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MERCAPTOMERIN AND WATER EXCHANGE IN CORTEX SLICES OF RAT KIDNEY

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It is now well known that when the metabolism of tissues is depressed, the cells swell, and that their original volume tends to be restored if metabolism is re-established. It is also known that the concentration of base in the active cell is in excess of the external concentration, the excess decreasing when metabolism is inhibited. References to the rather extensive literature will be found in papers by Robinson (1950, 1952), Whittam & Davies (1953) and Leaf (1956). These findings have evoked two divergent views. Thus Opie (1949), Whittam & Davies (1953) and Robinson (1956) postulate the existence of a water pump, whose action in the metabolizing cell is such as to maintain the concentration and osmotic pressure of the cell constituents above that of the external medium. Hence, when cell metabolism fails, the pump ceases to work, the cell swells and the concentration of base in cell water falls. An alternative view is advanced by Mudge (1951), Conway (Conway & McCormack, 1953; Conway & Geoghagen, 1955), Leaf (1956), and Maizels, Remington & Truscoe (1958), who hold that changes in cell volume are always accompanied by appropriate shifts of cation and anion. This second view gains strong support from the cryoscopic observations of Conway & McCormack (1953) which show that the osmolarity of the cells of fresh tissues is not in fact greater than that of the tissue fluids, but about the same. The distinction is an important one, and it is clear that the 'water-pump' theory would be strengthened if it could be shown that cells can transfer water without a corresponding movement of salts. Robinson (1956) attempts to demonstrate this in the following way: tissue slices are incubated in media whose constituents are unable to penetrate cells; if in the presence of a suitable inhibitor the cells still swell, it may be presumed that this is due to failure of the water pump. Robinson chose mercaptomerin as his inhibitor, and choline and sulphate as constituents of his media. He assumed that these were unable to penetrate renal cortex, and

the validity of his conclusions rests entirely on the correctness of these assumptions. It is the purpose of the present paper to show that in fact choline and sulphate do penetrate renal cells.

METHODS

Kidney cortex slices were treated as described by Robinson (1956) except for minor differences: (*a*) adult albino rats of about 450 g were used; (*b*) as the temperature of the laboratory was rather high, slices were cut and collected in glucose-free NaCl solution at about 10° C before transference to appropriate media; (*c*) the temperature of incubation was 37 not 38° C. The composition of the media is as in Robinson's paper, and the relevant data are shown in Table 1.

When slicing the cortex, the outermost slices were rejected as suggested by Whittam & Davies (1953), who showed that the outer slices of cortex contained less Na and more K than the inner, though the values of Na + K were similar in either situation. The thickness of slices was about 0·25 mm, six slices weighing about 100 mg. These seemed to suffer attrition on rocking in the water-bath, the ultimate weight of slices recovered being about 60 mg.

TABLE 1. Composition of media (after Robinson, 1956)

	Choline chloride medium (m-equiv/l.)	Sodium sulphate medium (m-equiv/l.)
Sodium	15	190
Potassium	5	6
Calcium	5	5
Magnesium	2	2
Chloride	140	0
Choline	130	0
Sulphate	2	183
Phosphate*	15	15
Nitrate	0	5
Freezing point of glucose-free medium (° C, Robinson's data)	−0·485	−0·435

Glucose (5·6 m-mole/l.) was added to all media before incubation).

* pH of the media was about 7·4. (If precipitation occurred, the medium was filtered.)

Water, Na and K content. 30–50 mg slices were weighed to 0·1 mg in platinum crucibles, dried during the night at 105° C and weighed again to obtain the water content. They were then ashed at 600° C after adding 0·1 ml. H_2SO_4 (2%, v/v), the residue dissolved in 0·01 N-HCl and the Na and K contents measured with a flame photometer. The S.E. for water contents measured in duplicate was ± 0·39% corresponding to ± 10 ml. water/kg dry wt. for 105 consecutive estimations.

When estimating Na and K in duplicate or triplicate, the values of Na seldom differed by more than ± 20 m-equiv/kg dry wt., whereas differences in K were less. It is probable that part of these variations represents real differences in the composition of slices from different parts of the renal cortex, because the variations of Na and K were often in opposite directions, so that the sums of Na + K from the various samples were approximately constant (cf. Whittam & Davies, 1953). It follows that variations in Na + K did not necessarily involve the sum of errors of Na and K. The S.E. for 76 sets of duplicate or triplicate observations were as follows: Na ± 11·2, K ± 6·8, and Na + K 11·0 m-equiv/kg dry wt., irrespective of the total amounts of Na, K or Na + K present.

