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The Regulation of Kidney Respiration by Sodium and Potassium Ions

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In biochemical studies on cellular energetics more attention has been devoted to energy production during intermediary metabolism than to energy utilization. Processes that utilize energy have not, as a rule, been investigated with a view to determining their role in the energy metabolism of the cell. Yet, when considering the cell as an integrated unit, it is evident that there must be interplay between energy production and utilization. The interrelationship between active cation transport and respiration is particularly suitable for the study of such a coupling, since active transport is continuous in the intact cell and does not require nervous or hormonal stimulation. Brain and kidney cortex, for example, have high rates of respiration and active transport, and the latter is a major energy-utilizing reaction in both tissues. In slices of these tissues evidence has been obtained that ion transport acts as a pacemaker of respiration, largely because decreases in the rate of uptake of K^+ ions, induced by means that were believed not to inhibit respiration directly, are nevertheless accompanied by parallel decreases in respiration (Whittam, 1961, 1962; Whittam & Willis, 1963). There is thus an interdependence of the rates of respiration and active movements of cations. Another parallelism is that between adenosine-triphosphatase activity and active cation transport (Skou, 1957; Post, Merritt, Kinsolving & Albright, 1960; Dunham & Glynn, 1961; Tosteson, Moulton & Blaustein, 1960; Wheeler & Whittam, 1962).

Thus on the one hand ion transport and respiration in slices are connected, and on the other hand there is a linkage between ion transport and adenosine-triphosphatase activity. The present experiments were designed to see whether this adenosine triphosphatase acts as a regulator of respiration, thus providing the mechanism by which active ion transport regulates respiration. Factors known to influence the rate of ion transport have therefore been investigated for their effects on the rates of respiration and of ATP hydrolysis in homogenates. Further, to see whether these factors affect respiration directly, their influence on the respiration of isolated mitochondria has been examined.

The results show that the respiration of rabbit-kidney homogenate is markedly affected by the concentrations of Na^+ ions, K^+ ions, Ca^{2+} ions and ouabain in the medium. A control of respiration has been demonstrated by an adenosine triphosphatase sensitive to these ions which is believed to be involved in the active transport of Na^+ and K^+ ions.

METHODS

Preparation of tissue suspensions. Adult rabbits of both sexes were stunned by a blow on the neck and bled from the throat. The kidneys were excised and, after removal of the capsule, each was bisected longitudinally and the exposed medulla cut away. The cortex (about 4 g.) was disrupted in about 12 ml. of ice-cold medium containing sucrose (0·25 M) and imidazole–HCl buffer, pH 7·8 (10 mM), in a Potter–Elvehjem-type homogenizer with a smooth glass tube and Teflon pestle (total clearance 0·01 in.). The pestle, revolving at 700 rev./min., was taken 20 times up and down through the suspension, which was then squeezed through a double layer of muslin and the volume adjusted to give a 20% (w/v, referred to wet wt. of tissue) suspension by the addition of more 0·25 M-sucrose medium.

Preparation of kidney mitochondria. A 10% (w/v) homogenate was centrifuged at 850*g* for 10 min. at 0° (MSE Major refrigerated centrifuge, 6886 head; 2300–2400 rev./

min.) to spin down the cellular debris and nuclei. This procedure was repeated on the supernatant liquid and the two sediments were discarded. The mitochondria were then sedimented at 5000*g* for 15 min., and, after being washed once in 0·25M-sucrose medium, they were resuspended and diluted with sucrose medium to give a final volume of about 15 ml. On the basis of dry weight the mitochondrial fraction constituted 15% of the homogenate.

Measurement of respiration. Portions (1·0 ml.) of 20% (w/v) homogenate (30–40 mg. dry wt.) or samples of a suspension of mitochondria (usually about 10 mg. dry wt./ml.) were added to manometer cups containing 3·0 ml. of medium, and the O_2 consumption was measured during incubation in air at 37°. The incubation medium contained (final concentrations) imidazole–HCl buffer, pH 7·8 (15 mM), tris–malate buffer, pH 7·8 (10 mM), $MgCl_2$ (2 mM), and Na^+ and K^+ ion concentrations and other additions as specified in the text. The medium was fortified with NAD (0·125 mM) and ATP (1·25 mM) to obtain linear rates of respiration. Incubation of homogenates was commenced 0·5–1 hr. after the removal of the kidneys from the animal, and that of mitochondria after 2·5–3 hr. Oxidative activity (Q_{O_2}) is expressed as μl. of O_2 uptake/mg. dry wt./hr.

Measurement of adenosine-triphosphatase activity. Reaction mixtures (1·9 ml.) were prepared in 10 ml. tubes and contained (final concentrations) imidazole–HCl buffer, pH 7·8 (15 mM), $MgCl_2$ (2 mM), various amounts of the ions under investigation and the tissue suspension (dry wt. 0·1–0·5 mg.). The tubes were shaken at 37° for 5 min. and the reaction was then initiated by the addition of 0·1 ml. of 40 mM-ATP (disodium salt) to give a final concentration of 2 mM. The reaction was stopped after 5 min. by the addition of 1·0 ml. of ice-cold 20% (w/v) trichloroacetic acid, and the tubes were placed in ice and water. After about 15 min. the precipitated protein was spun down and the orthophosphate in the supernatant measured by the method of Fiske & Subbarow (1925), during which procedure there is no appreciable hydrolysis of ATP (Bartlett, 1959).

Adenosine-triphosphatase activity, expressed as μmoles of orthophosphate released/mg. dry wt./hr., is subject to a measurement error of about 4%.

