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THE EFFECT OF SODIUM AND CHLORIDE IONS UPON SWELLING OF RAT KIDNEY SLICES TREATED WITH A MERCURIAL DIURETIC

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The present experiments were designed to investigate how the ionic composition of the medium influenced the oxygen consumption and the water content of slices cut from the kidney cortex of rats and treated with a mercurial diuretic (mercaptomerin) *in vitro*. Preliminary experiments, briefly reported by Robinson (1954), showed that mercaptomerin depressed the oxygen consumption of the slices and increased the percentage of water in them, and suggested that the uptake of water was not a direct consequence of the entry of sodium because it occurred when external sodium was replaced by choline. In order to analyse further the action of mercaptomerin, its effects were studied in two additional media; both of these contained sulphate instead of chloride, and most of the sodium in one of them was replaced by choline. The presence or absence of the common bulk ions of mammalian extracellular fluids was found to make surprisingly little difference to the behaviour of the slices, a finding which is out of harmony with a prevalent opinion that exchanges of water between tissue cells and their surroundings are largely secondary to exchanges of the common inorganic ions.

METHODS

Media

The ordinary medium used in these experiments was prepared by mixing 232 ml. 0·154 M-NaCl, 8 ml. 0·154 M-KCl, 6 ml. 0·110 M-$CaCl_2$ and 2 ml. 0·154 M-$MgSO_4$, and adding 25 ml. of a phosphate buffer prepared by bringing 75 ml. of 0·2 N-NaOH to pH 7·4 with 2 M-H_3PO_4 and diluting to 100 ml. with water. A low-sodium medium was prepared in exactly the same way but substituting 0·154 M choline chloride for the NaCl.

To prepare a chloride-free medium, 232 ml. of 0·103 M-Na_2SO_4 was substituted for the 0·154 M-NaCl, 8 ml. 0·103 M-K_2SO_4 for the KCl, and 6 ml. 0·110 M-$Ca(NO_3)_2$ for the $CaCl_2$. A low-sodium, chloride-free medium was made by substituting 232 ml. 0·103 M choline sulphate for the Na_2SO_4 in the chloride-free medium. The choline sulphate solution was prepared by rubbing the calculated weight (3·22 g) of solid silver sulphate in a mortar with 100 ml. of 0·206 M choline chloride until a portion of clear fluid removed by means of a filter stick contained less than 0·001 M chloride. It

will usually be convenient to refer to the extraordinary media by the names of their principal constituents.

The concentrations of the ions in these media are shown in round figures in Table 1. It will be seen that the two choline media contained about one-tenth the concentration of sodium found in normal extracellular fluids. Thus, although these media were not completely free from sodium, its concentration should have been low enough to reverse the concentration gradient which tends to drive sodium into cells in their normal environment. The cells of the renal tubular epithelium would be expected to contain more sodium than muscle cells, which make up so much of the cell mass of the body that they are usually regarded as typical, because they reabsorb each minute about half their own volume of a glomerular filtrate which has the composition of a typical extracellular fluid. Analyses by Whittam & Davies (1953) suggest that the cells of the guinea-pig's kidney cortex contain about 50 m-equiv Na/l. cell water, and there is no reason to expect figures of a different order for the rat. The two sulphate media contained 5 mM nitrate because the calcium was introduced into them as nitrate. Glucose (1 mg/ml.) was added to all media just before they were measured into the manometer flasks.

TABLE 1. Ionic composition of media (m-equiv/l.)

Principal constituent	Sodium chloride	Choline chloride	Sodium sulphate	Choline sulphate
Sodium	145	15	190	15
Chloride	140	140	0	0
Choline	0	130	0	175
Sulphate	2	2	183	183
Potassium	5	5	6	6
Calcium	5	5	5	5
Magnesium	2	2	2	2
Phosphate	8	8	8	8
Nitrate	0	0	5	5
Freezing-point of glucose-free solution	−0·48° C	−0·485° C	−0·435° C	−0·46° C

If all constituents had identical osmotic coefficients the activity of water should have been the same in all media, for the glucose-free salines all had the same osmolar concentration (298 osmole/l.). The osmotic coefficients of sodium and potassium sulphates are, however, known to be low compared with those of the chlorides (0·74 compared with 0·93 and 0·92 respectively according to Lifson & Visscher, 1944). Consequently the composition of the chloride-free media had to be a compromise. They had a higher concentration of sodium than normal extracellular fluids, but a lower osmotic pressure. The freezing-points of the glucose-free salines were determined by the method of Hervey (1955) and are shown in Table 1. The addition of 1 mg of glucose per ml. would be expected to depress the freezing-point by a further 0·03° C, so that on the basis of their freezing-points all the media as used were slightly hypotonic to normal serum.

