POTASSIUM ACCUMULATION IN THE PROXIMAL CONVOLUTED TUBULES OF THE FROG'S KIDNEY

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When the isolated kidney of the frog is soaked in solutions of varying ionic content, some ions are accumulated and others are lost by the tissue. It has been shown earlier (1) that similar changes occur with isolated muscle, the equilibrium conditions reached conforming with the predictions based on the assumption that the fibres are permeable to small anions and to potassium, but not to sodium. Such a permeability has been referred to as the standard permeability for ions (2) and applies to a wide variety of cells, though many exceptions may be instanced, in particular the special cells of the uriniferous tubules, or of the gills of fishes, which actively absorb or excrete Na ions.

In this connection impermeability of a cell to Na ions may be interpreted either as a complete blockage or a very slow escape, the latter associated with a similar very slow exit requiring some energy expenditure. From considerations of the available energy and the entrance rate of KCl into the frog's sartorius (1, 3) an associated entrance of NaCl would appear to be feasible only at about one-hundredth to one-thousandth of the rate of KCl entrance, and it may not occur appreciably fast when compared with the life of the fibres. Throughout some hours at room temperature or 24 hours in the cold impermeability to Na ions apart from whatever views we may entertain as to Na extrusion may be interpreted for frog muscle as a real or potential blockage.

It is of interest to note that the entrance of small anions, such as chloride, along with potassium has been shown by Wilde (4) for the muscle of live rats, and although he obtained a product of the K and Cl concentrations outside the fibre which was only 50 to 60 per cent of the product within, yet if his figures be corrected for the fact that inulin (used to measure the interspaces) does not enter the red corpuscles in the capillaries whereas chloride does, the products approach each other. In other words the Donnan relation is at least approximately obeyed.

As shown here for the isolated kidney the greater fraction of the cells behave in a manner similar to muscle fibres, and can accumulate potassium against a gradient, but other cells act differently and actively extrude sodium and chloride when the kidney tissue is immersed in Ringer's solution. This loss of sodium and chloride is accompanied by a diminution of volume of the cells concerned. Such decrease in volume and loss of sodium are prevented by cyanide.
In order to understand the equilibrium between the K-accumulating cells of the kidney—appearing to act in this way like muscle—and the Ringer solution, it is necessary to measure (a) the proportion of the kidney tissue which is intercellular space, (b) the region that is freely permeable to Na in a steady state after soaking in Ringer fluid. Of the two it will appear that this latter is the more important "space" determination in studying the K accumulation in the normal tissue as the difference of the water therein from the whole tissue water measures the volume of fluid in the K cells.

The two regions of the nephron are identified as the proximal and distal tubules. The cells of the proximal tubules are shown to have an ion permeability very similar to that of the sartorius muscle, being impermeable to Na but letting through K and Cl. The cells of the distal tubule are permeable both to Na and the smaller K ion (to the latter at least from the blood side) and because of this K cannot be appreciably accumulated therein. The water of such cells can be regarded as so much free interspace with concentrations the same as the external solution, when calculating concentrations in the proximal tubules. (If in fact the Na and K concentrations in the water of the distal tubule tissue are not quite the same as outside, the size of such tissue in the kidney after immersion is relatively so small that little error arises here from assuming that they are so.)

With such a preliminary statement of similarities and differences between muscle and renal tissue regarding K and Na ions, the authors would like to state that a previous reading of the paper on K accumulation in muscle (2) would appear advisable for an easy understanding of the theoretical section.

**Methods**

Kidneys from *Rana temporaria* were used throughout and treated as described for the sartorius muscle (2). Most of the experiments carried out refer to soakings in the cold (2–3°C.) in the modified Ringer solutions, and over a period of 24 hours. Others were carried out at room temperature, the kidneys being stirred by bubbling with a gas mixture for periods up to 3 hours.

**Immersion Fluids**

*Ringer-Barkan Fluid.*—For the long period immersions in the cold, the solution contained in small conical flasks (50 ml. volume each with 4 to 5 kidneys) was first prepared by bubbling the gas mixture (3 per cent CO₂ and 97 per cent O₂) through for 30 minutes, and transferring the well stoppered flasks to the refrigerator for 1 hour. The kidneys were then quickly immersed and the stoppered flasks replaced in the refrigerator.

The Barkan modification of the Ringer fluid (5) was chosen for reasons previously given (2) and it had the following standard composition:

- NaHCO₃ ........................................ 11.9 mm per litre
- Na₂HPO₄ ........................................ 0.67 “ “ “
- NaH₂PO₄ ........................................ 0.10 “ “ “
CaCl$_2$ ............................................. 1.8 mm per litre
Glucose ........................................... 3.9 “ “ “
a pH of 7.3 being reached by bubbling with the gas mixture (3 per cent CO$_2$, 97 per cent O$_2$). (The symbol mm is used throughout for millimols.)

To this was added the different NaCl and KCl quantities as required.

**Plasma-Salt Solution.**—Another fluid occasionally used simulated closely the inorganic composition of frog plasma. It is described in a previous communication (2).

**Hemoglobin-Ringer Fluid.**—The hemoglobin was prepared by dialysing heparinized rabbit blood, previously diluted 1 in 4, for several hours until practically free of the Cl ion and then making up with NaCl (to 0.65 per cent strength) and the usual Ringer ingredients (but without bicarbonate). KCl was also incorporated to the extent of 30 m.eq. per litre to prevent K losses and maintain the cell membranes in an apparently better condition, as to permeability, than without such addition.

**Inulin-Ringer Fluid.**—This was similar to the previous fluid but contained 1.5 per cent inulin instead of hemoglobin.

**Analyses.**—
Sodium and potassium were determined as previously described (1).
Chloride was determined by a microdiffusion method (6, 7).

**Hemoglobin.** Single kidneys were immersed—over 24 hours in the cold—in rather wide stoppered tubes containing a few milliliters of hemoglobin-Ringer fluid, then removed, surfaces quickly dried and transferred to 1 ml. hemoglobin-free Ringer solution in similar tubes for another 24 hours; after which 0.8 ml. was pipetted off into small centrifuge tubes, 0.2 ml. strong alkali added, mixed, and the contents warmed for about 5 minutes in the water bath, centrifuged, and the clear fluid examined spectrophotometrically. The original hemoglobin-Ringer fluid diluted 1/100 was used as a standard, and the blank values determined by immersion of the companion tissues in a similar way, hemoglobin being omitted.

**Inulin.** For experiments with single kidneys, immersions and diffusions were carried out as for the hemoglobin, 1.5 ml. of a 1.3 per cent Na$_2$SO$_4$ solution being used for the second 24 hours' soaking. For the inulin analyses, duplicate 0.5 ml. samples were used. These were transferred to the outer chambers of Conway units. Into the inner chamber of each unit was pipetted 1.3 ml. of N/50 Ba(OH)$_2$ containing 5 per cent of B.D.H. universal indicator and the lid (with fixative) placed in position. Into the outer chamber was then run 2 ml. of an oxidation mixture consisting of 40 per cent H$_2$SO$_4$ saturated with permanganate. (This mixture had been left exposed for some hours in a large beaker to allow the escape of CO$_2$ from traces of carbonate as impurity in the permanganate, and the mixture was made up in this way each day.) After 2 hours in unit, 1 ml. of the Ba(OH)$_2$ solution was removed from the central chamber into a small tube and titrated to a green colour with N/25 HCl from a Conway burette (7).

**RESULTS**

1. **Experiments to Investigate the Volume of the Intercellular Spaces in the Isolated and Immersed Kidney**

(a) **The Hemoglobin Ratio.**—This was investigated for thirty-five single kidneys soaked over 24 hours in the cold in the hemoglobin-Ringer fluid, the
hemoglobin diffused into the kidney being re-diffused out into Ringer fluid over another 24 hours. The mean value of the ratio—

\[
\frac{\text{Hemoglobin per kg. original weight}}{\text{Hemoglobin per kg. external fluid}}
\]

was \(0.175 \pm 0.004\) (giving standard deviation of mean). For similar experiments with \(N/250\) cyanide in the hemoglobin-Ringer solution the mean for twenty-one kidneys was \(0.174 \pm 0.005\).

### TABLE I

**Mean Ratio for the Immersed Kidneys**

\[
\text{Ratio} = \frac{\text{gm. per kilo original weight}}{\text{gm. per kilo external fluid}}
\]

<table>
<thead>
<tr>
<th></th>
<th>Active tissues</th>
<th>Inactive tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemoglobin</strong></td>
<td><strong>Ratios</strong></td>
<td><strong>Weights after/before</strong></td>
</tr>
<tr>
<td>Single kidneys</td>
<td>0.175 ± 0.004 (35)</td>
<td>0.81</td>
</tr>
<tr>
<td>in the cold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td>0.23 ± 0.012 (28)</td>
<td>0.85</td>
</tr>
<tr>
<td>Sets of 4</td>
<td>0.23 ± 0.02 (10)</td>
<td>0.82</td>
</tr>
<tr>
<td>kidneys, room</td>
<td></td>
<td></td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>0.26 ± 0.014 (26)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

The ± addition gives the standard deviation of mean. The figures in parentheses give the number of experiments.

