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Active Transport of Water, Sodium, Potassium and α -Oxoglutarate by Kidney-cortex Slices

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Previous experiments from this Department (Terner, Eggleston & Krebs, 1950; Krebs, Eggleston & Terner, 1951) and by Mudge (1951*a, b*) and Aebi (1953) have shown that slices of some animal tissues suspended in a saline medium may lose or gain potassium ions according to the conditions. The present work with kidney-cortex slices is a study of the movements of sodium ions and α -oxoglutarate ions (added as substrate) and of water which accompany the movements of potassium. Part of this work has been communicated to the Biochemical Society (Whittam & Davies, 1953).

EXPERIMENTAL

Material and procedure. As it was necessary to measure the fresh weight of tissue slices before incubation, contact between tissue and medium during the slicing had to be avoided. Guinea-pigs were stunned and bled from the carotids. The kidneys were at once removed, cut longitudinally and the cortex was sliced with a dry razor according to the method of Deutsch (1936). Slices, 0.3–0.4 mm. thick, were spread on hardened filter paper (Whatman no. 541) and rapidly blotted to remove blood, urine and cell debris. The outer slices were usually rejected; samples (100–200 mg.) of the inner slices were then weighed on a torsion balance and put into 2.0 ml. of medium in 15 ml. stoppered conical cups. In anaerobic experiments, a stick of yellow phosphorus was placed in the centre well. The cups were shaken at 130 oscillations/min. (amplitude 2 cm.) at

37° in a Dubnoff Metabolic Shaking Incubator (Precision Scientific Company, Chicago, U.S.A.), or at 0° in a mechanical rocker. After incubation the slices were removed, rapidly blotted and reweighed.

Media. Phosphate salines were used when media, gassed with O₂, were required at various pH values, which were checked with a glass electrode before and after incubation. They were similar to medium III of Krebs (1950*b*), without the addition of the organic substrates. In all other experiments, bicarbonate saline (Krebs & Henseleit, 1932) gassed with 5% CO₂ and either 95% O₂ or 95% N₂ at 37°, or 2.8% CO₂ and either 97.2% O₂ or 97.2% N₂ at 0° was used. The pH at both 37 and 0° was thus 7.4. The media used contained 6mm-K, 155–160mm-Na and usually 10mm α -oxoglutarate as substrate. A hypotonic (approx. 0.2 osm) medium was made by diluting 2 vol. of the medium with 1 vol. of water. (The osmolar concentration (osm) is obtained by adding together the molar concentrations of all the ions present, cf. Robinson, 1950.)

Water content of tissue. The tissue water was taken to be equal to the loss of weight on drying the tissue overnight at 105°.

Determination of α -oxoglutarate. The slices, or 1 ml. samples of the media, were each placed in 10 ml. centrifuge tubes containing 2.0 ml. of 10% (w/v) trichloroacetic acid (TCA). After breaking up the tissue with a glass rod the tubes were centrifuged. Samples of the supernatants were removed (0.1 ml. from the media, 1.0 ml. from the tissue extracts) and the volume made to 4.0 ml. with TCA. The tubes were then placed for 5 min. in a boiling-water bath to decompose any acetoacetic or oxaloacetic acids present (Markees, 1951; Lichtenbell & Florijn, 1952; Kogut & Podoski, 1953).

α -Oxoglutaric acid was estimated by the method of Goodwin & Williams (1952), which gave reproducible results only when freshly distilled diethyl ether was used for the extraction of the 2:4-dinitrophenylhydrazones.

Determination of sodium and potassium. Samples (0.4–0.5 ml.) of the TCA extracts of the tissue were diluted to 10.0 ml. either with 0.0382% (w/v) KCl (20 mg. K/100 ml.) or 0.84% (w/v) NaCl (330 mg. Na/100 ml.) for estimations of Na or K by flame photometry according to the method of Domingo & Klyne (1949) with the modifications of Turner *et al.* (1950). Samples of the TCA extracts of the medium (0.15 ml. for Na and 1.5 ml. for K) were also diluted to 10 ml. as above. In cases where the water content had been determined first, an amount of 16N-HNO₃ about equal to the wet weight of the tissue was added to the dried tissue and the mixture either left overnight at room temperature or kept for 30 min. at 100°. The acid digest was diluted to 15.0 ml. with water, and 5.0 ml. samples were used for analysis. For estimations of Na, 0.4 ml. of 0.955% (w/v) KCl and 4.6 ml. of water were added before flame photometry; for estimations of K, 0.5 ml. of 16.8% (w/v) NaCl and 4.5 ml. of water were added.

For estimations of Na in the medium or plasma, 0.05 ml. was diluted to 10.0 ml. with 0.0382% (w/v) KCl. For estimations of K in the medium or plasma, 0.5 ml. was diluted to 10.0 ml. with 0.84% (w/v) NaCl.