The demonstration of Hodgkin & Katz (1949) that the action potential of squid axons was abolished if external sodium was replaced by choline suggests that choline did not penetrate the axonal membrane. Less is known about other membranes. Trowell's (1935) observation that choline increased the oxygen consumption of rat liver slices considerably and of kidney slices much less might suggest that only slight penetration occurred. Choline chloride has been reported (Robinson, 1954) to be a little more effective than sodium chloride in causing osmotic shrinkage of slices which were not respiring; and the normal water content (here confirmed) of slices respiring freely in media made with choline salts suggests that choline did not enter the cells easily, for it is unlikely that a sodium pump would extrude it as effectively as sodium. Sulphate was intended to be a non-penetrating anion. Swan, Madisso & Pitts (1954) have reported it a good substance to use for measuring the volumes of mammalian extracellular fluids. The absorptive capacity of the renal tubule in many respects resembles that of the intestinal tract, where poor absorption is responsible for the well-known osmotic cathartic action of sulphates. Hence, although there is

evidence (Smith, 1951) that sulphate can be reabsorbed when its concentration in the plasma is low, it acts as an osmotic diuretic at higher concentrations, and all except a short segment of the nephron is probably impermeable to it. Although the ion product in the sulphate media was about twice the solubility product of calcium sulphate, no precipitate formed on standing, and it seems reasonable to assume that the concentration of calcium ions was not reduced sufficiently to affect the behaviour of the cells.

When mercaptomerin was to be used as an inhibitor, a 0·05 M solution of the disodium salt (Thiomerin sodium, John Wyeth Bros.) was prepared by dissolving 30 mg/ml. in water. Volumes of 0·005, 0·01, 0·02 and 0·055 ml. of this solution added to the medium in the manometric flasks to make a total volume of 2·7 ml. provided concentrations of Thiomerin of 0·9, 1·8, 3·7 and 10×10^{-4} M. It will be seen that even in the strongest of these solutions the concentrations of the principal ions were not altered by more than 2% when Thiomerin was added. The effect of dilution by the small quantity of water containing the Thiomerin was considered to be negligible. No difference was detected in a series of experiments using the ordinary medium if the Thiomerin was dissolved in 0·154 M-NaCl solution instead of in water.

Technique

The rats were all normal young adult males, weighing about 250 g, of the black and white hooded Lister strain maintained by the Department of Experimental Medicine. They were killed by a blow on the head and the kidneys were immediately removed and placed in a small volume of the ordinary medium (without glucose) at room temperature. Slices were cut a few minutes later by Cohen's (1945) modification of the method of Deutsch (1936) and washed in the same medium in a Petri dish at room temperature. They were removed from the dish after both kidneys had been sliced, and blotted as previously described (Robinson, 1950); groups of slices containing approximately 100 mg of renal cortex were weighed to the nearest mg on weighed watch-glasses or directly on a torsion balance, and immediately placed in the experimental media in the right-hand flasks of Barcroft manometers. The centre pots contained 0·3 ml. of 10% KOH and the usual roll of Whatman no. 40 filter-paper. The flasks were quickly gassed with pure oxygen at room temperature and then placed in the thermostat bath at 38° C. After 5 min shaking the taps were closed and oxygen consumption measured for a period which was almost always 1 hr. The slices were then quickly removed, blotted, transferred to weighed drying tubes, and weighed before and after drying during the night in an oven at 105° C. Their water content was expressed as the percentage by weight of water in the slices removed from the flasks at the end of the experiments. The rates of oxygen uptake are given in μl./hr/mg moist tissue placed in the flasks at the beginning of the experiment. The rate of oxygen uptake declined somewhat during experiments in which the medium contained Thiomerin. The rates shown in the Results are therefore those calculated for the last quarter-hour of each experiment, i.e. four times the volume of oxygen consumed during the last 15 min, divided by the weight of tissue placed in the flasks initially. It was considered that rates so calculated would be more satisfactory than rates averaged over the whole experimental period for comparison with figures for water content determined on the slices at the end of each experiment.