Concerning the adequacy of the soaking period of 24 hours, experiments with a 48 hour soaking period with subsequent 48 hour diffusion proved rather unsatisfactory owing to the development of some turbidity indicating tissue breakdown. The following experiment was also performed. Twelve kidneys first soaked for 24 hours in the cold were transferred to Ringer solution (about 25 times their volume) at room temperature and stirred gently by a mechanical stirrer. After 2 hours the diffusion had come to an end as evidenced by the fact that the ratio calculated after 30 minutes' stirring and examination of a sample of the fluid was 0.11 with ratios of 0.167 and 0.168 after 120 and 180 minutes. It is true that only the 24 hour entrance of hemoglobin in the cold
would emerge in these experiments, but the rapidity with which it comes out points to the adequacy of the soaking period, and such conclusions are in turn supported by the subsequent inulin investigations.

(b) The Inulin Ratio.—The inulin ratio was investigated in a similar manner to that of hemoglobin with single kidneys in the cold, but experiments with groups of kidneys were also made at room temperature. The mean value of the ratio for twenty-eight experiments is 0.23 ± 0.01 (Table I). With cyanide the figure increases slightly but scarcely significantly to 0.27 ± 0.02. At room temperature on the other hand (groups of four kidneys, bubbling for 2 hours in inulin-Ringer fluid) the mean change is from 0.23 to 0.32.

(The adequacy of the diffusion times was shown in the experiments at room temperature. After 60, 90, and 120 minutes the ratios were 0.214, 0.232, and 0.235, which give the means for three sets of seven groups of four kidneys.)

If instead of the inulin ratio as given above the relation be expressed as a “permeation” previously defined (8) and which may be written

\[
100 \times \frac{\text{gm. per kg. water after immersion}}{\text{gm. per kg. external fluid}}
\]

then the value for inulin in the cold is 32 and 36 at room temperature which may be compared with 41 for sections of rabbit kidney at 37°C. With cyanide at room temperature the value for the frog's kidney rises to 41 and for the cyanide-perfused rabbit kidney it was found to be 56.

II. Experiments to Investigate the Proportion of the Isolated Kidney Showing Free Entrance of NaCl

The measurement of this region is essential for determining the K-accumulating space, for wherever Na with accompanying Cl or HCO₃ ions enter freely, K cannot be accumulated, in the manner described for muscle (2).

It will be seen later that for the theoretical treatment it is the most important “space” determination. In volume it may be expected to be at least as great as the space indicated by the inulin ratio, and in fact for the isolated immersed kidney the Na ratio reaches a value but little different from that of inulin, but certain peculiarities such as a marked reduction in renal volume on immersion associated with similar reduction in the Na ratio require special consideration.

(a) Na and Cl Ratios for the Fresh Kidney with Respect to the Plasma Values.—The mean Na content of the fresh kidney is 41.2 ± 0.6 mM per kilogram (seventy-nine analyses of groups of four kidneys, Table II) and the mean Na content of plasma as shown in Table II is 103.8 mM per kg. This would mean a Na ratio of 0.397, or 0.38 with the external concentration referred to plasma water. For reasons considered later, 0.39 may be taken as close to the true mean value. Similarly the mean Cl content of the kidney is 30.1 ± 0.8 (twenty
analyses) and that of the plasma is 74.3, thus giving a ratio of 0.405. The Na and Cl ratios for the frog's kidney with respect to plasma are thus very

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Mean Values for Frog Kidney (Rana temporaria)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>Kidney</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>60.0 ± 0.6</td>
<td>2.5 (F)</td>
</tr>
<tr>
<td>Na</td>
<td>41.2 ± 0.6</td>
<td>103.8 (F)</td>
</tr>
<tr>
<td>Cl</td>
<td>30.1 ± 0.8</td>
<td>74.3 (F)</td>
</tr>
<tr>
<td>HCO₃</td>
<td>—</td>
<td>25.4 (F)</td>
</tr>
<tr>
<td>Water</td>
<td>816 gm./kg.</td>
<td>954 gm./kg. (S)</td>
</tr>
</tbody>
</table>

Each kidney analysis was carried out on four kidneys from four frogs. (F) represents Fenn's results (19) for plasma and (S) the figure of Schultz et al. as quoted by Fenn (19).

The ± values give the standard deviation of the mean.

<table>
<thead>
<tr>
<th>TABLE III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Values for Series of Kidneys Immersed for 24 Hours at 2-3°C. in Ringer Solutions with Fixed Na but Different K Concentrations</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>External concentrations</th>
<th>Renal concentrations</th>
<th>Weights after/before</th>
<th>Dried weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (mM/litre)</td>
<td>Cl (mM/litre)</td>
<td>Total (mM/litre)</td>
<td>K (mM/kg.)</td>
</tr>
<tr>
<td>6</td>
<td>82</td>
<td>192</td>
<td>30.8 (4)</td>
</tr>
<tr>
<td>12</td>
<td>88</td>
<td>204</td>
<td>57.1 (2)</td>
</tr>
<tr>
<td>18</td>
<td>94</td>
<td>216</td>
<td>62.3 (4)</td>
</tr>
<tr>
<td>30</td>
<td>106</td>
<td>240</td>
<td>71.1 (8)</td>
</tr>
<tr>
<td>60</td>
<td>136</td>
<td>300</td>
<td>88.0 (2)</td>
</tr>
<tr>
<td>90</td>
<td>166</td>
<td>360</td>
<td>111.0 (5)</td>
</tr>
<tr>
<td>120</td>
<td>196</td>
<td>420</td>
<td>123.4 (2)</td>
</tr>
<tr>
<td>150</td>
<td>226</td>
<td>480</td>
<td>144.0 (5)</td>
</tr>
<tr>
<td>180</td>
<td>256</td>
<td>540</td>
<td>167.8 (2)</td>
</tr>
<tr>
<td>210</td>
<td>286</td>
<td>600</td>
<td>184.2 (4)</td>
</tr>
</tbody>
</table>

All concentrations in the kidney are given as mM per kilogram of the weight before immersion. Bracketed figures give the number of experiments. There was constant Na concentration of 86 mM/litre in the immersion solutions. Total external diffusible anion is found by adding 13 to chloride values.

Similar, and may be compared with those for muscle, namely 0.22 for Na, and 0.14 for Cl, which show considerable relative difference.

(b) The Change in the Na Ratio for the Immersed Kidney and the Corresponding Volume Change.—When the kidneys are immersed for 2 hours at room temperature or for 24 hours in the cold (2-3°C.) the Na ratio falls and also the volume. As will appear from the data in Table III, (referred to again below), when the external concentration of the Ringer-Barkan fluid is 240 mM per litre...
and the external K value 30 mM (Na concentration = 86 mM per litre) the ratio has fallen from about 0.40 to 22.0/86 = 0.266 and the volume from 100 to 85 per cent. (Similar values are observed throughout the table with constant Na and different external K values, except where at the highest K concentrations, there is a relative increase in the Na ratio.) In the presence of N/250 cyanide no fall of Na ratio occurs, but rather an increase (as may be seen from data of Table IV), and provided the external potassium is not very high the volume of the kidney also increases. Volume changes over 2 hours for kidneys immersed in ordinary Ringer-Locke solution (0.7 per cent NaCl) at room temperature are shown in Fig. 1 (a limiting value being nearly reached within the period).

**TABLE IV**

Values as in Table III with the Immersion Fluids Containing N/250 Cyanide

<table>
<thead>
<tr>
<th>External concentrations</th>
<th>Renal concentrations</th>
<th>Weights after/before</th>
<th>Dried weights gms. per kg. of original tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>K mm/litre</td>
<td>Cl mm/litre</td>
<td>Total (c) mm/litre</td>
<td>K mg/kg.</td>
</tr>
<tr>
<td>6</td>
<td>82</td>
<td>192</td>
<td>35.5 (1)</td>
</tr>
<tr>
<td>12</td>
<td>88</td>
<td>204</td>
<td>35.0 (1)</td>
</tr>
<tr>
<td>18</td>
<td>94</td>
<td>216</td>
<td>46.3 (2)</td>
</tr>
<tr>
<td>30</td>
<td>106</td>
<td>240</td>
<td>64.6 (2)</td>
</tr>
<tr>
<td>60</td>
<td>136</td>
<td>300</td>
<td>80.6 (1)</td>
</tr>
<tr>
<td>90</td>
<td>166</td>
<td>360</td>
<td>97.8 (1)</td>
</tr>
<tr>
<td>120</td>
<td>196</td>
<td>420</td>
<td>130 (1)</td>
</tr>
<tr>
<td>150</td>
<td>226</td>
<td>480</td>
<td>152 (1)</td>
</tr>
<tr>
<td>180</td>
<td>256</td>
<td>540</td>
<td>—</td>
</tr>
<tr>
<td>210</td>
<td>286</td>
<td>600</td>
<td>184 (1)</td>
</tr>
</tbody>
</table>

All renal concentrations given as mm per kg. of weights before immersion. The immersion fluids contained a fixed Na concentration of 86 mm per litre.

