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# MOLECULAR CONCENTRATION OF KIDNEY CORTEX SLICES

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From the evidence obtained in a study of freezing-point depressions of frozen and powdered tissues maintained at 0° C (Conway & McCormack, 1953) it would appear that the intracellular concentration of mammalian tissues is the same as that of the plasma. This is also considered by Leaf, Chatillon, Wrong & Tuttle (1954) to be in agreement with their recent work on the distribution of a large water load in dogs. However, it has been suggested recently (Opie, 1949, 1954; Robinson, 1950, 1952) that the intracellular fluid is hypertonic and that the cells have to pump out water continuously in order to prevent swelling. As evidence for such a theory it has been shown that slices of renal cortex in isotonic saline swell under anaerobic conditions (e.g. at 0° C or in the presence of cyanide) owing to the uptake of water. At 37° C, in the presence of oxygen and absence of cyanide, this water is lost again (Stern, Eggleston, Hems & Krebs, 1949; Robinson, 1952; Whittam & Davies, 1953). Aebi (1950) has also demonstrated similar dependence of swelling on respiration with liver slices from the guinea-pig.

If the fluid within the cell remains isosmotic with the external fluid then the slices should give the same freezing-point depressions after immersion at 0° C and at 37° C. But if, on the other hand, water is actively extruded at 37° C, the slices at this temperature would have a much higher intracellular concentration, which should be evident from a marked difference in the freezing-point depressions. This has been investigated with the results described below.

Freezing-point depressions, water content, Na, K and Cl were determined in slices of rat kidney cortex, together with determinations of freezing-point depression of water content of slices of guinea-pig kidney cortex. The inulin 'space' for rat and guinea-pig cortical slices was also determined.

## METHODS

The animal was killed by a blow on the head and the kidneys removed immediately. The tissue was held between two pieces of frosted glass (moistened with saline) and slices of approximately 0·3 mm thickness were cut by hand with a razor blade, the outer slices being rejected. After

cutting, the medulla and cortex of each slice were separated with a blade, only the cortex being used. The slices were immediately placed in ice-cold isotonic saline (Robinson's $A_2$ solution, Robinson, 1950) and kept at 0° C for 60 min. After this period about half the slices were removed for analysis, the remainder being placed in saline at 37° C, bubbled with oxygen for 60 min and then removed for analysis. On removal from the saline the superfluous fluid was taken off each slice with filter-paper. For 'fresh tissue' values, the slices were cut without moistening with saline and analysed immediately.

*Cryoscopic measurements.* When freezing-points were to be determined the slices were frozen as quickly as possible with liquid oxygen, ground to powder in a mortar, and mixed with one volume of 0·95% NaCl. The procedure for freezing-point determinations was that described by Conway & McCormack (1953).

*Water, Na and K content.* For water content and Na and K determinations the slices, after removal from the saline, were placed in a platinum crucible, weighed and dried overnight at 105° C. The water content was taken to be the percentage weight lost in this procedure. The slices were then ashed, using some drops of 4N-$H_2SO_4$ and Na and K determined in the residue, using the Beckman flame photometer.

*Chloride content.* This was determined by a microdiffusion method (Conway, 1950).

*Inulin space determination.* Fresh cortical slices were placed in the saline fluid containing 0·1% inulin in solution, at 0° C or at 37° C with oxygen. After the usual period they were taken out, the surplus fluid removed, and re-immersed in 5 ml. of the saline, without inulin, for 30 min. They were then removed and inulin determined in the saline by the method of Ross & Mokotoff (1951). Inulin was also determined in the inulin saline fluid (diluted 50 times) after removing the slices. The space '$s$' was calculated from the following formula

$$\frac{50+sW}{sW}=I_0/I_a\,,$$

where $W$ is the weight of the kidney slices in grammes after immersion in the inulin fluid, $I_0$ the inulin concentration in the inulin saline after removing the slices therefrom, and $I_a$ the concentration in the saline after the second immersion.

It was shown that 30 min was quite sufficient time for the passage of inulin into the interspaces, as doubling this time made no difference to the results obtained.

