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ION MOVEMENTS AND OXYGEN CONSUMPTION IN KIDNEY CORTEX SLICES

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It is generally recognized that the oxygen consumption of cells is related to the amount of free energy being utilized by such processes as secretion, contraction, and the synthesis of complex molecules. Two questions arise concerning the nature of the coupling between energy release from metabolism and its utilization. First, what fraction of the cell's total metabolism is devoted to a particular energy-requiring process; and secondly, how is an increase in oxygen consumption elicited in order to ensure a supply of the requisite energy? This paper deals with these problems in connexion with the active movements of ions in kidney cortex slices.

In slices of kidney cortex from adult rabbits and guinea-pigs the establishment and maintenance of concentration gradients of sodium and potassium ions between intracellular and extracellular fluid depends on energy from respiration (Mudge, 1951*a*, *b*; Whittam & Davies, 1953). The evidence for this is chiefly the fact that active transport is stopped when the energy supply is decreased by the inhibition of respiration. Such studies have not revealed the fraction of the total respiratory energy expended on these ion movements or whether the latter in turn influence the rate of respiration. Kidney cortex is a particularly suitable tissue for studying the interdependence of ion transport and metabolism, because both its rate of respiration and the turnover rate of its potassium are high, so that a reduction in oxygen uptake which might result from stopping transport could easily be measured. The coupling between respiration and potassium transport in slices of kidney cortex has been investigated in the present study by comparing the effects of ouabain, sodium ion, α-oxoglutarate, and temperature on tissue potassium concentration and respiration during incubation at 25 and 38° C. A preliminary account of this work has been reported previously (Whittam & Willis, 1962).

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METHODS

Adult rabbits were stunned by a blow on the head and bled from the neck. An incision was made in the mid line and the kidneys were excised. After the capsule had been removed, each kidney was bisected longitudinally and the exposed medulla was cut away. The cortex was sliced freehand by the method of Deutsch (1936) with a razor blade moistened with medium. The outermost slice was discarded, because it contains more potassium and less sodium than the inner slices (Whittam & Davies, 1953). Unless otherwise stated, the slices were placed in a Petri dish containing about 30 ml. of the type of medium in which they were to be incubated. About 150 mg of tissue was transferred to 4·0 ml. of medium in cups of Warburg manometers, which were then gassed with 100 % oxygen. The oxygen consumption (Q_{O_2} = μl. O_2/mg dry wt./hr) was measured during incubation for about an hour at 25 or 38° C. A sample of unincubated tissue from the Petri dish and all the incubated samples were blotted on hardened filter paper (Whatman No. 542), quickly weighed on a torsion balance, and the dry weights determined by weighing the slices after they had been dried at 105° C during the night in tared beakers.

Media. The physiological saline medium, referred to as the normal medium, was similar to that of Robinson (1949), containing 155 mM-NaCl, 3 mM-$CaCl_2$ and 3 mM K phosphate, pH 7·4, giving a K concentration of 5·5 mM. Glucose (10 mM) was included and in addition, when its effect was investigated, α-oxoglutarate was added in a concentration of 10 mM. In some experiments with Na-free or Na-low media, choline chloride was substituted for NaCl.

Estimation of sodium and potassium. Sodium and potassium were estimated by internal standard flame photometry (Amoore, Parsons & Werkheiser, 1958) on samples of tissue which had been digested in concentrated HNO_3 as described by Whittam & Davies (1953).

RESULTS

The effects of ouabain

When slices were incubated in the normal medium, the tissue potassium concentration rose from an initial value of 220 ± 17 to 325 ± 18 μ-equiv/g dry wt., 7 expts., representing a net uptake of about 105 (Fig. 1). The water content of incubated slices was $3{\cdot}11 \pm 0{\cdot}04$ g water/g dry wt. and the potassium concentration in tissue water was, therefore, 100 μ-equiv/kg tissue water compared to 5·5 mM in the medium, indicating the maintenance of an eighteenfold difference between tissue water and medium.

As has been found in other tissues (see Weatherall, 1962), the presence of ouabain caused a reduction in the tissue potassium concentration. The lowest value of 90 ± 7 was found with 625 μM ouabain and represented a net change of -130 from the initial value and of -235 from the incubated control. The effect of ouabain increased with increasing concentration between about 1 and 100 μM.

The oxygen consumption of unpoisoned kidney slices fell from $11{\cdot}1 \pm 0{\cdot}3$ to $6{\cdot}4 \pm 0{\cdot}4$ on incubation in medium containing 625 μM ouabain. The ouabain-insensitive Q_{O_2} was therefore about 60 % of the control. A partial inhibition of Q_{O_2} was produced by those lower concentrations of ouabain which also had inhibitory effects on potassium uptake (Fig. 1), and the ouabain concentration for half-maximal inhibition would be about 15 μM.

In order to compare the relative effects of ouabain on Q_{O_2} and tissue potassium concentration, the results of Fig. 1 have been plotted in Fig. 2 as the 'per cent of the maximal effect'. It is clear that the relative effects of ouabain on the potassium uptake and Q_{O_2} were precisely the same, so that no concentration of ouabain caused an effect in one without the same proportional effect in the other.

Incubation with α-oxoglutarate. The addition of α-oxoglutarate to the medium causes a greater uptake of potassium by the tissue and a heightened Q_{O_2} in kidney slices of guinea-pigs (Krebs, Eggleston & Terner, 1951; Whittam & Davies, 1953). It was of interest to see if these effects also prevailed in rabbit kidney slices and whether each was subject to inhibition by ouabain. Figure 1 shows that the addition of α-oxoglutarate did not affect the tissue potassium concentration, indicating that the rabbit tissue is different in this respect from the guinea-pig tissue. On the other hand, Fig. 1 shows that in rabbit slices, as in guinea-pig, α-oxoglutarate caused an increase of approximately 40 % in the Q_{O_2}.

