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EFFLUX OF SODIUM FROM ANAEROBIC KIDNEY SLICES

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SUMMARY

1. Efflux of Na^+ into Na^+-free solutions, and of K^+ into K^+-free solutions and of Na^+ and K^+ into Na^+- and K^+-free solutions from cortical slices of the kidneys of rats has been measured at 25° under conditions in which both aerobic and anaerobic metabolism were blocked.

2. Most of the cell Na^+ was lost much more rapidly than any of the cell K^+, but a small amount of Na^+ was lost slowly.

3. Two estimates of the quantity of this 'slow' Na^+ suggested that it amounted to no more than 12% of the total cell Na^+ or 5% of the total tissue Na^+ (10–18 mequiv/kg dry wt.).

4. It is concluded that it is unlikely that only this small fraction of the tissue Na^+ could be inside the cell proper, and that the difference in rates of efflux of Na^+ and K^+ was possibly due to retention by the cells of some degree of K^+-preferring, Na^+-excluding properties.

INTRODUCTION

Efflux of most of the Na^+ was previously[1] found to be much more rapid than efflux of K^+ from cells of anaerobic cortical slices of the kidneys of rats and rabbits, suggesting that cells might retain a degree of K^+ selectivity in the absence of metabolism[1,2]. When Na^+ efflux was measured, however, the slices still contained small amounts of Na^+ at the end of the experiment. These measurements have now been repeated under different conditions which are more favourable to determining the amount and variability of this residual Na^+. The existence of an appreciable quantity of cell Na^+ lost at the same slow rate as cell K^+ would invalidate the tentative conclusions that were drawn from the striking difference in the rates of efflux of the two cations after metabolism had stopped.

It appears from the present results that the amount of this 'slow' Na^+ is probably so small that it should not affect the previous argument.

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METHODS

Preparation of tissue

Thin (0.1–0.15 mm) cortical slices from the kidneys of adult male hooded rats were cut by hand as described previously[2] and incubated for 15 min in 'ordinary' medium[3] at 25°. O_2 bubbling through the solution stirred it efficiently without damaging the very thin slices.

Media

'Ordinary' medium. The medium used for preparation of the slices contained (mequiv/l): Na^+, 145; K^+, 5; Mg^{2+}, 2; Ca^{2+}, 5; Cl^-, 140; SO_4^{2-}, 2; and 8 mM PO_4^{3-}.

Anaerobic receiving solutions. All the anaerobic solutions were saturated with N_2 to block aerobic metabolism and contained 1.0 mM iodoacetate to block glycolysis; they were buffered at pH 7.4.

The Na^+-free medium contained (mequiv/l): K^+, 100; Cl^-, 85; iodoacetate, 1; together with 8 mM PO_4^{3-} and 10 g/100 ml of polyethylene glycol 6000.

The K^+-free medium contained (mequiv/l): Na^+, 100; Cl^-, 85; iodoacetate, 1; together with 8 mM PO_4^{3-} and 8 g/100 ml of polyethylene glycol 6000.

The choline chloride medium (both Na^+- and K^+-free) contained (mequiv/l): choline ion, 286; Tris ion, 17.7; Cl^-, 300; iodoacetate, 1.

Procedure

Slices from the kidneys of 3 rats, pooled for 1 experiment, were transferred rapidly without blotting from the oxygenated ordinary medium at 25° to the appropriate anaerobic solution, also at 25°. To ensure that O_2 was absent, N_2 was bubbled constantly through this solution, which was stirred magnetically. At intervals of time slices were removed and separated into 2 lots for analysis, giving, usually, a final dry wt. of about 10 mg per sample. Towards the end of an experiment, however, when slices contained low concentrations of one or both cations, up to 30 mg was required.

Na^+ contamination. Towards the end of a Na^+-efflux experiment the amount of Na^+ remaining in a slice was so small that even slight contamination of either slice or solution with Na^+ at any subsequent point in the procedure led to serious error. All glassware was therefore eliminated from the apparatus. The concentration of ions that had already come out of slices was kept negligibly low by putting slices initially into a large volume (1 l) of well-stirred Na^+-free solution, most of which was quickly decanted after about 30 min when the slices had lost most of their Na^+, and replaced with 1 l of fresh Na^+-free solution at the same temperature. Na^+ and K^+ concentrations in the supernatant were measured at the end of each experiment.