These procedures gave the same values as those of Turner *et al.* (1950) which were designed for the determination of only either Na or K in the samples. Neither the TCA nor the HNO₃ in the quantities used interfered with the estimations. Each reading was preceded and followed by a standard solution, and at least four readings were taken of each sample in all cases. The standard error of the mean values for each sample was about 1%.

RESULTS

Sodium and potassium contents of kidney cortex and plasma in vivo

Table 1 gives values for the sodium and potassium contents of kidney-cortex slices before incubation. Inner slices of the tissue were found to contain more sodium (10.2 ± 3.0 μ moles/g. tissue), and less potassium (13.0 ± 2.3 μ moles/g. tissue) than the first outer slices, although the difference of the sums of sodium and potassium contents was not significant (2.8 ± 3.9 μ moles/g. tissue). The outer slices, which were uncut on one side, and different from the rest of the cortex, were not used in the following experiments. The sodium and potassium concentrations in plasma were $144 \pm 0.65(4)$ mM and $7.30 \pm 0.089(4)$ mM, respectively.

Changes in the sodium, potassium and water contents of kidney-cortex slices on incubation in vitro

Aerobic incubation at 37°. About one-fifth of the tissue potassium was replaced by sodium after 40 min. incubation when no substrate was added (Table 2). When 10 mM α -oxoglutarate was present, the potassium content of the tissue was the same as the initial one; the sodium content was increased.

Table 1. Sodium, potassium and water contents of guinea pig kidney-cortex slices before and after incubation in vitro

(Slices of tissue (0.1–0.2 g.) incubated 30–50 min. in 2.0 ml. of bicarbonate saline, pH 7.4, containing 10 mM α -oxoglutarate, and gassed with 5% CO₂ + either 95% O₂ or 95% N₂ at 37°, or 2.8% CO₂ + either 97.2% O₂ or 97.2% N₂ at 0°. Mean values given $\pm$ s.e. with number of observations in parentheses. The concentration gradient is the ratio: concentration in tissue water/concentration in external medium. For other details see text.)

Material	Experimental conditions	Temp. of incubation (°)	Amounts of cations in tissue (μ moles/g. tissue)			Water content of tissue (%)	Excess amounts of Na ⁺ + K ⁺ in tissue relative to medium		Concentration gradient of Na ⁺ + K ⁺ 1.21 $\pm$ 0.01*
			Na ⁺	K ⁺	Na ⁺ + K ⁺		μ moles/g. tissue	μ moles/g. water	
Outer slices	Before incubation	—	53.2 ± 2.3 (15)	89.4 ± 2.1 (17)	142.6 ± 3.3 (15)	76.9 ± 0.6 (4)	32*	25*	1.21 $\pm$ 0.01*
	Before incubation	—	63.4 ± 1.9 (14)	76.4 ± 0.9 (19)	139.8 ± 2.1 (14)	77.7 ± 0.2 (19)	27*	21*	1.18 $\pm$ 0.01*
Inner slices	Aerobic	37	90.2 ± 1.8 (24)	75.3 ± 1.6 (24)	165.5 ± 2.4 (24)	79.7 ± 0.3 (24)	46	37	1.29 $\pm$ 0.03
		0	112 ± 2.9 (13)	30.9 ± 0.7 (13)	142.9 ± 3.0 (13)	83.0 ± 0.5 (7)	11	9.2	1.07 $\pm$ 0.04
	Anaerobic	37	114 ± 2.2 (9)	27.5 ± 1.0 (9)	141.5 ± 2.5 (9)	82.7 ± 0.3 (4)	10	9.1	1.06 $\pm$ 0.03
		0	109 ± 2.9 (7)	32.2 ± 1.7 (7)	141.2 ± 3.3 (7)	83.5 (2)	8	9.0	1.05 $\pm$ 0.04

* These values are relative to plasma.

Table 2. *Changes in the sodium and potassium contents of guinea pig kidney-cortex slices on incubation in vitro*

(Slices of tissue (0.1–0.2 g.) incubated in 2.0 ml. of bicarbonate saline, pH 7.4; gas phase, 5% CO₂ + either 95% O₂ or 95% N₂ at 37°.)

