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# THE EFFECT OF TEMPERATURE ON THE TRANSPORT OF SODIUM AND POTASSIUM BY KIDNEY CORTEX SLICES

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The maintenance of differences in electrolyte composition between intracellular and extracellular fluids in kidney slices (predominantly tubular cells) is a process which requires the expenditure of metabolic energy (see review by Davies, 1954). Whittam & Davies (1954) have shown that the rates of uptake of $^{24}Na$ and $^{42}K$ were very much greater at 37 than at 0° C, as would be expected if the transport process was associated with a biochemical reaction rather than a physical process such as simple diffusion through a permeable membrane, on which the effect of temperature would be smaller.

It has been previously suggested (Cort & Kleinzeller, 1956, 1957; Kleinzeller & Cort, 1957*a*, *b*) that only Na is actively transported out of renal tubular cells (at the basal membrane), while K appears to behave more passively according to gradients of concentration and electrical potential across the cell membrane. An attempt has been made to examine this question further by observation of the effect of three different temperatures (15, 25 and 30° C) on the two fluxes of greatest physiological interest, the efflux of Na ($M'_{o,\,\mathrm{Na}}$) and the accumulation of K ($M'_{i,\,\mathrm{K}}$) during incubation of kidney cortex slices. (In this paper the symbol $M$ represents amount transported, whereas $M'$ represents the amount transported in unit time.) Change of temperature may be expected to influence the rate of transport at the cell membrane in a complex manner since permeability, active transport and potential gradient ($\Phi$) will probably all be affected. $\Phi$ cannot be independently varied in tissue slices, and has not been measured here. In order to minimize the effect of $\Delta T$ on $\Phi$ a kinetic, rather than a steady-state, approach has been adopted, the rate of transport being extrapolated to zero time by means of tangents to the curves of quantity transported versus time at the three temperatures involved. Since all the slices at the start of incubation are in approximately the same metabolic state and the same ionic environment, variation of $\Phi$ with $\Delta T$ should be at a minimum with a zero time approximation.

## METHODS

In order to have sufficient slices from a single animal for each set of temperature curves (about 200 slices are required for simultaneous measurement of $M_{o,\,\mathrm{Na}}$ and $M_{i,\,\mathrm{K}}$ at three temperatures on a six-point curve) dog kidneys were used. Five healthy adult mongrel dogs of either sex, body wt. about 10 kg, were killed by intravenous injection of about 100 ml. of air. Both kidneys were removed through a mid-abdominal incision as soon as consciousness was lost, i.e. incision was made within 5–10 sec, the kidneys were cut into quadrants, and placed immediately in dishes at 0° C for slicing.

For measurement of $M_{o,\,\mathrm{Na}}$ the slices (each of about 30 mg wet wt.) were leached for 2 hr in 0·154 M-$^{24}$NaCl, specific activity about 40 $\mu$c/ml., in order to achieve a high level of radioactive Na in cells. After leaching, the slices were washed thoroughly in non-radioactive 0·154 M-NaCl for about 30 sec to remove adherent activity, and then stored for a few minutes without medium in a moist atmosphere, still at 0° C, until placed in pre-warmed flasks with incubating medium (Krebs's Ringer-phosphate solution, buffered at pH 7·4, 3 ml./100–150 mg of slices) in a Dubnoff shaker. All media contained 0·005 M $\alpha$-ketoglutarate as substrate.

For measurement of $M_{i,\,\mathrm{K}}$ slices were leached in non-radioactive 0·154 M-NaCl for 2 hr, and incubated in 3 ml. of buffered (pH 7·4) Krebs's Ringer-phosphate medium, containing $^{42}K$ at about 1–2 $\mu$c/ml.

The isotopes were delivered in solid form as NaCl and KCl, and were purified by passing the prepared solutions through an ion-exchange column, using Dowex-50. The column for $^{24}$Na purification was pre-charged with Na to remove any K contamination, and that for $^{42}$K purification pre-charged with K to remove any Na contamination.