The addition of ouabain caused an inhibition of potassium uptake which was the same as with glucose. The oxygen consumption was also inhibited by ouabain, the Q_{O_2} with maximal inhibition being 7·7, which was not significantly different ($P > 0{\cdot}05$) from the comparable Q_{O_2} of slices without α-oxoglutarate. The inhibitory effects of 1–125 μM ouabain on potassium uptake and Q_{O_2} (Fig. 1) were again parallel in slices incubated with α-oxo-glutarate.

In the preceding experiments the extent of active transport was inferred from the potassium content of the slices; the effects of ouabain on their sodium and water content were also observed. The results are collected in Table 1. The tissue sodium concentration always increased in the presence of ouabain, accompanying the loss of potassium already described. The results in Table 1 also show that poisoning with ouabain always caused an increase in water content of 0·5–1·0 g water/g dry wt. Although this incidental finding was not pursued, to discover which ions accompany the water movement, it does confirm the expectation that specific inhibition of active ion transport by ouabain results in an uptake of water by the tissue which is similar to that caused by inhibition of respiration.

The effects of sodium ion

Experiments were done to test the effect of incubation in sodium-free medium on the tissue potassium concentration and oxygen consumption. The kidney slices were first placed in medium made up as usual, except that choline was used instead of sodium. When all the slices had been cut they were transferred to manometer cups containing fresh samples of the medium in which choline was substituted for sodium. The sodium and

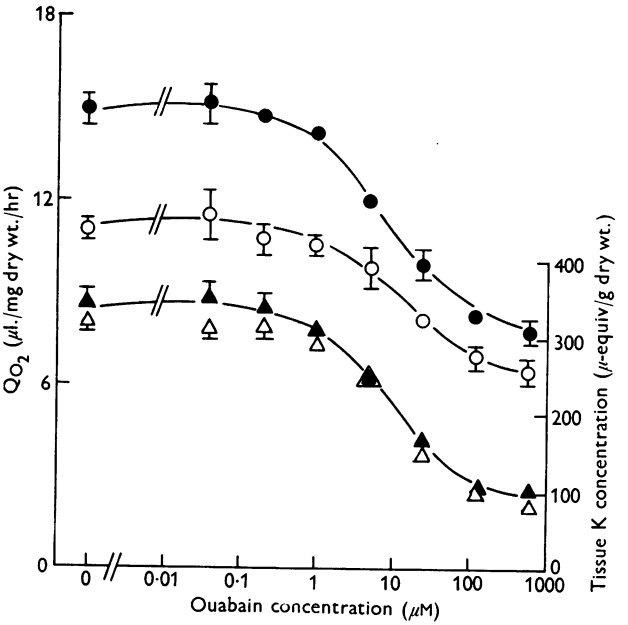

Fig. 1. Effect of ouabain on Q_{O_2} and tissue K concentration in slices of rabbit kidney cortex incubated for 1 hr at 38° C. Values represent means (±S.E. for 4 or more values) of 3–8 experiments. The potassium concentration before incubation was 220 μ-equiv/g dry wt. Q_{O_2} (–○–) and tissue K concentration (–△–) of slices incubated in the normal medium containing 155 mM-NaCl, 3 mM K phosphate, pH 7·4, 3 mM-$CaCl_2$ and 10 mM glucose; Q_{O_2} (–●–) and tissue K concentration (–▲–) of slices incubated in the same medium plus 10 mM α-oxoglutarate.

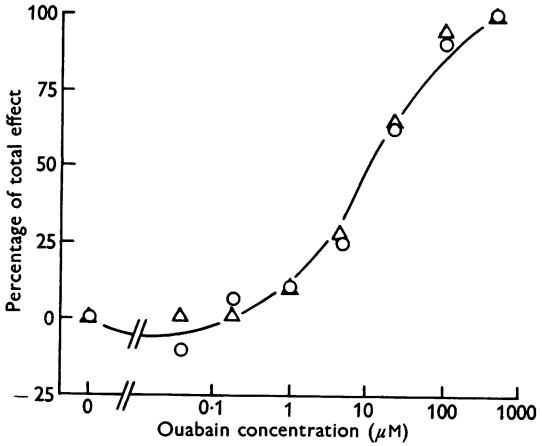

Fig. 2. The effect of ouabain on Q_{O_2} and tissue K concentration expressed as a percentage of the effect at ouabain concentration of 625 μM. The results are replotted from Fig. 1 for slices incubated in the normal medium. –△– tissue K; –○– Q_{O_2}.

potassium tissue concentrations were reduced by the preliminary treatment before incubation to about 60 and 200 μ-equiv/g dry wt. respectively, and this loss of cations was probably a result of their replacement by choline, to which kidney cells are permeable (Maizels & Remington, 1958).

TABLE 1. The effects of ouabain on the tissue Na concentration and water content of incubated kidney cortex slices

Temp. (°C)	Conditions	Cation concentrations (μ-equiv g dry wt.) Na	K	Water content (g H_2O/g dry weight)
38	Normal medium			
	Control	300	310	3·1
	+ouabain	480	100	4·1
	Change due to ouabain	+180	−210	+1·0
38	Normal medium + α-oxoglutarate			
	Control	290	350	3·1
	+ouabain	590	100	3·6
	Change due to ouabain	+300	−250	+0·5
25	Normal medium			
	Control	270	340	3·1
	+ouabain	460	130	3·8
	Change due to ouabain	+190	−210	+0·6

Slices were incubated in medium containing 155 mM-NaCl; K phosphate buffer, pH 7·4, 3 mM; 3 mM-$CaCl_2$ and glucose 10 mM. When added, α-oxoglutarate was 10 mM and ouabain 125 μM.

TABLE 2. The effects of incubation in Na-free medium on Q_{O_2} and tissue K and Na concentrations of kidney cortex slices

Metabolite	Conditions of incubation: Ouabain	Na	Q_{O_2} (ul./mg/hr)	Tissue K (μ-equiv/g dry wt)	Tissue Na (μ-equiv/g dry wt)
Glucose	−	+	12·2	300	290
Glucose + α-oxoglutarate	−	+	17·4	320	360
None	−	+	12·6	255	300
Glucose*	−	−	7·0	125	30
Glucose + α-oxoglutarate*	−	−	7·9	105	25
None*	−	−	7·0	120	10
Glucose*	+	−	7·0	125	50

In the upper three experiments slices were incubated at 38° C in the normal medium containing 155 mM-NaCl, K phosphate buffer, pH 7·4, 3 mM, and 3 mM-$CaCl_2$. The concentration of glucose and α-oxoglutarate, when added, was 10 mM. *In these experiments slices were incubated in medium containing choline instead of sodium. The concentration of ouabain was 125 μM.