Expt. no.	Experimental conditions	Concentration of α -oxo-glutarate in medium before incubation (mM)	Concentration of 2:4-dinitro-phenol in medium before incubation (mM)	Period of incubation (min.)	Amounts of cations in tissue (μ moles/g. tissue)					
					Before incubation			After incubation		
					Na ⁺	K ⁺	Na ⁺ + K ⁺	Na ⁺	K ⁺	Na ⁺ + K ⁺
1	Aerobic	0	0	40	56.0	78.8	134.8	74.5	63.3	137.8
		2	0	40	56.0	78.8	134.8	76.3	65.8	142.1
		4	0	40	56.0	78.8	134.8	75.4	73.7	149.1
		6	0	40	56.0	78.8	134.8	75.6	78.3	153.9
		8	0	40	56.0	78.8	134.8	74.3	78.5	152.8
		10	0	40	56.0	78.8	134.8	84.8	79.5	164.3
		10*	0	40	56.0	78.8	134.8	78.9	81.8	160.7
2	Aerobic	10	0	35	62.6	75.7	138.3	91.8	71.3	163.1
		10	0	50	62.6	75.7	138.3	87.6	71.8	159.4
		10	0.2	35	62.6	75.7	138.3	110	39.9	149.9
		10	0.2	50	62.6	75.7	138.3	112	35.4	147.4
		10	0.2†	50	62.6	75.7	138.3	107	42.6	149.6
		10	0	40	61.4	77.9	139.3	91.8	71.3	163.1
3	Aerobic	10	0	50	61.4	77.9	139.3	87.6	71.8	159.4
		10	0	90	61.4	77.9	139.3	88.9	56.6	147.5
		10	0	120	61.4	77.9	139.3	98.5	56.2	154.7
		10	0	240	61.4	77.9	139.3	130	27.2	157.2
		10	0	360	61.4	77.9	139.3	134	23.8	157.8
		10	0	2	68.2	76.1	144.3	95.8	47.1	142.9
		10	0	5	68.2	76.1	144.3	104	39.8	143.8
4	Anaerobic	10	0	16	68.2	76.1	144.3	116	29.2	145.2
		10	0	34	68.2	76.1	144.3	120	29.8	149.8
		10	0	120	68.2	76.1	144.3	131	14.5	145.5
		10	0	240	68.2	76.1	144.3	145	11.2	156.2
		10	0	360	68.2	76.1	144.3	141	9.8	150.8

* The saline also contained 20 mM glucose.

† 2:4-Dinitrophenol added after 35 min. incubation.

Table 3. *Effect of pH on sodium, potassium and water contents of guinea pig kidney-cortex slices on incubation in vitro*

(Slices of tissue (0.1–0.2 g.) were washed in three 10 ml. portions of phosphate saline of appropriate pH and then incubated for 40 min. at 37° in 4.0 ml. of phosphate saline containing 10 mM α -oxoglutarate; gas phase O₂; 0.2 ml. of 2N-NaOH in centre well. The concentration gradient is the ratio: concentration in tissue water/concentration in external medium.)

Experimental conditions	pH of medium during incubation	Amounts of cations in tissue (μ moles/g. tissue)			Water content of tissue (%)	Concentrations of cations in tissue water (μ moles/g. tissue water)			Concentration gradient of cations		
		Na ⁺	K ⁺	Na ⁺ + K ⁺		Na ⁺	K ⁺	Na ⁺ + K ⁺	Na ⁺	K ⁺	Na ⁺ + K ⁺
		Na ⁺	K ⁺	Na ⁺ + K ⁺		Na ⁺	K ⁺	Na ⁺ + K ⁺	Na ⁺	K ⁺	Na ⁺ + K ⁺
Slices before incubation		55.7	76.7	132.4	77.9	71.5	98.5	170.0	0.50*	13.5*	1.13*
Slices after incubation	3.6	112	9.1	121.1	84.1	133	10.8	143.8	0.86	1.35	0.88
	4.6	93.8	45.1	138.9	80.6	116	55.9	171.9	0.75	6.8	1.06
	5.7	83.5	58.7	142.2	79.5	105	73.8	178.8	0.67	10.0	1.10
	5.9	73.0	66.7	139.7	78.1	93.5	85.3	178.8	0.60	14.0	1.10
	6.9	77.7	83.8	161.5	80.9	95.0	104	199.0	0.61	18.2	1.23
	7.5	93.5	72.2	165.7	80.6	116	89.5	205.5	0.74	15.7	1.27
	7.6	88.6	71.2	159.8	79.7	111	89.2	200.2	0.71	15.7	1.24
	7.8	79.2	76.1	155.3	79.9	99.1	95.3	194.4	0.64	16.7	1.20
	8.3	98.0	47.7	145.7	82.3	119	58.0	177.0	0.76	7.0	1.09
	9.4	117	23.8	140.8	84.0	139	28.3	167.3	0.89	3.3	1.03

* These values are relative to plasma.

Glucose had no additional effect. After incubation in media with lower concentrations of α -oxoglutarate, the slices contained less potassium and more sodium than initially (Table 2).

