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TRANSPORT OF ALKALI CATIONS BY KIDNEY CORTEX SLICES

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While it is clear that Na is actively transported by the renal tubular cell, since Na flux normally occurs against a concentration gradient and Na extrusion from the cell can be inhibited by factors such as cold, anoxia, CN, etc. (MUDGE[1], DAVIES[2], CORT AND KLEINZELLER[3]) such a definite statement has not yet been possible with reference to other alkali cations. The specificity of any transport process may be tested by comparing the fluxes of molecularly or atomically related substances, *i.e.* optical isomers, substituted organic compounds, etc. In the specific problem at hand, Li^+ transport has been compared with Na^+ transport, and Rb^+ with K^+, as representing the closest analogues with the same chemical reactivity.

FOULKS, MUDGE AND GILMAN[4] have infused isotonic LiCl into dogs and have observed a rise in K excretion and urine pH as compared with a control infusion of NaCl, while MUDGE[1] and ANDERSON AND MUDGE[5], on the other hand, have shown, in experiments where Li was added to the incubation medium of kidney slices, that within wide limits Li^+ behaved much like Na^+ and did not change O_2 uptake or tissue HCO^-_3 content.

It has been suggested that K^+ transport may be a purely passive phenomenon (MUDGE[1], ANDERSON AND MUDGE[5], CORT AND KLEINZELLER[3]) since the uptake of K^+ by kidney slices from media with different concentrations of K^+ shows a direct relationship to K^+ concentration in the medium, each uptake curve being exponential in time. Rb behaves so much like K that it may be administered to K-deficient, alkalotic animals and will correct the extracellular alkalosis, and it appears that it may freely enter cells without signs of toxicity (RELMAN, ROY AND SCHWARTZ[6], ANDERSON AND MUDGE[5]).

The kinetics of Li^+ and Rb^+ transport have not been precisely determined however, nor have any of the authors cited actually directly analysed for either of the

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above two cations, so that arguments pertaining to the similarity of Li^+ and Na^+ transport, and Rb^+ and K^+ transport, have been to some degree speculative.

The present work presents some measurements of K^+ flux as a function of the concentration gradient of K, with the aid of $^{42}K^+$, and some further observations on the movement of Li, Na, K and Rb at varying external concentrations of these ions during both leaching (0°C) and incubation (25°C) of slices of rabbit kidney cortex.

METHODS

The basic techniques used with slices have already been described (CORT AND KLEINZELLER[3]) including analysis for tissue water, Na and K. Li was analysed from the same acid digest as Na and K, on a single-ended Zeiss flame photometer using a Schott (Jena) interference filter at 670 mμ. Standard solutions were made for Li containing, in addition, Na in the expected ratio to Li in the sample, so as to allow for any interference by Na emission despite the filter. Rb was not analysed directly. Variations in medium composition will be presented under the individual sections of the results.

In the radioactive experiments, counting was done with a special low-voltage halogen tube with an internal compartment permitting approximately 100% geometrical efficiency, and suitable for gamma and hard beta rays. The specific activity of the ^{42}KCl used was in the range of 4–7 mc/g.

RESULTS

The relation of K flux into cells ($M_{i,K}$) to K concentration in the medium

Slices were leached for 2 hours in physiological saline at 0°C, and then incubated in Krebs' Ringer Phosphate solution containing 10, 20 and 40 mequiv./l of ^{42}K. Fig. 1 shows the relative activity in 10^6 counts/100 g dry solids (*DS*)/sec at 10 (T_{10}) and 30 (T_{30}) min of incubation, and it can be seen that a fairly linear relation exists between $M_{i,K}$ and concentration of K^+ in the external medium ($C_{o,K}$) at any given point in time, and that the uptake curve is exponential up to isotonicity. This provides a more direct demonstration of this relationship than analysis of total tissue K^+ can provide ((MUDGE[1], CORT AND KLEINZELLER[3]) since the latter figure is the sum of opposite fluxes and previous content. This is consistent with the results of total tissue K^+ previously reported.

