Relations between Metabolism and the Rate of Turnover of Sodium and Potassium in Guinea Pig Kidney-cortex Slices

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It is now known that sodium and potassium ions exchange rapidly between cells and body fluids, and that the observed concentration gradients are not due to impermeability of the cell membrane (see reviews by Krogh, 1946; Ussing, 1949). It is also known that the production and maintenance of physiological concentration gradients of sodium and potassium in isolated kidney-cortex slices require respiratory energy (Krebs, Eggleston & Terner, 1951; Mudge, 1951*a*, *b*; Aebi, 1953; Whittam & Davies, 1953*b*).

In order to investigate the relation between the to-and-fro movements of sodium and potassium and the metabolism of this tissue, the turnover-rates of these ions have been measured in several steady-state conditions.

A preliminary account of this work has been presented to the Biochemical Society (Whittam & Davies, 1953*a*).

EXPERIMENTAL

Incubation of tissue. Guinea pig kidney-cortex slices were prepared and incubated as described by Whittam & Davies (1953*b*). In some experiments the tissue was placed directly into 2 ml. of bicarbonate saline, containing either $^{24}Na^+$ or $^{42}K^+$, in 15 ml. stoppered vessels, but it was usually pre-incubated for about 35 min. and then transferred to the radioactive medium.

Measurements of Q_{O_2} (μl./mg. dry wt./hr.) were made in Warburg manometers in the usual way.

Estimation of sodium and potassium. The tissue (approx. 150 mg.) was dissolved in 16 N-HNO_3 and diluted to 5 ml. with water (for details see Whittam & Davies, 1953*b*). Samples (1·0 or 2·0 ml.) were diluted to 10 ml. with either 0·0382% (w/v) KCl solution or 0·84% (w/v) NaCl solution. These solutions and samples of the medium were used for determination of Na^+ or K^+, respectively, with a flame photometer (Domingo & Klyne, 1949).

This procedure measures the total sodium and potassium, but the symbols Na^+ and K^+ have been used throughout to include any undissociated or un-ionized material which may be present.

Handling of $^{24}Na^+$ *and* $^{42}K^+$. Spectroscopically pure $^{24}Na_2CO_3$ and $^{42}K_2CO_3$ were supplied by the Atomic Energy Research Establishment, Harwell, and neutralized with HCl; enough was added to give samples of the medium with about 6000 counts/min. The tracer amounts of $^{24}Na^+$ and $^{42}K^+$ added in this way did not appreciably change the concentrations of Na^+ or K^+ in the medium. The radio-activity of medium and tissue was measured in an M. 6 liquid counter (Veall, 1948) and corrections were made for isotope decay, dead time and background counts. $^{24}Na^+$ was measured in solutions prepared by diluting 0·05 ml. of medium and 2·0 ml. of the acid digest of the tissue to 10 ml. with 0·9% (w/v) NaCl solution. $^{42}K^+$ was measured in solutions prepared by diluting 1·0 ml. of medium and 2·0 ml. of the acid digest of the tissue to 10 ml. with 0·1 N-KCl solution.

RESULTS

Exchange of the sodium of kidney-cortex slices on incubation in vitro

Non-steady-state exchanges. Table 1 shows the effect of various periods of aerobic and anaerobic incubation at 37° on the amount of exchange between sodium in the tissue and medium. The rate of exchange was higher aerobically, when the sodium content was lower, than anaerobically when the sodium content was higher. Under aerobic conditions there was nearly complete exchange (98%), and under anaerobic conditions 96% exchange in 2 hr. After 2 hr. aerobic and anaerobic incubation at 0°, there was an exchange of 92% of the tissue sodium. These results show that there was little, if any, non-exchangeable sodium in these kidney-cortex slices at either 0° or 37° in aerobic or anaerobic conditions.

Steady-state exchanges. After a preliminary incubation period of about 35 min., when the sodium content of the tissue had become approximately steady, the slices were placed in saline containing radioactive sodium. The uptake of this ion by the tissue during the following few minutes in aerobic conditions at 37° is shown in Fig. 1, and in anaerobic conditions at 37° and 0° in Fig. 2. There was in each case a rapid initial increase in the percentage exchange, but after about 1 min. the rate of increase became much slower.

The formula of Krebs *et al.* (1951) for the calculation of the steady-state turnover of material in a two-component system is applicable to the measurement of turnover-rates of the intracellular sodium only when a correction of the experimental measurements can be made for the amount of extracellular sodium present in the tissue. Since this amount was not known precisely, an alternative approach has been made to interpret the results.

Table 1. *Changes in the distribution of $^{24}Na^+$ between guinea pig kidney-cortex slices and medium during incubation at 37°*

(Tissue (0·1–0·2 g.) incubated in 2·0 ml. of bicarbonate saline containing $^{24}Na^+$ and 10 mM α-oxoglutarate; pH 7·4, gas phase 5% CO_2 and either 95% O_2 or 95% N_2.)