The time curves of the sodium and potassium contents during incubation with 10 mM α -oxoglutarate are shown in Fig. 1. There was an initial gain of sodium and loss of potassium during the first 5 min., but this was largely reversed after 20–30 min. incubation, after which a steady state was maintained for at least a further 20 min. The sodium, potassium and water contents in this steady state are also shown in Table 1. They represent changes from the initial state of $+26.8 \pm 2.6 \mu\text{moles/g.}$ tissue for sodium and $-1.1 \pm 1.9 \mu\text{moles/g.}$ tissue for potassium and $+2.0 \pm 0.4$ for the percentage water content. The increase in the sum of sodium and potassium contents was thus $+25.7 \pm 3.2 \mu\text{moles/g.}$ tissue and this was all due to a net uptake of sodium in excess of that required to balance the potassium changes. After incubations of more than 50 min. the sodium and potassium contents of the tissue progressively increased and decreased, respectively (Table 2).

Table 3 gives the sodium, potassium and water contents of tissue after 40 min. incubation in phosphate media of pH values between 3.6 and 9.4. The optimum pH for maintaining the approximate physiological concentrations of sodium, potassium and water was not critical and about pH 7. At this pH the potassium concentration of the tissue water was about 1600 % greater than that of the medium and the sodium concentration was about 40 % less than that of the medium. The sum of sodium and potassium concentrations in the tissue water was 23 % greater than that of the medium. This was similar to the effect observed after 40 min. incubation in physiological bicarbonate saline when the comparable figure was 29 % (Table 1); in both cases there was thus an increase in the sum of sodium and potassium concentrations of the tissue water.

At higher or lower pH values than 7 the concentration differences of sodium and potassium between the tissue water and medium became progressively smaller but did not completely disappear. Even at pH 3.6, there was no complete levelling out, the potassium concentration of the tissue water being 35 % greater than that of the medium and the sodium concentration 14 % less than that of the medium.

In contrast to the uneven distributions of the individual ions between the tissue water and medium, the sum of the sodium and potassium concentrations became progressively more constant at pH values increasingly higher or lower than 7. At pH 9.4 the sum of sodium and potassium concentrations in the tissue water and medium was approximately the same. At pH 3.6, however, the sum of the sodium and potassium concentrations of the

tissue water was 12 % less than that of the medium. This may be a result of the decrease in the number of anions of high molecular weight in the tissue.

2:4-Dinitrophenol caused the tissue to gain sodium and lose potassium when it was added after the slices had reached the steady state after 35 min. incubation (Table 2). When added at the start of the incubation it did not affect the initial leakages but prevented both the subsequent extrusion of sodium and the accumulation of potassium by the tissue.

Fig. 1. Changes in the sodium, potassium and α -oxoglutarate contents of guinea pig kidney-cortex slices during incubation *in vitro*. Slices of tissue (0.1–0.2 g.) incubated in 2.0 ml. bicarbonate saline, pH 7.4, containing 10 mM α -oxoglutarate and gassed with 5% CO_2 + 95% O_2 , temp. 37° .

Aerobic incubation at 0° . As at 37° , potassium was lost and sodium taken up at 0° , but at this temperature the tissue did not reverse these changes nor was there an increase in the sum of sodium and potassium contents. After from 30 to 50 min. incubation the potassium content was 40 % of the initial value (Table 1); after 6 hr. it was 26 % whilst slices frozen and thawed before incubation contained only 17 % of the initial amount (Table 4). Since the sum of the sodium and potassium contents remained the same as initially, the changes in the sodium content were equal and opposite to those of potassium. Thus the sodium content increased by 77 % after from 30 to 50 min. incubation and by 112 % after 6 hr. incubation, whilst previously frozen and thawed tissue contained 120 % more sodium than the initial tissue after 6 hr. incubation (Tables 1 and 4).

The ratio of the sum of sodium and potassium concentrations in tissue water/the sum of sodium and potassium concentrations in medium, which was 1.29 after incubation at 37°, was 1.07 after from 30 to 50 min. incubation at 0° and 1.05 after 6 hr. incubation at this temperature, whilst slices frozen and thawed before incubation at 0° gave a ratio of 0.99 (Table 4). Whilst the sum of the concentrations of sodium and potassium became the same in the tissue water and medium, the concentrations of the individual cations did not become equal. After 6 hr. incubation the potassium concentration of the tissue water was 160 % above that of the medium, and it was still 40 % greater for slices frozen and thawed before incubation (Table 4). The distribution of sodium between the tissue water and the medium was also uneven, the sodium concentration of the tissue water being lower than that of the medium by 13 % after about 40 min. incubation and 7 % after 6 hr. incubation.

