Sodium Extrusion and Potassium Uptake in Guinea Pig Kidney Cortex Slices

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ABSTRACT Slices from the cortex corticis of the guinea pig kidney were immersed in a chilled solution without K and then reimmersed in warmer solutions. The Na and K concentrations and the membrane potential V_m were then studied as a function of the Na and K concentrations of the reimmersion fluid. It was found that Na is extruded from the cells against a large electrochemical potential gradient. Q_{10} for net Na outflux was ~2.5. At bath K concentrations larger than 8 mM the behavior of K was largely passive. At the outset of reimmersion ($V_m > E_K$) K influx seemed secondary to Na extrusion. Na extrusion would promote K entrance, being limited and requiring the presence of K in the bathing fluid. At bath K concentrations below 8 mM, K influx was up an electrochemical potential gradient. Thus a parallel active K uptake is apparent. Q_{10} for net K influx was ~2.0. Dinitrophenol inhibited net Na outflux and net K influx, Q_{10} became <1.1 for both fluxes. The ratio between these fluxes varied. Thus at the outset of reimmersion the net Na outflux to net K influx ratio was >1. After 8 minutes it was <1.

INTRODUCTION

When kidney slices are immersed in a chilled solution, rich in Na, but with reduced K content, their cells lose part of their K and gain Na. The original composition is restored to an appreciable extent after reimmersion in a warmer Ringer's solution (1–3). Such soaking procedures, used extensively for other tissues (4, 5) provide a convenient way of reversibly altering the Na and K concentration of the kidney cells and of gaining information about their distribution and movements (6). Measurements of cell electrical potentials are necessary to define the electrochemical driving forces for the movements of these ions and to characterize their mode of transfer. Consequently, cell potentials and Na and K concentrations were estimated in guinea pig kidney cortex slices under various soaking conditions. It was found that Na is actively extruded from the cell interior by a mechanism that requires the presence of

K in the bathing fluid. The distribution of K was found to be passive, in reimmersion fluids with K activities higher than 8 mmole/kg. At lower K activities, despite a high degree of passive permeability to K, an active mechanism for the uptake of K by the cells is required to explain the K distribution. Na extrusion and K uptake are coupled, though this coupling is not rigid.

EXPERIMENTAL PROCEDURE

In this work, only the outermost slices from the kidney cortex (cortex corticis) have been used. They have only one surface section, hence their cells should be preserved better than those of the deeper slices. Their population, formed mainly by cells of the proximal tubules, is quite uniform. Thus, Walker and Oliver (7) found that 96 per cent of the tubules impaled at random from the kidney surface were proximal, only 4 per cent being distal. It should be noted that the outermost slices have been usually rejected in previous work (*cf* references 2, 21, 32) because their K content was higher and their Na content lower than that of the rest of the kidney cortex slices (2).

Fig. 1 illustrates the general experimental procedure. Healthy adult guinea pigs (*Cavia porcellus*) of the order of 1 kg in weight, were stunned and bled from the carotids. The kidneys were quickly removed, decapsulated, and sliced (8). The outermost slices, 0.2 to 0.3 mm thick, 100 to 200 mg in weight, were employed. They were divided in three groups: those from the first group were blotted, weighed, and separated for analysis of fresh tissue. Slices from the other two groups were dipped into the immersion medium at 0 to 3°C and shaken at 120 oscillations per minute (amplitude 2 cm) for 2 hours. The slices from the second group were then removed, blotted, weighed, and separated for analysis. The slices from the third group were transferred to the reimmersion media at 25°C, shaken similarly for 50 minutes and then taken out, blotted, weighed, and separated for analysis. Large amounts of media (about 200 ml) were used for immersion and reimmersion, so that only minimal changes in their composition could occur during the experiment.