The tissue sodium concentration was further reduced to about 30 μ-equiv./g during incubation at 38° C. Table 2 shows that during incubation in medium containing choline instead of sodium the slices did not reaccumulate potassium, the tissue concentration of which fell to about 120 μ-equiv/g. The oxygen consumption corresponded to a Q_{O_2} of 7–8. These values of tissue potassium concentration and Q_{O_2} were obtained regardless

of whether slices were incubated in medium containing glucose, α-oxoglutarate, or no metabolite at all. The consistency of the Q_{O_2} is in contrast to the variable values found in medium containing 155 mM-Na, in which α-oxoglutarate stimulated oxygen consumption by about 40 %.

Not only were the levels of tissue potassium concentration and oxygen consumption constant under various conditions of incubation in sodium-free medium, but they were also essentially the same as those produced by full ouabain inhibition in normal medium (i.e. Q_{O_2} of 6–8 and tissue

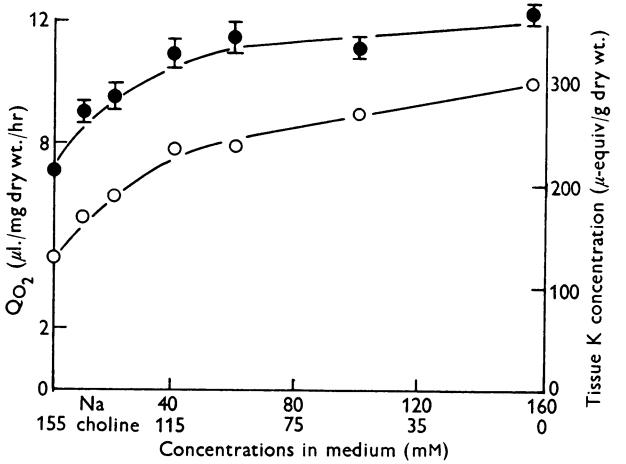

Fig. 3. Effect of Na concentration of the medium on the tissue K concentration (–○–) and the Q_{O_2} (–●–) of kidney cortex slices at 38° C. The medium contained 3 mM K phosphate, pH 7·4, 3 mM-$CaCl_2$ and 10 mM glucose with the concentrations of NaCl and choline chloride shown in the abscissa. Points indicate the mean (± S.E.) for 4 or more values.

potassium concentration of about 100 μ-equiv/g; Table 1; Fig. 1). Moreover, when slices were incubated in sodium-free medium with 125 μM ouabain, no further reduction occurred in tissue potassium concentration or in oxygen consumption. The endogenous Q_{O_2} of slices was about 12 in normal medium and it was decreased to about 7 both in sodium-free medium and in normal medium with ouabain (Table 2). The effects on respiration of sodium-free incubation and ouabain inhibition are therefore probably not related to a prevention of glucose uptake by the cells. The failure of ouabain to inhibit the oxygen consumption in the absence of sodium suggests that its effect on respiration in normal medium is mediated through an inhibition of transport. These findings suggest that both lack of sodium and the presence of ouabain in normal medium act upon a single factor, cation transport, which sets the pace of part of the cell's metabolism.

Since incubation of slices in sodium-free medium caused a fall in the tissue potassium concentration and in the oxygen consumption, the effects

were investigated of intermediate concentrations (from 0 to 155 mM) of sodium in the medium. Partial replacement of sodium by choline caused partial depression of oxygen consumption and potassium uptake alike, so that in 40 mM-Na, for example, the Q_{O_2} was 10·9 and the tissue potassium concentration was 234 μ-equiv/g compared with the values in the normal medium of 12·3 and 300 respectively (Fig. 3). The fall in oxygen consumption was closely correlated with the fall of tissue potassium.

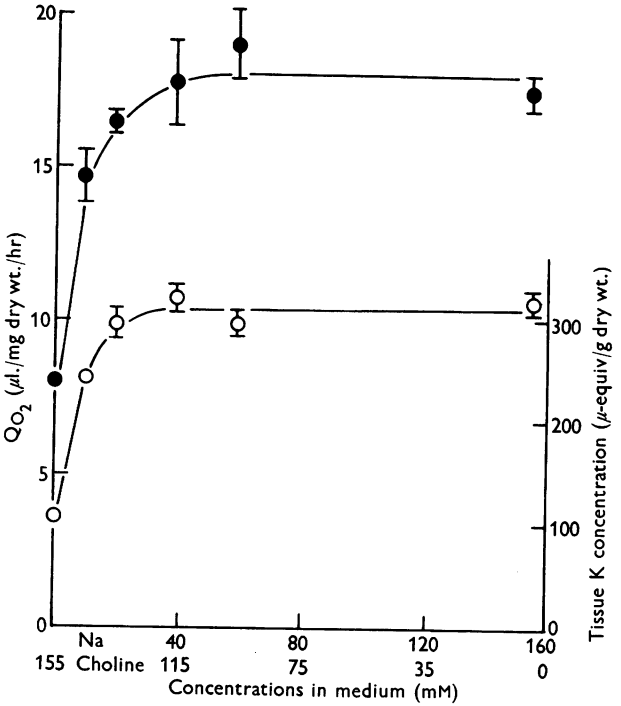

Fig. 4. Effect of Na concentration on tissue K concentration (–○–) and Q_{O_2} (–●–) of kidney cortex slices incubated with α-oxoglutarate. The medium contained 3 mM K phosphate, pH 7·4, 3 mM-$CaCl_2$, 10 mM glucose and 10 mM α-oxoglutarate, with the concentrations of NaCl and choline chloride shown in the abscissa. Points indicate the mean (± S.E. for 4 or more values).