Water contents of slices had to be measured before their ions could be extracted. During this process a slice depleted of Na^+ picked up significant quantities both from filter paper used for blotting and from the glass tube in which it was heated. Of the two batches of slices that were removed at any time, therefore, one was blotted, put into a weighed glass tube, and analysed for water and K^+, and the other put directly into a small plastic pot, extracted with 0.1 M HNO_3 and analysed for Na^+ and K^+. When slices were losing their Na^+ into Na^+-free medium, those slices put

without blotting into plastic pots took with them K^+-rich supernatant; they yielded neither water- nor reliable K^+-contents. Their K^+ was estimated, however, so that a correction could be made of the Na^+ reading on the flame photometer (see under *Determination of electrolytes*). When the anaerobic receiving solution contained choline chloride, K^+-contents could be obtained from both blotted and unblotted slices: the good agreement always found was a useful check on the method.

Analysis

Determination of water. The water contents of slices were obtained by weighing blotted slices before and after heating at 105° for 2 h (see ref. 4). The water was referred to a final dry wt. obtained by weighing slices that had been reheated for 2 h at 105° after extraction of their ions and choline chloride or polyethylene glycol 6000 with 0.1 M HNO_3.

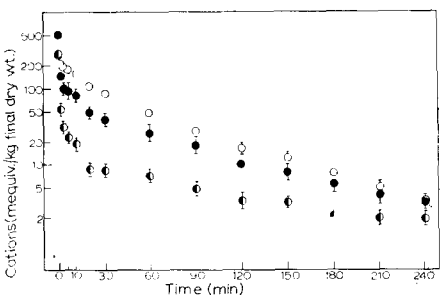
Determination of electrolytes. A batch of either wet or dried slices was extracted for 2 h in 3–5 ml 0.1 M HNO_3. Na^+ and K^+ were determined in these extracts using an EEL flame photometer with external standard made up in 0.1 M HNO_3. In some extracts where the concentration of K^+ was much higher than that of Na^+, K^+ interfered with Na^+ analysis. Correction curves were constructed from series of measurements of apparent Na^+ concn. in solutions of known Na^+ concn. to which had been added known concentrations of K^+. The ranges of concentrations encountered in the extracts (and therefore employed in the correction curves) were: Na^+, 0.01–0.05 mequiv/l; K^+, 0.1–2.0 mequiv/l. From these curves readings of the flame photometer for Na^+ concn. were corrected for K^+ interference. If the correction was more than 5% (as it was when the Na^+ concn. was less than 0.01 mequiv/l) the reading was discarded, and a value was obtained at a more favourable part of the correction curve by pooling enough slices to give a Na^+ concn. in the extract greater than 0.2 mequiv/l.

These corrections were necessary only when Na^+-free medium was the receiving solution. Interference of Na^+ in K^+ analysis or choline in either Na^+ or K^+ analysis was always negligible.

RESULTS

In Fig. 1 are compared the rates of loss of Na^+ and K^+ from anaerobic cortical slices under the following conditions. Na^+ loss was measured into the receiving Na^+-free medium, which contained 1.0 mM iodoacetate to block glycolysis, and polyethylene glycol 6000 to keep the water content of the slices approximately constant, as previous work[2] had shown that rates of loss of ions vary with water content. K^+ loss was measured into the corresponding K^+-free medium. Both cations, in Fig. 1, are referred to final dry wt. because the slices for which Na^+-contents were determined did not give water contents. Expressing concentrations in terms of tissue water makes no difference to the shapes of the curves, since the water contents were approximately constant except in the choline chloride medium (see Fig. 2).

As previously found[2], slices lost their K^+ relatively slowly (34% in 5 min; 61% in 20 min) with only a slight curvature in the log K^+–t plots, suggesting that the loss of K^+ was approx. first order. Most of the Na^+, on the other hand, was lost



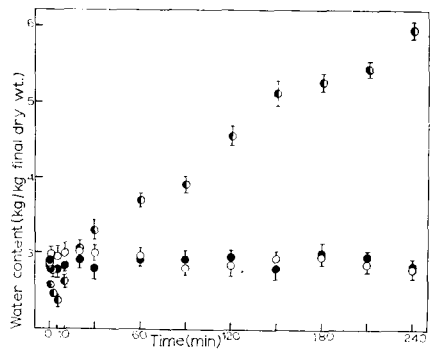


Fig. 1. Rates of loss of Na^+ into an anaerobic Na^+-free solution and of K^+ into an anaerobic K^+-free solution at 25°. Water contents of slices were approximately constant. ○, K^+ into K^+-free medium; ◐, Na^+ into Na^+-free medium; ●, Na^+-loaded slices into Na^+-free medium. Each point represents the mean and standard deviation of 6–8 determinations (top and bottom curves) or of 4 determinations (middle curve).