Bathing Media Unless indicated, all media contained, in mM: Na acetate, 9 (reference 1); $NaHCO_3$, 15; NaH_2PO_4, 0.6; Na_2HPO_4, 2.4; $MgSO_4$, 1.2; Na_2SO_4, 0.6; Ca gluconate, 1.0; glucose, 5. To this basic medium the required amounts of NaCl and KCl were added to obtain media with the Na, K, and Cl concentrations that are described below. The osmolarities were kept between 290 and 300 mOsM/liter by addition of sucrose when required. A mixture of 95 per cent O_2 and 5 per cent CO_2 was bubbled through the solutions during the experimental periods. The pH was maintained between 7.2 and 7.8 (1, 2).

A. IMMERSION MEDIUM Contained 150 mM Na, 0 mM K, and 120 mM Cl. For convenience, ( ) will denote concentrations and the (Na) and (K) of the bathing fluid will be written as ratios, (Na)/(K). The immersion medium is denoted as 150 (Na)/0(K).

B. REIMMERSION MEDIA WITH CONSTANT CATION CONCENTRATIONS Nine media were prepared, the sum of their (Na) and (K) being equal to 150 mM. The (Na)/(K)

were 150/0.3, 142/2.5, 145/5.3, 142/8, 136/14, 130/20, 116/34, 100/50, 78/72. The (Cl) was always 120 mM.

C. REIMMERSION MEDIA WITH FIXED (Na), BUT WITH VARIABLE (K) Eight media were prepared, their (Na) was always 142 mM. The K were 0.3, 1.1, 2.4, 4.6, 8.6,

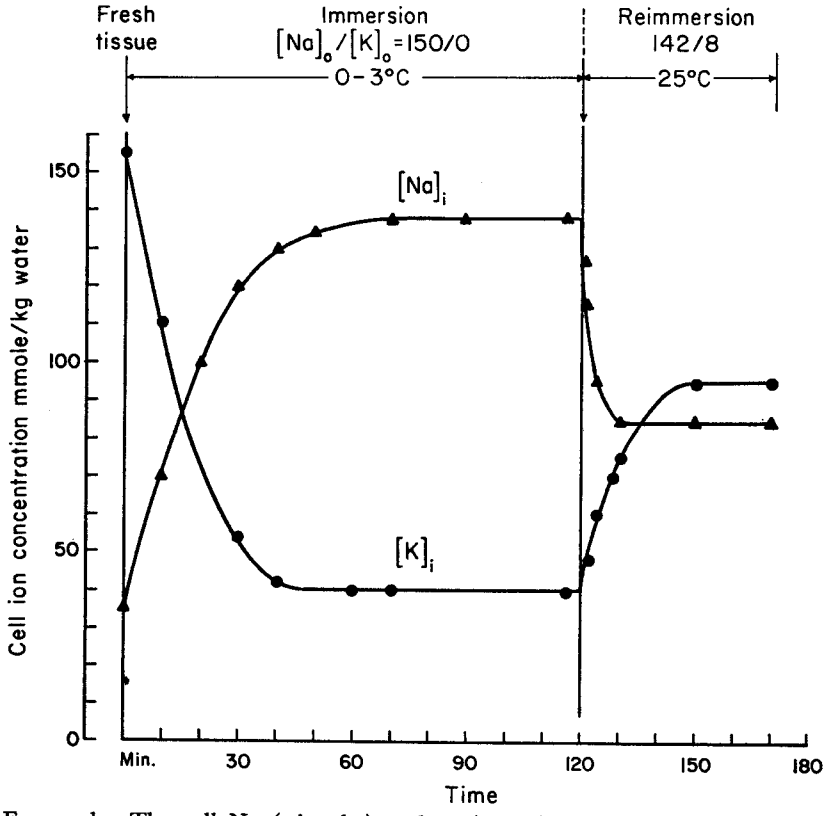

FIGURE 1. The cell Na (triangles) and K (circles) concentrations of the outermost slices from the guinea pig kidney cortex (cortex corticis), incubated *in vitro*, are shown as a function of time to illustrate the general experimental procedure. Some slices were separated for fresh tissue analysis. Others were immersed in a chilled medium without K. After 2 hours the slices were reimmersed in warm media in which Na and K were varied. In this particular case the reimmersion medium contained 142 mM Na and 8 mM K. Note that after reimmersion the rate of net Na extrusion is faster than that of net K uptake. About 10 minutes after reimmersion Na extrusion has practically stopped, while K uptake continues. A balanced state is reached after 30 minutes.