RESULTS

The results of all the experiments using Thiomerin, and of controls in the same media without inhibitor, are displayed as averages in Table 2, together with the standard errors of the means, and the numbers of experiments in each group. Thiomerin reduced the oxygen consumption of the slices in all four media, the inhibition was greater with larger than with smaller concentrations of Thiomerin, and the magnitude of the inhibition was of the same order in all media. The oxygen consumption of slices in the sodium sulphate medium was consistently less than that of slices in the other three media. Apart

TABLE 2. Effects of Thiomerin on oxygen consumption and water content of rat kidney cortex slices in various media

Concn. of Thiomerin M × 10^{-4}		Medium: Ordinary	Choline chloride	Sodium sulphate	Choline sulphate	Ordinary	Choline chloride	Sodium sulphate	Choline sulphate
		Oxygen consumption, μl./hr/mg				Water content of tissue, % by weight			
0	Mean	4·5	4·4	4·0	4·5	75·5	77·4	75·7	78·4
	S.E.	±0·06	±0·12	±0·09	±0·09	±0·15	±0·25	±0·22	±0·32
	Number	(27)	(19)	(10)	(12)	(27)	(19)	(10)	(12)
0·9	Mean	4·0	4·1	3·2	3·9	77·0	78·5	78·0	78·8
	S.E.	±0·06	±0·09	±0·06	±0·13	±0·30	±0·38	±0·28	±0·29
	Number	(11)	(12)	(10)	(10)	(11)	(12)	(10)	(10)
1·8	Mean	3·5	3·3	2·9	3·8	78·6	80·9	79·2	80·0
	S.E.	±0·09	±0·19	±0·03	±0·09	±0·36	±0·32	±0·32	±0·16
	Number	(12)	(10)	(10)	(10)	(13)	(10)	(10)	(10)
3·7	Mean	3·2	2·9	2·6	3·1	79·8	81·4	80·4	80·7
	S.E.	±0·08	±0·16	±0·03	±0·09	±0·28	±0·22	±0·25	±0·33
	Number	(13)	(10)	(10)	(11)	(13)	(10)	(10)	(11)
10	Mean	3·1	2·9	2·2	2·8	82·2	82·5	81·9	81·4
	S.E.	±0·08	±0·06	±0·07	±0·06	±0·17	±0·09	±0·16	±0·17
	Number	(13)	(12)	(9)	(12)	(13)	(12)	(10)	(12)

from this the rates of uptake of oxygen with each concentration of inhibitor were of the same order in all the media.

The right-hand half of Table 2 shows the changes in the water content of the slices which accompanied these alterations in oxygen consumption. The responses of the tissues in the four media again exhibited a common pattern with minor variations. The water content of the slices increased as their oxygen uptake was inhibited in all media, although the slices in the choline sulphate medium gained only about half as much water as the others in association with a comparable reduction in their oxygen consumption. The water content of these slices in the presence of the highest concentration of inhibitor was similar to those of the slices in the other media; but slices respiring in this medium contained more water than usual in the absence of any inhibitor. The slices in the sodium sulphate medium contained no more water than those in the ordinary medium, despite a consistently slower uptake of oxygen.

The figures in Table 2 show that the now familiar inverse relation between the water content and the oxygen consumption of kidney slices was still observed in spite of drastic changes in the bulk ions of the medium. More information about the effects of the ionic structure of the medium than is immediately apparent from Table 2 was obtained by plotting the water content of each group of slices against the corresponding rate of oxygen consumption for all concentrations (including zero) of Thiomerin in each of the four media. One of these four plots, that for control slices and those whose respiration was inhibited by various concentrations of Thiomerin in the sodium sulphate medium, is shown in Fig. 1. In the other three cases, although there was again a considerable scatter, the points also appeared to be distributed around straight lines. The lines indicated by the results in the different media did not, however, appear to be the same, although the clusters of points belonging to each line would overlap to produce a confusing cloud if all the points (corresponding to 245 pairs of observations) were plotted on the same scatter diagram. In order to see how far this cloud could be resolved into distinct components, a linear regression equation was calculated for water content (y) on oxygen uptake (x) for all experiments in each medium by the method of least squares (Mather, 1951). A similar regression equation was calculated for a further ninety-nine pairs of observations which had been obtained in previous work upon the effect of cyanide upon the water content and the oxygen consumption of kidney slices from male rats of the same strain, respiring in a medium which contained half as much calcium but was otherwise identical with the ordinary medium used in the present experiments (Robinson, 1950). The five regression equations with their standard errors and the comparisons of their regression coefficients are shown in Table 3 in descending order of their intercepts on the y-axis. Fig. 2 shows the five calculated regression lines, numbered to correspond with the equations in Table 3. These lines