From these experiments it will be seen that the original Na ratio in the kidney is about double that for hemoglobin or inulin ratios for the immersed kidneys, but that after immersion the value falls to near that of inulin and there is a corresponding loss of water.

The total average for the Na ratio after 24 hour immersions is 0.26, the average inulin ratio being 0.23.

(c) Relative Diffusion Rates of Na, Cl, and K from the Isolated Kidney into Isotonic Glucose.—On considering the high Na ratio of 0.39 for the fresh kidney and the comparative effect of cyanide, with the isolated kidney it would appear that Na is present not only in the intercellular spaces but a proportion is also intracellular. The question arises as to whether this intracellular Na is uniformly distributed throughout the renal cells or is restricted to some region of the nephron. The diffusion into isotonic glucose was designed to provide
data relevant to this question; for if Na is contained in appreciable amounts in cells which contain K, then since it is the larger ion, it could be expected to emerge relatively more slowly than K when immersions are made in K-free isotonic glucose. If, on the other hand almost all the Na emerges very rapidly while only a fraction of the K diffuses out, it would appear as the simplest explanation that the intracellular Na is confined to a separate region. Sartorius muscles were used for comparison.

These experiments were carried out with twenty kidneys (or sartorius muscles) immersed at room temperature in 3.8 per cent glucose and stirred by bubbling with oxygen, samples being taken at intervals for Na and K analyses, the tissues at the end being also analysed. For the chloride analyses groups of four kidneys were taken from a bath of twenty immersed kidneys as before, and analysed for chloride. Companion tissues were taken for the determination of the original chloride.

Fig. 2 illustrates the relative rates of emergence of Na, Cl, and K. It will be seen that nearly all the Na and Cl have left the kidney and diffuse out at practically the same rates, while only 10 per cent of the renal K is lost. After 60 minutes only 2 to 3 mm of the original 41 mm per kg. of Na are left. Comparing these results with those for the sartorius muscle it will be seen that about

![Graph](image-url)
40 per cent of the Na therein—approximately equivalent to the amount held in the fibres—is lost very slowly, while the free Na and Cl in the extracellular spaces are lost rapidly.

III. Experiments at 2-3°C. Showing KCl Accumulation, with Constant Na Concentration in the Immersion Fluid

For the sartorius muscle it was shown that large amounts of K as KCl could be accumulated with but little volume change if immersions were made in Ringer solutions in which the Na was held constant throughout the series and the KCl much increased. For the muscle series the constant [Na] in the Ringer fluid was 86 mm per litre being somewhat lower than the normal 104 mm per litre plasma. A small volume increase was therefore shown throughout, but largely independent of the K content (there being in fact a slight relative fall in weight as the external KCl was increased towards 300 mm per litre). Similar solutions were used for comparative purposes for the renal immersion, and the results are shown in Table III. The external solution had for these experiments the following composition (mm per litre) apart from the KCl increase:

<table>
<thead>
<tr>
<th></th>
<th>Na</th>
<th>HCO₃⁻</th>
<th>Ca</th>
<th>Cl</th>
<th>Glucose</th>
<th>P (as phosphate mixture)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>85.9</td>
<td>11.9</td>
<td>1.8</td>
<td>76.2</td>
<td>3.9</td>
<td>1.8</td>
</tr>
</tbody>
</table>

and the immersions were made as described under Methods.

The results are shown in Table III. The results for immersion in similar solutions containing N/250 sodium cyanide are shown in Table IV. It will be seen that K is largely accumulated both in the active and inactive kidneys, and to somewhat the same amounts. The Na changes are very different, there
being a mean loss of 18 mm per kg. with the active kidneys up to an external K of 180 mm per litre the results at each K level differing from this no more than 4 mm per kg. The corresponding mean weight loss is 16 per cent. On the other hand the cyanide kidneys show increase of Na throughout, the mean increase being approximately 16 mm per kg. Increases in volume are likewise shown when the external K is not very high.

TABLE V

(Immersion at 2-3°C. in Isotonic Mixtures with Varying K Concentrations)

<table>
<thead>
<tr>
<th>K (Potassium concentration in external fluid)</th>
<th>[Na] (Sodium concentration in external fluid)</th>
<th>100 [Na] + 3.7</th>
<th>Mean weight of 1 kg. of fresh kidney after immersion</th>
<th>Ranges and number of observations (each observation = weight of 4 kidneys)</th>
<th>K (Potassium concentration of immersed kidneys per kg. fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm/litre</td>
<td>mm/litre</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>107</td>
<td>0.89</td>
<td>0.81</td>
<td>0.75-0.83; 6</td>
<td>69 (2 observations)</td>
</tr>
<tr>
<td>20</td>
<td>97</td>
<td>0.97</td>
<td>0.80</td>
<td>0.76-0.83; 5</td>
<td>145 (2 observations)</td>
</tr>
<tr>
<td>30</td>
<td>87</td>
<td>1.08</td>
<td>0.86</td>
<td>0.82-0.93; 9</td>
<td>100 (2 observations)</td>
</tr>
<tr>
<td>40</td>
<td>77</td>
<td>1.21</td>
<td>0.91</td>
<td>0.84-0.93; 6</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>67</td>
<td>1.38</td>
<td>0.99</td>
<td>0.97-1.03; 6</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>57</td>
<td>1.59</td>
<td>1.06</td>
<td>1.02-1.11; 6</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>47</td>
<td>1.89</td>
<td>1.18</td>
<td>1.05-1.25; 6</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>37</td>
<td>2.34</td>
<td>1.34</td>
<td>1.26-1.46; 9</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>27</td>
<td>3.06</td>
<td>1.64</td>
<td>1.46-1.87; 14</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>17</td>
<td>4.40</td>
<td>2.00</td>
<td>1.89-2.08; 6</td>
<td></td>
</tr>
</tbody>
</table>

IV. Experiments Showing K Accumulation in Isotonic Solutions

When muscle is immersed in Ringer solution in which K is substituted for Na in equivalent relation, K is accumulated with large volume changes, following a theoretical relation up to a weight increase of about 70 per cent, or to about 100 per cent increase of the fibre water. Immersion in similar solutions was carried out with frogs' kidneys. The solutions had the following composition:

Total Na and K ........................................ 117 mm per litre
Cl .................................................... 107 mm per litre
HCO₃ ............................................... 11.9 mm per litre
Ca .................................................. 1.8 mm per litre
P (as phosphate mixture) .......................... 0.8 mm per litre
Phosphate ........................................ 3.9 mm per litre

These were also equilibrated with the gas mixture as before. The results for 24 hour immersions are shown in Table V, and it will be seen that a great relative increase of renal volume occurs as the Na content falls outside. The
mean weights range from 0.81 (of the original weight) with a K value of 10 mm per litre outside to 2.00 with 100 mm per litre external K. In Table VI it is shown that no further appreciable volume change occurs after a second 24 hour immersion.

### TABLE VI
(Immersions at 2–3°C. in isotonic mixtures as in Table V; comparison between weights referred to 1 kg. fresh weight after 24 and 48 hours for one set of forty kidneys—four in each mixture.)

<table>
<thead>
<tr>
<th>K</th>
<th>Weights after 24 hrs.</th>
<th>Weights after 48 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.83</td>
<td>0.83</td>
</tr>
<tr>
<td>20</td>
<td>0.82</td>
<td>0.83</td>
</tr>
<tr>
<td>30</td>
<td>0.84</td>
<td>0.84</td>
</tr>
<tr>
<td>40</td>
<td>0.92</td>
<td>0.91</td>
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<tr>
<td>50</td>
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<td>1.00</td>
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<tr>
<td>60</td>
<td>1.03</td>
<td>1.02</td>
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<tr>
<td>70</td>
<td>1.22</td>
<td>1.21</td>
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<tr>
<td>80</td>
<td>1.38</td>
<td>1.36</td>
</tr>
<tr>
<td>90</td>
<td>1.64</td>
<td>1.71</td>
</tr>
<tr>
<td>100</td>
<td>2.07</td>
<td>2.08</td>
</tr>
</tbody>
</table>

### TABLE VII
(Immersions at 2–3°C. isotonic mixtures as in Table V, but containing N/500 cyanide.)

<table>
<thead>
<tr>
<th>K</th>
<th>Mean weights of immersed kidneys referred to 1 kg. fresh weight</th>
<th>After 24 hrs.</th>
<th>After 48 hrs.</th>
<th>After 72 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td>0.97 (4)</td>
<td>1.05 (3)</td>
<td>1.11 (2)</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>0.98 (3)</td>
<td>1.02 (3)</td>
<td>1.09 (2)</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>1.05 (4)</td>
<td>1.13 (3)</td>
<td>1.18 (2)</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>1.09 (3)</td>
<td>1.17 (3)</td>
<td>1.19 (2)</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>1.17 (4)</td>
<td>1.25 (3)</td>
<td>1.28 (2)</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>1.23 (3)</td>
<td>1.33 (3)</td>
<td>1.42 (2)</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>1.31 (4)</td>
<td>1.44 (3)</td>
<td>1.50 (2)</td>
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<tr>
<td>80</td>
<td></td>
<td>1.46 (3)</td>
<td>1.58 (3)</td>
<td>1.65 (2)</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>1.53 (4)</td>
<td>1.70 (3)</td>
<td>1.79 (2)</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>1.54 (3)</td>
<td>1.65 (3)</td>
<td>1.78 (2)</td>
</tr>
</tbody>
</table>

Experiments were also carried out in which volume changes in similar isotonic solutions but containing M/500 or M/250 cyanide were investigated. Only the results with M/500 cyanide are given (Table VII) since those with M/250 cyanide did not appreciably differ therefrom. The results in Table VII show that the increase in volume is not ended after 24 hours but continues up to at least 72 hours.