Experimental conditions	Period of incubation (min.)	Na^+ content of tissue after incubation* (μmoles/g. tissue)	Specific activity of Na^+ in tissue (counts/min./μmole Na^+). After incubation	Specific activity of Na^+ in medium (counts/min./μmole Na^+) Before incubation	Specific activity of Na^+ in medium (counts/min./μmole Na^+) After incubation	Ratio: (specific activity of Na^+ in tissue/specific activity of Na^+ in medium) × 100 (% exchange)
Aerobic	0·27	78·0	776	1365	1255	61·8
	1·0	88·2	1050	1365	1280	82·1
	4·9	100	1080	1365	1140	94·8
	5·8	90·1	1160	1365	1235	94·0
	6·4	91·8	1220	1365	1250	97·7
	7·6	104	1025	1185	1170	87·6
	15·2	97·9	1160	1185	1215	95·5
	30·3	99·2	1149	1185	1136	101
	60·3	102	1095	1185	1153	95·1
	120·0	98·5	1100	1185	1130	97·4
	120·5	98·4	1208	1365	1230	98·4
Anaerobic	0·27	77·0	763	1325	1385	55·1
	0·5	82·5	992	1325	1285	77·2
	1·8	95·8	1120	1325	1300	86·3
	4·5	104	974	1325	1295	75·2
	7·7	114	1135	1261	1328	85·5
	15·2	125	951	1261	1207	78·8
	30·0	120	1106	1261	1225	90·4
	60·2	136	1095	1261	1237	88·6
	120·0	122	1176	1261	1218	96·7
	120·1	131	1250	1325	1300	96·1

* Sodium content of tissue before incubation = 58·9 μmoles/g. tissue.

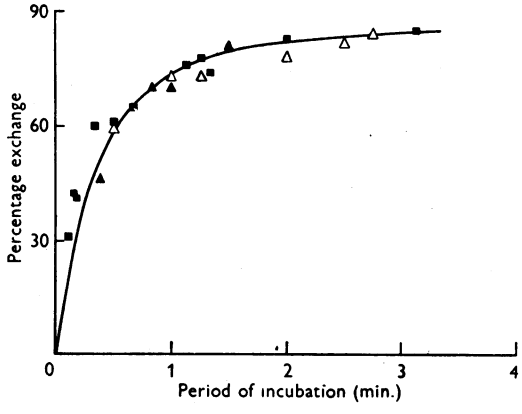

Fig. 1. The uptake of radioactive sodium by guinea pig kidney-cortex slices in steady-states at 37°. About 0·2 g. tissue incubated in 2·0 ml. of bicarbonate saline; gas phase, 5% CO_2 and 95% O_2; pH 7·4. After approx. 35 min. incubation the tissue was transferred to a similar saline solution containing ^{24}Na. ■, saline with 10 mM α-oxoglutarate; ▲, saline without additions; △, saline containing 10 mM α-oxoglutarate and 0·2 mM 2:4-dinitrophenol. The curve represents the function

$$P = 76[1 - \exp(-2{\cdot}65t)] + 24[1 - \exp(-0{\cdot}151t)],$$

where P is the percentage exchange of tissue sodium and t is the period of incubation in minutes in the radioactive medium.

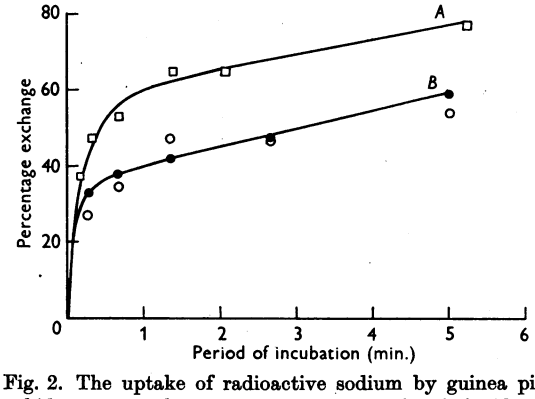

Fig. 2. The uptake of radioactive sodium by guinea pig kidney-cortex slices in steady-states at 37° and 0°. About 0·2 g. tissue was incubated in 2·0 ml. of bicarbonate saline containing 10 mM α-oxoglutarate at 37° (gas phase, 5% CO_2 and 95% N_2; pH 7·4) or at 0° (gas phase, 2·8% CO_2 and either 97·2% O_2 or 97·2% N_2; pH 7·4). After approx. 35 min. incubation the tissue was transferred to a similar saline solution containing ^{24}Na. □, anaerobic conditions at 37°; ○, aerobic conditions at 0°; ●, anaerobic conditions at 0°. Curve A represents the function

$$P = 55[1 - \exp(-4{\cdot}45t)] + 45[1 - \exp(-0{\cdot}134t)],$$

and curve B the function

$$P = 34[1 - \exp(-8{\cdot}28t)] + 66[1 - \exp(-0{\cdot}0954t)],$$

where P is the percentage exchange of tissue sodium and t is the period of incubation in minutes in the radioactive medium.