In order to maintain rigid timing of short intervals, flasks with medium were pre-warmed in the incubator, and the slices added to the shaking flasks. On removal from incubation, the contents of the flask were immediately poured through small glass funnels. Inserted into the stem of the latter were small glass rods, each with a hooked spiral at the upper end, so that the slices were caught on the spirals, and the medium immediately drained off below.

Since the presence of a considerable amount of $^{24}$Na in the extracellular compartment of the slices after washing would have to be corrected for in calculating fluxes at zero time ($t_0$), the following control experiment was carried out to estimate both the efficiency of washing and the rate of passive diffusion of labelled Na out of cells. Slices were leached in 0·154 M-$^{24}$NaCl at 0° C for 2 hr and washed for 30 sec in 0·154 M-NaCl at 0° C. They were then prepared for incubation in the same manner, but 'incubation' was carried out at 0° C in 3 ml. Krebs's Ringer-phosphate medium (pH 7·4). Further details were as described above.

Activity in the medium was measured in 0·1 ml. samples pipetted into plastic dishes of polyvinylchloride (so as to eliminate a correction factor due to reflexion of beta particles, necessary when metallic dishes are used) and quickly dried with an infra-red lamp (to eliminate a correction for self-absorption in the sample). These values were multiplied by a factor of 30 to calculate total activity in 3 ml. of medium. Tissue samples were weighed on a torsion balance, placed in 0·1 ml. 16 N-$HNO_3$ and heated for 5 min in a boiling water-bath, by which time they had dissolved. The samples were neutralized with conc. $NH_3$ and diluted to 3 ml., 0·1 ml. of which was used for measurement as above. Activities were corrected for background, decay and resolving time of the instrument, the ratio of measured counts to background counts being always greater than 10. Correction was also made for source geometry (see Cort & Kleinzeller, 1956) so that absolute values could be obtained (an end-window G.–M. tube was used) with further correction being made for air and mica absorption of the beta and gamma energy levels arising from disintegration of $^{24}$Na and $^{42}$K (data from standard tables). These activities were divided by the dry weight (mg) of tissue in the respective samples $\times 10^5$ (for conversion to activities per 100 g dry solids). When tissue activity was measured, parallel tissue samples were dried to determine relative water content, this value then being used to calculate the dry weight of tissue samples used for measurement of activity, where only a wet weight was directly determined. Total amount of transported cation

in a given time interval (in m-moles transported/100 g dry solids) was then calculated as follows:

$$M_{o,\ \mathrm{Na}(t_0\ \mathrm{to}\ t_x)} = \frac{\text{corrected total counts in medium at } t_x}{\text{corrected total counts in tissue at } t_x} \times \mathrm{Na}_{ICF},$$

where $\mathrm{Na}_{ICF}$ = m-moles of $^{24}$Na + $^{23}$Na/100 g dry solids (DS) in the intracellular compartment only.

$$M_{i,\ \mathrm{K}(t_0\ \mathrm{to}\ t_x)} = \frac{\text{corrected total counts in tissue at } t_x}{\text{corrected total counts in medium at } t_x} \times \mathrm{K}_{\mathrm{total}},$$

where $\mathrm{K}_{\mathrm{total}}$ = total $^{42}$K + $^{39}$K/100 g DS.