Sulphate was estimated by the method of Letenoff & Reinhold (1936) after the macerated slices had been extracted for 1 hr in water and then during the night after the addition of the uranium acetate reagent. For slices of about 40 mg, S.E. was ± 3·5%.

Choline was estimated by an adaptation of the potassium method of King, Haslewood, Delory & Beall (1942): this involves the precipitation of potassium cobaltinitrite, the cobalt then giving a green colour on the addition of choline chloride and Na ferrocyanide. In the present experiments

excess of cobalt nitrate and Na ferrocyanide were added to solutions containing unknown amounts of choline, and the colour developed was compared with choline standards.

Kidney slices were blotted, after immersion in the choline medium, weighed (about 50 mg), placed in a small test-tube containing 2·4 ml. water and shaken several times with a few tiny glass beads. After an hour 0·3 ml. Na tungstate (10%, w/v) and 0·3 ml. 2/3 N-H_2SO_4 were added to precipitate the proteins. Owing to the fact that there is a small loss of choline on precipitation, 'kidney-slice standards' were prepared as follows: 2·4, 1·8, 1·2, 0·6 and 0 ml. choline chloride solution (5 m-mole/l.) were placed in separate tubes and all made up to 2·4 ml. with water. About 50 mg untreated kidney slices were added to each and then the Na tungstate and sulphuric acid reagents. Unknowns and standards were filtered and 0·5 ml. filtrate added to 7 ml. cobalt nitrate solution (0·1%, w/v). 0·3 ml. Na ferrocyanide (2%, w/v) was then added, and the contents of the tube mixed immediately. The green colour which developed was then read in a photo-electric colorimeter at 605 mμ. It may be noted that when the colour of the simple standard was compared with the corresponding 'slice standard', there appeared to be about 12% loss in the weakest standard and about 2% in the strongest. The standard curve was approximately linear and estimations in triplicate agreed within ±3%. The method is unsuitable for red cells or for other material with a high content of soluble protein.

RESULTS

Penetration of choline into slices of renal cortex

Choline penetrates slices of renal cortex readily. When these are immersed in the choline chloride medium (Table 1), the concentration of choline in the water of the slice after dipping in the medium and immediate withdrawal being about 20% of the external concentration; after 10 min at 37° C it is about 55% and after 60 min it is about 75 or 80% of the external concentration. Table 2 shows the choline contents and the approximate concentrations in tissue water obtained by dividing the contents by 0·8. Entry of choline is effected in exchange for cell Na and K, loss of Na exceeding loss of K, presumably because loss of Na is facilitated and loss of K hindered by their respective mechanisms for active transport. In order to gain some idea of how much choline is actually intracellular, it is necessary to assign a value to the extracellular space, and a figure of 25% has been chosen, which is in rough agreement with the data of Robinson (1950), Conway & Geoghagen (1955) and Whittam (1956). On this basis it may be calculated that the true intracellular concentration of choline is about two thirds of the external concentration after 1 hr at 37° C.

TABLE 2. Penetration of choline into slices of the renal cortex of the rat incubated at 37° C in a choline chloride medium*

Expt.	Time (min)	Choline (m-mole/ kg wet wt.)	Na (m-equiv/ kg wet wt.)	K (m-equiv/ kg wet wt.)	Water % (w/w)
1	'0'	22	57	48	—
	10	60	—	—	—
	60	78	19	33	—
2	'0'	20	68	42	—
	10	54	—	—	—
	60	82	11	32	76

* For composition of medium see Table 1. Note 76% water (w/w) corresponds to 3160 g/kg dry wt.

It follows that the assumption that choline does not penetrate the cells of the renal cortex is inadmissible; but Robinson's conclusions based on his choline sulphate and sodium sulphate experiments might still be valid, if it could be shown that sulphate does not penetrate the cortical cells.

Penetration of sulphate into slices of renal cortex

Slices of cortex from the kidney of the rat were immersed in the sodium sulphate medium (Table 1) at 37° C. for varying periods up to 1 hr. Parallel observations were made using media without and with mercaptomerin. Table 3 shows the sulphate in m-equiv/kg tissue and also the concentration in m-equiv/kg cell water. For the present purposes, however, it is necessary to have a measure of absolute changes, that is, a measure of the amount of a substance moving in or out of a cell. Hence, in Table 3 sulphate (and water) are also expressed in relation to the dry weight of the tissue: this is the procedure

TABLE 3. Penetration of sulphate into slices of renal cortex of the rat incubated at 37° C in a sodium sulphate medium*