Determination of dry weight. The dry weight of a tissue suspension in sucrose, obtained by simple drying and weighing, includes the biological dry matter and the dry weight of sucrose. The correction for the weight of sucrose was often uncertain on account of its hygroscopic nature, and therefore could lead to considerable inaccuracy of the dry weight, particularly with dilute tissue suspensions. This difficulty has been avoided by the following method. Trichloroacetic acid (25%) was added to 2 or 3 ml. of tissue suspension in a weighed tube, to give a final concentration of 7%. The resulting precipitate was spun down, the supernatant fluid decanted and the sediment resuspended in water. The suspension was recentrifuged to obtain a tightly packed pellet, the supernatant removed and the tube drained. The dry weight of the sucrose-free sediment obtained this way was determined by weighing after drying overnight at 105°.

Materials. Ouabain (strophanthin-G), imidazole, choline chloride, trichloroacetic acid and α-oxoglutaric acid were laboratory-reagent products of British Drug Houses Ltd., Poole, Dorset; NAD was supplied by Boehringer und Soehne G.m.b.H., Mannheim, Germany; ATP (disodium salt) and tris (Sigma 7–9) were obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A.; L-malic acid was from L. Light and Co. Ltd., Colnbrook, Bucks.; all other compounds used were AnalaR reagents from British Drug Houses Ltd.

Solutions of ATP were buffered to pH 7·8 with saturated tris solution, and kept at 0–4°. Malic acid, α-oxoglutaric acid and succinic acid were used as solutions of their tris salts at pH 7·8. All solutions were made in glass-distilled water.

RESULTS

Sensitivity to Na^+, K^+ *and ouabain.* It is known that the respiration of slices of brain and kidney cortex (Whittam, 1961; 1962; Whittam & Willis, 1963) and of liver (Elshove & van Rossum, 1963) is inhibited by the removal of Na^+ ions from the medium or by the addition of the glycoside ouabain. To see whether respiratory control of homogenates is subject to the same inhibitions and activations that have been found in slices, portions of homogenate were incubated in media in which the concentrations of Na^+ and K^+ ions were varied.

Table 1 shows that with 102·5 mM-K^+ ion plus 55 mM-Na^+ ion the respiration was two- to three-fold greater than that with 155 mM-Na^+ ion plus 2·5 mM-K^+ ion, and some 30% greater than that with 152·5 mM-K^+ ion plus 5 mM-Na^+ ion. The homogenate containing 152·5 mM-K^+ ion plus 5 mM-Na^+ ion thus has a higher rate of respiration than one containing 155 mM-Na^+ ion plus 2·5 mM-K^+ ion, although ouabain is without effect on either. In contrast, when the two ions were present together at higher concentrations a marked inhibition by ouabain was observed; this was especially pronounced with 55 mM-Na^+ ion plus 102·5 mM-K^+ ion.

To elucidate further the nature of the dependence of Q_{O_2} on the concentrations of Na^+ and K^+ ions the respiration was examined at intermediate concentrations of these ions. Fig. 1 shows that on raising the concentration of K^+ ions there was a 60–70% increase in respiration, most of which occurred between 12·5 and 22·5 mM-K^+ ion, and which levelled out to a plateau between 32·5 and 52·5 mM-K^+ ion. In the presence of ouabain the Q_{O_2} at the plateau was some 50–60% greater than that with 155 mM-Na^+ ion plus 2·5 mM-K^+ ion, indicating that the stimulation caused by raising the K^+ ion and decreasing the Na^+ ion concentrations is only partly counteracted by ouabain.

Further increase in the K^+ ion concentration at the expense of the Na^+ ion concentration resulted in an additional rise in the respiration. This was entirely abolished by ouabain, or by decreasing the concentration of Na^+ ions to 5 mM. These results show that with high K^+ ion concentrations the addition of Na^+ ions stimulates respiration in a way that is completely sensitive to ouabain, whereas with a high Na^+ ion concentration the addition of

Table 1. *Sensitivity of the respiration of kidney-cortex homogenate to* Na^+ *and* K^+ *ions*

The sum of NaCl and KCl concentrations in the medium was held constant at 150 mM, variations in the concentration of each being made by interchanging the one ion for the other. Samples of homogenate (1·0 ml.) were incubated from 30 to 60 min. in 3·0 ml. of medium containing (final concentrations) tris–malate, pH 7·8 (10 mM), imidazole–HCl buffer, pH 7·8 (15 mM), $MgCl_2$ (2 mM), NAD (0·125 mM), ATP (disodium salt, buffered to pH 7·8 with tris) (1·25 mM), and the concentrations of Na^+ and K^+ ions indicated. These include contributions from ATP (2·5 mM-Na^+ ion) and from the homogenate itself (2·5 mM in each ion). The concentration of ouabain was 0·2 mM. The experiments shown are representative of a group of nine such experiments.

			Q_{O_2} (μl. of O_2/mg. dry wt./hr.)			
Concn. in incubation mixture (mM)		Na^+ ions ...	155	125	55	5
		K^+ ions ...	2·5	32·5	102·5	152·5
Expt. no.	ATP	Ouabain				
1	+	−	11·1	17·6	22·4	17·5
		+	11·3	15·7	17·9	17·8
2	+	−	9·4	21·4	29·0	19·0
		+	10·0	16·8	19·6	19·6
3	+	−	14·0	22·0	27·6	19·6
4	+	−	12·8	20·0	26·2	19·7
Choline in place of Na^+ ions ...			150	120	50	0
3	+	−	21·0	20·1	22·0	20·0
4	+	−	20·0	19·2	19·0	20·3

small amounts of K^+ ions stimulates respiration in a way that is largely insensitive to ouabain.

Replacement of Na^+ *ions with choline.* To see whether these changes persisted in the absence of added Na^+ ions to the homogenate, the oxygen uptake was measured in a medium made up with choline chloride and potassium chloride (Expts. 3 and 4 in Table 1). Choline had two effects: it prevented the additional rise in Q_{O_2} which was maximal at 55 mM-Na^+ ion plus 102·5 mM-K^+ ion and which was abolished by ouabain, and it also prevented the fall in respiration which was otherwise found at low K^+ ion concentrations. In contrast with results in controls in Na^+ ion–K^+ ion media, where the Q_{O_2} varied twofold (e.g. from 14·0 with 155 mM-Na^+ ion plus 2·5 mM-K^+ ion to 27·6 with 55 mM-Na^+ ion plus 102·5 mM-K^+ ion), the respiration in choline–K^+ ion media was unaffected by changes in the concentrations of these ions. The effects of Na^+ ions, K^+ ions, choline and ouabain on the respiration of homogenates raises the question of their site of action: whether they act on the mitochondria themselves or on extramitochondrial components that can exert some control of respiration.