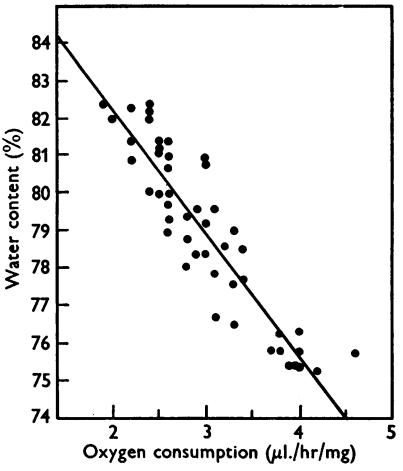

Fig. 1

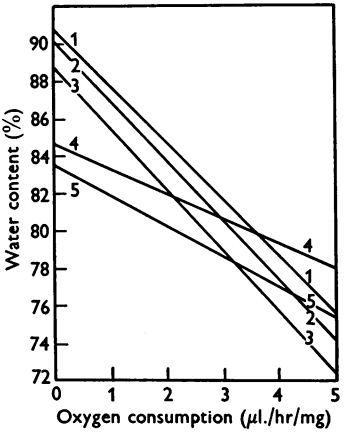

Fig. 2

Fig. 1. Water content and oxygen consumption of rat kidney slices incubated in sodium sulphate medium with various concentrations of Thiomerin. The straight line illustrates the calculated regression $y = 88{\cdot}84 - 3{\cdot}30x$.

Fig. 2. Regression lines corresponding to the equations in Table 3. Effect of Thiomerin: (1) in choline chloride medium; (2) in ordinary medium; (3) in sodium sulphate medium; (4) in choline sulphate medium. (5) Effect of cyanide in ordinary medium.

TABLE 3. Regression equations for water content (y) on oxygen consumption (x) in various media

	Pairs of observations
1. Inhibitor, Thiomerin; choline chloride medium $y = 90{\cdot}87 \pm 0{\cdot}11 - (3{\cdot}05 \pm 0{\cdot}14)\,x$	63
2. Inhibitor, Thiomerin; ordinary medium $y = 90{\cdot}15 \pm 0{\cdot}14 - (3{\cdot}20 \pm 0{\cdot}20)\,x$	78
3. Inhibitor, Thiomerin; sodium sulphate medium $y = 88{\cdot}84 \pm 0{\cdot}14 - (3{\cdot}30 \pm 0{\cdot}22)\,x$	49
4. Inhibitor, Thiomerin; choline sulphate medium $y = 84{\cdot}63 \pm 0{\cdot}16 - (1{\cdot}32 \pm 0{\cdot}23)\,x$	55
5. Inhibitor, cyanide; ordinary medium $y = 83{\cdot}57 \pm 0{\cdot}16 - (1{\cdot}64 \pm 0{\cdot}09)\,x$	99

Comparisons of regression coefficients

	t	Degrees of freedom	P
1 : 2	0·62	137	$\doteqdot 0{\cdot}5$
1 : 3	0·96	108	$\doteqdot 0{\cdot}3$
2 : 3	0·33	123	$\doteqdot 0{\cdot}7$
4 : 5	1·34	150	$\doteqdot 0{\cdot}2$
1 : 4	6·66	114	$<10^{-9}$
1 : 5	8·52	158	$<10^{-9}$
2 : 4	6·27	129	$<10^{-9}$
2 : 5	7·05	173	$<10^{-9}$
3 : 4	6·26	100	$<10^{-9}$
3 : 5	6·84	144	$<10^{-9}$