In Fig. 3 the kidney weights as ratios of the fresh weights are plotted against the reciprocals of ([Na] + 5.7) (the figure 5.7 corresponding to the small con-
POTASSIUM ACCUMULATION IN TUBULES OF FROG'S KIDNEY

The concentration of Ca and glucose). The upper line gives the results after 24 hours' immersion in cyanide-Ringer fluids and the lower the results without cyanide. It will be seen that the effect of cyanide is to raise the level of the line, but it has no influence on its slope. One rather interesting difference is the manner in which with cyanide the linear relation is somewhat abruptly departed from at a lower level of volume increase, showing that the differential permeability of the cells involved is then not so resistant to distension of the membrane.

![Graph](image)

**Fig. 3.** Mean weight of kidney after 24 hours' immersion at 2-3°C. in isotonic Ringer fluids, with varying Na and K concentrations, plotted against the reciprocal of \([\text{[Na]} + 5.7]\), [Na] being the external sodium concentration (mM per litre) and 5.7 the sum of the Ca and glucose concentrations.

The upper curve gives the results in the presence of \(\times/500\) sodium cyanide.

It is also of interest that though the renal volume increases with cyanide after 24 hours the slope of the line above considered remains unchanged (as would appear from plotting the results in Table VII).

**THEORETICAL SECTION**

*1. K and Na Regions of the Nephron*

When the frog's kidney is immersed in Ringer solution, as shown above the Na ratio falls from an initial value of 0.39 to 0.26, which is near the inulin...
From the high Na ratio of the fresh kidney, it is obvious that much of the Na is initially contained in cells of the nephrons, and is actively extruded. This is shown by the cyanide effect, when the Na ratio no longer falls but rises somewhat, with a corresponding increase in weight (provided the external K is not very high). The question then arises, is the Na present in all the cells of the nephron and actively extruded therefrom or is it present only in a fraction. It would appear that this can be answered effectively in the following way. If the Na is not present in the major fraction of the nephron and exists only in a special region the volume of which is indicated by the Na ratio, then the major fraction may be taken as impermeable to the Na ion, and if so it should act—at least within a certain range—practically as an osmometer for the Na ion concentration (even in the presence of KCl, with the membrane freely permeable to K and Cl ions).

Examining then how this relation is fulfilled, if we take the weight of the immersed kidney as \( W_i \) and subtract from this the Na ratio of 0.26 plus 0.16, (the latter being the mean weight of the solid material), we obtain \( W_i - 0.42 \). This may be expected to be proportional to the reciprocal of the Na ion concentration. We must take into account however, that a small amount of Ca is present in the fluid used, and also a little glucose, both adding to 5.7 mm per litre. By analogy with muscle the membranes of these renal cells may be expected to be impermeable to these as well as to the Na ion. Taking then the data of Table V where the total of the Na and K ion concentration remains the same (117 mm per litre, with total mm of 243 in the solution) but is relatively varied, and plotting \( (W_i - 0.42) \) against the reciprocal of the concentration \( ([Na] + 5.7) \) we get, up to an external K of 90 mm per litre a linear relation, the line of best fit passing through or very close to the origin. This will appear evident from Fig. 3 (lower curve) in which the line through the \( W_i \) values cuts the ordinate at 0.42. Attention may here be directed to the fact that the weight change of \( (W_i - 0.42) \) is from 0.34 to 1.18, a very large relative increase for cell water. The existence of this relation would seem explicable only by the effective impermeability to the Na ion of the greater fraction of the nephron, the remainder plus intercellular spaces being freely permeable to Na.

As supporting evidence we have the fact that Na and Cl diffuse together and almost completely from the immersed kidney within about 30 minutes after immersion in isotonic glucose as shown in Fig. 2. Within the same period the kidney loses only a small fraction of its potassium. This slow loss of K is like that from the muscle fibre when immersed in K-free solutions.

Can the conclusion be avoided that the larger fraction of the nephron is impermeable to Na? It might perhaps be entertained that the permeability in unidirectional and though the Na ions cannot pass back, they could still enter from the lumen side. This is scarcely tenable when we consider the long period of immersion and the practical certainty that NaCl will diffuse...
POTASSIUM ACCUMULATION IN TUBULES OF FROG'S KIDNEY

down through the glomeruli, and backwards up the lumen of the nephron as well as through the cellular region which is taken as permeable to the Na and Cl; and there is also the fact that, as seen from Fig. 3, up to high levels of distension cyanide does not appreciably affect the nature of the volume change resulting from reduced Na concentration, for the line of renal weight against the reciprocal of ([Na] + 5.7) retains the same slope.

In this first theoretical section we shall consider it sufficient then to conclude that there exists a large region of the nephron which has a permeability like that of the muscle fibre, impermeable to Na but permeable to K and Cl which can be greatly accumulated in the isolated kidney, and on the other hand there exists a smaller region which is freely permeable to Na and actively extrudes it from the cells in the immersed kidney decreasing very markedly in volume. It will be shown then that the theory used to explain K accumulation in the muscle fibre can in turn be very successfully used to explain K accumulation in the K region of the nephron. Before considering this, the manner of calculating K and Cl values in the K region will be dealt with.

Calculation of K and Cl in the Water of the K region

Fresh Kidneys.—For these the Na ratio may be taken as giving the region of free Na entrance. From Table II the mean Na content per kilogram is 0.412 ± 0.006 (seventy-nine analyses) and the mean plasma value is 103.8, from which the Na ratio is \[
\frac{41.2}{103.8} = 0.397 \text{ or } 0.40 \text{ for } 1 \text{ kg. (the Cl ratio is slightly higher, being 0.405). More strictly we should here consider the mm Na per kilogram of plasma water, which would mean dividing by 0.954 (Table II), but, at the same time we should need to multiply by a factor corresponding to the ratio of [Na] in extracellular water to that in plasma water. Since that is usually taken as 0.95 (though it may be expected to be nearer to unity in the frog) the two factors would largely cancel (or at least may be expected to do so within 1 or 2 per cent). A figure of 0.39 may be stated as probably very close to the true mean value. We have then for the mean value of [K] in 1 kg. of proximal tubule water \( k_1 \) of fresh kidneys:
\[
\begin{align*}
    k_1 &= \frac{k_o - 0.39k_p}{1.00 - W_d - 0.39} \\
    &= \frac{60.0 - 1.0}{1.00 - 0.184 - 0.39} = 139,
\end{align*}
\]
\( k_0 \) being the mm K per kilogram of the fresh tissue, \( W_d \) the dry weight of 1 kg. of kidney and \( k_p \) the K concentration in plasma water.

If the chloride content of the proximal tubule water be calculated in the same way, the amount is so small, that an error in the determination of the region of free Na and Cl entrance has a relatively large effect. For more
accurate results the Donnan relation may be assumed. The product of the
K and Cl concentrations in plasma water, from the data of Table II, is 204.
Since the K concentration in the proximal tubule water is 139, the Cl con-
ccentration is therefore 204/139 = 1.5 mM per litre.

This chloride calculation may be qualified by such consideration as that
the K in protein-free dialysates of dog's sera in vivo (9) shows a ratio with
respect to the plasma only 86 per cent that expected from the Na ratio, or that
for similar dialysates in vitro (10), the K ratio is 91 per cent that of the Na value
(a summary of such results is given by Peters (11)); but such qualifications even
if applying to the frog would have very little effect on the absolute figure for
Cl in the cell water of the K accumulating region of the kidney.

In a similar way it may be calculated that the concentration of the HCO3
ion in the water of the proximal segment is 0.5 mM per kg.

Immersed Kidneys.—All
concentrations giving direct analytical results,
measurements of weights and of dry weights are referred for uniformity and
convenience to 1 kg. of fresh weight of kidney.