The changes in sodium, potassium and water contents at 0° were reversed by subsequent incubation at 37° (Table 5). The initial potassium content

was completely restored. The sodium content fell but did not reach the original level, probably because of an uptake of α -oxoglutarate at 37° which, as will be shown later, may also account for the rise in the sum of sodium and potassium contents.

Anaerobic incubation. When the tissue was incubated under anaerobic conditions there was a rapid initial gain of sodium and loss of potassium similar to that under aerobic conditions, but whilst aerobically at 37° these changes were reversed on prolonged incubation, they became progressively greater anaerobically. The rate of change in sodium and potassium contents became slow after the first 30 min. incubation, whilst the sum of sodium and potassium contents remained about the same as that of the initial tissue after various periods of incubation (Table 2).

The tissue contained about 40 % of the initial potassium after from 30 to 50 min. incubation at either 37° or 0°. After 6 hr. incubation more potassium was lost at 37° than at 0°, the amounts then present being 13 and 23 % of the initial value, respectively. In all cases the change in sodium

Table 4. *Changes in the sodium, potassium and water contents of guinea pig kidney-cortex slices on incubation at 0°*

(Slices of tissue (0.1–0.2 g.) incubated for 6 hr. in 2.0 ml. of bicarbonate saline, pH 7.4, containing 10 mM α -oxoglutarate; gas phase, 2.8 % CO₂ + 97.2 % O₂ or 97.2 % N₂. Some slices, referred to as 'frozen' tissue, were alternately frozen on solid CO₂ and thawed four times before being incubated. The concentration gradient is the ratio: concentration in tissue water/concentration in external medium.)

Material	Treatment of tissue	Amounts of cations in tissue (μ moles/g. tissue)			Water content of tissue (%)	Concentration gradient		
		Na ⁺	K ⁺	Na ⁺ + K ⁺		Na ⁺	K ⁺	Na ⁺ + K ⁺
Slices before incubation		55.1	76.7	131.8	77.9	0.49*	13.3*	1.11*
Slices after incubation	Untreated, aerobic	117	20.3	137.3	84.8	0.93	2.6	1.05
	'Frozen', aerobic	121	13.0	134.0	87.4	0.95	1.4	0.99
	Untreated, anaerobic	111	17.8	128.8	85.3	0.89	2.4	0.98
	'Frozen', anaerobic	121	12.3	133.3	87.8	0.94	1.3	1.00

* These values are relative to plasma.

Table 5. *Reversibility of the changes in sodium, potassium and water contents of guinea pig kidney-cortex slices during incubation in vitro*

(Slices of tissue (0.1–0.2 g.) incubated in 2.0 ml. of bicarbonate saline, pH 7.4, containing 10 mM α -oxoglutarate, gassed with 5 % CO₂ + either 95 % O₂ or 95 % N₂ at 37°, or 2.8 % CO₂ + either 97.2 % O₂ or 97.2 % N₂ at 0°.)

Expt. no.	Material	Experimental conditions	Amounts of cations in tissue (μ moles/g. tissue)			Water content of tissue (%)
			Na ⁺	K ⁺	Na ⁺ + K ⁺	
1	Slices before incubation	—	62.8	74.9	137.7	79.1
	Slices after incubation	27 min. at 0° (aerobic)	109	32.6	141.6	84.5
		28 min. at 0° then 54 min. at 37° (aerobic)	99	73.6	172.6	80.1
2	Slices before incubation	—	57.3	77.3	134.6	76.8
	Slices after incubation	34 min. anaerobic at 37°	120	29.8	149.8	82.0
		15 min. anaerobic then 32 min. aerobic at 37°	83.4	83.0	166.4	77.2

content was approximately equal and in an opposite direction to the change in potassium content (Tables 1, 2 and 4).

The ratio of the concentration of sodium or potassium in tissue water/the concentration of sodium or potassium in the medium, changed towards unity but did not reach it at either 37° or 0° with increasing periods of incubation. The nearest approach to unity was after 6 hr. incubation at 37°, when the potassium of the tissue water was only 20 % more than that of the medium; at 0° the comparable value was 140 %. Thus, in anaerobic conditions there was a more uniform distribution of sodium and potassium between the tissue water and medium at 37° than there was at 0°. Hence, as was also the case with tissue incubated aerobically at 0°, the sum of the concentrations of sodium and potassium in the tissue water approached that of the medium, whilst the separate distributions of sodium and potassium remained uneven.

Table 5 shows that the swelling of tissue and the changes in sodium and potassium contents during anaerobic incubation at 37° were reversed when the conditions were made aerobic.