Respiration of mitochondria

Some indication of the purity of isolated mitochondria was obtained by examining them under the phase-contrast microscope. Their appearance revealed a homogeneous suspension free from nuclei or cell debris. Contamination of the mitochondria by microsomes would not, however, be shown by this examination on account of the small size of microsomal particles. The possibility of microsomal impurities in the mitochondrial preparations used in the following experiments could not therefore be excluded.

Sensitivity to Na^+ *ions,* K^+ *ions and ouabain.* To test whether Na^+ and K^+ ions had a direct effect on respiration, mitochondria from kidney cortex were incubated in media in which the concentrations of these ions were varied by interchanging one ion for the other. Fig. 2 shows that on raising the concentration of K^+ ions from 0 to 30 mM there was a 100 % increase in respiration, which occurred mainly between 10 and 20 mM-K^+ ion. Additional increases in the K^+ ion concentration resulted in no further change in respiration. Moreover, ouabain at a concentration of 100 μM was without effect, regardless of the Na^+ ion and K^+ ion concentrations.

Replacement of Na^+ *ions with choline.* When Na^+ ions were replaced by choline the respiration was no longer affected by changes in the K^+ ion concentration. Thus the presence of choline in the place of Na^+ ions prevented the decrease in respiration which was found in Na^+ ion-containing media when the K^+ ion concentration fell below 30 mM.

Adenosine-triphosphatase activity in homogenates and mitochondria

The response of the respiration of homogenates and mitochondria to Na^+ and K^+ ions suggests that there are two factors concerned in the control of respiration, one of which is located in the mitochondria and the other in an extramitochondrial factor. Thus the extramitochondrial control of respiration is rendered inoperative by ouabain, and the remaining respiration in homogenates is that controlled by the mitochondria. To see whether

these two regulatory mechanisms were the result of adenosine-triphosphatase activity, measurements were made of the rate of ATP hydrolysis in mitochondria and homogenates.

Sensitivity of homogenates to cations and ouabain. The results (Fig. 3) demonstrate that both Na^+ and K^+ ions are required together for stimulation of adenosine-triphosphatase activity. Under the conditions used activity was maximum with 10 mM-K^+ ion plus 154 mM-Na^+ ion, and fell gradually as the K^+ ion concentration was increased and that of Na^+ ions decreased. Maximal activity was 60–70 % higher than that observed with 154 mM-Na^+ ion or 150 mM-K^+ ion, or in the presence of ouabain. Ouabain thus counteracts the stimulating effect of Na^+ and K^+ ions together, but is without action if only one of these ions is present. Replacement of 150 mM- or 130 mM-Na^+ ion with choline, at K^+ ion concentrations of 0 and 20 mM respectively, resulted in a decreased rate of ATP hydrolysis, the magnitude of which was identical with that found with ouabain inhibition (Fig. 3). This shows that the increase in activity associated with a rise in K^+ ion concentration requires the presence of Na^+ ions, and that this is the component sensitive to ouabain.

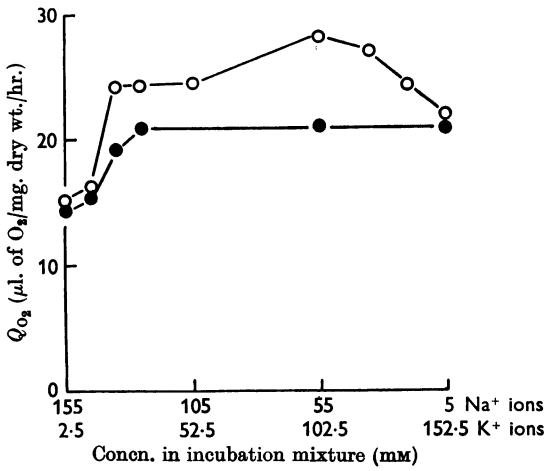


Fig. 1. Respiratory response of kidney-cortex homogenate to Na^+ ions, K^+ ions and ouabain. Samples (1·0 ml.) of homogenate were incubated for 20 min., during which time the rate of uptake of O_2 was constant. The medium in each cup (3·0 ml.) contained (final concentrations) tris–malate, pH 7·8 (10 mM), imidazole–HCl buffer, pH 7·8 (15 mM), $MgCl_2$ (2 mM), NAD (0·125 mM) and ATP (disodium salt, buffered to pH 7·8 with tris) (1·25 mM). The concentrations of Na^+ and K^+ ions shown on the abscissa include a 2·5 mM concentration of each from the endogenous ions in the homogenate, 2·5 mM-Na^+ ion from the ATP, and the remainder from NaCl or KCl in the medium. ○, Control; ●, with 0·2 mM-ouabain. The Figure illustrates a single experiment representative of a group of four such experiments.

Sensitivity of mitochondrial adenosine-triphosphatase activity to cations and ouabain. In contrast with the findings with homogenates (Fig. 3), interchanging the Na^+ ion and K^+ ion concentrations between 0 and 150 mM resulted in only a very small increase (about 10 %) in the adenosine-triphosphatase activity, from 12·5 to 14·0 µmoles/mg. dry wt./hr. in a typical experiment. Again, this increase was completely abolished in the presence of 100 µM-ouabain or by replacement of the Na^+ ions with choline. It is probable that this slight stimulation by Na^+ and K^+ ions is due to contamination of the fraction by microsomal particles, which possess a cation-sensitive adenosine-triphosphatase activity. Kidney mitochondria, unlike the homogenate, therefore appear to be insensitive to Na^+ ions, K^+ ions, choline and ouabain with regard to their adenosine-triphosphatase activity.