The Na ratio of 0.26 was considered above for measuring the volume of the
kidney freely permeable to the Na ion (and therefore, it may be assumed, to
the K ion outside the K region). The line drawn through the kidney weights
(as ratios of the fresh weights) minus 0.42 (0.26 plus the mean dry weight of
0.16) when plotted against the reciprocal of ([Na] + 5.7) passes through the
origin, or very close thereto, as may be judged from Fig. 3, and this makes
it reasonably certain that the figure of 0.26 is close to the true value for the
Na-permeable space. Its lower limit would in any case be the inulin ratio of
0.23 for wherever inulin goes it may be assumed that the Na ion would likewise
go, and it would in fact make little difference to the results whether 0.23 or 0.26
figures were used for the space freely permeable to NaCl, but clearly the 0.26
figure is the better.

We may write then:—

\[(\text{Water of K region per kilogram fresh kidney}) = W_i - W_d - 0.26 \quad (2)\]

where \(W_i\) = the weight after immersion and \(W_d\) the corresponding dry weight,
0.26 being that of the water freely permeable to Na.

Into this Na region it may be assumed that the external K—as a smaller
ion—will likewise have free entrance, but only to a concentration approximately
equal to the external value. With a mean dry weight of 0.16 (as ratio of the
fresh kidney weight) the mM per kilogram of water in the proximal tubule
\((k_1)\) may be written:—

\[k_1 = \frac{k_0 - 0.26k}{W_i - 0.42} \quad (3)\]

where \(k_0\) is the mM K per kilogram of the fresh tissue.
320 POTASSIUM ACCUMULATION IN TUBULES OF FROG'S KIDNEY

The mm Cl per kilogram water in the proximal tubule may be similarly calculated.

II. The Mechanism of Potassium Accumulation in the K Region of the Nephron

(a) Theory of the Potassium Equilibrium

The theory of such K accumulation has been already treated in some detail for muscle (2) and it is only necessary here to state the essential equations. It is a practical need in developing or using equations such as those for the volume of the cell water (Equation 7) to have a symbolism as compact as possible. The following symbols have been chosen, very similar to those used in dealing with muscle.

In “cell water” of the K-accumulating region

\[ k, l, h = \text{mm} \text{ K, Cl, and H ions per kg. of cell water.} \]
\[ \Sigma d = \text{mm diffusible anions per kg. of cell water.} \]
\[ \eta = \text{The “idiomolar” value, or total millimols of non-diffusible substance per kg. of the original cell water.} \]
\[ e = \text{The “electrostatic equivalent” of } \eta \text{ or the surplus of negative charges on the non-diffusible electrolytes, expressed as milli-equivalents per litre.} \]
\[ V = \text{Volume of water in the number of cells which for fresh tissue contain 1 litre or 1 kg. of cell water.} \]

For external solution:

\[ k, l, h = \text{mm} \text{ K, Cl, and H ions per litre external solution.} \]
\[ c = \text{total external concentration, as mm per litre.} \]
\[ \Sigma d = \text{mm diffusible anions per litre of external solution.} \]

From the previous considerations the cell water in the K-accumulating region of 1 kg. of fresh kidney is 1.00 - 0.18 - 0.39 = 0.43, and for the immersed kidney it is \( W_1 = 0.16 - 0.26 \) or \( W_1 = 0.42 \), so that \( V \) for the immersed tissue is \( V = 0.43 \).

Concerning the \( \eta \) value it seemed advisable to have a word which would indicate its nature, and the term “idiomolar value” is suggested as expressing the fact that the molecules referred to, are entirely retained within the cell itself. The \( e \) symbol is in turn termed the “electrostatic equivalent” of \( \eta \) since it determines the number of the K ions in excess of the small amount of diffusible anions, which are held electrostatically within the cell.

As in muscle the cell membrane in the K-accumulating cells of the kidney is considered to be permeable to cations and anions, but within certain size limits, so that whereas Na is excluded, K is allowed through; and though Cl passes the membrane, anions such as the esters of phosphoric acid do not. It was shown for muscle that much KCl could be accumulated without volume
change, the K ions moving against a concentration gradient to an equilibrium determined by the Donnan relation. At the same time there was no change in the Na value in the muscle which remained a constant and rather small fraction of that outside. This with the fact that cyanide does not affect the Na control of volume appears to be an exact proof of the relative Na impermeability in muscle, and it will be seen that similar conditions prevail for the cells of the K-accumulating region in the kidney.

(b) Accumulation of Potassium in the K Region with Fixed External Na Concentration

For immersed muscle with the mean $\eta$ value of 105.5, very little different from $\epsilon$ (= 103.5) it was shown that the volume of the fibre water was proportional to the sodium ion concentration, and it was only necessary to hold this value constant to maintain the value of $V$. Here the $\eta$ and $\epsilon$ values differ appreciably and though $V$ does not increase with the accumulation, it decreases somewhat, but in accordance with theoretical expectation.

The equation for the potassium concentration in the K-accumulating region (2) is:

$$k_1 = c/2 - (\eta - \epsilon)/2V$$

When Na is held a constant, though $V$ changes but little over the widest ranges of external K, and of K accumulation, it does change a little, and so also $\eta$ and $\epsilon$, but the total value of $(\eta - \epsilon)/2V$ is at all times small compared with $c/2$, consequently the slope of the line of $k_1$ values against the total external concentration is approximately $c/2$.

Where the slope of the line of $k_1$ values against $c$ cuts the ordinate at $c = 0$ the value of $(\eta - \epsilon)/2V = -28$ is obtained.

Equation 4 becomes therefore:

$$k_1 = c/2 + 28$$

The last two columns of Table VIII show the agreement between the experimental and theoretical values. From an external potassium value of 18 mm to 210 mm per litre, the divergence between the $k_1$ values and that from Equation 5 is less throughout than 5 per cent.

The $\eta$ and $\epsilon$ Values.—It will be seen from Table VIII that the mean value of $\eta$ is 76 for such immersed tissues, and varies from this with increasing accumulation no more than could be expected from the sampling error. The mean value of $\epsilon$ is 136 but there is clearly an increase with increasing "$k"$. This is to be expected theoretically, as discussed elsewhere for muscle (2) but with immersed muscle it is compensated by slight losses of non-diffusible material.

With regard to the $\eta$ and $\epsilon$ values for the fresh kidney, such may be calculated from the equations given under Table VIII where $V = 1.00$, "$c$" (for plasma)
POTASSIUM ACCUMULATION IN TUBULES OF FROG'S KIDNEY

= 233 (2), \( k_1 = 139 \), and \( \Sigma d_1 \), comprised almost entirely of Cl and HCO_3, has the value of \( 1.5 + 0.5 = 2.0 \) (the manner of calculating \( k_1 \), \( k_1 \), and \([HCO_3]\) values is given at the end of Theoretical section, I).

For the K region of the fresh kidney \( \epsilon \) is therefore 137 and \( \eta \) is 92.

### TABLE VIII

Values Derived from Table III

<table>
<thead>
<tr>
<th>( k )</th>
<th>( i )</th>
<th>( &quot;c&quot; )</th>
<th>( \nu )</th>
<th>( \eta )</th>
<th>( \epsilon )</th>
<th>( \epsilon )</th>
<th>( \Sigma d_1 )</th>
<th>( k_1 )</th>
<th>( k_1 ) from Equation 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>82</td>
<td>192</td>
<td>1.13</td>
<td>77</td>
<td>111</td>
<td>11.3</td>
<td>13.0</td>
<td>111</td>
<td>124</td>
</tr>
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<td>56</td>
<td>114</td>
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<td>28.7</td>
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<td>136</td>
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<td>106</td>
<td>240</td>
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<td>226</td>
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<td>139</td>
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<td>298</td>
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<tr>
<td>210</td>
<td>286</td>
<td>600</td>
<td>0.98</td>
<td>89</td>
<td>144</td>
<td>173</td>
<td>181</td>
<td>328</td>
<td>328</td>
</tr>
</tbody>
</table>

All concentrations are given as mm per litre.

\( \Sigma d_1 \) is obtained by assuming the small bicarbonate concentration to behave like chloride and adding its value then to \( i_1 \). Calculations of \( i_1 \) and \( k_1 \) are described in text (at end of Theoretical section, I).