fall into two quite distinct groups; within each group the regression coefficients are not significantly distinguishable, but each differs very significantly from any member of the other group. It is doubtful how much significance should be attached to the intercepts on the y-axis, because in the experiments with Thiomerin they depend upon extrapolation of the regression lines into the region beyond 50% inhibition of respiration where there were no experimental data, and hence involve the assumption that a linear regression equation would still adequately have described the trend of the points if the oxygen consumption had been inhibited more than 50%. Inhibitions greater than 90% were actually observed in the experiments using cyanide, which covered the whole range from normal to completely inhibited respiration more evenly; and here the regression did appear to be linear over the whole range (Robinson, 1950). However, even without extrapolation beyond the range of values actually observed in the experiments with Thiomerin, it is clear that the two groups of regression lines indicate quite different behaviour. The slopes of the first three lines are all approximately twice those of the remaining two. Moreover, it may be seen that slices whose respiration was half inhibited by Thiomerin in three of the four media contained about as much water as slices whose respiration had been almost completely inhibited by cyanide in the ordinary medium. Comparison of the two sets of experiments in the ordinary medium shows that a given inhibition of respiration produced by Thiomerin was associated with about twice as great an increase in water content as an inhibition of equal magnitude produced by cyanide (lines 2 and 5). The results of the experiments in other media showed that the inhibition of respiration by Thiomerin was accompanied by this greater increase in the water content of the slices when the medium contained a normal or higher concentration of either of the common extracellular ions, sodium (line 3) and chloride (line 1), but the increase in water content when both these ions were present together was not significantly greater than when either was present alone. This effect was not observed when the medium contained neither sodium nor chloride in high concentration; the increase in water content when respiration was inhibited by Thiomerin in the choline sulphate medium (line 4) was no greater than that produced by cyanide in the ordinary medium (line 5).

DISCUSSION

The differences disclosed by using different inhibitors and media must be related to the mechanism of the increase in water content which occurred whenever the oxygen consumption of the slices was depressed. This increase might have arisen from one or more of the following causes, listed in order of decreasing popularity:

(1) Failure of the active transport of sodium which normally keeps the concentration of this ion lower in the cells than in the surrounding extra-

cellular fluids. This would be followed by a net influx of sodium and an osmotic shift of water in the same direction.

(2) Failure to maintain the normal dynamic equilibrium of organic metabolites and storage substances of higher molecular weight, with a consequent increase in osmotically active solutes in the cells. If these were retained by the cell membranes they should lead to an osmotic shift of water into the cells. Such changes may be described as 'autolytic' by analogy with the somewhat similar changes which occur in dead tissues.

(3) Failure of a mechanism which opposes swelling by expelling water directly—not merely passively as a consequence of movements of extruded ions.

On the first two views, normally respiring cells are in osmotic equilibrium, and swell when respiration is inhibited in order to maintain equilibrium. The third view implies that respiring cells are not in equilibrium; they maintain their volume and a higher molecular concentration than their surroundings actively as a steady state, and swell when respiration is inhibited, not to maintain but to attain osmotic equilibrium. On all three views, the actual mechanism of the entry of water which increases the volume of the swelling tissue is osmosis—a passive movement of water down its activity gradient arising from the greater diffusive flux away from regions of higher activity.

Failure of ion transport

The extrusion of sodium is only one aspect of the transport of ions which maintains characteristic differences between intra- and extracellular fluids. It is now generally recognized that there is an inward transport of potassium, which would tend to promote swelling, as well as the outward transport of sodium which would oppose it; and inhibition of respiration interferes with the accumulation of potassium as well as with the extrusion of sodium. If equal numbers of sodium and potassium ions exchanged across the cell membranes when respiration was inhibited, only a trivial swelling attributable to the slightly greater osmotic activity of sodium (Hill, 1950) would be expected. Greater swelling might be expected from the finding of Mudge (1951) that although the *concentration* of potassium in the tissue water fell, and that of sodium rose to about the same extent, the total *amount* of (Na + K) in a portion of tissue increased. The number of sodium ions entering a cell was therefore greater than the number of potassium ions which left it, and the swelling appeared to be a simple consequence of slowing or stopping the outward transport of sodium. Since any loss of potassium would presumably diminish the swelling arising from uptake of sodium, a selective inhibitor of sodium transport should produce greater swelling than a general inhibitor of respiration.