The experimentally determined points in Figs. 1 and 2 are in good agreement with the curves representing the empirical functions,

$$P = 76[1 - \exp(-2{\cdot}65t)] + 24[1 - \exp(-0{\cdot}151t)]$$

for aerobic conditions with or without substrate and in the presence of 2:4-dinitrophenol at 37°;

$$P = 55[1 - \exp(-4{\cdot}45t)] + 45[1 - \exp(-0{\cdot}134t)]$$

for anaerobic conditions at 37°; and

$$P = 34[1 - \exp(-8{\cdot}28t)] + 66[1 - \exp(-0{\cdot}0954t)]$$

for aerobic and anaerobic conditions at 0°, where P is the percentage exchange of the tissue sodium, and t is the period of incubation in minutes. The figures before the square brackets represent the percentages of tissue sodium exchanging with the rate constants given within the brackets.

This shows that there are at least two major fractions of the tissue sodium, which exchange according to first-order processes. However, both the relative sizes and the velocity constants of these components changed with the experimental conditions. Aerobically at 37°, 50% equilibration was reached in 16 sec. by the rapidly exchanging fraction of the tissue sodium, and in 4·6 min. by the slowly exchanging fraction (Table 2).

The amount of sodium in the rapidly exchanging fraction at 37° in the presence of oxygen was 76%, which is surprisingly high. This fraction consisted of sodium indistinguishable from that of true extra-

Table 2. *Steady-state turnover-rates of* $^{24}Na^+$ *in guinea pig kidney-cortex slices during incubation*

(Tissue (0·1–0·2 g.) incubated in 2·0 ml. of bicarbonate saline containing 10 mM α-oxoglutarate; pH 7·4; gas phase at 37°, 5% CO_2 and either 95% O_2 or 95% N_2; gas phase at 0°, 2·8% CO_2 and either 97·2% O_2 or 97·2% N_2. Pre-incubated for 35–40 min. and then placed for various times in similar saline containing $^{24}Na^+$. Data derived partly from graph of percentage exchange against time. For further details see text and Fig. 1.)

Temperature of incubation ...	37°				0°	
Experimental conditions ...	Aerobic	Aerobic without added substrate	Aerobic with 0·2 mM 2:4-dinitrophenol	Anaerobic	Aerobic	Anaerobic
Na^+ content of tissue after incubation* (μmoles/g. tissue)	99·5	108	124	114	113	
Amount of rapidly exchanging Na^+ in the tissue (%)	76	76	76	55	34	
Space of tissue occupied by rapidly exchanging part of Na^+ ('outer' space) (%)	48·4	52·3	60·2	40·0	24·5	
Na^+ content of part of tissue occupied by slowly exchanging Na^+ (μmoles/g. tissue contained in 'inner' space)	46·0	54·3	74·4	85·6	98·8	
Ratio: Na^+ content of part of tissue occupied by slowly exchanging Na^+/Na^+ concentration in medium	0·293	0·346	0·474	0·546	0·630	
Rate constants of component parts of tissue Na^+ ($min.^{-1}$):						
Fast	2·65	2·65	2·65	4·45	8·28	
Slow	0·151	0·151	0·151	0·134	0·0954	
Time for half-exchange of the components of tissue Na^+ (min.):						
Fast	0·26	0·26	0·26	0·16	0·084	
Slow	4·6	4·6	4·6	5·2	7·3	
Turnover-rates of slowly exchanging parts of tissue Na^+ (%/min.)	16·2	16·2	16·2	12·1	8·94	
Exchange-rates of slowly exchanging parts of tissue Na^+:						
(μmoles/min./g. whole tissue)	3·92	4·20	4·80	6·21	6·67	
(μmoles/min./g. tissue contained in 'inner' space)	7·60	8·83	12·0	10·4	8·84	
Q_{Na^+} (μl. slowly exchanging Na^+/mg. dry wt. of tissue/hr.)	±26·0	±28·3	±32·3	±49·1	±52·8	

* Sodium content of tissue before incubation = 63·5 μmoles/g. tissue.

cellular sodium and if its concentration was similar to that of the medium, then the space occupied by it would be 48·4 % of the tissue and will be called the 'outer' space of the tissue. This contrasts with the 'inulin' space of kidney-cortex slices of 26 % (Robinson, 1950). It is of interest that the size of the 'outer' space was 40 % of the tissue anaerobically at 37° and only 24·5 % at 0°. The remaining 'inner' space therefore contained the amounts of sodium shown in Table 2 and as expected there was least sodium in the 'inner' space of the tissue in aerobic conditions at 37° and most when the energy supply was reduced either by 2:4-dinitrophenol, anoxia or incubation at 0°.