Expt.	Time (min)	Mercaptomerin added (m-mole/l. medium)	Water (g/kg dry wt. of tissue)	Sulphate (m-equiv/kg wet wt. of tissue)	Sulphate (m-equiv/kg cell water)	Sulphate (m-equiv/kg dry wt. of tissue)
1	15	0	3000	71	95	284
	15	1	2960	71	95	282
	60	0	3060	87	115	353
	60	1	4350	104	128	555
2	0	0	3450	28	36	124
	10	0	3450	65	84	289
	60	0	3370	73	94	319
	60	1	4130	98	122	503
3	0	0	3700	23	29	108
	60	0	3350	74	96	322
	60	1	4320	104	128	554

* For composition of medium see Table 1.

followed by Leaf (1956), and is based on the obvious fact that the number of cells in a tissue is proportional to their dry weight, irrespective of the hydration of the cells. This procedure has also been followed in a subsequent paper.

Table 3 shows that slices of renal cortex, even in mercaptomerin-free media, gain sulphate at first rapidly and later at a slower rate. Thus after dipping and immediate withdrawal ('0' min) the sulphate concentration is already about 30% of the 1 hr value, and at about 15 min it is approximately 80% of the 1 hr figure. At 60 min the sulphate content of the actual cells is about 45 m-equiv/kg, and the true intracellular concentration about 65 m-equiv/kg water. In the presence of mercaptomerin the true content is about 75 m-equiv/kg cells and the true concentration about 100 m-equiv/kg cell water. Mercaptomerin also causes an increase in the amount of sulphate per cell, the excess over that gained by mercaptomerin-free preparations in 1 hr corres-

ponding to between 100 and 250 m-equiv/kg dry weight; some examples are shown in Table 3. It is probable that increase in the rate of entry of sulphate in the presence of mercaptomerin is associated with increased entry of base (Table 4), since the latter is also evident when mercaptomerin is added to cortex slices incubated in sodium chloride media.

Effects of mercaptomerin on the Na, K and water content of renal cortex slices incubated in sulphate-rich media

In order to obtain as many observations as possible from the kidneys of a single rat, large animals were used and individual observations made on 30–50 mg tissue: for observations made in duplicate or triplicate this corresponds to between 6 and 10 slices of original tissue (i.e. before rocking). Owing to the small amount of tissue used, variations in replicates are somewhat large, but to some extent this is due to real differences in the composition of slices (see Methods). In these circumstances the term 'standard variation' might be more applicable than 'standard error', but for convenience the latter term is retained. The extent of this error, which seemed to be largely independent of the total amounts of Na, K or Na + K present, is discussed in the section on Methods.

When cortex slices are removed from the simple NaCl solution in which they have been leached at about 10–12° C, the concentration of Na + K in tissue water is 1·8 times the external concentration (S.D. ± 0·031 for 30 observations). On transferring the slices to the sulphate medium, the external concentration is raised from 150 to 190 m-equiv/l., and when the slices are removed immediately from this medium, R or the Na + K concentration ratio (tissue water: medium water) is only 0·9, the ratio increasing to about 1·08 after 60 min; three out of six experiments are shown in Table 4. It may be presumed that substitution of sulphate in the external medium for the more rapidly penetrating chloride will transpose the asymmetry at '0' min, R then being less than 1; thereafter the asymmetry tends once more to be reversed as sulphate penetrates the cells, and R progressively rises above unity, reaching its highest observed value at 60 min.

If the Na + K values in m-equiv/kg dry weight are compared after incubation at '0' and 60 min in the mercaptomerin-free sulphate medium, it will be seen that there is a fall (Table 4), cell water decreasing at the same time. This arises because although both Na and K move against their respective concentration gradients, absolute loss of Na exceeds absolute gain of K.

In the presence of mercaptomerin, gain of Na after 60 min at 37° C exceeds loss of K and there is an absolute gain of Na + K and water. It must be presumed that entry of base is accompanied by entry of sulphate. Table 3 supports this, though in only one experiment were observations actually made in parallel: here sulphate and base gained by slices containing mercaptomerin

respectively exceeded the corresponding gains in unpoisoned media by 236 ± 20 and 220 ± 16 m-equiv/kg.

With regard to individual changes in Na and K, these are best seen when observations are made at 0 and 60 min and at some intermediate time. Experiments such as No. 3 (Table 4) suggest that the apparent effect of mercaptomerin on Na and K is rather slight for the first 20 min or so of incubation, Na and K moving against their concentration gradients. But at some time between 20 and 60 min, Na increases markedly while K is rather little affected, though the influence of mercaptomerin on K transfer is shown by the fact that at 20 and 60 min the content is less than in the unpoisoned control.