Mercurial diuretics have been considered to exert a rather specific inhibitory action upon the active transport of sodium by the renal tubular epithelium,

and this does not appear to be confined to any one segment of the nephron (Grossman, Weston, Borun & Leiter, 1955). The experiments in ordinary media (Fig. 2) showed that Thiomerin produced greater swelling than cyanide, as though it exerted some more specific effect besides depressing the uptake of oxygen, but the hydrating effect of Thiomerin was as great (Fig. 2) in the choline chloride medium as in ordinary media and cannot then have been due to entry of sodium.

Both the ordinary and the choline chloride media contained chloride in a higher concentration than has been reported in most cells, and Whittam (1956) and Leaf (1956) have both drawn attention to the large amounts of chloride which may enter tissue slices along with sodium when metabolism becomes abnormal; indeed the increase in tissue chloride may greatly exceed the net gain of sodium plus potassium. Conway (1956) also stressed the importance of chloride entering cells with sodium as potassium is lost, and Leaf (1956) concluded that the swelling of guinea-pig kidney slices when metabolism was suppressed arose from the uptake of isotonic sodium chloride. If the same mechanism operated in rat kidney slices, replacement of chloride in the medium by sulphate ought to have reduced the swelling, but Fig. 2 shows that swelling was as great in the sodium sulphate medium, which contained no chloride, as in media containing mainly sodium or choline chloride. Even the swelling of slices in the choline chloride medium could not be due directly to entry of chloride unless either choline entered with it or chloride entered unaccompanied by cations and without a corresponding loss of intracellular anions. Similarly, swelling in the medium made with sodium sulphate could only be due to entry of sodium if sulphate entered too or sodium entered unpaired. Passive movements of unpaired ions are virtually impossible and alterations in pH which might have been caused by exchanges with H^+ or OH^- ions were not detected in the present experiments. Perhaps the strongest argument against the free entry of choline and sulphate is the surprisingly normal oxygen consumption and water content of slices respiring freely in the extremely abnormal ionic environments provided by the modified media. This seems to imply that if choline and sulphate could enter cells freely, then the sodium pumps could also transport choline, or there were chloride pumps which could also pump sulphate.

Finally, Fig. 2 shows that although Thiomerin caused only about one half as much swelling in the choline sulphate medium as in the other media, yet it caused as much as cyanide had done in ordinary media. Such swelling in the absence of the common external ions cannot be explained by the failure of known systems for ionic transport.

Autolysis

Slices might have been expected to behave similarly in the different media if the swelling arose from events inside the cells which were relatively independent of the composition of the external fluid. Robinson (1950) mentioned that cells might swell under anaerobic conditions because the number of organic molecules inside them increased, but did not pursue this because the magnitude of the change was so uncertain. Conway, Geoghegan & McCormack (1955), seeking the cause of a progressive depression of the freezing-point observed by Conway & McCormack (1953) in tissues ground in the frozen state, found that labile cell constituents broke down surprisingly quickly even at 0° C. The freezing-point of ground rat kidney had been found to fall from −0·48 to about −0·7° C in 20 min, and now it was found that enough adenosine triphosphate and hexose phosphate broke down and enough organic acids and new non-protein nitrogen were formed to increase the molecular concentration by nearly 100 m-osmole/l. in an hour. This was a maximum estimate, for compounds classed as non-protein nitrogen were assumed to have 1 gram-atom of N per osmole although more than half of them were listed as nucleotides, nucleosides, purines and peptides.

If the same changes occurred in the intact cells of incubated slices as in the contents of cells whose structure had been disorganized by grinding, they could account for a considerable part of the observed swelling, provided that the products of autolysis remained in the cells. They would have to be retained up to 2 hr to account for the stability of the swelling observed by Robinson (1950). Yet Deyrup's (1953*a*) observation that rat kidney slices did not swell in isosmotic solutions of disaccharides suggests that if autolysis occurred, the products were not kept in the cells; and the further observation (Deyrup, 1953*b*) that swelling was not reversed by re-supplying oxygen in isotonic dextrose also seems to rule out autolysis as the major cause of reversible swelling. Leaf (1956) invoked no contribution from autolysis to account for the swelling of slices of guinea-pig kidney; but Conway & McCormack (1953) had found no evidence of autolysis in this tissue after grinding, though its curious absence does not seem to have been confirmed by chemical analysis.