In this paper 'turnover-rate' means the number of ions exchanging/min./100 ions present; 'exchange-rate' means the μmoles of ions exchanging/min./g. tissue. The turnover-rates of the sodium in the 'inner' spaces have been calculated using the formula (problem 1, case 3) of Stewart (1953). The rates were the same in all the aerobic conditions at 37°, despite large differences in the sodium content. The turnover-rate in anaerobic conditions was 25 % lower than that in aerobic conditions, whilst at 0° the rate was 45 % lower. The exchange-rates (in μmoles/min./g. tissue contained in the 'inner' space) at 37° were approximately proportional to the amount of sodium present. These results mean that at 37° the turnover-rate in the steady-state was largely independent of the amount of sodium in the tissue and hence of the energy supply (cf. Table 6).

Exchange of the potassium of kidney-cortex slices on incubation in vitro

Non-steady-state exchanges. Under aerobic and anaerobic conditions at 37° there was complete exchange between the potassium of the tissue and the medium (Table 3). The potassium was also completely exchangeable in the presence of 2 mM 2:4-dinitrophenol in the medium, or in the absence of substrate. As with sodium, the rate of exchange was more rapid aerobically than anaerobically. In contrast, the potassium content of the tissue was lower anaerobically than aerobically.

Steady-state exchanges at 0°. An unexpected result was obtained on aerobic and anaerobic incubation at 0° in medium containing $^{42}K^+$ from the start; about 20 and 40 %, respectively, of the tissue potassium had not exchanged even after 10 hr. The amount of exchange, however, increased with the period of incubation (Fig. 3) and was greater aerobically than anaerobically. These facts are in agreement with the assumption that there are two forms of intracellular potassium, one exchanging more rapidly than the other. The potassium content of the tissue did not alter much after the first 2 hr.

Table 3. *Changes in the distribution of* $^{42}K^+$ *between guinea pig kidney-cortex slices and medium during incubation at* 37°

(Slices of tissue (0·1-0·2 g.) incubated in 2·0 ml. of bicarbonate saline containing $^{42}K^+$ and 10 mM α-oxoglutarate; pH 7·4; gas phase 5 % CO_2 and either 95 % O_2 or 95 % N_2.)

Experimental conditions	Period of incubation (min.)	K^+ content of tissue after incubation* (μmoles/g. tissue)	Specific activity of K^+ in tissue (counts/min./μmole K^+). After incubation	Specific activity of K^+ in medium (counts/min./μmole K^+) Before incubation	Specific activity of K^+ in medium (counts/min./μmole K^+) After incubation	Ratio: (specific activity of K^+ in tissue/specific activity of K^+ in medium) × 100 (% exchange)
Aerobic	2·0	42·2	722	2330	1680	43·0
	4·0	51·4	1040	2330	1900	54·8
	8·0	57·5	1250	2330	1750	71·4
	16	53·0	1470	2330	1745	84·4
	32	59·0	1630	2330	1800	90·6
	64	53·7	1510	2330	1610	94·0
	90	46·6	1640	2330	1630	100
	119	47·2	1550	2330	1540	100
	240	27·2	1580	2330	1540	103
	359	19·5	1780	2330	1760	101
Anaerobic	2·0	39·2	607	2800	2210	27·5
	4·0	32·7	926	2800	2210	41·9
	8·0	31·9	935	2800	1950	47·9
	16	29·2	995	2800	1900	52·4
	34	20·5	1280	2800	1950	65·7
	64	18·6	1380	2800	1970	70·1
	90	17·2	1350	2800	2000	67·5
	120	16·5	1440	2800	1950	74·0
	240	11·2	1690	2800	1640	103
	357	9·8	1860	2800	1880	99·0

* Potassium content of tissue before incubation = 76·2 μmoles/g. tissue.

of incubation and approximately steady-state conditions were established, with the tissue potassium content higher than that of the medium. The rates of exchange of the two forms of intracellular potassium can be represented by simple first-order kinetics. Aerobically the experimental points fit the curve

$$P = 37[1 - \exp(-0{\cdot}228t)] + 63[1 - \exp(-0{\cdot}00194t)];$$

anaerobically the points fit the curve

$$P = 37[1 - \exp(-0{\cdot}160t)] + 63[1 - \exp(0{\cdot}00069t)].$$

These curves were obtained from two different kinds of experiments; the fast rate from experiments in which the tissue was pre-incubated before the addition of radioactivity and the slow rate from experiments where the radioactivity was added at the beginning. These conditions were selected to make the overall time of incubation as short as possible. The slowly exchanging fractions of the tissue potassium had the very long times for half exchange of 6 hr. aerobically and 17 hr. anaerobically (Table 4). The rates for the more rapidly exchanging fractions of the tissue potassium at 0° are given in Table 4. In contrast to sodium, the rates for the rapidly exchanging fraction are far slower than would be expected for extracellular potassium.