9.2, 12, and 16 mM. The (Cl) were 112, 113, 114, 117, 121, 121, 124, and 128 mM, respectively. Four other reimmersion media were prepared. Their (Na)/(K) were 148/0.3, 85/0.3, 148/13, and 85/13. Their (Cl) were 118, 55, 131, and 68 mM, respectively.

D. REIMMERSION MEDIA CONTAINING 5 mM OF 2,4-DINITROPHENOL Media such as those described in *b* and *c* were prepared, but with the following variations: 2,4-

dinitrophenol (9) was added to a concentration of 5 mM, and 24 mM of $NaHCO_3$ instead of 15, glucose and acetate were not included. A gas mixture of 95 per cent air and 5 per cent CO_2 was bubbled through these solutions.

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

Extracellular Space Inulin space was chosen as a measure of the extracellular space. This compound was included in the immersion fluid and the volume of tissue water into which it had diffused was measured using a resorcinol method (10).

Electrolyte Analysis After the dry weight had been obtained, the slices were immersed in 2 ml of 1 N HNO_3 and shaken for 48 hours at room temperature. Na and K concentrations were determined by flame photometry (11) in aliquots of the extraction fluid.

Electrical Potential Difference Measurements The exploring and indifferent electrodes were symmetrical saturated calomel electrodes. The recording instrument was a voltmeter, 10^{14} ohm input impedance model 200-B (Keithley Instruments, Inc., Cleveland, Ohio). The exploring micropipettes (12) were drawn in a puller (13), filled in the cold with water by capillarity (14) and later with 3 mM KCl (15). Their resistance and tip potential were checked frequently in the course of the experiments to see whether they fell within the range of 3 to 30 megohms and of 0 to −5 mv, respectively (16). The indifferent electrode was connected to the bathing solution by an agar bridge.

One or two tissue slices were firmly mounted betwen fiberglass nets in a lucite chamber. Thus, the reimmersion medium circulated freely around the slices, to and from a reservoir, by means of a stream of the gaseous mixture already described. A jet of the same gas was blown on the fluid surface. During penetration only about 0.3 mm of the fluid was allowed above the slices. The potentials were selected when they had been established by a sudden jump to a stable level (15). About 5 readings were taken in each slice examined. The membrane potential has the conventional inside-outside sign, the cell interior being negative.

Diffusion Delay The influence of diffusion delay within the slices may be estimated using the following equation for diffusion in a plane sheet (17, 18)

$$1 - (C_t/C_b) = (8/\pi^2) \exp(-D\pi^2 t/x^2)$$

C_t/C_b is the tissue to bath concentration ratio of the probing molecule. D is the diffusion coefficient. The value of 8.6×10^{-5} cm²/min. measured for the diffusion of urea in kidney tissue *in vitro* (19) will be used. This is about one order of magnitude smaller than the diffusion coefficients for urea and KCl in free solution and should include all the restrictions that the tissue offers to diffusion. x is the thickness of the diffusion layer, and t the time. The center of a slice 0.2 mm thick will be 99 per cent equilibrated with the medium in 0.5 minute (which is the shortest time interval for our measurements). In the same time, a slice 0.3 mm thick will be 99 per cent equilibrated

at a depth of 0.1 mm, and 90 per cent at its center. Therefore, an analysis of the whole slice made at this time will give a figure within 2 per cent of that calculated assuming complete equilibration.