The uptake of K and Rb

Since K^+ leaves cells during leaching it was of advantage to vary the concentration of K^+ and Rb^+ in the medium only during incubation, when there is normally a flux of K^+ back into cells. The media were composed as shown in Table I. Although attempts were made to analyse directly for Rb^+ content with both flame and chemical methods, no sufficiently sensitive method was available to deal with the quantities involved in a single sample of slices representing only 25–30 mg of dry wt of tissue. Since it has been shown that Rb^+ in the same concentration produces no change in O_2 consumption or tissue HCO^-_3 (intracellular pH?) (ANDERSON AND MUDGE[5]) and the administration of Rb^+ to K^+-deficient rats repairs the alkalosis (transport of Rb into cells?) without signs of metabolic toxicity (RELMAN *et al.*[6]), it was assumed that Rb^+ can replace K^+ in cells, and the level of Rb in the tissue was assumed to be equal to the difference between the K^+ tissue level in the A and B samples of each series as

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TABLE I

THE COMPOSITION OF INCUBATION MEDIA IN THE Rb EXPERIMENTS

Elements other than K and Rb are in the usual concentrations of Krebs' Ringer Phosphate solution

	K mequiv./l	*Rb* mequiv./l	*Σ* mequiv./l
A	10	—	10
B	5	5	10
A	20	—	20
B	5	15	20
A	40	—	40
B	5	35	40

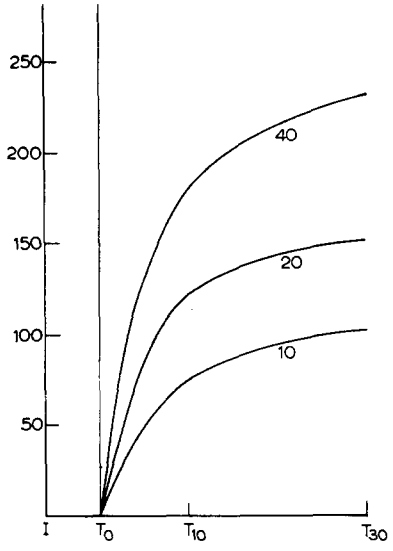

Fig. 1. The relative activity of kidney cortex slices after incubation in a medium containing 10, 20 and 40 mequiv./l of ^{42}KCl. The individual curves are labelled according to the external concentration of K. Ordinate: 10^6 counts/100 g dry solids/sec. Abscissa: time, *I* referring to initial values, the period *I* to T_0 representing 2 h of leaching at 0° C, T_0, T_{10} and T_{30} referring to 0, 10 and 30 min of incubation at 25° C, respectively.

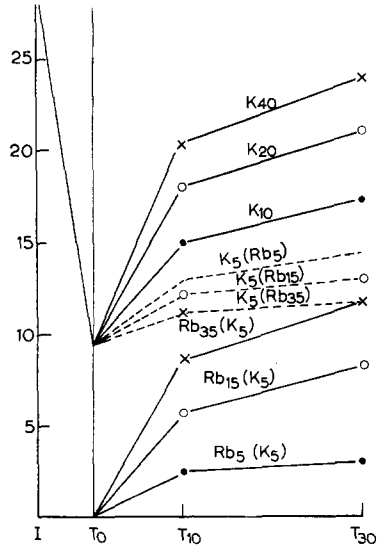

Fig. 2. Tissue levels of K and Rb in mequiv./100 g dry solids (*DS*) (ordinate) during leaching and incubation with various combinations of K and Rb in the incubation medium. Each curve is labelled with the various proportions of K and Rb in the medium, and is preceded by the symbol (without brackets) that the tissue level curve represents, while the bracketed figure represents the concentration in mequiv./l of the other cation. Abscissa: see Fig. 1.

listed in Table I. Both the analysed K^+ results and the *assumed* Rb results are plotted for a typical experiment in Fig. 2.

The K^+ results here confirm the nature of K^+ uptake as a function of external concentration. If $C_{o\,K}$ remains the same (5 mequiv./l) in combination with a varying $C_{o,Rb}$ (5, 15, and 35 mequiv./l) there appears to be a slight inverse relationship between Rb^+ and K^+ uptake, not of sufficient magnitude to suggest a competitive transport process across the membrane, but rather suggesting a limit to the possible total sum of $K^+ + Rb^+$ that a given quantity of cellular material can obtain.

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There was no difference in tissue water content between controls and either the Rb or the Li experiments.