Incubation in hypotonic medium. Swelling of kidney slices on incubation in saline medium (40 min.; 37°), as might be expected, was greater in hypotonic medium (0.2 osm) than isotonic medium (0.3 osm), the increases in water content from the initial state being 3.6 and 2.0 %, respectively. Despite this difference in swelling, the excess in the sum of sodium and potassium contents of the tissue relative to the medium was about 37 μ moles/g. tissue in each case. The sums of the concentrations of sodium and potassium in the tissue water were 38 and 29 % greater than those of the medium after incubation in 0.2 and 0.3 osm media, respectively, and these values are similar to those reported by Robinson (1952a) for the ratio of 'fixed base' between tissue water and suspending medium.

To find the rates of change of sodium, potassium and water contents, slices were pre-incubated under aerobic conditions at 37° for 35 min. in an isotonic medium, in order to allow the completion of the recovery processes, and then transferred to the hypotonic medium at 0°. The subsequent changes in sodium, potassium and water contents at 0° are shown in Fig. 2. The swelling of the tissue and the decrease in the sum of sodium and potassium contents were concomitant and complete within about 10 min., although slow changes in individual sodium and potassium contents still occurred even after 2 hr. There was a very rapid extrusion of sodium against a gradient before the subsequent uptake.

The sum of sodium and potassium concentrations of the tissue water at 0° in 0.2 osm medium was 23 % greater than that of the medium, whereas in isotonic medium (0.3 osm) the comparable figure

was 7 %. Thus, despite the fall in the sum of sodium and potassium contents of the tissue on transfer to the hypotonic medium, the excess of these ions over the content in the medium was 22 μ moles/g. tissue; in isotonic medium this excess was only 9.2 μ moles/g. tissue (Table 1). This difference would not be expected if the excess of cations were unexchangeably bound in the tissue.

Fig. 2. Changes in sodium and potassium contents and relative swelling of guinea pig kidney-cortex slices on incubation at 0°. Slices of tissue (0.1–0.2 g.) incubated in 2.0 ml. of isotonic (0.3 osm) bicarbonate saline, pH 7.4, at 37° for 35 min. and then transferred to 2.0 ml. of hypotonic (0.2 osm) bicarbonate saline, pH 7.4, at 0°. Relative swelling is $(100 - W)/(100 - W')$, where W and W' are water contents of the slices before and after incubation respectively.

Transport and metabolism of α -oxoglutarate

The α -oxoglutarate content of fresh guinea pig kidney cortex was 0.12 μ mole/g. tissue (cf. Krebs, 1950a; LePage, 1950; Frohman, Orten & Smith, 1951) and was unaltered when the tissue was sliced. Table 6 and Fig. 1 show the amounts of α -oxoglutarate accumulated and metabolized by incubated tissue slices. During aerobic incubation at 37° for 40 min., the concentration of α -oxoglutarate in the tissue water reached a level 2.6 times greater than that of the medium. The quantity of α -oxoglutarate accumulated by the tissue during this time was 12.0 ± 0.6 μ moles/g. tissue. Under the

Table 6. *Accumulation and metabolism of α -oxoglutarate in guinea pig kidney-cortex slices during incubation in vitro*

(Slices of tissue (0.1–0.2 g.) incubated 40 min. in 2.0 ml. of bicarbonate saline, pH 7.4, containing 10 mM α -oxoglutarate; gas phase, 5% CO₂ + either 95% O₂ or 95% N₂ at 37°, or 2.8% CO₂ + either 97.2% O₂ or 97.2% N₂ at 0°. Determinations of α -oxoglutarate were made on incubated tissues and on media before and after incubation. The concentration gradient is the ratio: concentration in tissue water/concentration in external medium. Mean values are given $\pm$ s.e. The figures in parentheses represent the number of observations.)

Experimental conditions	Temp. of incubation (°)	Accumulation of α -oxoglutarate in tissue (μ moles/g. tissue)	Amount of α -oxoglutarate in medium after incubation (μ moles/ml.)	$Q_{\alpha\text{-oxoglutarate}}$ (μ l./mg. dry wt./hr.)	Concentration gradient of α -oxoglutarate
Aerobic	37	12.0 $\pm$ 0.6 (13)	5.8 $\pm$ 0.4 (15)	-8.9 $\pm$ 0.9 (15)	2.6 $\pm$ 0.2
Anaerobic	37	3.1 (3)	7.6 (3)	-4.7 (3)	0.49
Aerobic	0	2.3 (3)	8.4 (3)	-3.1 (2)	0.33
Anaerobic	0	2.1 (2)	9.1 (2)	-1.1 (2)	0.28

same conditions, the increase in the sum of sodium and potassium contents was 25.7 ± 3.2 μ moles/g. tissue (see earlier). So the ratio of this increase/increase in α -oxoglutarate content was 2.1 ± 0.3 . This means that equivalent net quantities of monovalent cation and divalent anion had been accumulated. However, this net uptake of anion must be less than the gross uptake, because during this period 53.3 ± 5.5 μ moles α -oxoglutarate/g. tissue had been metabolized. This is more than 4 times the amount found in the tissue after incubation.