Although the breakdown of labile substances could account for similarities in the behaviour of the slices in different experiments, it probably could not account for the whole of the swelling, or for the large differences which were observed. Thus the 30% increase in solutes reported by Conway *et al.* (1953) would have to account for a 50% increase in cell water in chilled slices, and a 74% increase in cell water in slices incubated with cyanide (Robinson, 1950). The greater increase produced by Thiomerin would be more difficult to explain, especially since Conway & McCormack found that mercuric chloride inhibited the autolytic processes disclosed by their cryoscopic measurements.

Failure of direct transport of water

Swelling in excess of that attributable to autolysis and the failure of ion transport could be explained by failure of water pumps, but this view became less probable when Conway & McCormack (1953) published the first cryoscopic measurements to give what seemed intuitively to be the correct answer, and gave reasons for rejecting the results of all other workers. A further objection to the hypothesis that intracellular fluids in respiring tissues are kept hypertonic has been that it implies the presence of considerable amounts of unrecognized organic substances in the cell water (Conway, 1956). If, however, concentration is raised by extrusion of water, the 'extra' solutes need be neither organic nor unfamiliar. Ljungberg (1947) and Whittam (1956) found high concentrations of chloride in some renal cells. Robinson (1954) called attention to the high concentration of (Na+K) found by Aebi (1953) in respiring rat kidney cortical slices—probably too high to attribute to non-diffusing anions in the cells—and suggested that the last word could not be said on the transport of water until adequate cryoscopic measurements had been made upon such slices.

Conway & Geoghegan (1955) found by the method of Conway & McCormack (1953) that rat kidney slices, incubated at 37° C for an hour, had a freezing-point of −0·60° C, not significantly different from that of slices incubated at 0° C; and also, in the light of the earlier work, not significantly different from that of the medium (−0·53° C). Unlike slices studied by Aebi and by Robinson, however, the slices incubated at 37° C had first been kept for an hour at 0° C; and chemical analysis (Conway & Geoghegan, 1955, Table 2) showed that the concentration of (Na + K) in the cell water was (50 + 38)/0·578 = 152 m-equiv/kg, whereas Conway & Geoghegan calculated that Aebi's analytical figures (for slices which had not been kept on ice for an hour before being incubated at 37° C) would, on the same basis, have given 291 m-equiv (Na+K)/kg of cell water. The cells in Aebi's experiments therefore had a concentration of (Na+K) about 90% greater than the medium, and about 90% greater than the cells of slices which had been found cryoscopically to have the same molecular concentration as the medium. Hence although the difference between the water contents of the slices incubated at 0° and at 37° C by Conway & Geoghegan may have been entirely due to autolytic changes and faults in the transport of ions, it remains possible that under the more physiological conditions of Aebi's experiments there was a further concentration of ions brought about by active extrusion of water, although this has still not been established by unexceptionable measurements of total molecular concentration.

Apart from adding to the evidence that living cells may increase their water content and swell, regardless of external osmotic pressure, when their metabolism is impaired by a variety of adverse circumstances, the present

work leads to few positive conclusions. It has become clearer that more mechanisms than one are involved in the swelling, and unlikely that it can be wholly explained by the failure of specific processes transporting common ions and by autolytic changes. It is hoped to gain further information from the analysis of slices incubated in the modified media which were used in the present experiments, as well as from the behaviour of slices which are being studied in non-aqueous media to eliminate the effect of external osmotic pressure. Meanwhile anisotonic secretions continue to be formed, a wide variety of living organisms sustain osmotic gradients, and complete certainty that 'Tissue osmotic pressure *in vivo* equals that of the blood plasma' (Wilde, 1955) may yet be premature.

SUMMARY

1. The oxygen consumption and the water content of rat kidney cortical slices in the presence of the mercurial diuretic mercaptomerin (Thiomerin) were measured at 38° C in an ordinary medium and in media modified by replacing sodium chloride by choline chloride, sodium sulphate or choline sulphate.

2. Thiomerin in concentrations up to 0·001 M depressed the oxygen consumption of the slices to a similar extent in all the media.

3. The slices took up water when respiration was inhibited in all media.

4. About twice as much water was taken up for a given depression of oxygen consumption when the medium contained either sodium or chloride as when both were deficient; and no more water was taken up if both were present together.

5. In media containing either sodium or chloride or both, the swelling produced by Thiomerin was about twice as great as had accompanied the inhibition of respiration by cyanide in media made with sodium chloride.