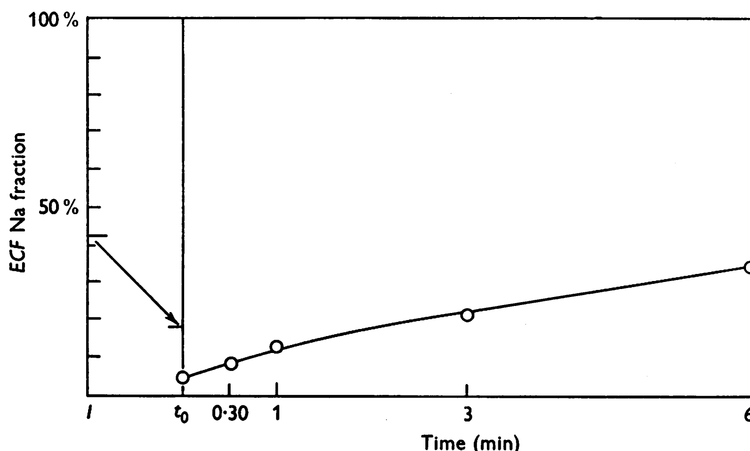


Fig. 1. Diffusion of $^{24}$Na out of tissue slices into Krebs's Ringer-phosphate medium during contro 'incubation' at 0° C (after 2 hr in 0·154 M-$^{24}$NaCl at 0° C and subsequent washing in 0·154 M-NaCl at 0° C). Ordinate, activity of medium as % of total tissue activity at the start of 'incubation'; abscissa, $I$ = initial period before leaching; the period $I$–$t_0$ = 2 hr; the marks in the $I$–$t_0$ portion of the graph indicate the changes in percentage extracellular fraction of Na during leaching.

Tissue electrolytes were estimated by flame photometry, as described by Cort & Kleinzeller (1956). Correction for the intracellular partition of total tissue Na was calculated on the basis of the data of Conway, Geoghegan & McCormack (1955) and Whittam (1956) that 25 % of tissue water is extracellular before both and after leaching at 0° C, as follows:

$$\mathrm{Na}_{ICF} = \mathrm{Na}_{\mathrm{total}} - [\mathrm{Na}]_{\mathrm{med.}} \times 0{\cdot}25\, W_{\mathrm{total}},$$

where $\mathrm{Na}_{\mathrm{total}}$ = total tissue Na in m-moles/100 g DS, $[\mathrm{Na}]_{\mathrm{med.}}$ = concentration of Na in the medium in m-moles/l., and $W_{\mathrm{total}}$ = total tissue water in ml./100 g DS (calculated from wet and dry wt., Cort & Kleinzeller, 1956). Average percentage values of extracellular tissue Na calculated on this basis are shown in Fig. 1.

Standard techniques were used for the measurement of $Q_{O_2}$, as previously described (Cort & Kleinzeller, 1956)

## RESULTS

Fig. 1 shows the rate of appearance of $^{24}$Na in the medium during a control 'incubation' carried out at 0° C (inhibition of active transport) under otherwise identical leaching conditions as with slices incubated at higher temperatures. Time intervals in this case were 30 sec, 1, 3 and 6 min of shaking after leaching.

Activity is expressed as a percentage of the average tissue activity (counts/min/100 g DS) after leaching in $^{24}$NaCl and washing in NaCl, but before 'incubation' at 0° C. This tissue level was set at 100%. The curve appears to be exponential, and on extrapolation to $t_0$, yields a value of the order of 4% of total tissue activity. It has been assumed that this initial value at $t_0$ represents the residual extracellular $^{24}$Na left in the slices after washing. The average percentage of total slice Na present in the extracellular compartment before and after leaching is indicated in the same figure (42 and 18%, respectively). The inherent error of the entire calculation is certainly greater than 4%, so that the correction factor for residual $^{24}$Na in the extracellular compartment may be neglected.

For incubation at higher temperatures, typical curves of $M_{o,\mathrm{Na}}$ and $M_{i,\mathrm{K}}$, expressed as m-moles/100 g DS versus time of incubation, are presented in Figs. 2 and 3, respectively. Samples were withdrawn for analysis at 30 sec, 1, 2, 4, 8 and 15 min for the three temperatures used, and tangents were drawn to approximate maximum transport rate at $t_0$. The differences between the two sets of curves show (1) a shift of the entire family of $M_{o,\mathrm{Na}}$ curves up from the $M_{i,\mathrm{K}}$ curves, indicating that $M_{o,\mathrm{Na}}$ starts off at a far higher rate than $M_{i,\mathrm{K}}$ at any of the temperatures used; and (2) that the differences between individual temperature curves for the two cations show that the same $\Delta T$ has a greater effect on $M_{o,\mathrm{Na}}$ than on $M_{i,\mathrm{K}}$. $Q_{10\,(15-25^\circ\,\mathrm{C})}$ has been calculated for the tangents drawn, and the following average values were obtained:

| | $Q_{10\,(15-25^\circ\,\mathrm{C})}$ |
|---|---|
| $M'_{o,\mathrm{Na}}$ | 2·02 |
| $M'_{i,\mathrm{K}}$ | 1·32 |
| $Q_{O_2}$ | 3·01 |

After the third minute of incubation, $Q_{10,M'_{i,\mathrm{K}}}$ approaches the values obtained for $Q_{10,M'_{o,\mathrm{Na}}}$. $Q_{10,M'_{o,\mathrm{Na}}}$ and $Q_{10,O_2}$ fall within the usual range (2–3) for biochemical reactions. $Q_{10,M'_{i,\mathrm{K}}}$ is significantly below this range in the first minutes of incubation.