Expression of Results To correlate ion concentrations across the cell membrane, the results were expressed as concentrations in the cell water using the following

TABLE I
WATER CONTENT OF KIDNEY CORTEX SLICES

Conditions	gm H_2O/100 gm tissue*
Fresh tissue	78±0.2 (60)
After 2 hrs'. immersion (0–3°C) in medium 150 (Na)/0 (K)	82±0.3 (60)
After 50 min. reimmersion (25°C) in	
Media containing 0.3–16 mM K	78±0.3 (40)
Medium 115 (Na)/34 (K)	80±0.5 (10)
Medium 100 (Na)/50 (K)	82±0.6 (8)
Medium 78 (Na)/72 (K)	83±0.5 (7)
Media 85 (Na)/13 (K) and 85 (Na)/0.3 (K)	79±0.5 (20)
Media containing 5 mM DNP	85±0.6 (60)

* Mean ± standard deviation. Number of slices given in parentheses.

TABLE II
SODIUM AND POTASSIUM CONCENTRATIONS IN KIDNEY CORTEX SLICES

	Tissue concentrations*		Cell water concentrations		No. of slices
	mmole/kg tissue		*mmole/kg water*		
	Na	K	Na	K	
Fresh tissue	56±2.5	85±3	35	156	40
After immersion‡	114±4	23±2	137	40	40

* Mean ± standard error.
‡ 2 hrs. at 0–3°C in medium 150 (Na)/0 (K).

equation (13)

$$(X)_i = ((X)_t - v \cdot (X)_o)/(w - v)$$

where $(X)_t$, $(X)_o$, and $(X)_i$ are the ion concentrations (in millimoles per kilo) in the tissue, bathing fluid, and cell water, w is the water content of the slice, expressed as a fraction of the final wet weight of the slice (Table I), and v is the extracellular fraction of the slice.

Extracellular Space In agreement with Robinson (20) and Whittam (21), the inulin space was found to be a constant fraction of the weight of the slice, 26 ± 0.6 gm/100 gm tissue (standard deviation, 83 measurements). This value was also found to be unaffected by the conditions of incubation, in spite of the increase in the water

content of the slices observed after immersion in the cold or in media with high K concentration (see Table I).

Time Course of Leaching in Cold Media Preliminary experiments showed that slices immersed in the cold (in the medium containing 150 (Na)/0(K) lost K and gained Na, reaching a steady level in 60 to 90 minutes (Fig. 1). A leaching time of 2 hours was used in all the experiments described in this paper. The analyses at the end of this time are given in Table II, with those of fresh tissue. They agree with the values of Whittam and Davies (2) and of Aebi (3).

Experiments in the Balanced State Most of the present work has been carried out in balanced states (22) because their quantitative treatment is relatively simple. The influx of each ion equals its outflux and the net volume flow is zero. A monovalent cation that distributes passively, singly, and independently across a membrane with an electrical potential difference V_m, is described in the balanced state by the following equation (5):

$$a_i = a_o \exp(-FV_m/RT) \quad (1)$$

where a_i and a_o are the cation activities in the cell and bathing medium, R is the gas constant, T is the absolute temperature, and F is Faraday's constant.

The differences in concentration between cells and media are maintained by known physicochemical forces and by some special cellular activity (transport mechanism) that balances the leaks due to diffusion (23). The work performed by this transport mechanism in the transference of one equivalent of a cation (5) is given in the balanced state by the following expression (24, 25):

$$W = RT \ln (a_i/a_o) + FV_m \quad (2)$$

RESULTS

Cell Na and K Concentrations As a Function of Their Concentrations in the Reimmersion Media, in the Balanced State

The slices to be analyzed were removed from the bath after 50 minutes of reimmersion at 25°C. A balanced state had been achieved in only 30 minutes, as may be seen for the medium containing 142 (Na)/8 (K) in Fig. 1 (*cf* also references 1–3).