\( \eta \) and \( \epsilon \) are calculated from equations of Boyle and Conway (1).

\[
\eta / V = c - k_1 - \Sigma d_1 \\
\epsilon / V = k_1 - \Sigma d_1
\]

(c) Potassium Accumulation in Isotonic Mixtures (Causing Large Volume Increases)

The volume of the cell water is governed by the equation (1, 2)

\[
V^2(c^3 - 42kd) - 2\nu Vc + (\eta^3 - \epsilon^3) = 0
\]

whence

\[
V = \frac{\frac{\eta c}{c^3 - 42kd} + \frac{\epsilon c}{\epsilon^3 - 42kd} \times \sqrt{\left(1 - \frac{\eta^3}{c^3}\right) - \left(1 - \frac{\epsilon^3}{c^3}\right)}}{2}
\]

For the immersed sartorius muscle \( \eta \) and \( \epsilon \) differ very little so that the equation for \( V \) reduces to

\[
V = \frac{2\eta}{c - 42kd/c}
\]

and this in turn to:

\[
V = \frac{\eta}{[\text{Na}] + 5.7}
\]
since the value of \( c - 4\Sigma kd/c \) is practically identical with \( 2([\text{Na}] + 5.7) \) or twice the sum of the concentrations of the non-diffusible constituents in the immersion fluid. Thus for the isotonic mixtures when the external K changes from 10 to 90 mM per litre, \( "c" \) being 243 and \( d = 120 \), the value of \( c - 4\Sigma kd/c \) changes from 223 to 65 and \( 2([\text{Na}] + 5.7) \) from 225 to 65.

When \( \eta \) and \( \epsilon \) differ very appreciably in the proximal tubules the fuller equation could be expected to give better results. It is a fact, however, that owing to the small increase of \( \epsilon \) with increasing external K concentrations, a simple linear equation again applies very well, though not quite identical in form with the above relation for muscle. It arises in the following way. The value of \( V \) in Equation 7 may be written as:

\[
\frac{\eta}{c - 4\Sigma kd/c} \cdot \text{multiplying } 1 + \sqrt{\left(1 - \left(1 - \frac{\epsilon}{\eta}\right)^2\right)\left(1 - \frac{4\Sigma kd}{c^2}\right)}
\]

Even with the ratio \( \epsilon/\eta \) fixed as the mean value of 136/76 this multiplicand changes only from 2.74 to 2.27 when the external value of \( k \) changes from 10 to 90 mM per litre and \( V \) increases over three times. When, however, a change of \( \epsilon \) from 114 to 155 occurs over the same range of \( k \) then the value of the multiplicand changes only from 247 to 237 or about 4 per cent, and this is further decreased by the slight difference in value between \( c - 4\Sigma kd/c \) and \( 2([\text{Na}] + 5.7) \). The result is that the following relation

\[
V = \frac{1.24\eta}{[\text{Na}] + 5.7} = \frac{94}{[\text{Na}] + 5.7}
\]

is very well obeyed over a wide range as shown in Table IX.

(The increase of the \( \epsilon \) value from about 114 to 155 as K changes from 10 to 90, and the \( k_1 \) value remains approximately at 130 mM per litre, may be deduced from a curve through the \( \epsilon \) values of Table VIII against the \( k_1/k \) ratio, since this latter determines the \( k_1/k \) ratio and so the changes in \( \epsilon \). These are in turn due to changes in the ionization of the non-diffusible constituents.)

Also, if we consider the relation in terms of \( W_\epsilon \), the actual weight of the whole kidney (immersed), since

\[
V = \frac{W_\epsilon - 0.42}{0.43}
\]

where 0.43 is the water in the proximal tubule cells of the fresh kidney (or 1.00 – 0.18 – 0.34), then from Equation 10

\[
W_\epsilon = \frac{40}{[\text{Na}] + 5.7} + 0.42
\]
POTASSIUM ACCUMULATION IN TUBULES OF FROG'S KIDNEY

which describes the lower curve in Fig. 3 for the immersed kidneys without cyanide.

This gives the fuller theoretical reasons for the linear relation of Fig. 3.

The question may here be briefly considered why it is that with $\eta = \epsilon$ in immersed muscle, with relatively low external K values, the expected increase in $\epsilon$ with rising $k$ has not an appreciable effect on the relation

$$V = \frac{2\eta}{c - 42k\epsilon/c}$$

There is firstly the fact that the muscle membrane permeability breaks down at a lower K level than the kidney K cells, at about 70 m$\text{M}$ K per litre outside,

| TABLE IX |
| Values Derived from Table V |

<table>
<thead>
<tr>
<th>$k$</th>
<th>[Na] $10^7$</th>
<th>Volume $V$ of cell water in proximal tubules $V = 1.0$ for fresh tissue</th>
<th>$V$ as calculated from Equation 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>107</td>
<td>89 $10^7$</td>
<td>91</td>
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<tr>
<td>80</td>
<td>37</td>
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<td>214</td>
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<tr>
<td>90</td>
<td>27</td>
<td>306 $10^7$</td>
<td>284</td>
</tr>
<tr>
<td>100</td>
<td>17</td>
<td>440 $10^7$</td>
<td>367</td>
</tr>
</tbody>
</table>

and from the kidney analogy $\epsilon$ might be expected to change from 1.14 to 1.45, an increase of 1.28 times; hence from the full equation there would be a $V$ value only 6 per cent greater than that from Equation 8 or 9. It would appear also that $\epsilon$ in immersed muscle does in fact alter much less than the above amount, partly because the ionization of the non-diffusible constituents within it changes less with rising pH than the renal K cells and partly because the increase theoretically expected is offset by slight losses of non-diffusible constituents (2).

(d) Potassium Accumulation and the Donnan Equilibrium

$$k \times l = k_1 \times l_1$$

or the product of the potassium and chloride concentrations within the cell water of the K region should be equal to that outside (strictly it is the activity products which should be equal, but for such univalent electrolytes and a $\mu$ value in the cells probably not much different from that of the external...
solution the concentration products may be expected to differ but little on each side of the membrane).

Table X (data from Table VIII) shows the relation of the ion products. It will be seen that from a $k$ value of 30 mM per litre onwards the relation applies very well. The result is thus similar to that found for muscle except that in the kidney a slightly higher external K value is necessary before the relation applies exactly. For muscle it was pointed out that the cell membrane in the isolated tissue was inefficient with low external K owing to the high concentration ratios across it. A similar explanation may be advanced for the membranes of the renal K cells, and true equilibrium may not be reached with relatively low K concentrations in the immersion fluid. It may also be considered that at such low external K values the proportion of total chloride contained in the space outside the K-accumulating region is so high that even slight changes in the estimate of this space have a relatively great effect on the estimated Cl content as used for the Donnan product.

### III. The Identification of the K and Na Regions of the Nephron as the Proximal and Distal Convoluted Tubules

The existence of a relatively large K region of the kidney impermeable to Na, and a smaller region permeable to Na actively extruding it and losing weight, at once suggests that the two regions are the proximal and distal segments of the nephron. In support of this three reasons may now be advanced which would appear conclusive.

1. It has been shown above that when the kidney is immersed in Ringer solution the cells of the K region (like skeletal muscle fibres) can be greatly swollen in accordance with theoretical expectation, when the Na of the solution is largely replaced by K. No such swelling, but rather a decrease occurs with the cells of the Na region. When the kidney in turn is perfused with such
Ringer solutions containing high K and low Na concentration swelling of the
kidney is observed, and here while no increase in volume of the Na cells should
occur the relatively large reduction in volume observed in the immersed tissue
is not to be expected. This is so because a supply of NaCl is being maintained
from the lumen side (though with the high K solution its concentration is
reduced below the normal). When after some hours' perfusion the fluid is
suddenly replaced by suitable fixative, and the tissues examined histologically
(to be described in another paper) the cells of the proximal tubule are found
much swollen and those of the distal tubule a little shrunken. The following is
typical of the result obtained. Taking the mean cross sectional areas of the
proximal tubule minus that of the lumen to be 100 in the control kidney, or
kidney perfused with normal Ringer solution (total strength of 243 mm per
litre), then for a series of measurements (7 to 10 in each case) there is a mean
change from 100 to 135 on perfusing with Ringer solution containing 70
m. eq. K per litre and 47 m.eq. Na per litre. The cross-sectional area of the
distal tubule changes from 106 to 91, or shows some reduction instead of an
increase.

If the increase in length of the proximal tubule is similar to the increase in
cell diameter, this would mean a total volume increase of 58 per cent or 70 to
80 per cent increase in the cell water. This accords with theoretical expectation
for the swelling of the K region, and it may be concluded that the proximal
tubule is the K region investigated above. Such direct evidence is in turn
supported by the two following points.

2. The relative size of the K region to the whole renal tissue in the isolated
kidney is the same (within the limit of error involved) as Huber's measure-
ments of the proximal tubule (Table XI), and there is a similar correspondence
between the Na region and the distal tubule, though, owing to its small size
and the large intercellular volume, the margin of error involved for the distal
tubule is relatively greater.

Huber's measurements (12) show that the proximal tubule in the isolated
immersed tissue is 85 per cent of the whole nephron and the distal tubule

<table>
<thead>
<tr>
<th>TABLE XI</th>
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<tbody>
<tr>
<td>Dimensions</td>
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<td>mm.</td>
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<td>-----------------</td>
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<tr>
<td>Proximal tubule</td>
</tr>
<tr>
<td>Distal tubule</td>
</tr>
<tr>
<td>Glomerulus</td>
</tr>
<tr>
<td>Collecting tubule</td>
</tr>
<tr>
<td>Neck segment</td>
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<tr>
<td>Intermediate segment</td>
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</table>
9 per cent. If the glomerulus be excluded from the nephron the figures are 88 and 9 per cent respectively.

