated for by the relatively perfect CO₂ saturation and by the very strict precautions against any loss of CO₂. The sodium and total base methods were found satisfactory. The probable maximal error of either method is ± 3 m.-eq. per liter. Inasmuch as the "calculated" sodium or total base was estimated from data subject to the same error, a difference between the "found" and "calculated" might conceivably be as large as 6 m.-eq. but in most instances the error of the method could not account for a difference greater than 3 to 4 m.-eq. In Experiment 5 where the greatest disturbance of electrolyte equilibrium was produced 6 to 8 per cent of the added base might have crossed the cell membrane undetected. However, in five of the eight present experiments, the difference between found and calculated is less than 2 m.-eq. per liter and the possible undetected transfer of base varies from 1 to 5 per cent. This error is insignificant in comparison with any similar experiments that have been reported.

If the cell membrane were permeable to both sodium and potassium, it is difficult to explain the almost exclusive separation of potassium within the cells and sodium in the serum. If this peculiar distribution were due to a selective permeability on the part of the membrane for potassium, it is quite incredible that the electrolyte changes in the first three experiments would show such close agreement when equimolar amounts of a similar potassium and sodium salt were added. For these reasons, it would appear that red blood cells in vitro are impermeable to both sodium and potassium and that probably when they are in circulation they remain impermeable to these cations. At present, it seems impossible to explain the peculiar distribution of sodium and potassium between cells and serum on any basis which assumes a trans-
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transfer of these cations across the membrane of red blood cells in circulation.

It is interesting to note that in the last five experiments where the serum protein determinations are believed to be reliable there is a close agreement between the ratio of serum volume change based on the hematocrit determinations and on the serum protein determinations (see Table V).

### SUMMARY.

It was believed on theoretical grounds that the red blood cells were impermeable to the cations sodium and potassium within the limits of total base change found in human disease. CO₂ saturation experiments support this belief, for in the reestablishment of electrolyte equilibrium, the changes can practically all be accounted for by the transfer simply of water and anions across the cell membrane. But Hamburger found that when he added Na or K salts or water to blood, both basic and acid ions appeared to traverse the cell membrane. In this paper are reported the results obtained on repeating some of Hamburger's experiments. Human blood equilibrated at 40 mm. of CO₂ in air at 38°C. was used. Sodium and potassium salts of chloride and carbonate were added in concentrations approximating the extremes found in human disease. To other samples sufficient water was added to cause a 30 per cent dilution of the serum. Complete electrolyte determinations, including sodium and total base, were done and the transfer of H₂O, CO₂, and Cl across the cell membrane was demonstrated. In no instance was there any evidence of a shift of base across the cell membrane, and when equimolar amounts of a like Na and K salt were added to specimens of the same blood, the

### TABLE V.

**Comparison of Ratios of Serum Volume Change as Determined by Hematocrit and Serum Protein.**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Serum volume₁</th>
<th>Serum volume₂</th>
<th>Serum protein₁</th>
<th>Serum protein₂</th>
</tr>
</thead>
<tbody>
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<td>4</td>
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<td></td>
<td>93.8</td>
<td>94.6</td>
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<td>95.2</td>
<td></td>
<td>93.8</td>
<td>94.6</td>
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<td></td>
<td>93.8</td>
<td>94.6</td>
</tr>
<tr>
<td>8</td>
<td>76.9</td>
<td></td>
<td>78.2</td>
<td></td>
</tr>
</tbody>
</table>
electrolyte changes were practically identical. Hamburger's experiments have been reviewed and certain criticisms offered. It is believed that the experiments presented very definitely indicate an impermeability of the red blood cell to the cations sodium and potassium.

CONCLUSIONS.

1. The human red blood cells appear to be impermeable to the cations sodium and potassium during electrolyte readjustments caused by either the addition of sodium or potassium salts of chloride or carbonate or the addition of water. Equilibrium is reestablished, apparently, by the transfer of H₂O, CO₂, and Cl across the cell membrane.

2. When equimolar amounts of a similar sodium and potassium salt are added to blood the changes in the distribution of water, CO₂, and Cl are quantitatively practically identical in the two analyses.

3. No explanation is offered for the extraordinary distribution of potassium and sodium between the cells and their surrounding fluid for the cell membrane is apparently equally impervious to both of these cations.

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