Fig. 2 shows the results for slices reimmersed in media with constant cation concentration. In the medium containing 150 (Na)/0.3 (K), cell K and Na concentrations were the same as those observed at the end of immersion in cold solution. In the medium containing 148 (Na)/2.5 (K), the cells markedly gained K and lost Na. This entrance of K and loss of Na increased as the K concentration was raised and the Na concentration lowered in the reimmersion media. The movement of Na, accompanied by that of K in the opposite direction, has already been described (1–3).

To ascertain whether these changes were a result of the rise in the K concentration in the reimmersion media or of the diminution in Na, slices were reimmersed in media with fixed Na concentration. These experiments (Fig. 3) showed that the ionic shifts depended on the changes in the K and

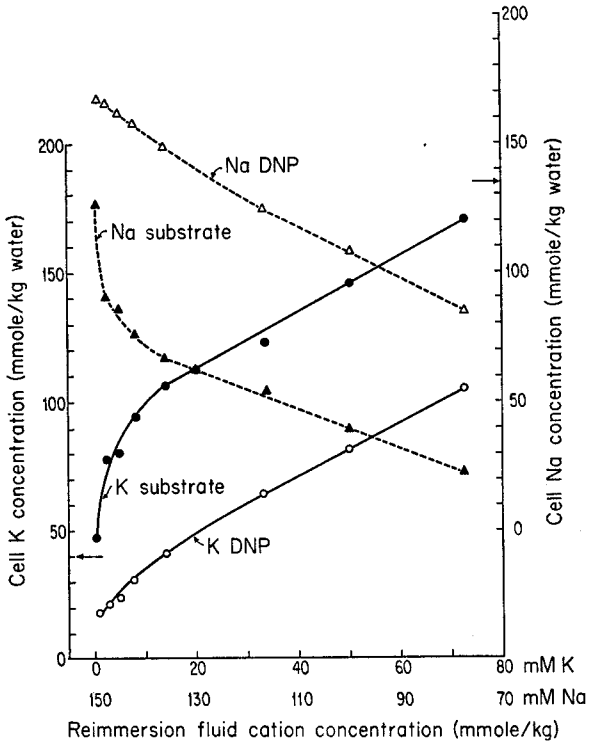

FIGURE 2. The cell Na (triangles) and K (circles) concentrations as a function of the Na and K concentrations of the reimmersion media, as analyzed at the end of reimmersion. The total cation concentration of the reimmersion media was constant. The arrows point to cell concentrations prior to reimmersion (end of immersion). Each point is the average of 4 to 6 slices. Results with the DNP-treated slices are also shown. The DNP-treated slices continue to lose K after reimmersion, and gain Na (similar inhibitory action had been reported in references 2 and 9). This indicates that immersion at low temperature did not block all active transport processes. The membrane potential at the end of immersion ranged between 50 and 60 mv.

not in the Na concentration in the reimmersion media. Thus, at a fixed external K concentration, a large change in the external Na concentration produced only a small change in cell Na. In contrast, at a fixed external Na concentration, small changes in the external K concentration markedly influenced the cell Na concentration. However, changes in the external K concentration could directly influence the cell cation concentration, or, indirectly, through changes in the membrane potential. The membrane potential was, therefore, measured to evaluate these two possibilities.

Membrane Potential As a Function of the K Concentration in the Reimmersion Media

The membrane potential was measured in the balanced state after the slices had been reimmersed for 50 minutes at 25°C in the media with constant cation concentration. The results are presented in Table III and in Fig. 4. Activities were calculated using the activity coefficient for KCl in free solution (26).

A continuous line has been drawn according to the Nernst equation and represents the K equilibrium potential E_K. Therefore, the membrane potential largely depends upon the K activities, as in the *Necturus* kidney (27–29, 11). However, at low external K activities (higher ratios) the membrane potential is less negative than the K equilibrium potential.