Slices were also incubated in medium containing 10 mM α-oxoglutarate and glucose, and sodium in various concentrations. In the sodium-free medium α-oxoglutarate was added as the Tris salt. In the medium containing 10 mM-Na, half the α-oxoglutarate was added as the Tris salt and half as the Na salt, and the latter provided the only sodium in the medium. In the presence of α-oxoglutarate, even 10 mM-Na caused 64 % of the total possible increase in the tissue potassium and 69 % of the maximal increase in oxygen consumption, so that the sodium concentration required for half-maximal stimulation was about 7–8 mM for each (Fig. 4).

In Fig. 5 the results given in Figs. 3 and 4 have been replotted to show the virtually linear correlation between tissue potassium concentration and the Q_{O_2} of slices incubated in various concentrations of sodium. With glucose a rise in tissue potassium concentration of 100 μ-equiv/g dry wt. was accompanied by an increase in the Q_{O_2} of 3, whereas with α-oxoglutarate the Q_{O_2} rose by 5 for each increase of 100 in the tissue potassium.

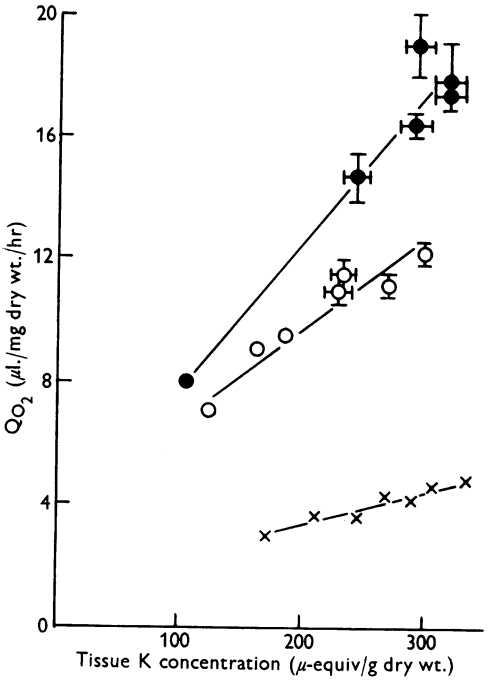

Fig. 5. Relations of Q_{O_2} and tissue K concentration in slices of kidney cortex incubated in various concentrations of Na. These values are based on the experiments illustrated in Figs. 3, 4 and 7. -○- 38° C, glucose (Fig. 3); -●- 38° C, α-oxoglutarate + glucose (Fig. 4); -+- 25° C, glucose (Fig. 7).

The dependence of the tissue sodium concentration on the medium sodium concentration was also determined after incubation in media containing graded concentrations of sodium. The tissue sodium concentration increased linearly with increasing medium sodium concentrations with glucose as metabolite (Fig. 6). In the presence of α-oxoglutarate, however, the tissue sodium concentration at each level of sodium in the medium was about 70 μ-equiv/g dry wt. higher than in the comparable slices incubated with glucose, so that on raising the sodium in the medium from 0 to 10 mM the tissue sodium concentration rose abruptly from 30 to 120 μ-equiv/g. The elevated tissue sodium concentrations found in the presence of α-oxoglutarate are compatible with an active uptake of α-oxoglutarate

with which sodium is taken up passively (cf. Whittam & Davies, 1953). These results show that the tissue potassium concentration and the oxygen consumption are dependent upon the sodium concentration in the medium, which, in turn, determines the tissue sodium concentration.

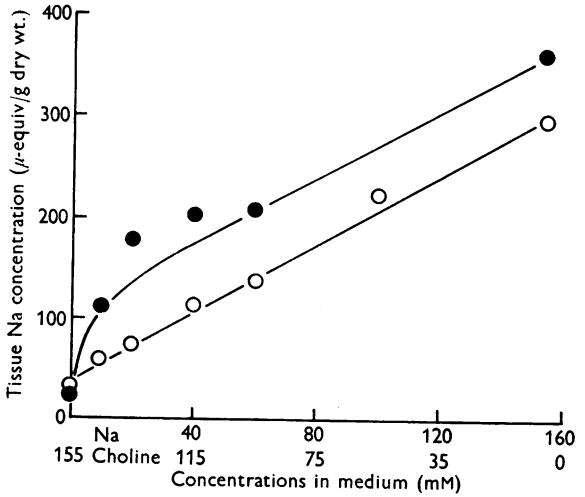

Fig. 6. Tissue Na content of kidney cortex slices after incubation in various concentrations of Na. –○– 10 mM glucose in medium; –●– 10 mM α-oxoglutarate + 10 mM glucose in medium. The medium contained 3 mM K phosphate, pH 7·4; 3 mM-$CaCl_2$ with the concentrations of NaCl and choline chloride shown in the abscissa.

Effects of temperature

Slices were incubated at 25° C in the usual medium in the manner previously described. The tissue potassium concentration at this temperature was 335 ± 5 μ-equiv/g (12 cases), which may be compared with an over-all mean value of 325 ± 18 obtained at 38° C. Mudge (1951*b*) found that 25° C was the optimal temperature for the accumulation of potassium by rabbit kidney cortex slices. Although potassium was thus maintained at a high level at 25° C, the Q_{O_2} fell to $4{\cdot}7 \pm 0{\cdot}1$ (11). This decrease from a mean value of 11·1 at 38° C represents a Q_{10} of 2·0, which is similar to Q_{10} values of 2·5 and 1·8 obtained in kidney cortex slices of rat (Robinson, 1950) and guinea-pig (Whittam, 1956) respectively.

Effect of external sodium at 25°C. Incubation of slices in medium containing choline instead of sodium caused a fall in the tissue potassium to 172 ± 11 μ-equiv/g (7), whereas incubation in this medium at 38° C caused the tissue potassium to fall to 124. The Q_{O_2} at 25° C in the medium containing choline instead of sodium was $3{\cdot}0 \pm 0{\cdot}1$ (8) as compared to 4·7 in the normal medium, a reduction of 36 %; at 38° C the corresponding figures were 7·0 and 11·1, a reduction of 43 %. As at 38° C, increasing concentrations

of sodium in the medium at 25° C caused a parallel increase of potassium uptake and of oxygen consumption, which was half-maximal at about 30 mM-Na (Fig. 7). Thus the qualitative effects of the substitution of choline for sodium in the medium and of its partial replacement were the same at 25 as at 38° C.