In contrast, much less α -oxoglutarate was found in the tissue than in the medium and much less was metabolized during aerobic or anaerobic incubation at 0°, or anaerobic incubation at 37° (Table 6).

DISCUSSION

Electrochemical equivalent of potassium movements.

The experiments of Terner *et al.* (1950) and Krebs *et al.* (1951) have shown that brain- and kidney-cortex slices lose potassium but can largely recover it when the suspending medium contains L-glutamate and glucose, or α -oxoglutarate. These findings raise the question of the electrochemically equivalent change which must accompany the movement of potassium to maintain electro-neutrality. The present data indicate that potassium ions are exchanged for sodium ions: the movement of potassium is accompanied by a movement of sodium in the opposite direction (see also Mudge, 1951*a, b*, and Aebi, 1953).

Effect of energy supply. Conditions which either reduce energy production (anoxia, cooling) or energy utilization (addition of 2,4-dinitrophenol) prevent the transport of potassium and sodium in the recovery period. These movements, which are against concentration gradients and in opposite

directions, thus represent an active transport. Unlike red blood cells, kidney slices cannot obtain sufficient energy for active transport from anaerobic glycolysis, as might be expected from the relatively low rate of glycolysis in kidney cortex. Unlike brain, kidney does not require glucose for optimum energy production, which is again expected, because kidney contains much greater quantities of oxidizable substrates than brain.

Concentration differences not dependent on energy supply. There remains an uneven distribution of sodium and potassium between tissue water and medium, even in the virtual absence of energy supplies, namely at 0° or anaerobically. This indicates that concentration gradients of some sodium and potassium ions can be maintained by mechanisms not requiring the expenditure of energy. This applies to about 12% of the tissue sodium and 4% of the tissue potassium. The phenomenon may be due to a Donnan effect or, what is not fundamentally different, to properties of cell constituents similar to those of ion-exchange resins. A simple Donnan effect would require that the ratios of the sodium concentration in tissue water/the sodium concentration in medium, and the potassium concentration in tissue water/the potassium concentration in medium, are the same. In fact, that for potassium is greater. However, such effects are also known in the case of some exchange resins and are attributed to preferential binding of potassium due to the smaller radius of the hydrated ion (Boyd, Schubert & Adamson, 1947).

Transport of α -oxoglutarate. The movement of sodium cannot all be explained as a counterbalance of potassium movement because more sodium than required can be taken up by the tissue under aerobic conditions. The surplus sodium can be accounted for as the cationic equivalent of α -oxoglutarate which

is accumulated concomitantly. An accumulation of α -oxoglutarate in the tissue cannot be explained by a Donnan effect. Being an anion, the α -oxoglutarate would be expected to be in lower concentration inside than outside the tissue, whilst the reverse holds. As the concentration gradients were only found in respiring tissue at 37°, the accumulation of α -oxoglutarate must also represent a case of active transport.

Intracellular osmotic pressure. Earlier workers have generally assumed that the intracellular fluid is isotonic with extracellular fluid (see Peters, 1944), but the work of Stern, Eggleston, Hems & Krebs (1949) and of Robinson (1950, 1952*b*) who showed that animal tissues swell when the energy supply is cut off, and of Opie (1949) who showed that this swelling can be prevented by placing tissues in hypertonic solutions, suggests that tissues are in fact hypertonic and have to pump out water continuously to prevent swelling. This interpretation has not been accepted by Conway & McCormack (1953) who found the freezing-point depression of fresh guinea pig tissues to be the same as that of plasma. A high intracellular osmotic pressure is also suggested by the fact that the sum of the concentrations of sodium and potassium in the tissue water can be much greater than in plasma (Conway & Hingerty, 1946; Davies, Davies, Francis & Whittam, 1952; Aebi, 1953). Since some of the tissue water is extracellular with concentrations of sodium and potassium similar to those in plasma, this sum must be even greater in the cell water. It is, however, not known to what extent cations are osmotically inactive within the cell. It is feasible that they are partly rendered inactive by immobile polyvalent anions (Kern, 1938), but it is unlikely that such anions play a major role in the distribution of electrolytes because they could hardly account for the characteristic behaviour of the tissue water and electrolytes, such as the changes which occur on addition of inhibitors, lowering of temperature and cessation of respiration.

Further work is therefore needed to measure the osmotic pressure in isolated tissue slices under a variety of conditions to see whether the changes in water content and ionic gradients are in fact compatible with a constant osmotic pressure across the cell wall, in agreement with the conclusion of Conway

& McCormack (1953), or signify a difference in the osmotic pressure across the cell wall, as postulated by Robinson (1950).