Correlation of respiration and adenosine-triphosphatase activity

The inhibition of respiration and adenosine-triphosphatase activity in homogenates produced by different concentrations of ouabain (0–400 µM) is shown in Fig. 4. The Q_{O_2} is plotted against the rate of orthophosphate release from ATP for each concentration of ouabain used. The linearity of the plot shows that the relative effects of ouabain on Q_{O_2} and on adenosine-triphosphatase activity were

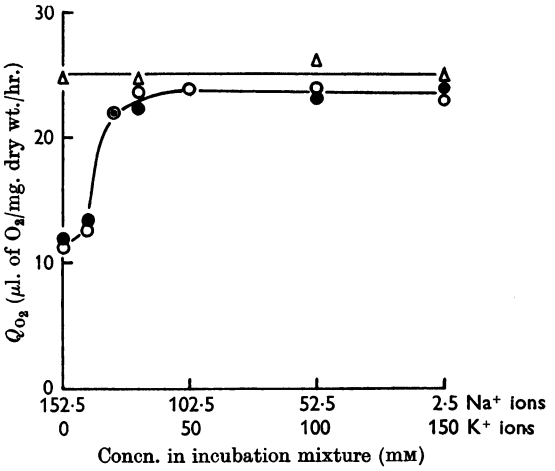


Fig. 2. Respiratory response of kidney-cortex mitochondria to Na^+ and K^+ ions. Mitochondria (about 10 mg. dry wt.) were incubated for 30 min. in a medium containing (final concentrations) tris–malate, pH 7·8 (10 mM), imidazole–HCl buffer, pH 7·8 (15 mM), $MgCl_2$ (2 mM), NAD (0·125 mM), ATP (disodium salt, buffered to pH 7·8 with tris) (1·25 mM) and the NaCl and KCl concentrations indicated. The Na^+ ions in the ATP solution have been included in the total. ○, Control; ●, with 0·1 mM-ouabain; △, Na^+ ions replaced by choline. The Figure illustrates a single experiment typical of three such experiments.

the same: no concentration of ouabain caused an effect on one without the same proportional effect on the other.

Nature of the rise in the rate of respiration at low concentrations of K^+ ions

The rise in respiration of homogenates on increasing the K^+ ion concentration from 2·5 to 32·5 mM appears to be due to an effect on mitochondria that is not associated with enhanced adenosine-triphosphatase activity. The effect is also found with substrates other than malate, and since it is observed when succinate is the substrate it is evidently not dependent on the oxidation of substrate by an NAD-linked reaction. Table 2 shows that the stimulation of respiration by 30 mM-K^+ ion was found with glucose, malate, α-oxoglutarate and succinate. In the absence of added K^+ ions the Q_{O_2} varied from 10·7 to 12·2, whereas with 32·5 mM-K^+ ion the rate had increased from 15·7 to 20·9; it is therefore unlikely that the stimulating effect of K^+ ions is due to facilitation of a stage in substrate oxidation.

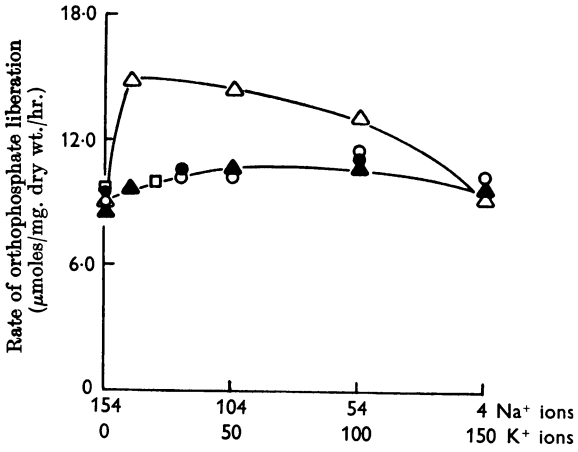


Fig. 3. Synergic stimulation of adenosine-triphosphatase activity in homogenates by Na^+ and K^+ ions, and its inhibition by ouabain and Ca^{2+} ions. Samples of homogenate (0·1–0·5 mg. dry wt.) were incubated for 5 min. in a reaction mixture containing imidazole–HCl buffer, pH 7·8 (15 mM), $MgCl_2$ (2 mM), ATP (disodium salt, buffered to pH 7·8 with tris) (2 mM), and the concentrations of Na^+ and K^+ ions indicated. The Na^+ ions added in the ATP solution have been included in the concentrations shown on the abscissa. The reaction was stopped by adding 1·0 ml. of 25% ice-cold trichloroacetic acid and the precipitated protein spun down. Samples from the supernatant were analysed for orthophosphate by the method of Fiske & Subbarow (1925). △, Control; ▲, with 0·2 mM-ouabain; □, Na^+ ions replaced with choline; ○, with 1 mM-$CaCl_2$; ●, with 1 mM-$CaCl_2$ and 0·2 mM-ouabain. The experiment illustrates a single experiment representative of five such experiments.