For the kidney immersed in Ringer solution with ordinary K content (and total molecular concentration of 243 mm per litre) the weight falls to 0.74, the total solids being 0.16 and the Na ratio 0.26. Taking the Na ratio to measure the total water in the intercellular spaces plus that in the Na cells, the water of the K region is 0.74 - 0.16 = 0.26 or 0.32. If we take the inulin ratio of 0.23, in the cold, to measure the intercellular space, the total water in the nephron is 0.35, so that the water of the K region is 91 per cent of the water in the cells of the whole nephron tissue, and the K tissue in turn may be presumed to have this proportion also of the whole nephron. The water of the Na region would then be 9 per cent of the total nephron water.

As considered above the Na region decreases much in volume on immersion, the Na ratio falling from 0.39 to 0.26 with a marked loss in weight of the kidney. If we subtract the inulin ratio from these figures the water of the region would change from 0.16 to 0.03. The question arises then—is there any direct evidence that a diminution of this order occurs in the size of the distal tubule? This we can provide in conjunction with Huber’s data. As given above the relative cross-sectional area of the cells of the proximal and distal tubules after perfusing with Ringer solution of ordinary type is 1.0 to 1.06, but from Huber’s data after immersions the ratio of the diameter is 1.0 to 0.4 and the area therefore 1.0 to 0.16. This change is of the expected order.

3. The experiments of Walker, Hudson, Findley, and Richards, (13) show that while the Cl content of the flowing urine in the frog’s kidney remains unchanged along the proximal tubule (samples being obtained by cannulating the nephron), it falls steeply when the distal tubule is reached, and with it no doubt the Na ion concentration. The distal tubule at least must be freely permeable to Na and Cl, and the curve of concentration with distance from the glomerulus suggests a marked difference in behaviour to the Na ion in the two segments, for the Cl concentration remains unchanged along the proximal tubule and falls steeply from the beginning of the distal tubule. If then, there are two regions of the nephron comprising practically its entire length, the larger impermeable to Na and the other permeable, this latter must be the distal tubule, and clearly the other very large segment can only be the proximal tubule.

Interpretation of the Hemoglobin, Inulin, and Na Ratios

The average inulin and Na ratios for the active kidney in the cold were found to be 0.23 and 0.26. The question is, what exactly does the inulin ratio measure? The indications are that, at least in the cold, it measures the interspaces of the frog kidney, in which may be included the lumen volume of the nephrons and glomeruli. The glomerular membrane as is well known from
the work of the Richard's school is freely permeable to inulin, and over 24 hours inulin could be expected to reach an equilibrium value in the lumen spaces.

That no appreciable amount of inulin is adsorbed is very probable from the analogy with muscle, where the inulin ratio gives a value as low as 0.09 and it is scarcely possible that appreciable adsorption could occur, when the interspace volume is compared with that from other methods (2). In the cold the effect of cyanide on the kidney is to increase slightly, but scarcely significantly the inulin ratio, this changing from $0.23 \pm 0.012$ to $0.27 \pm 0.016$ (giving the standard deviation of the means), whereas the Na ratio changes from $0.26 \pm 0.014$ to $0.55 \pm 0.023$. This indicates that the distal tubules are—in the cold—impermeable, to inulin or so slightly permeable as makes little difference to the results discussed. At room temperature the cyanide effect on the inulin ratio is much greater, changing from $0.23 \pm 0.020$ to $0.32 \pm 0.028$.

Concerning the hemoglobin ratio, this may be taken as measuring approximately the interspaces of the kidney apart from the lumen volume of the nephrons, basing this view on the impermeability of the glomerular membranes to hemoglobin and that diffusion into the lumen from other regions is either debarred or of negligible rate. It may be added that the exact interpretation given of the hemoglobin ratio makes no difference in the general treatment of the results throughout the paper; and the same applies even to the inulin ratio except in so far as this may be used to calculate the water of the Na cells in the active kidney by subtracting from the Na ratio.

**DISCUSSION**

From the experiments described it is clear that the frog's nephron is divisible into two main cellular regions, in one of which K can be accumulated, and without volume increase if the external Na be maintained constant. This region of the kidney contains no appreciable sodium and conversely the other region contains no appreciable potassium (or only of plasma level) while its Na content is at or close to the Na value of the external fluid. These regions have been identified as the proximal and distal tubules (though the K-accumulating region may include the comparatively very small collecting tubule—amounting to 2 to 3 per cent of the nephron). The cell membranes of the first are permeable to potassium but not to the sodium ion. The membranes of the latter are permeable to Na ions and to the smaller K ion (to the latter at least from the blood side). The process of accumulation follows well the theoretical treatment already outlined for muscle, and specially notable is the correspondence of the volume changes with the theoretical requirement. The production of these large volume changes and their measurement is very simple and affords a ready means of testing the underlying theory. In measuring the volume changes and weighing the tissues after immersion no undue pressure
should be exerted, especially on the highly distended tissues, a fixed routine of
light surface drying on filter paper being advisable.

The Effect of Cyanide.—For the isotonic Ringer solutions where K is sub-
stituted for Na with consequent large volume changes, the effect of cyanide is
strikingly shown by means of the linear relation of volume to the reciprocal of
the external non-diffusible constituents as appears in Fig. 3. Cyanide does
not change the slope of the line up to an external concentration of 70 mm
per litre. This means that it affects neither the differential permeability of the
K cells nor does it affect their volume. It appears, however, to render their
membrane system less resistant to high distension, for with cyanide the dif-
ferential permeability breaks down when the cell water has about doubled,
whereas without cyanide this does not happen until an increase of nearly four
times occurs. While not affecting the volume nor differential permeability of the
K cells (up to high limits of distension) cyanide has a great effect on the
renal volume as a whole as shown in Fig. 3 arising from the fact that it con-
verts the active contraction into a slow distension. The line through the
points after 24 hours cuts the ordinate at 0.62. This will include 0.18 (mean
dry weight of the cyanide kidney) leaving 0.44, and subtracting the inulin
ratio of 0.23 as representing the total intercellular spaces, this leaves 0.21, so
that the water of the Na cells instead of decreasing markedly from the fresh
value of 0.16, as in the active kidney, has somewhat increased. After 48 hours
this water in the cyanide kidney increases to 0.27. Now such distension
agrees with the conclusions drawn that the cells are quite permeable to Na and
K ions, but that in their active state they are capable of keeping Na and water
steadily extruded and decrease in volume. When normally functioning in the
animal their volume would appear to be associated with a steady influx of Na
ions from the lumen side.

It will be seen that the fortunate appearance of a practically exact linear
relation of renal volume to the reciprocal of ([Na] + 5.7) throws much light on
the nature of the cyanide effect, and also confirms the measurement of the
renal tissue outside the K region.

For such passive accumulation of K in the proximal tubules the question
arises as to its possible relation with the normal active excretion of potassium
or other substances—a question arising not only for the frog's kidney, but as a
general one for active renal excretion. That it has an important relation may
be considered likely, but a treatment of such implications will be deferred until
later, the further discussion here being confined to a consideration of the ab-
sorption of water from the urine as it flows down the proximal tubules in the
normal animal.

The Question of Water Absorption in the Proximal Tubule of the Frog’s Kidney

The proximal segment in the immersed frog’s kidney comprises 85 per cent
of the volume of the whole nephron, and it would appear to be about 70 per
cent of the nephron volume when normally functioning in the animal. Whether in the latter case water is absorbed or not is of special significance if only from the analogy with the mamalian kidney, in the proximal tubule of which about 80 per cent of the whole water absorption in the nephron would occur if we explained the glucose increase after phlorhizin as due to the absorption of fluid.

It has been shown that no diminution of chloride occurs down the proximal segment in the frog's kidney (13) nor does any significant change of pH occur in the same region (14).

If the cells of the proximal tubule are impermeable to Na ions, as shown for the immersed kidneys, there can be no appreciable absorption of water, for if water be absorbed, chloride and bicarbonate must follow in similar concentration to maintain the lumen concentration constant, and sodium is the only cation available with the required concentration to accompany the chloride and bicarbonate ions.

Such an argument might be met by the objection that some section only of the proximal tubule cells need be concerned with the Na absorption, but since the process (or apparent water absorption) is progressive down the tubule, we should need to picture Na cells scattered between the K cells, but from volume considerations they could constitute at most only a small fraction of the whole. When the proximal tubule is largely swollen by low Na and high K perfusions or immersions they might be expected to appear as localised constrictions, but so far we have noticed none such, and the picture of such scattered Na cells seems a highly unlikely one. Without unduly labouring the question it will be seen that the facts of K and Na exchanges are against the view of water absorption in the proximal tubule.