6. It is concluded that the swelling of the slices when their metabolism was impaired cannot be explained by the failure of specific transport processes for common ions; and reasons are given for regarding the question of active transport of water as still open.

I wish to thank Mr L. A. R. Luff for determining the freezing-points.

REFERENCES

AEBI, H. (1953). Elektrolyt-Akkumulierung und Osmoregulation in Gewebsschnitten. *Helv. physiol. acta*, **11**, 96–121.

COHEN, P. P. (1945). Methods of preparing animal tissues. In UMBREIT, B. H. & STAUFFER, J. F., *Manometric Techniques*. Minneapolis: Burgess.

CONWAY, E. J. (1956). Fundamental problems in the hormonal control of water and salt-electrolyte metabolism. *Mem. soc. Endocrin.* no. 5, part II, pp. 3–24. Cambridge University Press.

CONWAY, E. J. & GEOGHEGAN, H. (1955). Molecular concentration of kidney cortex slices. *J. Physiol.* **130**, 438–445.

CONWAY, E. J., GEOGHEGAN, H. & MCCORMACK, J. I. (1955). Autolytic changes at zero centigrade in ground mammalian tissues. *J. Physiol.* **130**, 427–437.

CONWAY, E. J. & MCCORMACK, J. I. (1953). The total intracellular concentration of mammalian tissues compared with that of the extracellular fluid. *J. Physiol.* **120**, 1–14.

DEUTSCH, W. (1936). An improvement of Warburg's method for cutting tissue slices for respiratory experiments. *J. Physiol.* **87**, 56–57 *P.*

DEYRUP, I. (1953*a*). A study of the fluid uptake of rat kidney slices *in vitro*. *J. gen. Physiol.* **36**, 739–749.

DEYRUP, I. (1953*b*). Reversal of fluid uptake by rat kidney slices immersed in isosmotic solutions *in vitro*. *Amer. J. Physiol.* **175**, 349–352.

GROSSMAN, J., WESTON, R. E., BORUN, E. R. & LEITER, L. (1955). Factors influencing the course of mercurial diuresis during pitressin infusion in normal subjects. *J. clin. Invest.* **34**, 1611–1624.

HERVEY, G. R. (1955). A method for determining the freezing points of biological fluids. *Analyst*, **80**, 284–289.

HILL, D. K. (1950). The volume change resulting from stimulation in a giant nerve fibre. *J. Physiol.* **111**, 304–327.

HODGKIN, A. L. & KATZ, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **108**, 31–77.

LEAF, A. (1956). On the mechanism of fluid exchange of tissues *in vitro*. *Biochem. J.* **62**, 241–248.

LJUNGBERG, E. (1947). On the reabsorption of chlorides in the kidney of the rabbit. *Acta med. scand.*, **127**, Suppl. 186, 1–189.

LIFSON, N. & VISSCHER, M. B. (1944). Osmosis in living systems. In GLASSER, O., *Medical Physics*, pp. 869–892. Chicago: Year Book Publishers Inc.

MATHER, K. (1951). *Statistical Analysis in Biology*, 4th ed. London: Methuen.

MUDGE, G. H. (1951). Studies of potassium accumulation by rabbit kidney slices: effect of metabolic activity. *Amer. J. Physiol.* **165**, 113–127.

ROBINSON, J. R. (1950). Osmoregulation in surviving slices from the kidneys of adult rats. *Proc. Roy. Soc.* B, **137**, 378–402.

ROBINSON, J. R. (1954). Secretion and transport of water. *Symp. Soc. exp. Biol.* **8**, 42–62.

SMITH, H. W. (1951). *The Kidney: Structure and Function in health and disease.* New York: Oxford University Press.

SWAN, R. C., MADISSO, H. & PITTS, R. F. (1954). Measurement of extracellular fluid volume in nephrectomized dogs. *J. clin. Invest.* **33**, 1447–1456.

TROWELL, O. A. (1935). Choline and liver respiration. *J. Physiol.* **85**, 356–374.

WHITTAM, R. (1956). The permeability of kidney cortex to chloride. *J. Physiol.* **131**, 542–554.

WHITTAM, R. & DAVIES, R. E. (1953). Active transport of water, sodium, potassium and oxoglutarate by kidney cortex slices. *Biochem. J.* **55**, 880–888.

WILDE, W. S. (1955). Transport through biological membranes. *Annu. Rev. Physiol.* **17**, 17–36.