## RESULTS

*The inulin space.* Twenty determinations of the inulin space were carried out, twelve on rat kidney slices and eight on slices from guinea-pig kidney. With each animal one determination was carried out on the slices after 1 hr at 0° C and one on the slices after removal from 0 to 37° C for 1 hr. No difference within the sampling error was found between the inulin space for the rat and the guinea-pig kidney but the values at 0° C differed from those at 37° C. The mean inulin space for slices at 37° C was 0·21 kg/kg tissue, the S.E. being 0·014. For 0° C the mean was 0·16 kg/kg tissue, with a S.E. of 0·011. The difference between the means is $0·05 \pm 0·018$, which may be taken as significant since it is about three times its S.E. These results differ somewhat from those of Robinson (1950) who gave the inulin space as 0·26 for the rat kidney and reported no difference between the values at 2–3° C, when the slices were swollen, and at 37° C when they were at or near normal volume. The difference may arise from some change in conditions, and from the fact that we used 0·1% inulin, whereas Robinson used 1%. (In Robinson's curve of the

determination of the inulin space with time in the saline at 0° C there is a secondary slow increase.)

It may also be noted that the mean inulin space in our determinations, when referred to 1 kg of the original or fresh tissue, is in fact about the same at 0° C as at 37° C, so that there is no appreciable change in the actual space for a given number of cells or a fixed weight of fresh tissue.

*Changes in the water content of the rat cortical slices.* In Table 1 the mean water content per kg of fresh cortical tissue is 769 ± 7·8 ml. On immersing the slices in the saline mixture described (0·30 osmolar) for 1 hr at 0° C, the water content becomes 831 ± 3·6 ml./kg. Then on placing for 1 hr in the saline mixture at 37° C it becomes 774 ± 7·7.

TABLE 1. Rat kidney cortical slices; changes after immersion in 0·30 osmolar saline for 60 min at 0° C and then at 37° C

| | Immersed at 0° C | Immersed at 37° C | Fresh tissue |
|---|---|---|---|
| Water content (g/kg of tissue) | 831 ± 3·6 (15 obs.) | 774 ± 7·7 (10 obs.) | 769 ± 7·8 (15 obs.) |
| Dry material (g/kg) | 169 | 226 | 231 |
| $F_t$ values | 0·59 ± 0·011 (14 obs.) | 0·61 ± 0·017 (11 obs.) | — |
| $F_t$ value (median) | 0·58 | 0·60 | — |
| Na content (m-equiv/kg) | 116 ± 5·3 (14 obs.) | 78 ± 3·9 (15 obs.) | 56 ± 3·9 (15 obs.) |
| K content (m-equiv/kg) | 24 ± 2·8 (16 obs.) | 38 ± 1·9 (15 obs.) | 56 ± 3·5 (16 obs.) |
| Cl content (m-equiv/kg) | 55·5 ± 4·3 (6 obs.) | 53·3 ± 5·5 (6 obs.) | 31 (4 obs. range 27–34) |

If it be assumed that the total solid material remains approximately constant, the changes can be represented as per kg of original fresh tissue. Thus 1 kg of fresh tissue slice swells at 0° C to 231/169 or 1·367 kg. This swollen tissue contains 831 × 1·367 g (= 1136 ml.) of total water. Of this 1·367 × 160 (= 219 ml.) is interspace water and therefore 917 ml. is cellular water, indicating a 64 % increase from 559 ml. (769 − 210 ml.). The tissue placed subsequently in saline at 37° C loses nearly all this excess water. Thus per kg original tissue it decreases from 1·367 to 1·022 kg, the total water being 1·022 × 774 = 791 ml. Of this, 1·022 × 200 (= 204 ml.) is interspace water leaving 587 ml. as cellular water which has lost 330 ml. (i.e. 917 − 587 ml.).