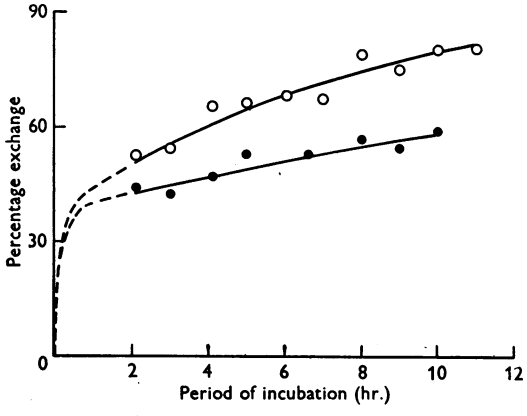

Fig. 3. The uptake of radioactive potassium by guinea pig kidney-cortex slices at 0°. About 0·15 g. tissue was incubated in 4·0 ml. of bicarbonate saline containing $^{42}K^+$ and 20 mM α-oxoglutarate at 0°; gas phase, 2·8% CO_2 and either 97·2% O_2 or 97·2% N_2; pH 7·4. ○, aerobic conditions; ●, anaerobic conditions. The curves represent the functions

$$P = 37[1 - \exp(-0{\cdot}228t)] + 63[1 - \exp(-0{\cdot}00194t)]$$

and

$$P = 37[1 - \exp(-0{\cdot}160t)] + 63[1 - \exp -0{\cdot}00069t)]$$

for aerobic and anaerobic conditions, respectively. P = the percentage exchange of the tissue potassium and t is the period of incubation in minutes. (For further details see text.)

Table 4. *Exchange of $^{42}K^+$ in guinea pig kidney-cortex slices during incubation at 0°*

(For measurements of the rates of the rapidly exchanging potassium, tissue (approx. 0·2 g.) was incubated in 2·0 ml. of bicarbonate saline containing 10 mM α-oxoglutarate, pH 7·4 (gas phase 2·8% CO_2 and either 97·2% O_2 or 97·2% N_2), for 30–40 min. and then placed for from 2 to 6 min. in a similar saline containing $^{42}K^+$. For measurements of the rates of the slowly exchanging potassium see Fig. 3. For further details see text.)

Experimental conditions	Period of incubation (min.)	Component of tissue K^+	Amount of K^+ in component (%)	K^+ content of tissue after incubation* (μmoles/g. tissue)	Rate constant of tissue K^+ ($min.^{-1}$)	Time for half-exchange of tissue K^+ (hr. min. sec.)	Turnover-rate of tissue K^+ (%/min.)	Exchange-rate of tissue K^+ (μmoles/min./g. tissue)	Q_{K^+} (μl. K^+ exchanging/mg. dry wt. of tissue/hr.)
Aerobic	40	Fast	37	30·9	0·228	0 3 02†	18·1	2·07	±15·7
Anaerobic				28·7	0·160	0 4 20†	12·7	1·35	±10·2
Aerobic	From 120 to 600	Slow	63	24·5	0·00194	5 57 0	0·155	0·0239	±0·212
Anaerobic				18·7	0·00069	16 40 0	0·0622	0·00732	±0·0656

* K^+ content of tissue before incubation = 76·1 μmoles/g. tissue.
† Calculated for K^+ in 200 mg. tissue.

If this fraction were contained in a space having the same potassium concentration as the medium, the space would be twice the volume of the whole tissue slices. Since this is impossible there must be at least two species of intracellular potassium at 0° in addition to extracellular potassium.

Steady-state exchanges at 37°. Preliminary experiments showed that in contrast to the results at 0°, all the potassium at 37° behaved as a single species. The turnover-rates were therefore measured according to Krebs *et al.* (1951). As at 0°, the extracellular potassium was neglected and this introduced an error of less than 6 %.

In spite of a twofold difference in the amount of potassium present, the turnover-rates under all conditions which were aerobic and at 37° were about the same (Table 5), whilst under anaerobic conditions the rate was 28 % lower. The similar turnover-rates found with and without 2:4-dinitrophenol are in contrast with the different amounts of potassium in the tissue. Thus, whilst the amount of ion is regulated by the energy supply, the turnover at 37° under aerobic conditions appears to be independent of it.

The 'outer' space of the slices contained sodium exchanging at a rate indistinguishable from that of the sodium in the extracellular space of the tissue. The potassium contents of the 'inner' spaces (Table 5) have been calculated on the assumption that this 'outer' space contains potassium also at the same concentration as the medium. These values show that there was a large reduction in the potassium content of the 'inner' space when the supply of energy from respiration was inhibited (with 2:4-dinitrophenol) or abolished (by anoxia).