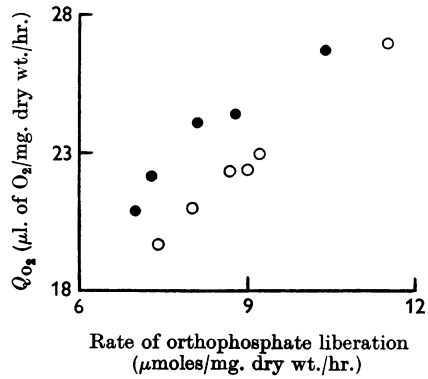


Fig. 4. Inhibition by ouabain of respiration and adenosine-triphosphatase activity in kidney-cortex homogenate. The O_2 consumption was measured on samples of homogenate incubated in the presence of tris-malate buffer, pH 7·8 (10 mM), imidazole–HCl buffer, pH 7·8 (15 mM), $MgCl_2$ (2 mM), ATP (disodium salt, buffered to pH 7·8 with tris) (1·25 mM), NAD (0·125 mM), NaCl (50 mM), KCl (100 mM) and ouabain at concentrations in the range 0–400 μM. Adenosine-triphosphatase activity was measured in the same homogenate after diluting tenfold and allowing the coarse fibres to settle out. The reaction mixture contained $MgCl_2$ (2 mM), ATP (disodium salt, buffered to pH 7·8 with tris) (2 mM), imidazole–HCl buffer, pH 7·8 (15 mM), and the appropriate concentrations of NaCl, KCl and ouabain. The amount of orthophosphate liberated was determined after incubating for 5 min. at 37°. All points are means of duplicate determinations. The Figure illustrates two experiments typical of four such experiments.

Table 2. *Stimulation by Na^+ and K^+ ions of the respiration of kidney homogenates with different substrates*

Samples (1·0 ml.) of homogenate were incubated in a medium containing (final concentrations) imidazole–HCl buffer, pH 7·8 (15 mM), $MgCl_2$ (2 mM), NAD (0·215 mM), ATP (disodium salt, buffered to pH 7·8 with tris) (1·25 mM), and the concentrations of NaCl and KCl indicated. Substrates were present in 10 mM concentration, and with the exception of glucose were used as solutions of the acid neutralized to pH 7·8 with tris. The Table illustrates one of two experiments showing the same result.

Concn. in medium (mM)	KCl	NaCl	Q_{O_2} (μl. of O_2/mg. dry wt./hr.) 0	30	Percentage increase in Q_{O_2}
Substrate					
Glucose			12·0	18·6	55
L-Malate			12·2	20·9	68
α-Oxoglutarate			10·7	17·4	63
Succinate			11·5	15·7	36

Table 3. *Dependence of respiration of kidney-cortex homogenates on the relative concentrations of* Na^+ *and* K^+ *ions*

Samples (1·0 ml.) of homogenate were incubated for 40 min. in 3·0 ml. of medium containing (final concentrations) tris–malate, pH 7·8 (10 mM), imidazole–HCl buffer, pH 7·8 (15 mM), $MgCl_2$ (2 mM), ATP (disodium salt, buffered to pH 7·8 with tris) (1·25 mM), NAD (0·125 mM), and the concentrations of Na^+ and K^+ ions indicated. These include contributions from ATP (2·5 mM-Na^+ ion) and from the homogenate itself (2·5 mM in each ion). Where necessary choline chloride was added in place of Na^+ or K^+ ions to maintain osmolarity.

Expt. no.	Concn. of Na^+ ions (mM)	Concn. of K^+ ions (mM)	Concn. of choline (mM)	$[Na^+]:[K^+]$ ratio	Q_{O_2} (μl. of O_2/mg. dry wt./hr.)
14	105	12·5	40	8·4	8·6
	55	12·5	90	4·4	27·5
	5	12·5	140	0·4	27·8
23	5	2·5	150	2·0	21·0
	5	32·5	120	0·15	20·1
	5	102·5	50	0·05	22·0
	5	152·5	0	0·033	20·0
22A	155	2·5	0	62	12·5
	145	12·5	0	11·6	13·5
	135	22·5	0	6·0	17·5
	125	32·5	0	3·85	19·5
	55	102·5	0	0·54	19·4
	5	152·5	0	0·033	18·9

Table 4. *Inhibitory effects of* Ca^{2+} *ions on the respiration of kidney-cortex homogenate*

Samples (1·0 ml.) of homogenate were incubated for 40 min. in 3·0 ml. of medium containing (final concentration) tris–malate, pH 7·8 (10 mM), imidazole–HCl buffer, pH 7·8 (15 mM), $MgCl_2$ (2 mM), NAD (0·125 mM), and the concentrations of Na^+ and K^+ ions indicated, in which the endogenous concentrations of each are included. ATP (disodium salt, buffered to pH 7·8 with tris) (1·25 mM) and $CaCl_2$ (1 mM) were present where shown. The results are taken from a single experiment, typical of three such experiments.

Concn. of K^+ ions in medium (mM)	Concn. of Na^+ ions in medium (mM)	Q_{O_2} (μl. of O_2/mg. dry wt./hr.)			
		Ca^{2+} ions absent		Ca^{2+} ions present	
		ATP absent	ATP present	ATP absent	ATP present
2·5	152·5	9·0	10·5	6·0	9·0
32·5	122·5	11·0	21·5	6·5	9·5
102·5	52·5	14·0	25·5	6·5	10·0

Effect of relative concentrations of Na^+ *and* K^+ *ions.* To investigate the possible dependence of the rate of respiration on the relative concentrations of Na^+ and K^+ ions, the respiration was measured when the concentration of one ion was held constant while the other was varied, osmolarity being maintained by the addition of choline (Table 3). When the Na^+ ion concentration was constant at 5 mM and that of K^+ ions varied, respiration was maintained at a constant high level. On the other hand, when the K^+ ion concentration was kept at 12·5 mM and the Na^+ ion concentration was increased from 55 to 105 mM, there was a 70% fall in Q_{O_2}. Comparison of the respiratory rates in several experiments reveals a dependence of the Q_{O_2} on the $[Na^+]:[K^+]$ ratio. When the $[Na^+]:[K^+]$ ratio was less than 4–7 the respiration was high, whereas with ratios greater than this there was a fall in Q_{O_2}. The results of previous experiments (e.g., Expt. 22A in Table 3), where the concentrations of Na^+ and K^+ ions were interchanged, agree with this pattern, in showing a fall in respiration with ratios greater than 4–6. These results do not exclude an absolute requirement for less than 2·5 mM-K^+ ion which was always present from endogenous sources.