The ratio of moles of Na cations extruded per mole of oxygen atoms utilized has been calculated both for maximum transport rates at $t_0$ and at $t_{8\,\mathrm{min}}$ when the slices are approaching a steady state. The average ratio at maximum transport rates was 33:1 at 25° C, approaching 50:1 at 15° C. When the curve of reaction rate flattens out after the third minute of incubation, the average ratio at $t_{8\,\mathrm{min}}$ was 3·8:1.

The combined average data for $M'_{o,\mathrm{Na}}$, $M'_{i,\mathrm{K}}$ and $Q_{O_2}$ have been set down on an Arrhenius plot of $T^{-1}$ versus the natural logarithm (ln) of the reaction rate, as shown in Fig. 4. All the points plotted lay on straight lines, the steepest slope belonging to $Q_{O_2}$, the next steepest to $M'_{o,\mathrm{Na}}$ and the shallowest to $M'_{i,\mathrm{K}}$. There was also a significant separation of the two cation transport curves.

On the assumption that Na and K transport can be treated as biochemical reactions, the usual thermodynamic formulae have been applied to these data, as follows:

$$-R\frac{\mathrm{d}\ln k}{\mathrm{d}(T^{-1})}=E_{\mathrm{act.}}=\Delta H^{*}+RT=-R\phi,$$

where $R$ is the gas constant, $k$ is the reaction rate constant, $E_{\mathrm{act.}}$ the activation energy of the 'active complex' of Na (or ? K) with a carrier molecule (this

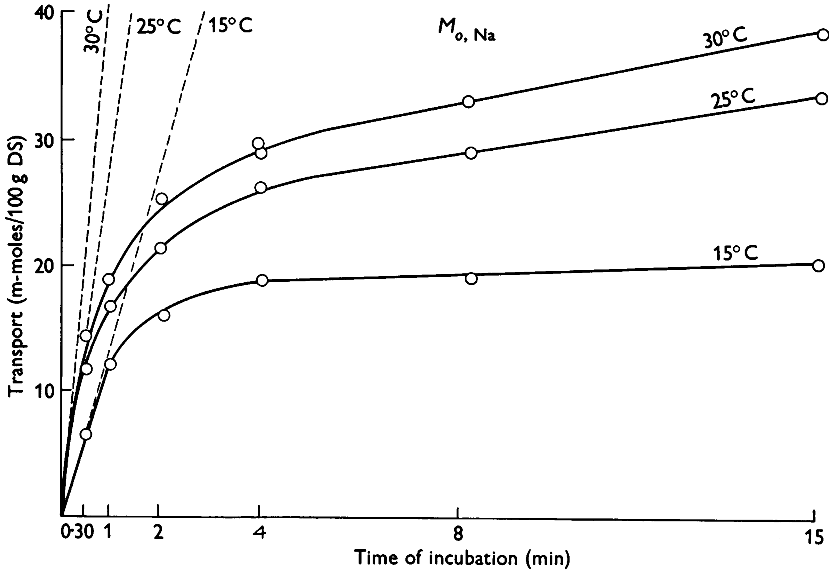


Fig. 2. Extrusion of Na from kidney cortex slices at 15, 25 and 30° C. Ordinate, transport (m-moles/100 g dry solids); abscissa, time of incubation.