*The $F_t$ values of the cortical slices.* The results of the determination of the freezing-point depressions of the tissue water ($F_t$) in the slices are given in Table 1, for fourteen observations on the slices at 0° C and for eleven observations on the slices at 37° C. The mean $F_t$ changes from 0·59 ± 0·011 at 0° C to 0·61 ± 0·017 at 37° C. The difference here is not significant. The medians or most representative values for the series are 0·58 and 0·60. The values are somewhat greater than the freezing-point depression of the external fluid

(0·53). However, it has been shown in a previous communication (Conway & McCormack, 1953) that with maintenance at 0° C for up to 20 min the freezing-point depression increases by $0{\cdot}0103t$, where $t$ is the time in minutes. After 5 min, which is about the time required for a freezing-point determination by the method described, the $F_i$ value could be expected to be approximately 0·58 so that no significant change from this occurs in the slices.

Na, K *and* Cl *values*. From the data in Table 1, the derived values of Table 2 have been obtained. The data for Na, K and Cl content of the cell water have been calculated per kg of original tissue as described above.

TABLE 2. Rat kidney cortical slices; data from Table 1 expressed per kg fresh tissue and referred to the cell water

| | Immersed at 0° C, m-equiv/kg fresh tissue | Immersed at 37° C, m-equiv/kg fresh tissue | Change on transferring from 0 to 37° C |
|---|---|---|---|
| Na | 159 | 80 | −79 |
| K | 33 | 39 | +6 |
| Cl | 76 | 55 | −21 |
| Total water (ml./kg fresh tissue) | 1140 | 793 | −347 |
| | m-equiv in the cell water per kg fresh tissue | m-equiv in the cell water per kg fresh tissue | Change on transferring from 0 to 37° C |
| Na | 128 | 50 | −78 |
| K | 31 | 38 | +7 |
| Cl | 44 | 24 | −20 |
| Cell water (g/kg fresh tissue) | 920 | 578 | — |

It will be seen that, on changing from 0 to 37° C, the slices lose 78 m-equiv Na and 20 m-equiv Cl from the cell water while gaining 7 m-equiv K. The total loss for Na, Cl and K is thus 91 m-equiv per kg of original tissue. With osmotic equilibrium this should account, at most, for $91 \times 1000/300$ ml. = 303 ml., though if some of the cations were bound by colloidal or protein molecules, the figure would be less.

As shown above the total water lost was 330 ml. or a minimum of 8 ($=27 \times 0{\cdot}30$) extra m-mole would appear to be lost from the cell water per kg of original tissue (as by re-synthesis of larger molecules).

*Data for the guinea-pig kidney*. In Table 3 the data for guinea-pig slices are summarized. It will be seen that swelling of the cortical slices occurs on immersion at 0° C and the slices lose water on placing again in saline at 37° C. The water changes are much less than for the rat kidney. One kilogram of fresh slices changes to 1·24 kg at 0° C, the extra water being lost again on placing in saline at 37° C, with oxygen bubbling.

The mean $F_i$ values for the slices at 0° C and at 37° C ($0{\cdot}53 \pm 0{\cdot}02$ and $0{\cdot}54 \pm 0{\cdot}02$) are the same as the value for the external fluid ($=0{\cdot}53$), within the sampling error.

The absence of any increase above the external value for the slices at both temperatures agrees with previous observations (Conway & McCormack, 1953) in which no increase with time was found on maintaining the frozen and powdered tissue at 0° C for more than 30 min, so that in the 5 min required for the freezing-point determination no increase should be expected.

TABLE 3. Guinea-pig kidney cortical slices; changes after immersion in 0·30 osmolar saline for 60 min at 0° C and then at 37° C

| | Immersed at 0° C | Immersed 37° C | Fresh tissue |
|---|---|---|---|
| Water content (g/kg tissue) | 823±6·4 (5 obs.) | 800±6·2 (5 obs.) | 781±4·3 (5 obs.) |
| Dry material (g/kg) | 177 | 200 | 219 |
| $F_t$ value | 0·53±0·02 (6 obs.) | 0·54±0·02 (6 obs.) | — |
| $F_t$ value (median) | 0·52 | 0·53 | — |

## DISCUSSION

From the results obtained, it would appear that swelling of the rat kidney slices when their metabolism is practically stopped by immersion in saline (0·30 osmolar mixture) at 0° C can be attributed to the following factors: first, to the failure of the sodium pump either in all the cells of the slices or in the cells of some special region, and, second, to the breakdown of labile substances as described in the previous communication (Conway, Geoghegan & McCormack, 1955). The failure of the sodium pump allows the accumulation of Na in the following ways:

(*a*) $Na^+$ ions enter with $Cl^-$ ions.