TABLE 4. Na, K and water contents of slices of renal cortex incubated at 37° C in sulphate-rich media* with and without mercaptomerin

Expt.	Mercaptomerin (m-mole/l. medium)	Time (min)	Water (g/kg dry wt.)	R	Tissue cation (m-equiv/kg dry wt.) Na	K	Na + K
1*a*	0	0	4040	0·93	522	211	733
b†	0	60	3370	1·02	402	288	690
c†	1	60	4050	1·04	574	259	833
2*a*	0	0	3940	0·88	487	187	674
b	0	60	3290	1·05	448	230	678
c	0·125	60	3690	1·06	553	213	766
d	0·25	60	3710	1·08	393	191	784
e	0·5	60	4030	1·08	661	191	852
f	1	60	4380	1·12	793	167	960
3*a*	0	0	3800	0·91	508	168	676
b	0	20	3160	1·04	427	216	643
c	1	20	3100	1·04	448	192	640
d†	0	60	3100	1·08	433	220	653
e†	1	60	3800	1·10	635	180	815

* For composition of medium see Table 1.

R = ratio of the concentrations of Na + K in the water of tissue and medium.

† = observations in triplicate; others in duplicate. Each single observation requires about three slices. The means of the extreme values of replicates are shown.

The standard errors (76 sets of duplicate or triplicate observations) are as follows: water ± 10 ml./kg dry weight; Na ± 11·2, K ± 6·8 and Na + K 11·0 m-equiv/kg dry weight, irrespective of the total amounts of Na, K or Na + K present.

DISCUSSION

It is known that tissues swell when their metabolism is inhibited, the volume tending to return to normal if metabolic activity is re-established. This is true of erythrocytes (Maizels, 1943, 1949), of brain slices (Stern, Eggleston, Hems & Krebs, 1949), of kidney cortex slices (Mudge, 1951; Whittam & Davies, 1953), and of mouse ascites tumour cells (Maizels *et al.* 1958). When the cells swell, the amount of Na + K per cell rises, but the concentration of Na + K in cell water falls; when the cells shrink to their normal volume, Na + K per cell falls while the concentration of Na + K rises. With actively metabolizing cortex slices in a NaCl medium, the ratio of Na + K in tissue water to the external

concentration of Na + K is about 1·27 (Maizels & Remington, 1958). If it be assumed that 25% of the tissue consists of intercellular space, then the true concentration ratio for the actual cells is about 1·4. The evidence in favour of such a high ratio being the direct result of cation transport, rather than the result of an active device for moving (or pumping) water, without corresponding movements of anions and cations, is discussed by Leaf (1956) and need not be considered further here.

It is, however, necessary to consider Robinson's (1956) experiments in favour of a water pump. These show that tissue swelling is induced by mercaptomerin, even when incubation is conducted in media consisting mainly of choline chloride, sodium sulphate or choline sulphate. Robinson argues (on somewhat tenuous grounds) that it is likely that neither choline nor sulphate penetrates the cells of the renal cortex, and hence entry of water without osmotically active material must be due to paralysis of the water pump by mercaptomerin. The arguments are not conclusive, and in the present paper it has been shown that in fact both sulphate and choline penetrate quite rapidly. It follows that Robinson's experiments cannot be accepted as evidence in favour of a water pump. Indeed in our experiments, swelling of slices is accompanied by an appropriate increase of Na + K and of sulphate. It is of interest to note that according to Deyrup & Ussing (1954) kidney slices actively accumulate sulphate provided that the external medium is rich in K and poor in Na. The data (see their Fig. 3) suggest that without our sulphate medium the internal and external sulphate concentrations should not differ greatly. The present paper shows this to be the case.

One other matter is worth of emphasis: both sulphate and choline penetrate cortex slices fairly rapidly. This is true also of lithium which at '0' min is about 15% of the 1 hr figure when the temperature of the medium is 3° C, 25% at 10° C and 30% at 37° C (Maizels & Remington, 1958). This initial gain is largely at the expense of tissue Na and not of K, and suggests the possibility that much of the lithium gained is localized at first in the tissue spaces. In any case, the findings suggest that washing slices to remove contaminants (e.g. radioactive tracers in an external medium) is unlikely to prove satisfactory.

SUMMARY

1. Choline and sulphate readily penetrate slices of renal cortex at 37° C.

2. Swelling of slices in media containing mercaptomerin is not necessarily due to the inhibition of a device for pumping water from the cells, because increase in tissue water is accompanied by an appropriate increase in anion and cation.

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