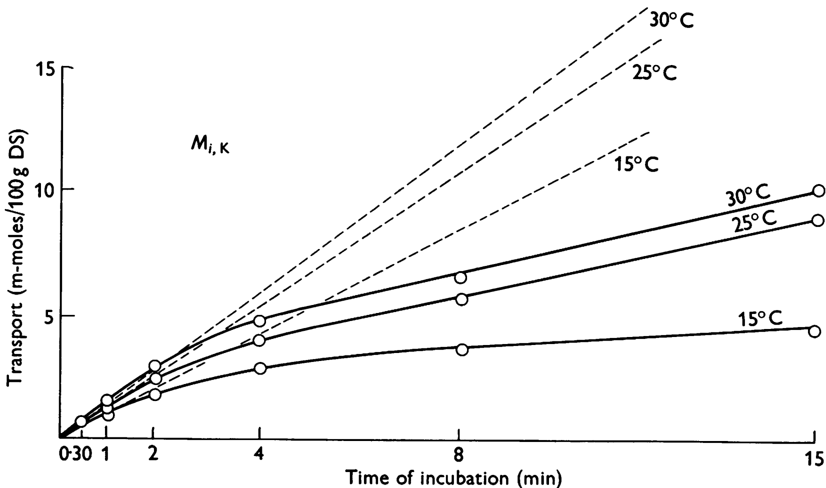


Fig. 3. Accumulation of K by kidney cortex slices at 15, 25 and 30° C. Other conditions as Fig. 2.

complex accomplishing the actual transport process), $\Delta H^*$ = the enthalpy change of active complex formation, $T$ = absolute temperature, and $\phi$ is the slope of the line on the Arrhenius plot, $d \ln k/d(T^{-1})$. Since all slices had statistically the same content of Na and K at the beginning of incubation,

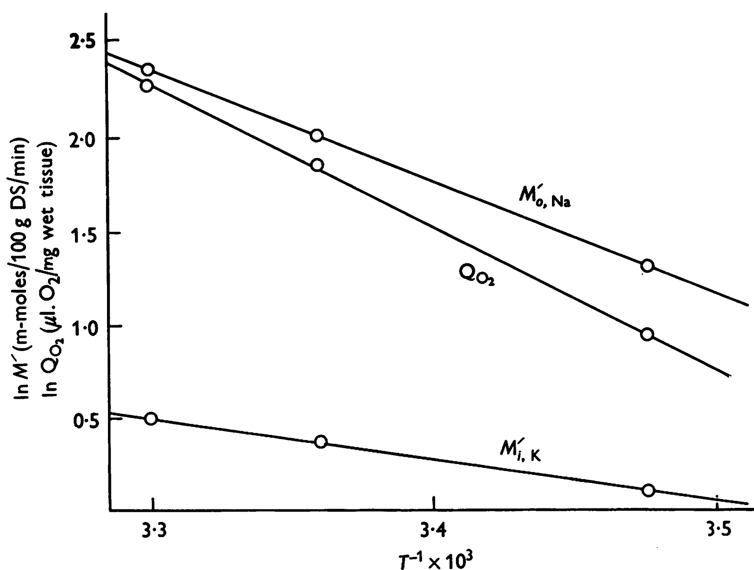


Fig. 4. Arrhenius plot of natural logarithm of rate of transport versus the reciprocal of temperature. Na extrusion, K accumulation and $O_2$ utilization by kidney cortex slices incubated at 15, 25 and 30° C.

regardless of incubation temperature, the reaction velocity constants ($k$ in the Arrhenius equation) may be replaced by the observed fluxes $M'_{o,\text{Na}}$ and $M'_{i,\text{K}}$, respectively. The results of the calculations of average activation energies for $M'_{o,\text{Na}}$ and $M'_{i,\text{K}}$ are:

| | $E_{\text{act.}}$ |
|---|---|
| $M'_{o,\text{Na}}$ | – 12·6 kcal/mole |
| $M'_{i,\text{K}}$ | – 4·8 kcal/mole |

while the order of magnitude for $\Delta H^*$ is 600 kcal for $M'_{o,\text{Na}}$. The $E_{\text{act.}}$ for $M'_{o,\text{Na}}$ falls within the usual limits of 10–20 kcal/mole for a biochemical reaction, while the value for $M'_{i,\text{K}}$ is significantly below this.