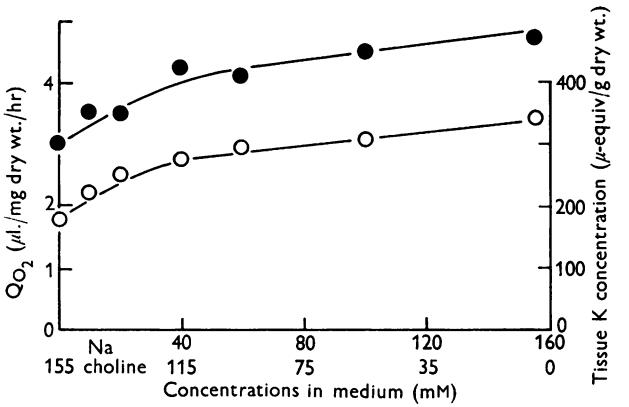

Fig. 7. Effect at 25° C of Na concentration in the medium on Q_{O_2} (-●-) and tissue K concentration (-○-). The medium contained 3 mM K phosphate pH 7·4; 3 mM-$CaCl_2$ and 10 mM glucose with the concentrations of NaCl and choline chloride shown in the abscissa.

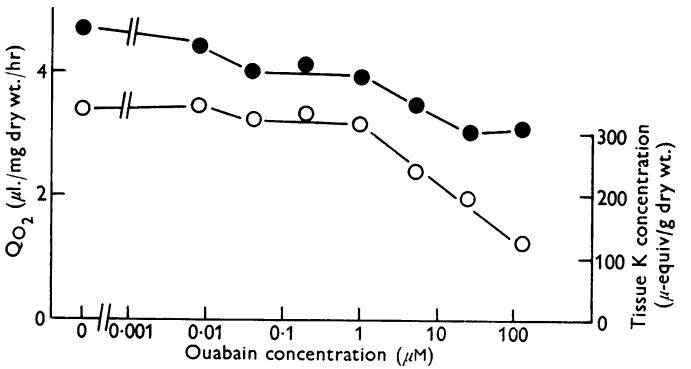

Fig. 8. Effect of ouabain at 25° C on Q_{O_2} (-●-) and tissue K concentration (-○-) in kidney cortex slices.

Effect of ouabain at 25° C. The tissue potassium concentration was not significantly affected by a concentration of ouabain less than 5 μM, but 25 μM ouabain caused a fall in tissue potassium content to about the level produced by sodium-free incubation (195) (Fig. 8). A higher concentration (125 μM) caused a further, statistically significant ($P < 0{\cdot}01$) reduction to 126. The Q_{O_2} at 25° C was already depressed in comparison with the value at 38° C and was further decreased by ouabain.

The sodium content of slices after incubation in normal medium at 25° C was similar to that at 38° C (270) and ouabain caused increases in the tissue sodium and water content which were also virtually the same as at 38° C. Orloff & Burg (1962) have also found inhibitory effects of ouabain on cation transport at 25° C in rabbit kidney cortex slices.

Rate of potassium transport in relation to oxygen consumption

The foregoing results record the K content of the slices after incubation for 1 hr. Accumulation of K may be complete before this and the rate of uptake of K has therefore been estimated from net movements and compared with O_2 uptake to see how the two rates are related.

Rate of net uptake. Figure 9 illustrates one experiment. Tissue slices were first shaken for 30 min at 38° C in 155 mM choline chloride, 3 mM K phosphate, pH 7·4, 3 mM-$CaCl_2$ and 10 mM glucose. At the end of this time the K content of some slices was analysed and had fallen to 125 μ-equiv/g dry wt. (zero time in Fig. 9*B*). 0·24 ml. of M-NaCl was then tipped into 3·76 ml. of the choline chloride medium containing further slices of tissue, to give a sodium concentration of 60 mM, and slices were withdrawn for analysis 3, 6, 10, 16 and 25 min later. The results are shown in Fig. 9*B*. The oxygen consumption following the addition of sodium was measured in slices from the same kidney incubated concurrently in manometer flasks and compared with the O_2 consumption of slices kept in the choline chloride medium without the addition of sodium (Fig. 9*A*). The uptake of K (Fig. 9*B*) was initially rapid, being 13 μ-equiv/g dry wt/min during the first 3 min. The associated increase in oxygen consumption was 80 μl./g dry wt/min or 3·6 μ-mole/g/min. The ratio of the rate of potassium uptake (in μ-equiv/g/min) to the associated increment in the rate of oxygen consumption (in μ-moles/g/min) was $13/3{\cdot}6 = 3{\cdot}6$.

By this procedure the rate of potassium uptake and the associated change in oxygen consumption were measured at 38° C in 20, 60, and 100 mM-Na, and also at 25° C in 100 mM-Na. The results are shown in Table 3. In order to avoid excessive hypertonicity in experiments with 100 mM-Na, the slice was pre-incubated in the side arm with 1 ml. choline chloride medium, which was tipped into the main compartment containing 3 ml. normal medium to create an isotonic medium with 100 mM-Na.