Effect of oxygen uptake of kidney-cortex slices on the concentration gradients of sodium and potassium

Table 6 shows that at 37°, 10 mM α-oxoglutarate increased the Q_{O_2} of guinea pig kidney-cortex slices by 40 %. This concentration of α-oxoglutarate also led to a 15 % increase in the concentration gradients of sodium and potassium (Tables 2 and 5). The 60 % increase of the Q_{O_2} in the presence of 0·2 mM 2:4-dinitrophenol was accompanied by falls of 62 and 45 % in the concentration gradients of sodium and potassium (cf. Mudge, 1951*b*).

Relations between metabolism and sodium and potassium turnover

Table 7 gives the relations between the rate of respiration of kidney-cortex slices and the concomitant rates of exchange of sodium and potassium. The ratio, Q_{cation}/Q_{O_2}, was higher for respiration with endogenous substrate than with α-oxoglutarate, and much higher than the ratio in the presence of 0·2 mM 2:4-dinitrophenol. However, Tables 2 and 5 show that the turnover-rates of

Table 5. *Steady-state turnover-rates of* $^{42}K^+$ *in guinea pig kidney-cortex slices during incubation at* 37°

(Tissue (0·1–0·2 g.) incubated in 2·0 ml. of bicarbonate saline containing 10 mM α-oxoglutarate, pH 7·4 (gas phase, 5 % CO_2 and either 95 % O_2 or 95 % N_2), for 35–40 min. and transferred to a similar saline containing $^{42}K^+$ for from 2 to 6 min. Radioactivity of medium was measured before and after incubation. Turnover-rates calculated using formula of Krebs *et al.* (1951). For further details see text.)

Experimental conditions	K^+ content of tissue after incubation* (μmoles/g. tissue)	K^+ content of part of tissue not occupied by rapidly exchanging Na^+ (μmoles/g. tissue contained in 'inner' space)	Ratio: K^+ content in slowly exchanging Na^+ space/K^+ concentration in medium	Rate constant of tissue K^+ ($min.^{-1}$)	Time for half-exchange of tissue K^+† (min.)	Turnover-rate of tissue K^+ (%/min.)	Exchange-rate of tissue K^+ (μmoles/min./g. tissue)	Q_{K^+} (μl. K^+ exchanging/mg. dry wt. of tissue/hr.)
Aerobic	76·3	143	25·1	0·206	3·37	15·9	12·1	±80·0
Aerobic without added substrate	64·2	128	22·5	0·206	3·37	15·6	10·0	±66·2
Aerobic with O_2, 2 mM 2:4-dinitrophenol	34·6	78·4	13·8	0·206	3·37	16·9	5·85	±39·3
Anaerobic	23·1	34·7	6·1	0·145	4·78	11·5	2·66	±21·0

* Potassium content of tissue before incubation = 78·5 μmoles/g. tissue.
† Measured when the amount of K^+ in the medium was the same as the amount of K^+ in the tissue.

sodium and potassium remained nearly identical in these aerobic conditions. The lowered efficiency in the presence of α-oxoglutarate and 2:4-dinitrophenol is therefore apparently due to increments of respiration (Table 6) which do not affect the turnover-rates.

Table 6. *Oxygen uptake by guinea pig kidney-cortex slices*

(Slices of tissue (approx. 0·15 g.) placed in 4 ml. of phosphate saline (medium III of Krebs, 1950, without the addition of organic acids), gassed with O_2. CO_2 was absorbed by 0·2 ml. 2N-NaOH in the centre well and Q_{O_2} was measured with standard Warburg manometers.)

Additions to saline	Temp. (°)	Q_{O_2} (μl./mg. dry wt./hr.)
None	37	−16·8
10 mM α-oxoglutarate		−23·6
10 mM α-oxoglutarate and 0·2 mM 2:4-dinitrophenol		−37·8
10 mM α-oxoglutarate	0	−0·68
None		−0·54

Table 7. *Quotients relating the rate of respiration of guinea pig kidney-cortex slices to the rates of exchange of sodium and potassium*

(Calculated from results in Tables 2 and 4–6.)

Temp. (°)	Experimental conditions	Ratio: $\pm\frac{Q_{cation}}{Q_{O_2}}$ Na+	K+
37	Aerobic, with 10 mM α-oxoglutarate	1·10	3·39
	Aerobic, no added substrate	1·69	3·47
	Aerobic, with 10 mM α-oxoglutarate and 0·2 mM 2:4-dinitrophenol	0·86	1·04
	Anaerobic	∞	∞
0	Aerobic	77·7	0·31* 23†
	Anaerobic	∞	∞

* This refers to the slowly exchanging part of the tissue K^+.

† This refers to the rapidly exchanging part of the tissue K^+.

The effects of oxygen and temperature on the turnover-rates of sodium and potassium are shown in Table 8. The turnover-rates in aerobic conditions were 30–40% higher than those in anaerobic conditions. The effect of temperature on the slowly exchanging part of the tissue potassium was very large, the increase aerobically being 104-fold and anaerobically 184-fold.