## DISCUSSION

A question as to the identity of the real fluxes, $M'_{o,\text{Na}}$ and $M'_{i,\text{K}}$, with the measured changes may be raised, owing to the complication of the presence of extracellular fluid in the slices, amounting to some 25% of total slice water. In other words, how much of the measured changes are due to pure diffusion of isotope in the extracellular space of the slice? Although kidney tissue *in vivo*

has three fluid compartments—intracellular fluid, interstitial and intravascular fluid, and intratubular fluid—under conditions of tissue slice experiments there are only two compartments, intracellular and extracellular fluid. Since the long axis of the tubules lies at right angles to the long axis of the slice, and the length of the tubules is considerably greater than the average thickness of a tissue slice (0·4 mm) it may be assumed that all tubules open into the medium and come into rapid equilibrium with it. The data in Fig. 1 allow extrapolation of the curve of the control 'incubation' to $t_o$, indicating that the relative quantity of extracellular $^{24}$Na present which might interfere with the calculation of maximal initial transport rates amounted to some 4% of total slice activity. Since this is at least of the order of magnitude of the error of the entire flux calculation, it can be neglected. The fact that the calculated $Q_{10}$ and $E_{act.}$ figures came out in the usual range for a biochemical reaction supports this contention. The curve in Fig. 1 represents the rate of diffusion of $^{24}$Na out of cells through the extracellular compartment into the medium at 0° C, while the extrapolated $t_0$ value represents the amount of $^{24}$Na still left in the extracellular compartment after washing. In the case of the measurement of $M_{i,K}$ the presence of extracellular labelled K cannot have produced any serious quantitative error, since only some 1% of total tissue K is actually present in the extracellular compartment at any given time. Both measurements have therefore been taken to represent a reasonable approximation to the actual fluxes indicated, within our given experimental error.

For further discussion of the results, it will be convenient to set up a model reaction for at least Na extrusion from renal tubular cells:

$$\begin{array}{ccccc} ICF & & \text{MEMBRANE} & & ECF \\ Na^{+} & \xrightarrow{\;①\;} & \overbrace{Na^{+}-X^{-} \rightarrow Na^{+}-X^{-}} & \xrightarrow{\;③\;} & Na^{+} \\ & & \uparrow \; \nwarrow ② & & \\ & & En. & & \end{array}$$

where $X^-$ is an anionic component of the membrane (presumably lipoid in nature) capable of specifically binding $Na^+$, *ICF* refers to intracellular fluid, *ECF* to extracellular fluid, and *En.* refers to a thus far unspecified source of metabolic energy. The steps of the process are labelled 1 to 3. According to such a model there are at least two 'chemical' (1 and 3) and three thermodynamic (1 to 3) processes directly involved only in the outward movement of Na across the cell membrane. Such a model represents the simplest possible set of phenomena to explain movement against an electrochemical gradient. The nature of the Na-carrier bond is immaterial to the discussion. It is the purpose of this discussion, therefore, to consider with what validity we may treat Na extrusion as a series of biochemical reactions, and whether K accumulation in cells can be similarly treated as a separate biochemical process *per se*.

The derived values for both $Q_{10,\,M'_{o,\,Na}\,(15-25°\,C)}$ and $E_{act.}$ for Na extrusion lie

within the accepted range for biochemical reactions. It must be stressed that these values apply to the series of processes 1 to 3 in the above diagram, or, perhaps better stated, to the rate-limiting process of this series. $M_{i,K}$ is more complex, however. The fact that neither $Q_{10,M'_{i,K}}$ nor $E_{act.}$ lie within an acceptable range for a biochemical reaction makes it doubtful that this process can be considered as such. K is certainly not bound to the Na 'carrier' in the manner envisaged for the red blood cell by Maizels (1954) and Glynn (1956), since the reciprocal fluxes of these two cations are not equal, and leaching results in a rise in the sum of Na + K in renal tubular cells, this level returning towards normal on 30 min of incubation (Whittam & Davies, 1954, Cort & Kleinzeller, 1956). This inequality of reciprocal flux is true for the entire period of incubation up to 30–40 min, when a steady state occurs. It would appear that the two main arguments for a direct carrier-linked reciprocal transport of Na and K across the red cell membrane, i.e. stoichiometrically equal quantities of reciprocal transport and an absence of Na extrusion when there is no K present in the medium, are not conclusive. A lack of Na extrusion in the absence of K in the medium is also true for kidney cortex slices (Cort & Kleinzeller, 1956), but this can also be explained by an indirect interaction of two different mechanisms for the two cations, i.e. by changes in potential gradient across the membrane, and the differences between red blood cell and renal tubular cell transport may be due to differences in movement of other charged particles (anions) across the membrane.