In another series of experiments the rate of potassium loss was measured in slices after tipping ouabain into normal medium (Fig. 10). When ouabain was added to a concentration of 125 μM, the time course of the fall in tissue potassium concentration allowed the calculation of the rate of loss in the first 4 min, which, in the experiment illustrated in Fig. 10*B*, was 11 μ-equiv/g/min. The associated fall in oxygen consumption was measured

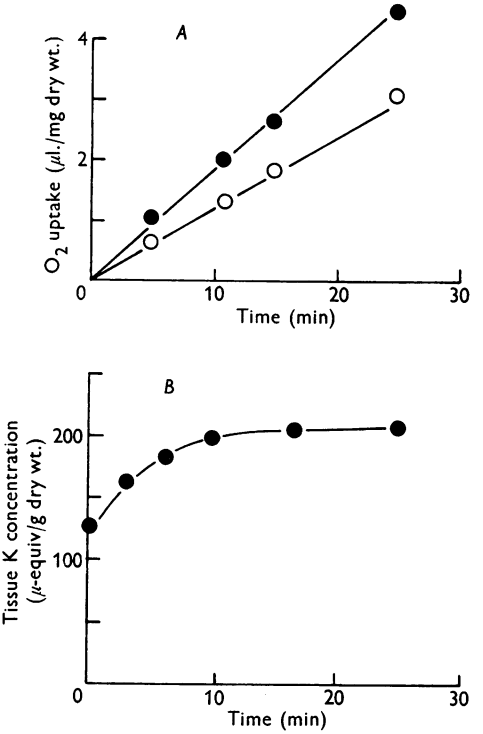

Fig. 9. Uptake of K and O_2 by incubated kidney cortex slices at different times after adding NaCl to choline chloride medium at 38° C. *A*. O_2 uptake in choline chloride medium (–○–) and in choline chloride medium with 60 mM-NaCl (–●–). *B*. Tissue K concentration of slices collected at timed intervals after adding NaCl (–●–). For details see text.

TABLE 3. Comparison of rate of net potassium movement and associated change in oxygen consumption

Temperature (°C)	Operation and conditions	No. of cases	Rate of change of tissue [K] (μ-equiv/g/min)	Change in rate of oxygen consumption (μ-mole/g/min)	K:O_2 (μ-equiv/μ-mole)
38	Na added to final concn. of (mM)				
	20	1	5·6	2·1	2·7
	60	3	9·5	3·1	3·3
	100	4	11·1	3·8	3·1
38	Ouabain added to final concn. of (μM)				
	25	1	−6·7	−1·9	3·5
	125	2	−12·5	−2·6	4·8
25	Na added to final concn. of 100 mM	3	5·6	1·4	4·0
25	Ouabain added to final concn. of 125 μM	2	−5·0	−1·1	4·9

These are the results of experiments like those of Figs. 9 and 10.

in parallel slices and in this experiment (Fig. 10*A*) was 55 μl./g/min or 2·5 μ-mole/g/min, giving a K:O_2 ratio of 4·5.

The rates of net uptake and loss of tissue potassium shown in Table 3 depended upon the concentration of sodium or ouabain and upon temperature. Thus, at 38° C the rate of potassium uptake was doubled by increasing the medium sodium concentration from 20 to 100 mM, and similarly the rate of net loss was approximately doubled by increasing the

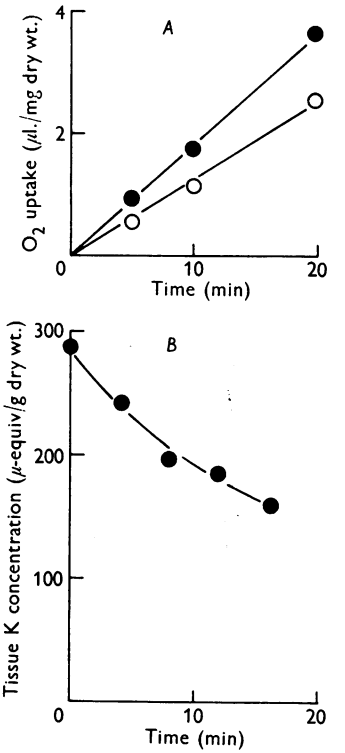

Fig. 10. Loss of K and reduction of O_2 uptake in incubated kidney cortex slices at different times after adding ouabain to normal medium at 38° C. *A*. O_2 uptake in normal medium without ouabain (–●–), and in normal medium with 125 μM ouabain (–○–). *B*. Tissue K concentration of slices collected at timed intervals after adding ouabain (–●–).

ouabain concentration from 25 to 125 mM. It is therefore significant that the rate of net potassium gain induced by 100 mM-Na was about equal to the rate of loss caused by 125 μM ouabain both at 25 (5·0–5·6) and at 38° C (11·1–12·6). The effect of temperature is shown by the fact that at 25° C the rate of potassium uptake in 100 mM-Na and the rate of loss in 125 μM ouabain were half the comparable values at 38° C (Table 3). Consequently, after adding sodium the maximal concentration of tissue potassium was

attained at about 20 min at 25° C compared with about 10 min at 38° C. These findings are in accord with those of Mudge (1951*a*), who found that at 25° C in rabbit kidney cortex slices maximal tissue potassium concentration was reached in 20–30 min when uptake was initiated by adding potassium to sodium-rich potassium-free medium. Berndt & Le Sher (1961) also found that the time required for maximal accumulation of potassium by the same tissue was doubled at 25° C compared with 38° C.

The principal finding revealed in Table 3 is that under all conditions the change in the rate of oxygen consumption was related to the rate of potassium uptake or loss. This proportion, which is expressed as the ratio $K:O_2$, varied from 2·7 to 4·9 (Table 3) and may be regarded as reasonably constant in view of the twofold variation in rate of net loss or gain of potassium produced by varying the temperature or the concentration of ouabain or sodium. A part of the oxygen consumption in slices of rabbit kidney cortex would therefore appear to be coupled in a roughly stoichiometrical manner with the rate of net potassium accumulation.