The transport of Li and Na

This can be observed much more satisfactorily, both because of the direct estimation of Li^+ and because during leaching Na^+ enters cells, to be extruded again during incubation, so that we are able to follow transport across the cell membrane in both directions. If Li^+ is added to the incubation medium at T_1 at a concentration of 64 mequiv./l (Na^+, 90 mequiv./l) we see (Fig. 3, left) that Li^+ immediately enters cells at a time when Na^+ is being extruded. The curves of Na^+ extrusion, in comparison to controls in which Na^+ concentration in the medium was 154 mequiv/l show that $M_{o,Na}$ may be a function of the concentration gradient of Na^+ across the cell membrane. If, on the other hand, the leaching solution in the period I to T_0 is ½ Li^+, ½ Na^+, and the incubation medium is ordinary Krebs' Ringer Phosphate (KRP) (Fig. 3, right), then, with a reduced $C_{o,Na}$, Li^+ differentially diffuses into cells when active transport is reduced by cooling. During incubation, this Li concn. gradient is reversed, and the tissue content promptly falls, while intracellular Na rises to the level usually attained at T_{30} in such experimental conditions (CORT AND KLEINZELLER[3]).

In order to determine whether the fall in tissue Li during incubation (Fig. 3) is active or passive, experimental conditions were arranged so that the ratio of intracellular Li/extracellular Li would be less than 1.0 at T_0, so as to determine whether Li can move against a concentration gradient. Slices were leached in an isotonic solution containing both LiCl and NaCl, Li^+ concentration being set at 0, 10 and 20 mequiv./l. The incubation medium contained 64 mequiv of Na+ replaced by Li^+. Fig. 4 shows that during 2 hours leaching at 0°C, Li^+ entered cells to a level of 5.2 mequiv./100 g *DS* at $C_{o,Li}$ 10 mequiv./l and 8.6 mequiv./100 g *DS* at 20 mequiv./l C_o.

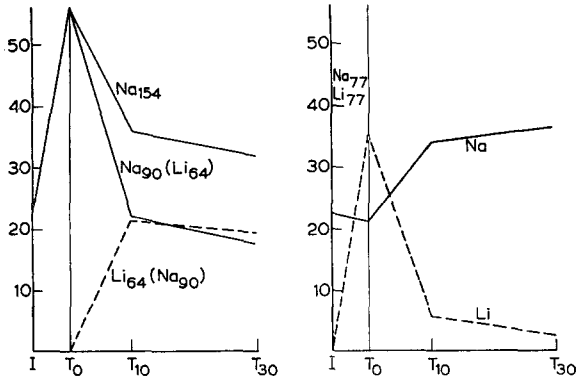

Fig. 3. Tissue levels of Na and Li in mequiv./100 g *DS* (ordinate) during leaching and incubation in media with varying proportions of Na and Li (each curve is labelled with the appropriate concentrations). The full line represents Na content, the dashed line Li content. The figures on the left-hand side of the right-hand diagram indicate the composition of the leaching medium. Abscissa: see Fig. 1.

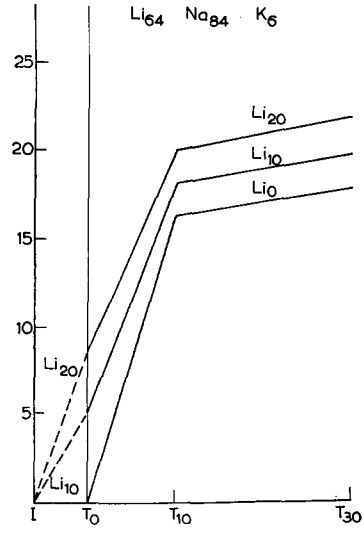

Fig. 4. Tissue levels of Li during leaching in media of varying Li content (each curve is labelled with the concentration of Li in the *leaching* medium in mequiv./l) and incubation in a medium whose composition is indicated at the top of the diagram. Abscissa: see Fig. 1.

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When incubated at 25° C at a $C_{o,Li}$ of 64 mequiv./l, it may be seen that Li continued to rise in a parallel fashion exponentially in time, the curves being displaced by the amount of Li^+ in cells at T_0. Thus Li does not under these conditions move against the concentration gradient here achieved. The total figures for such an experiment are included in Table II to show that, as has been similarly observed in all such experiments, when Li^+ is added to the normal complement of cations, despite the maintenance of a constant total osmolarity, each ion does not exactly equilibrate with itself, so that the sum of $Na^+ + K^+ + Li^+$ in cells is higher than the sum of $Na^+ + K^+$ in the control slices.

Measurement of Q_{O_2} showed no difference between experimental and control slices in the Li conc. range used.

TABLE II

THE TISSUE CONTENT OF Na, K AND Li IN THE EXPERIMENT DEPICTED IN FIG. 4, TO DEMONSTRATE THE CHANGE IN TOTAL CATION CONTENT (Σ) BROUGHT ABOUT BY THE ADDITION OF Li TO THE MEDIUM

For time symbols, see legend, Fig. 1. Electrolytes are in mequiv./100 g dry solids.