DISCUSSION

The exchangeability of the sodium and potassium of guinea pig kidney-cortex slices

Effects of respiration. It is useful to distinguish between the mechanisms which cause net movements of ions and those which maintain steady-state exchanges. These may be identical or different (Davies & Krebs, 1952).

Net movements of sodium into and of potassium out of the cell are in accordance with the concentration gradients and are unlikely to require energy supplies. However, the reverse process, the net movements of these cations in opposite directions against concentration gradients, must require energy whatever the electrical potential difference across the cell wall. Much work with isolated tissue slices has, in fact, shown that this process requires a supply of energy from respiration.

Respiration is also required to maintain a steady-state in the tissue in which the sodium and potassium concentrations are approximately physiological (Terner, Eggleston & Krebs, 1950; Krebs *et al.* 1951; Mudge, 1951*a*, *b*; Aebi, 1953; Whittam & Davies, 1953*b*). Changes in the metabolic state of the tissue lead to the establishment of steady-states with different sodium and potassium concentrations. Since in all these steady-states there is no net transport of ions but simply a to-and-fro exchange, it is possible that the steady-state turnover could take place without a direct energy supply. Such a turnover of ions occurs, e.g. between the sodium form of a cation-exchange resin in a neutral, dilute

Table 8. *Effect of oxygen and increase of temperature on the turnover-rates of sodium and potassium in guinea pig kidney-cortex slices*

(Calculated from results in Tables 2, 4 and 5.)

Cation	Ratio: turnover-rate in aerobic conditions/turnover-rate in anaerobic conditions: 0°	37°	Ratio: turnover-rate at 37°/turnover-rate at 0°: Aerobic	Anaerobic	Ratio: increase of turnover-rate due to O_2 at 37°/increase of turnover-rate due to O_2 at 0°
Na^+	1·00	1·34	1·81	1·35	>20‡
K^+	2·5* 1·42†	1·38	104·0* 0·88†	184·0* 0·91†	47* 0·82†

* This refers to the slowly exchanging part of the tissue K^+.

† This refers to the rapidly exchanging part of the tissue K^+.

‡ This was calculated after making allowance for the maximum possible error in the measurement of the turnover-rates.

solution of sodium salts, in Donnan systems and in 'exchange diffusion' as suggested by Ussing (1949). An alternative mechanism is that concentration gradients are maintained by 'active', i.e. energy-fed transport, which counteracts the effects of 'leakage' (cf. Krebs *et al.* 1951).

The results presented here of measurements of the uptake of radioactive sodium and potassium ions by isolated slices of guinea pig kidney-cortex in various steady-state conditions have shown that about 75 % of the aerobic turnover-rates of both these cations are apparently independent of respiration, although the maintenance of the physiological concentration gradients of these ions certainly depends on respiration.

The absence of effect of 2:4-dinitrophenol on the aerobic turnover-rates suggests that the effects of respiration are not mediated by high-energy phosphate bonds, whilst Table 8 shows that there is no direct proportionality between respiration and the turnover-rates.

Effects of temperature. At 37° all the potassium was uniformly exchangeable and the most remarkable effect of incubation at 0° was the appearance of a major fraction of intracellular potassium with a very slow turnover-rate. Similar results were found by Harris (1952) who showed that 20 % of the potassium of frog muscle exchanged very slowly at 0°, and by Mudge (1952) who found that 40 % of the potassium of rabbit kidney-cortex slices was not exchangeable even after 4 hr. incubation under anaerobic conditions at 25°. This effect of incubation at 0° on the potassium turnover of kidney-cortex slices cannot be directly related to respiration because it also occurred anaerobically.

At 0°, however, the sodium in the 'inner' space of the tissue exchanged far faster than the slowly exchanging fraction of the intracellular potassium. It is therefore unlikely that the slowness of this potassium turnover is due to a non-specific decrease in permeability.

Extracellular space of kidney-cortex slices

A large fraction of the tissue sodium exchanged at very rapid rates and was indistinguishable from the sodium in the spaces outside the cells. If this sodium is contained in a space with the same sodium concentration as the medium, it would occupy about 50 % of the tissue at 37°. This 'outer' space would be 24·5 % at 0° which compares well with the 'inulin' space of 26 % found by Robinson (1950).

The high values of the 'outer' space at 37° suggest that the barrier to the exchange of the rest of the sodium is not the outer cell membrane but may lie within the cell. In fact, this barrier may be associated in part with mitochondria which may be concerned in active transport (Bartley & Davies, 1952, 1954; Macfarlane & Spencer, 1953; Spector, 1953; Stanbury & Mudge, 1953). At 0°, with much-reduced metabolism, the rapidly exchanging fraction of the tissue sodium is the same as the 'inulin' space. It may be that the apparent extracellular space of other tissues is also affected by metabolism and the method of measurement.