Fig. 2. Variation with time of the water contents of slices put into 3 anaerobic solutions at 25°. ◐, choline chloride; ○, Na^+-free medium; ●, K^+-free medium. Each point represents the mean and standard deviation of 6–8 determinations.

in the first few min (93% in 5 min; 98% in 20 min). After this initial rapid efflux of Na^+, slices lost their remaining small quantity of Na^+ rather more slowly, apparently, than their larger remaining content of K^+.

This slow loss of Na^+ was not detected in previous experiments[2] because (1) Na^+ contamination occurring during blotting of slices must have been at an approximately constant level which was sufficiently great to mask the slight decrease after 20 min (at a rate of about 1.5 mequiv/kg final dry wt. per h), and (2) Na^+ was sufficiently concentrated in the receiving solution (0.5–1 mequiv/l) to make influx of the same order as efflux toward the end of an experiment. This would further reduce the apparent gradient of Na^+ loss.

Slices loaded with Na^+

In order to determine whether the small amount of slow Na^+ observed in Fig. 1 could be increased, slices were stirred for 90 min in K^+-free medium. During this time there was a large increase in Na^+ (from 290 to 540 mequiv/kg final dry wt.) and a large decrease in K^+ (from 270 to 28 mequiv/kg final dry wt.). The middle curve in Fig. 1 shows the rate at which slices lost this amount of Na^+ into Na^+-free medium, the receiving solution used also for the bottom curve of Fig. 1. Significantly more Na^+ was lost at the slow rate.

Receiving solutions containing choline chloride

When Na^+-free medium was the receiving solution the concentration of Na^+ in the supernatant at the end of an experiment ranged up to 0.3 mequiv/l. This concentration could not readily be reduced, as it was due to a small and variable contamination of the polyethylene glycol 6000 with Na^+. After 3 h the concentration of Na^+ in Na^+-depleted slices in Fig. 1 was only 0.7 mequiv/kg tissue water. With an external concentration of 0.3 mequiv/l net flux outward must have been

considerably less than absolute efflux; this could account for the flattening of the log Na^+–t curve toward the end of the experiment where there was no longer a significant decrease in Na^+-content (bottom curve, Fig. 1). No such flattening occurs in the curve for K^+, which cation was never more concentrated in the supernatant than 0.005 mequiv/l.

Polyethylene glycol 6000 was therefore omitted from the receiving solutions, and slices permitted to swell during loss of their ions. In order that rates of loss of the two cations from slices of varying water content should be strictly comparable, the losses of Na^+ and K^+ into Na^+- and K^+-free choline chloride solutions were measured concurrently. Concentrations of Na^+ and K^+ in the supernatant were both less than 0.01 mequiv/l at the end of these experiments. When choline chloride was used as the receiving solution, conditions were in every way identical for the two cations; differences in their rates of loss could not, therefore, be attributed to differing experimental conditions.

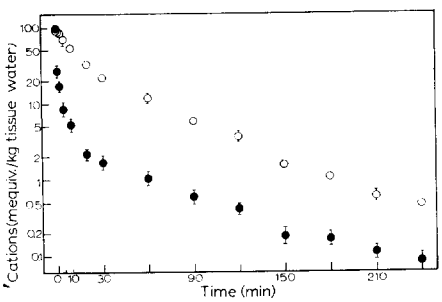


Fig. 3. Rates of loss of Na^+ and K^+ measured concurrently into Na^+- and K^+-free anaerobic choline chloride solutions at 25°. ○, K^+; ●, Na^+. Each point represents the mean and standard deviation of 6–8 measurements.

Fig. 3 shows these results. This time cations are expressed in mequiv/kg tissue water in spite of the fact that this was a quantity derived from measurements on 2 separate batches of slices. This method of plotting was adopted because concentrations in water are more meaningful in a rate process, and because it reduces the curvature caused by changing water content (see Fig. 2) in plots of log cations/kg final dry wt. against time.

As in Fig. 1 K^+ was lost approximately exponentially and relatively slowly (the concentration decreasing 29% in 5 min, 68% in 20 min); most of the Na^+ was lost rapidly in the first few min (the concentration decreasing 93% in 5 min, 98% in 20 min). Here the slow loss of Na^+ following the first 20 min can be seen to continue almost parallel to the slow loss of the residual K^+; flattening of the curve seems to have disappeared with the appreciable concentration of Na^+ in the external solution.

DISCUSSION

These results confirm the previous observations[1] that these cells lose most of their Na^+ much more rapidly than their K^+. They also show, however, that there is a small amount of Na^+ that is lost slowly.