Leaching medium	*Time*	*Na*	*K*	*Li*	Σ	*Incubation medium*
	I	20.4	22.4	—	42.8	
						64 mequiv./l Li
NaCl	T_0	46.8	13.2	—	60.0	6 mequiv./l K
Na, 154 mequiv./l	T_{10}	24.9	14.1	16.5	55.5	84 mequiv./l Na
	T_{30}	21.5	13.8	18.0	53.3	
						anions as in Krebs'
LiCl,	T_0	48.7	12.6	5.1	66.4	Ringer Phosphate.
10 mequiv./l	T_{10}	25.5	14.3	19.5	59.3	
	T_{30}	20.8	14.6	19.0	54.4	
LiCl	T_0	46.8	9.4	8.6	64.8	
Li, 20 mequiv./l	T_{10}	24.6	12.0	22.0	58.6	
	T_{30}	20.6	14.2	20.0	54.8	

DISCUSSION

We are principally concerned with two physiological processes of cation transport—those for Na^+ and K^+—in the renal tubular cell. Li^+ and Rb^+ are used only to throw some light on the nature of the physiological processes. It would appear clear that the active transport process for Na^+ is specific for that cation, since Li^+ cannot be transported against a concentration gradient of the order of 1:3 to 1:6, while Na^+ can under similar circumstances. Since no concentration ratios between 1:1 and 1:3 were established, it cannot be stated that Li is under no circumstances actively transported, for, in fact, USSING[7] has shown in frog skin that Li^+ can move against a slight concentration gradient. The latter also shows, however, that in frog skin Li^+ can by no means substitute for Na^+, and that it cannot be transported at the same rate or against the same order of gradient. The argument has been previously presented that this specific active extrusion of Na^+ is located at the basal membrane of the tubular cell (CORT AND KLEINZELLER).

Interpretation of the results with Rb^+ substitution for K^+ is hindered by our failure to find an available method for the micro-estimation of Rb directly. If the

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assumption is allowed that the control tissue level of K minus experimental K level (where K^+ concn. in control medium = K^+ + Rb^+ concn. in experimental medium) may be used as an approximation of the level of tissue Rb^+, it would appear, in agreement with the work quoted above, that Rb^+ may replace K^+ within the cell with little metabolic effect, and that Rb^+ equilibrates with itself across the cell membrane with a similar exponential curve as does K^+ with varying external levels of both cations. This fact, plus the nature of the uptake curve itself, further suggests that both cations, along with Li^+ to some degree, penetrate the membrane by a diffusion process alone.

It has been consistently observed that for any given electrolyte osmolarity of the incubating or leaching media, the substitution of a new cation, such as Li^+, for an equivalent osmolarity and equivalence of Na^+ or K^+ leads to a raised total content of $Na^+ + K^+ + Li^+$ within the cell. This effect is only of the order of 5%, but quite consistent. It may well be due to specific binding of cations within the cell or a change in Coulomb forces, and does not appear to be of sufficient magnitude to suggest the presence of a specific active transport process over and above that for Na^+.

SUMMARY

Slices of rabbit kidney cortex were leached for 2 h at 0° C and incubated with O_2 for 30 min at 25° C under the following conditions:

(1) Leaching in physiological saline, incubation in Krebs' Ringer Phosphate (KRP) with varying concentrations of $^{42}K^+$.

(2) Leaching in physiological saline, incubation in KRP with varying proportions of K^+ and Rb^+.

(3) Leaching in physiological saline and various combinations of LiCl and NaCl, incubation in KRP again with varying proportions of Li^+ and Na^+.

The uptake by cells of Na^+, K^+, $^{42}K^+$ and Li^+ have been measured, while the tissue uptake of Rb^+ has been indirectly estimated.

It has been found that:

(1) Li^+, K^+, $^{42}K^+$ and Rb^+ all equilibrate with themselves across the cell membrane exponentially in time, in linear relation to the external concentrations of the specific cation.

(2) Li^+ penetration of the cell in the concentrations used was not associated with a change in Q_{O_2}.

(3) Li^+ cannot be transported out of cells against a concentration gradient in the range of C_i/C_o = 1:3 to 1:6, in contrast to Na^+.

It may be concluded that:

(1) The active transport of Na^+ out of cells is a specific process for the renal tubular cell.

(2) Li^+, K^+ and Rb^+ to a large measure passively diffuse across the cell membrane, subject to concentration gradients and Coulomb forces alone.

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