Effects of Ca^{2+} *ions on homogenates*

Ca^{2+} ions are known to have profound effects on biological processes, especially those in which Na^+ and K^+ ions are involved. It was decided, therefore, to examine their action on the Na^+ ion-plus-K^+ ion-dependent respiration and adenosinetriphosphatase activity of kidney homogenate.

Respiration. The effect of 1 mM-Ca^{2+} ion on respiration was measured at different concentrations of Na^+ and K^+ ions in the presence and absence of added ATP (Table 4). The respiratory stimulation by K^+ ions, both in the presence and in the absence of added ATP, was inhibited by Ca^{2+} ions. When the K^+ ion concentration was increased from 2·5 to 32·5 mM in the absence of ATP, the Q_{O_2} increased from 9·0 to 11·0. In the presence of Ca^{2+} ions the Q_{O_2} was lower (6·0) with 2·5 mM-K^+ ion and increased only to 6·5 on raising the K^+ ion concen-

tration to 30 mM. The abolition by Ca^{2+} ions of the response to 32·5 mM-K^+ ion was more pronounced in the presence of ATP, when the Q_{O_2} increased from 10·5 to 21·5 in a Ca^{2+} ion-free medium and only from 9·0 to 9·5 when Ca^{2+} ions were present. Further increase in the K^+ ion concentration to 102·5 mM caused an additional increase in respiration which was also abolished by 1 mM-Ca^{2+} ions. Thus the increment in Q_{O_2} due to K^+ ions in the absence of Ca^{2+} ions was from 11·0 to 14·0, but in the presence of Ca^{2+} ions there was no increase.

The results also show that the respiratory stimulation by ATP was counteracted by Ca^{2+} ions. The 100 % increase in Q_{O_2}, from 11·0 to 21·5, caused on adding ATP with 32·5 mM-K^+ ion was diminished by Ca^{2+} ions to a 50 % rise, from 6·5 to 9·5. With 102·5 mM-K^+ ion the 70 % rise on the addition of ATP decreased to 35 % when Ca^{2+} ions were present.

These findings show that Ca^{2+} ions affect the respiration of the homogenate in two ways: they prevent the stimulation when the K^+ ion concentration was raised from 2·5 to 32·5 mM, and they inhibit the additional pacemaker operating at concentrations of about 100 mM-K^+ ion plus 50 mM-Na^+ ion.

Adenosine-triphosphatase activity. Adenosine-triphosphatase activity was measured to see if it was similarly affected by Ca^{2+} ions. The results (Fig. 3) show that the adenosine-triphosphatase activity in the presence of 1 mM-Ca^{2+} ion was the same at different concentrations of Na^+ and K^+ ions, in contrast with the synergic stimulation by these ions in the absence of Ca^{2+} ions. Thus the adenosine-triphosphatase activity in the presence of Ca^{2+} ions was identical with that found when either 2·5 mM-Na^+ ion or 2·5 mM-K^+ ion was present, or in the presence of ouabain. When Ca^{2+} ions and ouabain were present together, the inhibition was the same as that found with each separately. It therefore seems that Ca^{2+} ions at a concentration of 1 mM inhibit only the adenosine-triphosphatase activity sensitive to Na^+ ions, K^+ ions and ouabain.

A comparison of the effects of Ca^{2+} ions on the rates of adenosine-triphosphatase activity and of respiration indicates a correlation between the two processes, in that Ca^{2+} ions counteract the increase in both which is sensitive to ouabain and dependent on the combined presence of Na^+ and K^+ ions.

DISCUSSION

Interdependence of adenosine triphosphate hydrolysis and respiration

The results show that the hydrolysis of ATP, catalysed by fragments of cell membranes, is sensitive to stimulation by Na^+ and K^+ ions and to inhibition by ouabain and Ca^{2+} ions. In the homogenate this kind of activity is responsible for 40 % of the total hydrolysis of ATP (Fig. 3), and is associated with an increased rate of respiration that is likewise sensitive to inhibition by Ca^{2+} ions (Fig. 3 and Table 5) and by ouabain (Table 2). Thus partial inhibitions of this adenosine-triphosphatase activity by submaximal concentrations of ouabain are accompanied by similar relative decreases in the rate of respiration, indicating that the latter is regulated by the adenosine-triphosphatase activity. The ADP or orthophosphate resulting from this hydrolysis of ATP evidently stimulates oxygen consumption, which is thus coupled in an obligatory manner to the adenosine-triphosphatase activity associated with the active movements of ions.

A puzzling feature of the ouabain-sensitive components of respiration and adenosine-triphosphatase activity is that each is activated maximally by different Na^+ ion and K^+ ion concentrations. It is conceivable that this arises from the different conditions of assay: up to 400 times more tissue is used for measurement of respiration than is used for measurement of the rate of ATP hydrolysis, and the concentrations of endogenous compounds that affect the processes may likewise be very different. In spite of the disparity in ionic concentrations needed to elicit maximum activity, both processes show identical ouabain sensitivity at the Na^+ ion and K^+ ion concentrations required for optimum respiration.

Besides the Na^+ ion-plus-K^+ ion-activated adenosine-triphosphatase activity, the extramitochondrial fraction of the homogenate shows activity insensitive to Na^+ ions, K^+ ions and ouabain, which comprises some 45 % of the total, the remaining 15 % being found in the mitochondria. The results show that when the tissue is treated as mildly as possible, so as to give a homogenate in which respiration and adenosine-triphosphatase activity can be measured, most of the latter is found outside the mitochondria. Scheme 1 summarizes the connexions between active cation transport, adenosine-triphosphatase activity and respiration suggested by the results of the present study and by work with kidney slices (Whittam & Willis, 1963).