Kinetics of ion exchange

In these experiments the system was not in a steady state, but Na^+ was exchanging for K^+ or for choline, and K^+ was exchanging for Na^+ or choline. There is, therefore, no manageable rate equation which can be used to determine the amount of slow Na^+ that was initially present. The following 2 approximate methods, however, should give its order.

(1) Assume that the cell contains different compartments of both Na^+ and K^+ and that for each kind of cation an equation can be written of the kind $-dc_i/dt = kc_ic_0'$ where c_i is the concentration of the cation in the tissue water, and c_0' the concentration of the exchanging ion in the receiving solution. Since c_0' = constant, this equation can be written $-dc_i/dt = k'c_i$ where k' is a new constant. In this equation it is assumed that the other terms in the rate equation are negligible compared with the concentration term. The curves of Figs. 1 and 3 can then be analysed into sums of exponentials, giving the amount of each kind of ion and its specific rate constant. Table I shows the result of this kind of analysis for Fig. 3 where the ions were lost under identical conditions so that their rates of loss should be strictly comparable, and where influx was negligible throughout the experiment.

TABLE I

SUM OF EXPONENTIALS SOLUTIONS FOR CURVES OF FIG. 3, GIVING APPROXIMATE AMOUNTS OF CATION IN EACH COMPARTMENT, AND THE APPROXIMATE TIME OF HALF LOSS FROM THAT COMPARTMENT

$t_{\frac{1}{2}}$, 2.303 $\log 2/k$ where k is the specific rate constant.

Percentage intracellular K^+	*K^+-content (mequiv/kg final dry wt.)*	*$t_{\frac{1}{2}}$ (min)*	*Percentage intracellular Na^+*	*Na^+-content (mequiv/kg final dry wt.)*	*$t_{\frac{1}{2}}$ (min)*
25	69	49	3	4	72
75	207	12	4	6	21
			93	135	1.7

Intracellular Na^+ in this table is equal to the total tissue Na^+ less the Na^+ contained in the usual inulin space[5,6] (26% of the weight of the wet tissue) which KLEINZELLER, JANACEK AND KNOTKOVA[7] reported to be the same as the Na^+ space. It has been assumed that the extracellular Na^+ calculated in this way was all lost in the first min; this assumption could lead only to an underestimate of the rate of Na^+ loss. The striking point which emerges from this analysis is that only 7% or 10 mequiv/kg final dry wt. of the cell's initial Na^+ was lost at rates comparable with the rates of loss of any of the cell's K^+. The time of half loss of the rest of the cell's Na^+ (93% or 135 mequiv/kg final dry wt.) was at least an order of magnitude smaller.

(2) An alternative solution which probably gives a maximal estimate of the initial slow Na^+, assumes that all the cell K^+ was lost at one rate (*i.e.* from one compartment), but not strictly exponentially. Since the shapes of the curves for Na^+ and K^+ are similar after about 20 min, it is assumed that the time course for the loss of slow Na^+ was similar to that of K^+ before this time also; *i.e.* the time course of the K^+ loss is taken as a pattern for the time course of the slow Na^+ loss. The problem then is to extrapolate the curve for slow Na^+ from $t = 20$ min to $t = 0$ min.

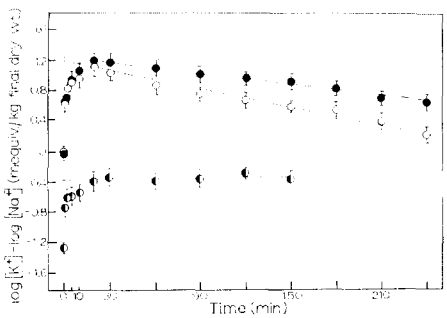


Fig. 4. Curves derived by subtracting the Na^+ curves from the K^+ curves in Figs. 1 and 3. The straight lines were calculated by the method of least squares. ○, top and bottom curves, Fig. 1; ◐, top and middle curves, Fig. 1; ●, Fig. 3.

This extrapolation is made simpler in Fig. 4 by plotting log K^+—log Na^+ against time. Straight lines have been fitted by the method of least squares to the points from 20 min onwards: these are assumed to represent values of log K^+—log Na^+ (slow). Extrapolation of these straight lines to $t = 0$ gives estimates of the amount of slow Na^+ initially present according to this model.

Table II gives the results of these extrapolations. The amounts of slow Na^+ obtained by this method are rather higher than those in Table I, but still remarkably low. It is interesting that the value obtained when choline chloride was the receiving solution agrees quite well with the value obtained under completely different conditions with K^+- and Na^+-free medium as the receiving solutions. The value for slices loaded with Na^+ shows that the amount of slow Na^+ can be increased, but that it remains at about the same low percentage of the total intracellular Na^+, suggesting that it might be held behind a permeability barrier.