SUMMARY

1. In extending previous work (Turner *et al.* 1950; Mudge, 1951*a, b*) it was found that slices of guinea pig kidney cortex lost 40 % of their initial potassium content to, and gained an extra 60 % sodium from, a saline medium during the first 5 min. of aerobic incubation at 37° and pH 7.4. These changes were reversed after 30 min. with 10 mM α -oxoglutarate as substrate.

2. During aerobic incubation at 37° for 40 min. the sum of sodium and potassium contents of the tissue rose by 25.7 ± 3.2 μ moles/g. tissue, whilst the content of dibasic α -oxoglutarate rose by the equivalent amount of 12.0 ± 0.6 μ moles/g. tissue. The sum of sodium and potassium concentrations in the tissue water was 1.29 times greater than that in the medium; the α -oxoglutarate concentration was 2.6 times greater. During this time 53.3 ± 5.5 μ moles α -oxoglutarate/g. tissue were metabolized.

3. All concentration gradients of sodium, potassium and α -oxoglutarate between slices and medium were reduced or disappeared during aerobic or anaerobic incubation at 0°, or anaerobic incubation at 37°.

4. The dry weight of the tissue decreased during incubation by 9.0 % at 37° in O₂ and by from 22 to 26 % at 37° in N₂ or at 0° in either O₂ or N₂.

5. During incubation of tissue in a hypotonic medium at 0°, the water content increased and the sum of sodium and potassium contents fell concomitantly. Movements of sodium and potassium separately continued after the movement of water was complete.

6. These results confirm that in guinea pig kidney-cortex slices the prevention of swelling, and the production and maintenance of sodium and potassium concentration gradients are dependent upon a supply of energy from respiration.

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The Enzymic Hydrolysis of Acid Anhydrides

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According to recent investigations (Burgen, 1949; Wilson & Bergmann, 1950) tetraethyl pyrophosphate (TEPP) and related inhibitors of esterases are enzymically split into a phosphonium ion, which combines with the nucleophilic group in the active surface, and a negative ion, e.g. the diethyl phosphate ion. The combination 'enzyme-P(OR)₂' is

thus analogous to the complex 'enzyme-C—R',

$$\begin{array}{c} \text{O} \\ || \\ \text{enzyme}-\text{C}-\text{R} \end{array}$$

which we assumed as intermediate in the hydrolysis of esters (Bergmann, Wilson & Nachmansohn, 1950). The main difference between the two combinations is that the enzyme-acyl complex is spontaneously split by the solvent, regenerating the active form of the enzyme, whereas the enzyme-phosphonium compound is stable, at least for the duration of a laboratory experiment.

The superiority of TEPP over trialkyl phosphates in the inhibition of esterases (Aldridge & Davison, 1952) shows that the anhydride linkage is split faster by esterases than the ester group in derivatives of phosphoric acid. It should therefore be expected that anhydrides of carboxylic acids will also be easily attacked by these enzymes. The experiments described in the present investigation substantiate this prediction.

MATERIALS AND METHODS

Substrates and inhibitors. The fatty acid anhydrides were commercial samples and were purified by fractionation *in vacuo*. Dibenzyl acetyl phosphate was prepared essentially as described by Bentley (1948), but the reaction with keten was carried out in the absence of solvent, the liquid end product serving as solvent for dibenzyl hydrogen phosphate. Tetrabenzyl pyrophosphate was synthesized by the method of Kenner, Todd & Weymouth (1952).

Enzyme preparations. Liver esterase was prepared from dog's liver by ammonium sulphate fractionation, as described earlier (Bergmann & Shimoni, 1952). The yellow solution, obtained after two subfractionations, contained 16.6 mg. protein/ml., as determined by precipitation with 20% (w/v) trichloroacetic acid. When diluted 1:100 and with diacetin 0.43 M as substrate, this enzyme liberated 5.3 μ moles CO₂/ml./hr.

Acetylcholine esterase was prepared from the electric organ of *Torpedo marmorata*. The final preparation, when diluted 1:40 000 and with acetylcholine 3.3 $\times 10^{-3}$ M as substrate, hydrolysed 6 μ moles/ml./hr. For some of our experiments the enzyme from *Electrophorus electricus* was used, which in a dilution of 1:1200 hydrolysed 6.6 μ moles acetylcholine/ml./hr.

Manometric methods. The buffer used as medium for these experiments contained 0.1 M-NaCl, 0.026 M-NaHCO₃ and 0.04 M-MgCl₂. The pH was adjusted to 7.3. The gas phase consisted of 95% air and 5% CO₂. In the experiments with toluene as the organic phase, the solution of anhydride in this solvent (0.1–0.3 ml.) was placed in the side bulb of a