On the other hand, we must conclude that if no water be absorbed in the proximal tubule, then the increase of glucose concentration after phlorhizin is necessarily an active excretion. Such has been regarded as inconceivable (e.g. Walker and Hudson (20); Walker et al. (18)) whether for teleological reasons, or otherwise. But if we consider the reverse process of glucose secretion into the blood from the urine, and this to depend on phosphorylating and dephosphorylating mechanisms (in accordance with Lundsgaard's views (15, 16) or modified versions) and an orientation of cell metabolism such as developed by Danielli (17), and long entertained in similar form in this laboratory, it would seem by no means inconceivable that an inhibitor of carbohydrate metabolism unequally concentrated within the cell, might reverse the direction of glucose passage.

Besides the phlorhizin effect, the collection rates of fluid down the tubule are held to support the view of water or fluid absorption. This is considered below.

The Proximal Tubule of the Mammalian Kidney.—Our investigations concerning the K equilibrium etc. have not been extended to the mammalian
E. J. CONWAY, O. FITZGERALD, AND T. C. MACDOUGALD

kidney, but provisionally we may assume that the proximal tubule has the same permeabilities as found for the amphibian kidney. If this is so, and no water be absorbed down the proximal tubule, it is an essential requirement that the Na ion concentration should not change, but correspond to that in a plasma ultrafiltrate. This is the result found by Walker et al. (18).

Concerning the collection rates found down the tubule the following very simple relations may be firstly considered. If, on cannulating any part of the tubule, and registering the flow, we write "G" for the rate of glomerular filtration throughout the collection, "S" the amount of fluid absorbed as calculated from the glucose concentration after phlorhizin (assuming this to be due to water absorption), and "D" an extra amount of fluid absorbed not included in "S" (owing to possible back diffusion of glucose) then the difference in rates of collection from two tapping at different sites appears as:--

\[(G_1 - S_1 - D_1) - (G_2 - S_2 - D_2)\]

which may be written:--

\[(G_1 - G_2) + (S_1 - S_2) + (D_2 - D_1)\]

(the symbols \(G_1\), \(S_1\), and \(D_1\) referring to the tapping nearer to the glomerulus).

If we consider these symbols as representing average figures in a number of collections, then fluctuations in \(G\), and in resistance due to the collecting pipettes, etc. will tend to disappear. \(G_1\) will then be necessarily, if only very slightly, greater than \(G_2\) and \(D_2\) will tend to be greater than \(D_1\), so that \((S_1 - S_2)\) will give a minimal mean value for the water absorption between the sites of collection. The actual mean water absorption should be greater. This argument is independent of the question of the resistance to flow offered by the pipettes, or as to whether the collection rates correspond to normal rates of flow down the tubules. With a sufficient number of results it should answer decisively the question as to whether the glucose increase after phlorhizin is due to fluid absorption. This should amount to more than about 60 per cent down to half the length of the proximal tubule. As reproduced in the first series of Table XI Walker et al. (18) have summarised their own data for rats obtained in experiments of great manipulative elegance. It will be seen that they find 1.7 c.mm. for the first fifth, 1.1 for the second, and 0.8 c.mm. per hour for the third fifth. This would appear to support their views, but on examination the apparent effect is entirely due to one very aberrant figure (No. 12 of their series), the deviation of which from the mean is about four times the standard deviation for its group. This, included in the arithmetical mean of the first three figures gives the high value for the collection rate from the first fifth of the proximal tubule. The medians from the same data are shown in the second series of Table XII, and no effect of diminished rates down the tubule is apparent. The next two series give the means and medians for the guinea pig,
for which glomerular collection data are also available, and it will be seen that
no diminution appears on passing from the glomerulus to the middle of the
proximal tubule or about as low as it has been found feasible to collect the
urine. Applying a stricter statistical treatment the correlation between the
collection rates and distance down the tubule (taking the glomerular
collection as at zero distance) is \(-0.03 \pm 0.21\) for the twenty-three guinea pig
results, and no decrease with distance can be deduced from the results. For
the twenty-three rat experiments (omitting No. 12) the correlation is \(-0.11
\pm 0.21\), and the regression equation shows a very doubtful fall of 17 per
cent in the collection rates midway down the proximal tubule, which may be compared with the expected fall of more than 60 per cent. The correlation of the
whole forty-seven collection rates from rats and guinea pigs with tubular
distance is \(-0.18 \pm 0.15\), and for the forty-six results omitting the aberrant
value it is \(0.05 \pm 0.15\). On the other hand there are high correlations (order of
+0.80) between creatinine (or glucose after phlorhizin) concentration ratios
and the tubular distance. In such results commented on above, the total
variability of the collection rate is not high, being only 40 to 45 per cent as a
coefficient of variation, and it will include the variability of glomerular flow,
pitette resistance, etc. It is easy to show that changes in these variables from
tubule to tubule, with an underlying relation of distance down the tubule to
amount of water absorption cannot then account for the low correlations ob-
served between collection rates and sites of collection. They would at most
reduce it from \(-1.00\) to \(-0.75\) or from \(-0.80\) to about \(-0.60\). The position
remains essentially the same if a logarithmic relation of collection rate to dis-
tance is considered instead of a linear.

If similar figures be examined for the frog (20) there is not the same exact
measurement of tubular distance as for the mammal, but for the fifteen re-
results listed for the proximal tubule collections, the mean and median for the
seven rates down to the first half of the tubule are 0.37 and 0.35 c.mm. per hour,

<table>
<thead>
<tr>
<th>Table XII</th>
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<tbody>
<tr>
<td><strong>Average Collection Rates of Tubular and Glomerular Urine</strong></td>
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<tr>
<td><strong>Animal</strong></td>
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<tr>
<td>Rat</td>
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<tr>
<td>Guinea pig</td>
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Data taken from Walker et al. (18).
and for the seven collections at the end 0.46 and 0.39 c.mm. per hour, showing a slight but scarcely significant increase. For the frog it is true that the rate of glomerular collection—as judged in this series by three experiments—is 0.72 c.mm. per hour (median value). Such a difference may be possibly due to the higher pressures at the head of the tubule (the glomerulus being here succeeded by a short, but comparatively narrow neck). The difference is no doubt also emphasised by the selection of the most suitable glomeruli for puncture; that is, the most distended.

It may be reasonably concluded that the observed collection rates do not support the view that fluid is absorbed in the proximal tubule, but rather the contrary that there is no fluid absorption therein; and this agrees with the deductions from the permeability studies described in the present paper.

**SUMMARY**

1. In a manner similar to that of the sartorius muscle, the isolated kidney of the frog can accumulate K against a gradient to upwards of three times its normal concentration.

2. The K-accumulating region is identified as the proximal tubule, which in the isolated tissue immersed over 24 hours in the cold (2–3°C.) amounts to about 90 per cent of the nephron minus the glomerulus. In the fresh tissue it constitutes about 70 per cent.

   The cells of the proximal tubule are impermeable to Na, but freely permeable to K and Cl.

3. The distal tubule in the isolated kidney does not accumulate K over the external concentration. The cells are permeable to Na which they actively extrude. This extrusion of Na goes parallel with a loss of osmotically associated water amounting to about 15 per cent of the weight of the fresh kidney, but varying somewhat with the conditions.

4. The accumulation of K in the proximal tubules is in accordance with the equations established for the sartorius muscle, and, as theoretically expected, there is no volume increase (but rather a small decrease) with the large accumulations, when the external Na concentration is maintained throughout.

5. With K accumulation in isotonic mixtures large volume changes occur as K is progressively substituted for Na. Over the range of external K concentration of 10 to 100 mM per litre the weight of the whole kidney changes to 2.5 times and the water of the cells of the proximal tubules increases to over four times. Up to an external K value of 90 mM per litre the mean weight of the kidney shows a linear relation when plotted against the reciprocal of the Na concentration plus the small glucose and Ca concentration. This relation is interpreted theoretically.

6. The effect of cyanide in the isotonic mixtures is to prevent the contraction of the distal tubules and to cause swelling of the same. It does not affect the
volume, volume changes, or differential permeability of the proximal tubule. At the same time the membranes of the proximal tubule cells lose their characteristic permeability at a lower level of distension in the presence of cyanide.

7. The mean Na ratio for the kidney after 24 hours' immersion in the cold is 0.26 ± 0.014 (giving standard deviation of mean). The ratio is defined as Na/kg. of original tissue

\[
\text{Na/kg. external fluid}
\]

For the fresh kidney the mean ratio is 0.39 ± 0.006.

8. The mean inulin ratio (28 observed in the cold) is 0.23 ± 0.012 and the same value for 10 observed at room temperature. At room temperature—2 hour immersion—the ratio is increased by cyanide to a mean of 0.32 ± 0.028, but only a slight increase is caused by cyanide in the cold.

9. The mean hemoglobin ratio after 24 hours' immersion in the cold is 0.17 ± 0.004 and is unaffected by cyanide.

We wish to express our gratitude to the Irish Medical Research Council for a grant-in-aid, and to Dr. P. Boyle for assistance in the analyses.

REFERENCES