TABLE II

AMOUNTS OF SLOW Na^+ DERIVED BY EXTRAPOLATION OF THE STRAIGHT LINES IN FIG. 4 TO $t = 0$

$t_{\frac{1}{2}}$ of the remainder of the cell Na^+ (total intracellular Na^+ less slow Na^+) is a rough estimate, but lies within the range indicated.

Receiving solutions	*Slow Na^+-content (mequiv/kg final dry wt.)*	*Percentage total intracellular Na^+*	*Estimated $t_{\frac{1}{2}}$ of remainder (min)*
Na- and K^+-free medium	18	12	1.5–2.2
Choline chloride	15	10	0.8–2.0
Na^+-loaded slices in Na^+- and K^+ free medium	66	15	0.7–2.2

The significance of the different rates of loss

That 2 such similar cations could diffuse from poisoned cells at such different rates suggests that either they were initially in different compartments with widely differing barriers to diffusion, or that their activities were not nearly equal to their concentrations.

Compartmentation. If the separation of cations were into different compartments within one cell, the K^+-containing compartments, which presumably would be bounded by the membranes which actively transport Na^+, would contain only 7–12% of the cell Na^+; the rest of the cell Na^+ (127–135 mequiv/kg final dry wt.) should be outside these membranes, but within the region of tissue not available to inulin. Wherever this Na^+ might be, it should, presumably, be at a concentration not very different from that in the extracellular solution; *i.e.* it should be associated with about 0.9 kg of water per kg final dry wt., leaving only 0.9, instead of the usual estimate of 1.8 kg/kg final dry wt. for the rest of the cell containing all the K^+. Using cortical slices from the kidneys of guinea pigs, WHITTEMBURY[8] showed that the measured membrane potential was equal to the K^+ equilibrium potential unless the external K^+ concn. was too low. If cellular K^+ were twice as concentrated as he calculated, however, the expected equilibrium potential would be 59 log 2 or 18 mV more negative. This same difficulty is encountered if the compartments are considered to be in different kinds of cells.

Activity differences. An alternative explanation of the different rates of loss of most of the Na^+ and K^+ is that both ions were initially in the same regions of the cell, but that the activity of K^+ was lower than its concentration, or the activity of Na^+ was higher than its concentration, or both activities were anomalous. Such differences could arise from residual K^+-preferring, Na^+-excluding properties of the cell as a whole[1,2,12].

Slow Na^+

There is evidence of a small amount of slowly exchanging Na^+ in cortical cells. KLEINZELLER AND KNOTKOVA[9] found that 10% of the total tissue Na^+ had not exchanged after incubation of rabbit-kidney slices aerobically for 45 min at 25° and 37°. BURG, GROLLMAN AND ORLOFF[10] found in aerobically incubated separated renal tubules of the rabbit that, whereas K^+ exchange seemed to be complete in about 1 h, only 97% of the Na^+ had exchanged after 90 min. More measurements after about 30 min could have revealed a slowly exchanging compartment containing only about 10% of the total cell Na^+ (in the same way that many measurements from 30 to 240 min have been necessary to reveal the existence of slow Na^+ in these experiments). Similarly the results of WHITTAM AND DAVIES[11] are consistent with the existence of a very small slowly exchanging compartment of Na^+. At 37° all the K^+ exchanged both aerobically and anaerobically but only 98% (aerobically) and 96% (anaerobically) of the tissue Na^+ in cortical slices of the kidneys of guinea pigs.

If it is assumed that this very small amount of slowly exchanging Na^+ can be identified with the similar amount of slow Na^+ in the present experiments it becomes even less probable that only the slow Na^+ was inside the cell proper; *i.e.* inside the Na^+-transporting membranes. BURG, GROLLMAN AND ORLOFF[10], who neglected this slow Na^+, found that the bulk of the cell Na^+ was divided between 2 compartments of about equal size (86.4 and 74.6 mequiv/kg final dry wt.) and they calculated that the observed rate of reabsorption in the kidney would require the flux from the faster of these 2 compartments. The flux from the third, smallest and most slowly exchanging compartment would clearly be much too slow.

The amount of slow Na^+, then, seems too small to be considered the total intracellular Na^+, and therefore too small to invalidate the previous suggestion[1] that

the striking difference in the rates at which anaerobic cortical cells lost Na^+ and K^+ might be due to retention by the poisoned cells of a diminishing K^+ selectivity.

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