SUMMARY

1. The uptake of radioactive sodium by aerobic guinea pig kidney-cortex slices in a steady-state at 37° can be represented by the empirical function

$$P = 76[1 - \exp(-2{\cdot}65t)] + 24[1 - \exp(-0{\cdot}151t)],$$

where P is the percentage exchange, and t is the time in minutes. Anaerobically at 37° the function is

$$P = 55[1 - \exp(-4{\cdot}45t)] + 45[1 - \exp(-0{\cdot}134t)],$$

and at 0°, aerobically and anaerobically,

$$P = 34[1 - \exp(-8{\cdot}28t)] + 66[1 - \exp(-0{\cdot}0954t)].$$

This means that sodium exchanged as at least two fractions and that both the relative amounts and the turnover-rates of the fast and slow fractions of tissue sodium changed with temperature and rate of respiration.

2. The rapidly exchanging tissue sodium apparently occupied an 'outer' space of about 50 % of the tissue volume at 37° and 25 % at 0°.

3. All the tissue intracellular potassium exchanged uniformly at 37° both aerobically and anaerobically with rate constants of 0·206 and 0·145 min.$^{-1}$, respectively. In contrast, the uptake of radioactive potassium at 0° in a steady-state can be represented by

$$P = 37[1 - \exp(-0{\cdot}228t)] + 63[1 - \exp(-0{\cdot}00194t)],$$

and anaerobically at 0° by the function,

$$P = 37[1 - \exp(-0{\cdot}160t)] + 63[1 - \exp(-0{\cdot}00069t)].$$

This means that at 0° the intracellular potassium exchanged as at least two fractions.

4. Respiration increased the turnover-rates (% per min.) of sodium and potassium at 37° by only 34 and 42 %. Thus, about 75 % of the aerobic turnover-rates are independent of respiration. The turnover-rates in O_2 were not affected by absence of substrate or the presence of 2:4-dinitrophenol despite major changes in the respiration rate and the amounts of cations present.

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Dietary Carotene and the Degree of Esterification of Vitamin A in the Milk and Blood of Cows

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In a previous paper (Chanda & Owen, 1952) the authors showed that depriving cows of carotene caused an increase of the vitamin-A alcohol in their milk and that this increase was enhanced by imposing thyroxine treatment simultaneously with the carotene deprivation. These increases were interpreted as indicating a draft by the cow from the stores of vitamin A in the liver. In the previous paper the effects of carotene deprivation on the composition of milk were studied but no analyses of blood were made. In the present experiment the β-carotene, vitamin A ester and vitamin-A alcohol were studied in both the blood and milk of a further four cows on diets which provided intakes of carotene varying from a mere trace to more than 2000 mg./cow/day.

EXPERIMENTAL

The experiment lasted 20·5 weeks and was divided into seven periods, all except the last of which are shown in Fig. 2. In periods 1, 3 and 6, each of four Ayrshire cows received diet 1, which was free from carotene and consisted of (parts by weight): oats, 6; bran, 1; field beans, 2; decorticated-earthnut meal, 1. Diet 2, which was fed during period 2, consisted of: oats, 18; beans, 12; dried grass, 9; lucerne meal, 4. In periods 4 and 5, diet 3 was fed. It consisted of: oats, 18; beans, 12; dried grass, 7; lucerne meal, 5. Diet 1 contained no carotene, whilst diets 2 and 3 contained carotene as a natural constituent of the dried grass and lucerne meals. The concentrate ration for each period was prepared in a single batch. On all diets, straw and beet pulp were fed as roughage. As in a previous experiment (Chanda, Clapham, McNaught & Owen, 1952), analyses showed that faecal carotene became negligible after 7 to 8 days of carotene deprivation.

In 24 hr. representative samples of Sunday's and Wednesday's milk, the partition of vitamin A between alcohol and ester and the content of carotene were determined. On the last day of each period and also in the middle of period 6 these same determinations were made on the blood of each

Table 1. *Carotene intake and carotene content of production rations*

Cow no.	Period	Food intake* (kg./day)	Carotene (mg./100 g. food)	Carotene intake (mg./day)
1	2	9·05	2·96	268
	4	10·50	5·26	552
	5	10·50	6·28	659
	7†	9·00	22·61	2035
2	2	9·07	2·96	269
	4	10·29	5·26	541
	5	10·50	6·28	659
	7†	9·50	22·61	2148
3	2	9·04	2·96	268
	4	10·50	5·26	552
	5	10·50	6·28	659
	7†	8·70	22·61	1967
4	2	8·13	2·96	241
	4	8·77	5·26	461
	5	8·47	6·28	532
	7†	8·30	22·61	1877

* Not including oat straw and beet pulp fed according to appetite.
† Grazing.