While an active process for $M_{i,K}$ is not consistent with these data, it cannot be stated whether K accumulation is a simple diffusion process. In an ideal diffusing system, $Q_{10}$ would be expected to lie between 1·01 and 1·02. But in a biological system, a rise in $T$ of 10° C will produce a fall in viscosity of up to 20 %, so that a $Q_{10}$ of 1·32, as determined for $M_{i,K}$, might be explained as the effect of $\Delta T$ on a pure diffusion process were sufficient data available. On the other hand, since $M_{o,Na}$ is greater initially than $M_{i,K}$, it might be expected that $\Phi$ (the electrical potential difference) would increase with temperature, and this might play a role in increasing the values of K accumulation above those predicted on the basis of diffusion alone. It is interesting in this regard that Teorell (1953) gives a range of values of 2–5 kcal/mole for the activation energies of particles being passively transported across ionic membranes by 'exchange diffusion', the measured $E_{act.}$ for $M'_{i,K}$ falling within this range. It is suggested, therefore, that K accumulation (as well as K loss) in renal tubular cells is a process which follows electrochemical gradients alone.

Na extrusion is a process which, if we limit our consideration to the above processes 1 to 3 alone, decreases entropy. The entropy of the entire system would, of course, be increasing owing to the utilization of metabolic energy for transport.

It is most probable that a lipoprotein cell membrane has a fairly high charge density, and that some element of 'ion exchange' will occur at the membrane

surface. But although a 'cation exchange' mechanism has been proposed to explain Na reabsorption in the renal tubules (Na supposedly 'exchanges' for K or H moving in the opposite direction, so that $M'_{i,\mathrm{Na}} = M'_{o,\mathrm{K}} + M'_{o,\mathrm{H}}$ at the tubular membrane of the tubular cell) and has been vigorously supported by Pitts (1952), Berliner, Kennedy & Orloff (1951), Black & Emery (1957) and many others, it is very difficult to see how such a mechanism could transport Na against an electrochemical gradient, or how it could explain the above thermodynamic data, or the lack of specificity of K accumulation (Cort & Kleinzeller, 1957; Kleinzeller & Cort, 1957*b*). The 'exchange diffusion' theory

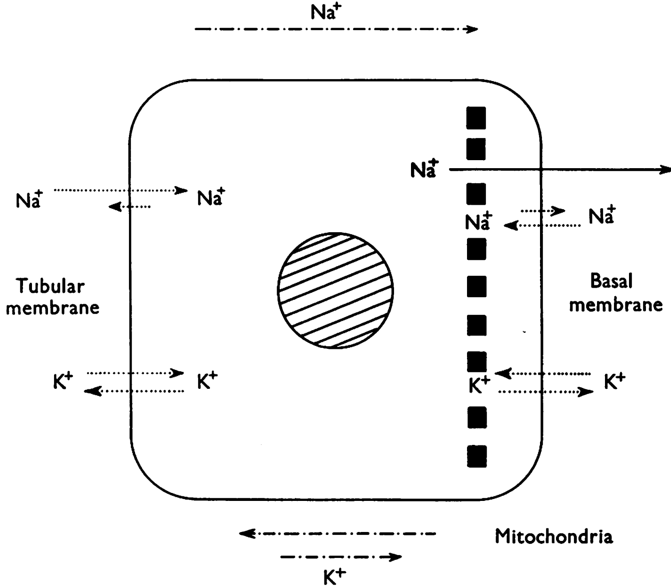


Fig. 5. Suggested scheme for Na and K transport by the renal tubular cell. A solid arrow indicates active transport, a dotted arrow indicates passive transport, and a dot-dash arrow indicates the possible direction of net transport across the renal tubular cell. Mitochondria are included to indicate the basal membrane of the cell, as opposed to the tubular membrane. All arrows are meant to have a vector connotation, i.e. length of arrow indicates the relative magnitude of the process.