DISCUSSION

The variation in the rate of oxygen consumption during rest and activity in muscle and secretory tissues has led to the general concept of a basal rate, characteristic of a resting cell, and of a variable rate, determined by the demands for energy during activity (see Barcroft, 1934). Part of the oxygen consumption is therefore ultimately controlled by the extent to which energy-requiring processes are operating. The results of the present study with kidney cortex slices conform to this general notion. At a given temperature the oxygen consumption consists of a roughly constant basal rate, which is insensitive to ouabain and sodium, and of a variable rate above this level which is inhibited by ouabain and stimulated by sodium. Ouabain and sodium act primarily upon cation transport, and their effects on suprabasal oxygen consumption are proportional and parallel to that action. The process of active transport therefore appears to regulate part of the respiration of kidney cortex cells, thereby ensuring the availability of the energy required for its own continuation. Regulation of a part of cellular respiration by active transport has been demonstrated previously in frog skin (Zerahn, 1956; Ussing, 1959) and sartorius muscle (Mullaney, 1961; Conway & Mullaney, 1961), toad bladder (Leaf, Page & Anderson, 1959), and mammalian brain slices (Whittam, 1961, 1962). Such regulation is also involved during sodium reabsorption in mammalian kidneys (Kramer & Deetjen, 1960; Lassen, Munck & Thaysen, 1961; Kiil, Auckland & Refsum, 1961) and during metabolic recovery from post-tetanic potentiation in frog sciatic nerve (Connelly, 1959).

It has been assumed in this and earlier studies that the fraction of respiration thus controlled provides a measure of the cell's energy devoted exclusively to active transport. Two possible objections to this assumption should, however, be considered. On the one hand, the energy required for transport might be less than that represented by the inhibition of respiration by ouabain and by its stimulation with sodium. This would be the case if there were other energy-requiring processes influencing respiration which were also sensitive to sodium and ouabain. No experiment has yet been devised to determine the existence of such a fraction. Conversely, cation transport might require *more* energy than is supplied by the fraction of oxygen consumption subject to the effects of ouabain and sodium. Thus, either part of the basal respiration or energy derived from glycolysis might be necessary to support the maximal rate of active cation transport. Since glycolysis sustains no appreciable active transport in slices of adult rabbit kidneys (Mudge 1951*b*), the error deriving from its contribution is negligible in this tissue. The possibility that some of the energy from basal respiration might be used for active transport cannot, however, be excluded.

With these qualifications it is possible to state that the fraction of respiration controlled by and supplying the energy for active transport in slices of rabbit kidney cortex is about 35–45 % of the total at temperatures of 25 and 38° C with glucose and calcium in the medium. In order to estimate the energy required to support a specific amount of active transport it is necessary to compare a rate of transport with an associated change in the rate of oxygen consumption. Such a comparison is important because it may provide a clue to the mechanism of active transport in relation to its utilization of energy. In a variety of conditions the net movement of about four equivalents of potassium (2·7–4·9) was associated with the uptake of one mole of oxygen (Table 3). When this ratio is compared with analogous ratios obtained for sodium and potassium by various authors using different methods in a number of tissues (Table 4), it can be seen that the $K:O_2$ ratio obtained for kidney slices is similar to the $Na:O_2$ ratio of frog sciatic nerve and sartorius muscle, and to the $K:O_2$ ratio of mammalian brain slices. On the other hand, these values are considerably less than the $Na:O_2$ ratio found for transport across frog skin and toad bladder, and for sodium reabsorption in mammalian kidney *in situ*. It may be pertinent that the experiments in the latter group were concerned with transcellular transport, whereas those in the former group relate to transport between the inside and outside of cells. It is difficult to say at present whether this distinction accounts for the disparity between the values of $K:O_2$ found with kidney slices *in vitro* and of the $Na:O_2$ with whole kidneys. Another possibility is that a difference exists between the $K:O_2$

and $Na:O_2$ ratios under the same conditions. Using an approach altogether different from ours, Lassen & Thaysen (1961) found a $Na:O_2$ ratio of 25 in slices of rabbit kidney cortex, which is in agreement with results obtained *in situ*. On the other hand, rough estimations of the rate of sodium gain with inhibition by ouabain obtained in the present study, although complicated by swelling and of little quantitative value, nevertheless provided values which are qualitatively similar to the rate of loss of potassium in the same experiments and gave, therefore, $Na:O_2$ ratios similar to the $K:O_2$ values.

TABLE 4. Utilization of energy for active transport of Na and K

Organism	Tissue	Cation (equiv:mole O_2) K:O_2	Na:O_2	Cation (equiv:mole ATP) K:ATP	Na:ATP	Source
Dog	Kidney (*in situ*)	—	24	—	4·0	Kiil *et al.* (1961)
Toad	Skin	—	22	—	*ca.* 4·0	Zerahn (1961)
	Bladder	—	16·5	—	*ca.* 3·0	Leaf *et al.* (1959)
Frog	Skin	—	16–20	—	*ca.* 3·0	Zerahn (1956, 1961)
	Muscle	—	4	—	0·7	Conway & Mullaney (1961)
	Nerve	4–5	4–5	0·7	0·7	Connelly (1959)
Squid	Nerve (giant axon)	—	—	—	0·7*	Caldwell *et al.* (1960)
Man	Red blood cells	—	—	0·7*	—	Whittam (1958); Glynn (1956)
Rabbit	Brain cortex (slices)	6	—	1	—	Krebs *et al.* (1951); Whittam (unpublished data)
	Kidney cortex (slices)	3–5	—	0·5–0·8	—	This paper
Guinea-pig	Kidney cortex (slices)	5	—	0·9	—	Whittam & Davies (1954); Whittam (unpublished data)
	Seminal vesicle (slices)	2	—	0·3	—	Breuer & Whittam (1957); Whittam & Breuer (1959)
	Brain cortex (slices)	7·5	—	1·25	—	Cummins & McIlwain (1961)

* Utilization of ATP was directly measured in these studies. The other cation: ATP ratios have been calculated from the cation:O_2 ratios, on the assumption of a P:O ratio of 3.

Rates of Na and K movements were determined by a variety of methods in different tissues. In all cases the fraction of suprabasal oxygen consumption *associated with* the ion movement was used to calculate the cation :O_2 ratio.