(*b*) $Na^+$ ions enter in exchange for $H^+$ ions when, through the formation of new non-colloidal molecules, increased anionic groups such as $R.COO^-$ are formed.

(*c*) $Na^+$ ions enter, as in (*b*), when new anionic groups appear on the colloidal molecules chiefly through increase of pH.

(*d*) $Na^+$ ions exchange for $K^+$ ions.

It may be added that it is also very probable that there will be a variable loss of normally non-diffusible material due to permeability changes, thus providing another factor in the total change.

Of these four points only (*a*) and (*b*) will cause an increase of molarity in the cell water. It will be seen from the results that if the slices be considered for convenience as exchanged from 37 to 0° C (with reversal of the changes on transferring from 0 to 37° C), then the cell water (per kg fresh tissue) gains 20 m-equiv $Cl^-$ which may be considered as accompanied by 20 m-equiv $Na^+$. Also, 7 m-equiv of $K^+$ are exchanged for 7 m-equiv $Na^+$ leaving 51 m-equiv $Na^+$ (78 − 20 − 7) to be accounted for otherwise. A little of this may possibly be connected with phosphate entrance, but it may be assumed that it is nearly

all due to exchange with $H^+$ ions from the new anionic groups. There is no evidence at present for the relative assignment to colloidal or non-colloidal molecules.

Mudge (1951) found that under the conditions which he employed the sum of the Na and K concentration in the cell water remains constant as the change is made from 0 to 37° C. From our results the concentration per litre of cell water was found to be 173 m-equiv at 0° C and 153 m-equiv at 37° C. However, the difference only favours Mudge's conclusion against the water pump theory.

The volume changes in guinea-pig cortical slices on transferring from 0 to 37° C are much less than for the rat kidney. This is to be expected from the fact shown previously (Conway & McCormack, 1953) that when guinea-pig kidney (as opposed to rat kidney) is frozen, powdered, mixed with an equal volume of saline and kept at 0° C there is no appreciable change in molecular concentration over a period of 30 min.

Deyrup (1953*a*, *b*) has investigated the swelling in various media of cortical slices from the rat kidney, and considers her findings incompatible with the view that the cells are normally hypertonic with respect to the plasma. Results with glucose, either with or without NaCl, and with the disaccharides are difficult to explain on any simple basis but are probably explicable by a combination of effects on the Na pump, the formation of new molecules (or inhibition of the metabolism), differences in permeability to glucose and disaccharides, and changes of permeability induced by the media.

Reference may here be made to recent work of Aebi (1953) on the total Na and K concentrations in rat kidney cortical slices incubated for 1 hr in a Ringer fluid containing 11 m-equiv K and 158 m-equiv Na/litre. The mean figures found for K and Na were 71·1 and 133·7 m-equiv/kg of tissue, which would give 291 m-equiv (Na and K) per kg of cell water (calculating this as indicated in the present text). If isosmotic equilibrium existed in the outer fluid this would leave about 50 m-mole/kg water for other cell constituents.

*Comparison with the frog kidney.* Like the cortical slices of the mammalian kidney, the isolated frog kidney swells considerably when its metabolism is inhibited by cyanide (Conway, FitzGerald & Macdougald, 1946). Unlike the mammalian tissue, placing in Ringer fluid at 0–3° C does not cause it to swell over 24 hr, presumably by insufficiently inhibiting the metabolism. Such kidneys in the cold have still an active metabolism, and extrude sodium and some chloride. Immersed in Ringer–Locke solution containing 0·7 % NaCl at room temperature, after 2 hr the mean weight falls to 0·83 of the original weight while if 0·002 M-potassium cyanide is present the kidneys increase to 1·06 times the original weight after 2 hr immersion.