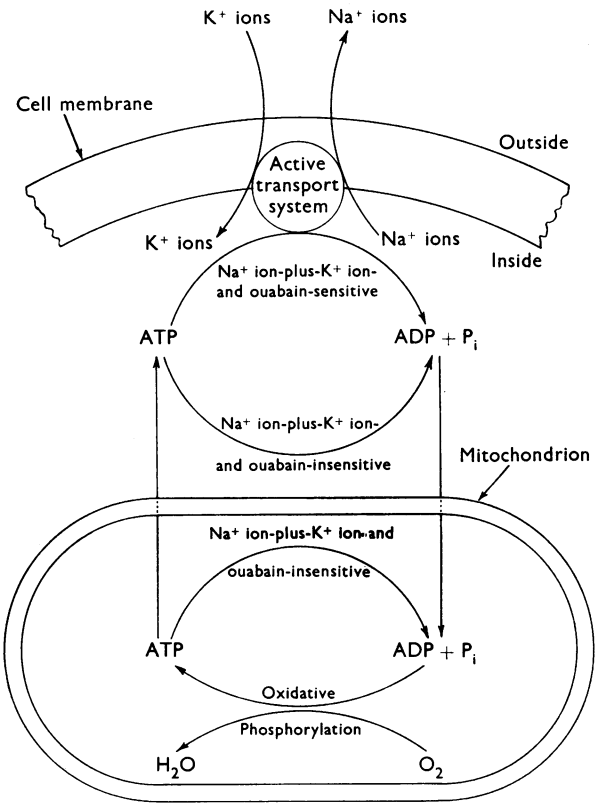
An important basis for Scheme 1 is that the rate of respiration of mitochondria is governed by the concentration of ADP or orthophosphate (see Chance & Williams, 1956). The rate of formation of both ADP and orthophosphate is regulated by the activity of energy-utilizing processes, and ion transport, acting as a membrane adenosine triphosphatase, will therefore be one of the factors controlling their concentration in the cell.

Comparison of studies on homogenates and slices

A comparison can be made of the effect of Na^+ ions, K^+ ions, Ca^{2+} ions and ouabain on the

three aspects of cell function: respiration, active transport and adenosine-triphosphatase activity (Table 5). The respiration of homogenates and slices is affected in a similar way by Na^+ ions, K^+ ions and ouabain, and it is evident from this that the mechanism regulating this component of respiration in slices is still operative in the disrupted tissue. There is a similar close resemblance of the adenosine-triphosphatase activity in the homogenate to that in a membrane-containing fraction (Wheeler & Whittam, 1962).

The combined presence of Na^+ and K^+ ions is required for active transport of either of these ions,



Scheme 1. Coupling between active transport, respiration and adenosine-triphosphatase activity in rabbit kidney cortex. Oxidative phosphorylation in mitochondria provides ATP required to support active transport and other energy-requiring processes in the cell. The ADP resulting from the hydrolysis of this ATP stimulates oxygen consumption, which is therefore coupled in an obligatory manner to active ion transport. The hydrolysis of the ATP by the membrane adenosine triphosphatase represents the overall fission of ATP, and does not exclude the possibility that some other reaction coupled at ATP breakdown, e.g. an oxidoreduction reaction, might be still more closely linked with transport.

and also for the increase in respiration accompanying increased rates of transport. Maximal activity of both the adenosine triphosphatase and the respiration of the homogenate is likewise dependent on the presence of both ions. Further, a similar correspondence can be seen in the effects of ouabain on each of the three processes: inhibition of ion transport is accompanied by parallel decreases in the respiration of slices, and in homogenates, and the decreases of adenosine-triphosphatase activity and of respiration are likewise parallel.

The respiration of mitochondria did not respond to Na^+ ions plus K^+ ions and to ouabain in the same way as did that of slices and homogenates. Ouabain had no effect on mitochondria, suggesting that its inhibitory action in homogenates is through an action of an extramitochondrial factor. A similar conclusion can be drawn for the concerted action of Na^+ and K^+ ions on the homogenate respiration compared with the absence of effect on mitochondria. By analogy with studies on other tissues the locus of action of Na^+ ions plus K^+ ions and of ouabain is most probably the cell membrane.

Effect of Ca^{2+} *ions.* In homogenates, the large decrease in the rate of respiration caused by Ca^{2+} ions contrasts sharply with its lack of effect on the respiration of kidney-cortex slices incubated for a similar period (Cutting & McCance, 1947; Robinson, 1949; Krebs, 1950). Further, Ca^{2+} ions have little effect on the active transport of Na^+ and K^+ ions in slices (Kleinzeller & Cort, 1960), whereas they inhibit the Na^+ ion-plus-K^+ ion-sensitive adenosine triphosphatase in the homogenate. This difference in the effects of Ca^{2+} ions on slices and in homogenates may be the result of the difference in accessibility of intracellular components to Ca^{2+} ions in the two tissue preparations. It may be significant that only the ouabain- and Na^+ ion-plus-K^+ ion-sensitive component of the adenosine-triphosphatase activity is inhibited by 1 mM-Ca^{2+} ion (Fig. 3); in the presence of ouabain this concentration of Ca^{2+} ions does not further diminish the activity. This feature has also been observed in brain-cortex homogenate (Whittam & Blond, 1964).