Wilde (1955) has pointed out that the tubular secretion which kidney cells perform *in situ* might persist *in vitro* even after slicing the cortex and would therefore complicate the interpretation of results obtained in kidney slices. Thus, if some of the cells in the slices were actively transporting potassium *out* as well as *in*, the measurement of net potassium uptake by the slices would only represent a fraction of the total active potassium transport, and the oxygen required for this fraction would therefore be over-estimated. A similar criticism would also apply to the $K:O_2$ ratios

based on the rate of net potassium uptake if the rate of uptake during the initial period of measurement was accompanied by a high rate of potassium loss. That such errors did not in fact prevail to a significant extent in the present study is suggested by the finding that the $K:O_2$ ratios based on the rate of net potassium loss were very similar to those based on the rate of net potassium uptake. Kidney cortex slices contain a varied population of cells and no attempt has been made to extrapolate these results to renal function *in vivo*. The partial regulation of oxygen consumption by ion transport, however, illustrates the importance of the latter in the energetics of cells which maintain concentration gradients across their membranes.

The question of how respiration and active transport are interconnected depends upon how respiratory energy is utilized by active transport, for

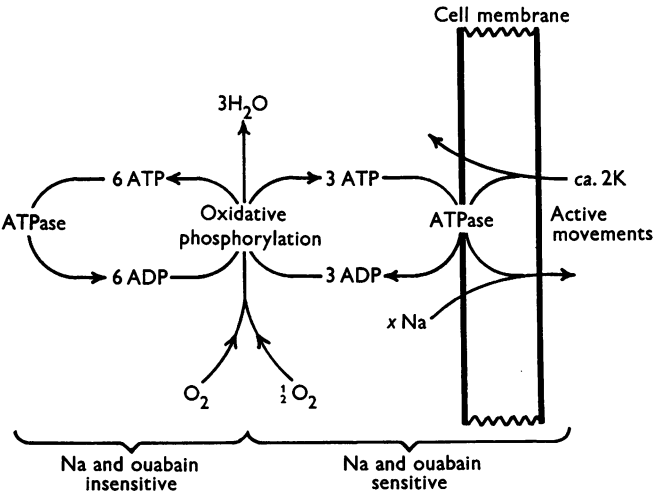

Fig. 11. Scheme for the coupling between active transport and respiration in slices of rabbit kidney cortex. Oxidative phosphorylation in mitochondria provides ATP required to support active movements of Na and K and other energy-requiring processes in the cell. In a medium containing 155 mM-Na, a third to a half of the oxygen consumption is devoted to active ion movements, so that the relative utilization of ATP is approximately as shown. Assuming a P:O ratio (phosphorylation quotient) of 3, the hydrolysis of about 3 moles of ATP is associated with the accumulation of 2 equivalents of K ions and with the outflow of an undetermined number (x) of equivalents of Na ions. The hydrolysis of this ATP depends upon an ATPase, which is sensitive to stimulation by Na ions and to inhibition by ouabain, as are the active movements of Na and K ions. The ADP resulting from this hydrolysis stimulates oxygen consumption, which is therefore obligatorially coupled to active ion movements. The 'ouabain-sensitive ATPase' represents the over-all hydrolysis of ATP to ADP and does not exclude the possibility that some other reaction coupled to ATP, such as an oxido-reduction reaction, might be still more closely linked with transport. The ouabain-insensitive ATPase represents the hydrolysis of ATP due to other reactions utilizing ATP.

presumably the control of oxygen consumption operates through a chemical intermediate common to the two. Such a link is probably adenosine triphosphate (ATP), which seems to be a source of energy for ion transport in red blood cells and squid giant axons (Gardos, 1954; Whittam, 1958; Hoffman, 1960; Caldwell, Hodgkin, Keynes & Shaw, 1960), as it is for muscular contraction (Cain & Davies, 1962). The scheme in Fig. 11 summarizes a mechanism by which active transport at the cell membrane could exert an effect as a pace-maker of oxygen consumption in mitochondria. Utilization of the energy of ATP involves its hydrolysis to ADP, which in turn is re-phosphorylated by the process of oxidative phosphorylation. It is now well known that ADP specifically stimulates oxidative phosphorylation and oxygen consumption, and the series of reactions in Fig. 11 provides an explanation for the feed-back control of oxygen consumption by energy-requiring processes (see Krebs, 1962). Chance & Williams (1956) suggested that these reactions could explain the 'anion respiration' of plant cells and similar suggestions have been made for cation transport in animal tissues (Ussing, 1959; Quastel, 1961; Whittam, 1961). This concept is given support by the present results and by the discovery of an adenosine triphosphatase (ATPase) which is located in the cell membranes of a variety of tissues, including kidney cortex, and which is stimulated by the combined presence of sodium and potassium and inhibited by ouabain (cf. Wheeler & Whittam, 1962). Thus, all the components exist for a pace-maker hypothesis regarding the regulation of respiration by active transport.

SUMMARY

1. Slices of rabbit kidney cortex were incubated in saline media and their K content and O_2 uptake measured.

2. Various concentrations of ouabain caused parallel reductions in the oxygen consumption and the tissue potassium concentration.

3. The presence of 10 mM α-oxoglutarate in the medium produced a heightened Q_{O_2}, but had no effect on the changes produced by ouabain.

4. Ouabain caused tissue slices to swell and to take up sodium from the medium.

5. When slices were incubated in medium in which choline was substituted for sodium, their Q_{O_2} and tissue potassium concentrations were reduced to constant levels. The presence of graded concentrations of sodium in the medium caused a parallel increase in potassium uptake and oxygen consumption.

6. The effects of sodium on tissue respiration and potassium concentration were qualitatively the same at 25° C as at 38° C, although the control Q_{O_2} was reduced to 4·7 at this temperature. Oxygen consumption was

more sensitive to ouabain at 25° C, however, while potassium uptake was less so.

7. The rate of net potassium gain induced by sodium and of net potassium loss induced by ouabain in normal medium was proportional to the associated change of tissue oxygen consumption under a variety of conditions. The number of equivalents of potassium moved per mole of oxygen varied from 2·7 to 4·9.

8. It is concluded that oxygen consumption of kidney cortex slices consists of two fractions—a constant basal rate and a variable rate which is related to the energy requirements for potassium uptake and sodium extrusion. The rate of active cation transport appears to be a factor in the control of tissue respiration, compatible with the hypothesis of an obligatory coupling between ATP production and utilization.

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