of Teorell (1953) can explain a limited degree of passive transport against a slight concentration gradient, but not of the order found in the renal tubular cell. It would seem far more likely to attribute different basic mechanisms for the reciprocal movement of the two cations, interrelated only by Coulomb and osmotic changes in the cell. Since $M_{o,\mathrm{Na}}$ appears to be the only clearly active process of the four possibilities $M_{o,\mathrm{Na}}$, $M_{i,\mathrm{Na}}$, $M_{o,\mathrm{K}}$, $M_{i,\mathrm{K}}$, and as far as is known from whole-animal work Na is only reabsorbed to a greater or lesser extent (there is no evidence for secretion) it would appear logical to assign this active process to the basal membrane of the tubular cell, as depicted in Fig. 5.

The arguments of Ling (summarized, 1956) and Troshin (1956) against active transport of Na would appear to have some validity only for a non-secreting tissue such as muscle, but would not be able to explain the far greater level of Na flux through a secreting cell such as the renal tubular cell.

The molar ratio of Na ions transported to oxygen utilized has been measured at near steady-rate conditions in muscle by Conway (1954) and in kidney by Davies (see review, 1954). Both have found values near to 4, which Conway takes as support for his redox theory of Na pumping. Similar figures have also been attained here for near steady-state conditions, i.e. after the third minute of incubation after leaching, but at maximum transport conditions ($t_0$) this ratio is of the order of 33:1 at 25° C, with a higher ratio at lower temperatures. Even greater ratios at low temperatures have been found for Na transport in frog skin (Snell & Leeman, 1957). Until it can be shown, therefore, that there is more than one mechanism for Na extrusion by the renal tubular cell, it is unlikely that this process is directly related to $O_2$ utilization. It would appear that in the initial minutes of incubation there is a stored source of energy for Na extrusion, the rate at which this can be utilized after the third minute of incubation then being dependent on $O_2$ utilization. An alternative explanation is the possibility that more than one mole of Na is transported per one mole of high-energy bonds. Such a possibility has to be considered in view of the observation by Whittam & Davies (see Davies, 1954) that in mitochondria from kidney cortex the Na:$O_2$ ratio is of the order of $10^4$.

## SUMMARY

1. The rates of $^{24}$Na extrusion from cells and uptake of $^{42}$K have been measured in dog kidney cortex slices at short time intervals after a period of leaching. Incubation was carried out at 15, 25 and 30° C.

2. A zero time approximation was made by drawing tangents to the curves of transport versus time, to obtain an estimation of maximum transport rates.

3. $Q_{10}$'s were calculated, and the data set down on an Arrhenius plot of natural logarithm of transport versus the reciprocal of temperature, in order to calculate activation energies and enthalpy changes, on the assumption that such transport can be treated as a biochemical reaction.

4. The data for maximum rates of Na transport come within the accepted range for a biochemical reaction, the data for maximum rates of K accumulation do not.

5. On incubation after leaching, renal tubular cells extrude Na at a faster rate than they accumulate K, and Na transport is more affected by $\Delta T$ than K transport. This applies for the first few minutes of incubation.

6. The ratio of moles of Na transported to moles of $O_2$ utilized at maximum reaction rates can be as high as from 33 to 50:1, this ratio falling to the region of 4:1 only after the third minute of incubation.

7. It has been argued from these results that:

(*a*) a 'cation exchange' mechanism, proposed for the reabsorption of Na in the renal tubules, cannot explain transport against existing electrochemical gradients or the thermodynamic data presented above;

(*b*) Na is actively extruded from renal tubular cells by a series of biochemical reactions not directly or 'carrier' linked to K transport;

(*c*) it would appear most logical to assign this transport of Na to the basal membrane of the tubular cell, since there exists no evidence for Na secretion into the urine;

(*d*) K transport across the tubular cell membranes follows concentration and electrochemical gradients alone; and

(*e*) the energy source for Na extrusion from tubular cells is not directly related to oxygen utilization.

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