In spite of qualifications inherent in work with homogenates, there is some quantitative agreement between these results and those obtained in intact slices (Whittam & Willis, 1963). Thus, for example, the relative magnitudes of inhibition by ouabain of the Q_{O_2} and adenosine triphosphatase in homogenates is remarkably similar to that of the Q_{O_2} and K^+ ion accumulation in slices. Also, on the removal of Na^+ ions from the medium a 40 % fall in both the Q_{O_2} of slices and the adenosine-triphosphatase activity of homogenates was found. The combined results on adenosine-triphosphatase activity and the respiration of homogenates on the one hand,

Table 5. *Comparison of the effects of* K^+ *ions,* Na^+ *ions, ouabain and* Ca^{2+} *ions on different types of kidney-cortex preparation*

	Slice		Homogenate		Membrane fraction	Mitochondria	
Function ...	Active transport	Oxygen uptake	Oxygen uptake	Adenosine-triphosphatase activity	Adenosine-triphosphatase activity	Adenosine-triphosphatase activity	Oxygen uptake
Agent							
K^+ ions	Stimulates Na^+ ion transport	←	Synergic stimulation with Na^+ ions	→	→	No effect	Stimulates
Na^+ ions	Stimulates K^+ ion transport	←	Synergic stimulation with K^+ ions	→	→	No effect	Opposes K^+ ion stimulation
Ouabain	Inhibits Na^+ ion and K^+ ion transport	←	Inhibits stimulation due to Na^+ ions plus K^+ ions	→	→	No effect	No effect
Ca^{2+} ions	←No effect (when Ca^{2+} ions in medium)→		←Inhibits stimulation due to Na^+ ions plus K^+ ions		→	—	Opposes K^+ ion stimulation

and the K^+ ion uptake and respiration of slices on the other, therefore suggest that about 30–40 % of the energy production is devoted to active transport, and that this is the fraction that is in turn controlled by the active transport process. It should be noted, however, that this estimate of the percentage of the energy used for active cation transport refers specifically to these experiments and those with slices in which malate or glucose is the sole metabolite added. Under these conditions the energy consumed by other processes, e.g. chemical synthesis, is probably small. On the other hand, when the rate of synthesis is high, the total demand for energy will be raised and its rate of production will be correspondingly increased. The proportion of the total energy output that is utilized by active cation transport will then be lower, although the absolute consumption will remain unchanged.

Mitochondrial respiration

The results in Fig. 2 throw light on the question of whether Na^+ ions, K^+ ions and ouabain affect respiration directly. Ouabain was without effect on the respiration and the rate of ATP hydrolysis by mitochondria, giving evidence that its inhibition of the respiration in the homogenate was due to an action at an extramitochondrial site. This conclusion is in keeping with the independent demonstration that the locus of action of ouabain is the external surface of the squid giant axon (Caldwell & Keynes, 1959) and the erythrocyte membrane (Glynn, 1957; Dunham & Glynn, 1961).

Although Na^+ and K^+ ions were without influence on the adenosine-triphosphatase activity of mitochondria, they did affect the respiration. The K^+ ion-stimulated respiration of mitochondria differed from that of the homogenate in not being inhibited by ouabain or dependent on Na^+ ions. It does not appear that access of substrate was a factor limiting the Q_{O_2}, nor was the stimulation dependent on a particular substrate (Table 2). Two possible explanations would appear to be, first, that the accumulation of K^+ ions by mitochondria, which is an energy-dependent process (Gamble, 1957), may result in an increased Q_{O_2}. This is somewhat improbable, since K^+ ion concentrations that stimulate oxygen uptake do not stimulate the rate of ATP hydrolysis in the mitochondria, and, further, the respiratory response to K^+ ions is counteracted by Na^+ ions, whereas Na^+ ions do not affect the mitochondrial energy-dependent binding of K^+ ions (Stanbury & Mudge, 1953). A second explanation is that K^+ ions might increase the magnitude of the respiratory response to ADP or orthophosphate, perhaps by enhancing the tightness of coupling of oxidative phosphorylation to respiration.

SUMMARY

1. A study has been made of the effect on the respiration and adenosine-triphosphatase activity of rabbit-kidney-cortex homogenates and mitochondria of some factors (Na^+ ions, K^+ ions, Ca^{2+} ions and ouabain) that influence respiration and ion movements in tissue slices.

2. The Q_{O_2} of the homogenate showed a dual response to variations in K^+ ion and Na^+ ion concentrations. First, when the K^+ ion concentration was increased from 2·5 to 32·5 mM there was a marked rise in Q_{O_2}, the magnitude of which was dependent on the relative concentrations of Na^+ and K^+ ions; it was not prevented by ouabain or the absence of Na^+ ions, and was found with different respiratory substrates. Secondly, a further increase in K^+ ion concentration caused an additional rise in Q_{O_2} which was abolished by ouabain or the absence of Na^+ ions. Ca^{2+} ions prevented both responses to increased K^+ ion concentrations.

3. The respiration of mitochondria was stimulated by K^+ ion concentrations up to 30 mM; ouabain or the lack of Na^+ ions was without effect. A further increase in K^+ ion concentration did not alter the respiration.

4. Adenosine-triphosphatase activity in homogenates was stimulated by Na^+ and K^+ ions together, and was inhibited by ouabain or Ca^{2+} ions and by the absence of either Na^+ or K^+ ions; that of the mitochondrial fraction was unaffected by Na^+ ions, K^+ ions or ouabain.

5. Concentrations of ouabain giving submaximal inhibition caused parallel decreases in the oxygen consumption and adenosine-triphosphatase activity of homogenates.

6. It is concluded that an extramitochondrial adenosine triphosphatase, sensitive to Na^+ ions, K^+ ions, Ca^{2+} ions and ouabain, is one of the pacemakers of the respiration of kidney cortex.

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Repressors of Sulphate Activation in *Escherichia coli*

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The formation of the enzyme system (ATP–sulphate adenylyltransferase, EC 2.7.7.4, and ATP–adenylylsulphate 3′-phosphotransferase, EC 2.7.1.25) that catalyses the synthesis of PAPS* from inorganic sulphate and ATP (Lipmann, 1958) is repressed in *Escherichia coli* by the addition of L-cystine to the growth medium (Pasternak, 1961, 1962). The suggestion (Peck, 1962) that these enzymes are not repressed by cystine needs revision. The addition of probable intermediates in the reduction of sulphate to cysteine and of other sulphur-containing compounds also represses the sulphate-activating system (Ellis & Pasternak, 1962). The experiments reported below make it likely that a common metabolite, namely cysteine, is the actual repressing agent in every case (Ellis & Pasternak 1962).

* Abbreviation: PAPS, adenosine 3′-phosphate 5′-sulphatophosphate.