From curves through the data given in tables 3 and 4 of the communication referred to above (Conway *et al.* 1946) the following mean values (referred to 1 kg of fresh tissue) may be given: the K, Cl, Na and weight values after 24 hr

immersion in the Ringer–Barkan fluid, are 49, 24, 22 and 0·88 respectively. With cyanide (0·004 M-KCN) the values were 32, 40, 55 and 1·18, with an external value for K of 2·5 m-equiv/l. and a total external concentration of 185 m-equiv/l. Thus in cyanide the kidneys have 33 m-equiv Na and 16 m-equiv Cl more but 17 m-equiv less K than the kidneys in the Ringer-Barkan fluid without cyanide. The total Na entering without Cl is (33 − 16) or 17 m-equiv, which is the same as the K lost. This balance, however, must be considered with respect to the possible entrance also of 2·5 m-equiv $HCO_3^-$, as calculated from the external ratio of Cl and $HCO_3$ ion concentrations. The total increase of (Na + K + Cl) is 32, or allowing for bicarbonate, 34·5. The total external concentration is 185, or 180 omitting the KCl which may be considered as osmotically inactive with K and Cl freely diffusing across the membrane. Thus about 190 ml. water per kg may be expected to enter with the change in inorganic ion balance but in fact 300 ml. enter per kg of the original tissue and the excess may be accounted for by the formation of new molecules due to the breakdown of labile substances with the metabolic inhibition produced by cyanide.

With the frog kidney it was shown that in such electolyte exchanges one was dealing with two nephron regions, identified as the proximal and distal tubules. Nearly all the Na and Cl in the fresh kidney (apart from the interspace) is contained in the distal tubule and is actively excreted therefrom when the kidney is immersed in Ringer fluid with resulting great decrease in volume of the distal tubule. Cyanide inhibits the passing out of Na and the Na and Cl ions enter freely into the distal tubule causing considerable swelling. The proximal tubule appears to be little affected by the cyanide. Either Na enters very slowly into the cells or the pumping mechanism in these cells is not affected by cyanide. In the earlier paper the proximal tubule cells were considered impermeable to Na ions. This may be interpreted rather as a virtual impermeability (due to the Na pump) like that of the muscle fibre membrane.

## SUMMARY

1. In confirmation of the results of other workers, renal cortex slices from rats and guinea-pigs immersed for 1 hr in 0·30 osmolar saline mixture (Robinson, 1950) at 0° C, swelled considerably.

2. On subsequently immersing the slices in a similar saline at 37° C, and bubbling with oxygen for 1 hr, the slices lost water to about the normal value. The ratio of the tissue water at 0° C to that at 37° C per kg fresh tissue was 1·43:1 for rat kidney, and 1·24:1 for guinea-pig.

3. There was no difference (within the sampling error) between the total molecular concentration of the renal cortex slices at 0° C and at 37° C, in the case of rat and guinea-pig, as was shown by the freezing-point depression. For the guinea-pig this was the same as that of the external fluid. For the rat

kidney the freezing-point depressions at 0 and 37° C were both somewhat raised, but no more than could be expected from the rapid autolytic breakdown during the period required for the determination (Conway & McCormack, 1953).

4. The loss of water from the renal cortex of the rat on transferring from 0 to 37° C with oxygen was shown to be partly due to the excretion of Na ions, accompanied by Cl and possibly by some other anions or in exchange for H ions, the remainder being considered due to reduction of intracellular molecules by the resynthesis of labile complexes.

5. The mean inulin space for the slices was determined, using 0·1 % inulin in immersion fluid. In the case of both rat and guinea-pig renal cortex it was found to be $0{\cdot}16 \pm 0{\cdot}01$ g/g tissue at 0° C, and $0{\cdot}21 \pm 0{\cdot}014$ at 37° C. Little or no change in the actual space per kg of fresh tissue resulted from the changes in cell water.

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