TABLE III

MEMBRANE POTENTIALS OF THE CELLS OF KIDNEY CORTEX SLICES AFTER REIMMERSION

Reimmersion medium concentrations		Cell water concentrations		Membrane* potential
Na	K	Na	K	V_m
mmole/kg	*mmole/kg*	*mmole/kg*	*mmole/kg*	*−mv*
149	1	115	57	73±2.2 (11)
148	2	95	68	70±1.1 (19)
145	5.3	83	85	63±0.8 (67)
140	10	72	99	55±1.0 (46)
130	20	64	113	43±0.8 (33)
110	40	48	134	29±0.8 (44)
78	72	22	170	23±1.0 (24)
40	110			3±0.6 (26)

* Mean ± standard error. Number of impalements is given in parentheses.

The Distribution of K

Fig. 5 presents the same observations plotted in a different manner. The experimental and calculated cell K activities coincide in reimmersion media with K activities larger than 8 mmole/kg. Therefore, K distributes passively across the cell membrane at such outside K activities. At external K activities lower than 8 mmole/kg, the K activities within the cell are higher than those expected from the membrane potential, with the differences between the experimental values and those calculated from equation 1 increasing at the lowest outside K activities. Thus the difference is about 10 mmole/kg cell water at an outside K activity of 6 mmole/kg, about 15 mmole at an outside K activity of 4 mmole/kg, and about 30 mmole/kg cell water at an outside K activity of 1 mmole/kg.

The Distribution of Na

The experimental values for cell Na activities are much lower than those calculated for a passive Na distribution, as may be seen in Fig. 6. Activities were calculated using the activity coefficients for NaCl in free solution (26). These results indicate, as is generally accepted, that Na is extruded from the cell up an electrochemical potential gradient; *i.e.*, actively.

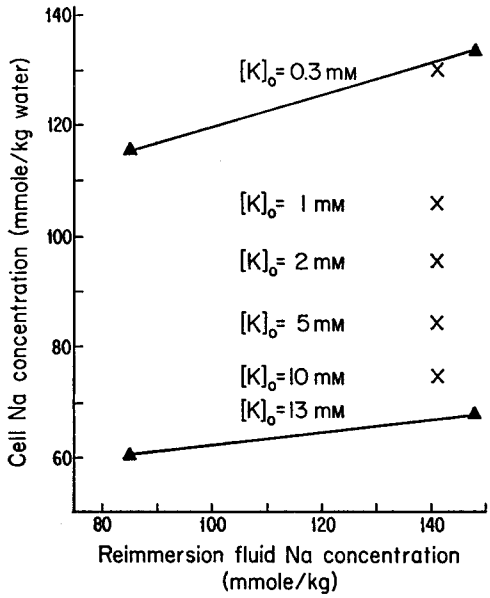

FIGURE 3. The cell Na concentration as a function of the Na concentration of the reimmersion media, as analyzed at the end of reimmersion. Results with slices reimmersed at the same K concentration are joined by a line. Note that changes in the reimmersion media K concentration have much greater influence on the cell Na concentration than changes in the reimmersion media Na concentration. X corresponds to an external Na of 142 mM. Each point is the average of 4 to 6 slices. Cell K concentrations are not given. They were practically superimposable with those of Fig. 2.

DISCUSSION

It has been already mentioned that the membrane potential is less negative than the potassium equilibrium potential at low outside K activities (Fig. 4). This observation has the same meaning as that seen in Fig. 5, which shows that the cell K activities are higher than those calculated from the membrane potential when the outside K activities are lower than 8 mmole/kg. There are three possible explanations for these differences. (*a*) that other ions besides potassium contribute to the membrane potential, (*b*) that some K is bound within the cells, so that the cell K concentration measured chemically would lead to an overestimation of the K equilibrium potential, or (*c*) that the cells take up K actively.

It could also be suggested that the leakage rate of K is restricted at low external K concentrations. However, this would require that other ions contribute to the membrane potential, since our observations have been made in the balanced state. The cells of the proximal tubule of the *Necturus* kidney

are largely permeable to K and only slightly to Na (11, 29). This slow diffusion of Na inward could lower the membrane potential. Thus, the membrane potential would be less negative than the K equilibrium potential. This does

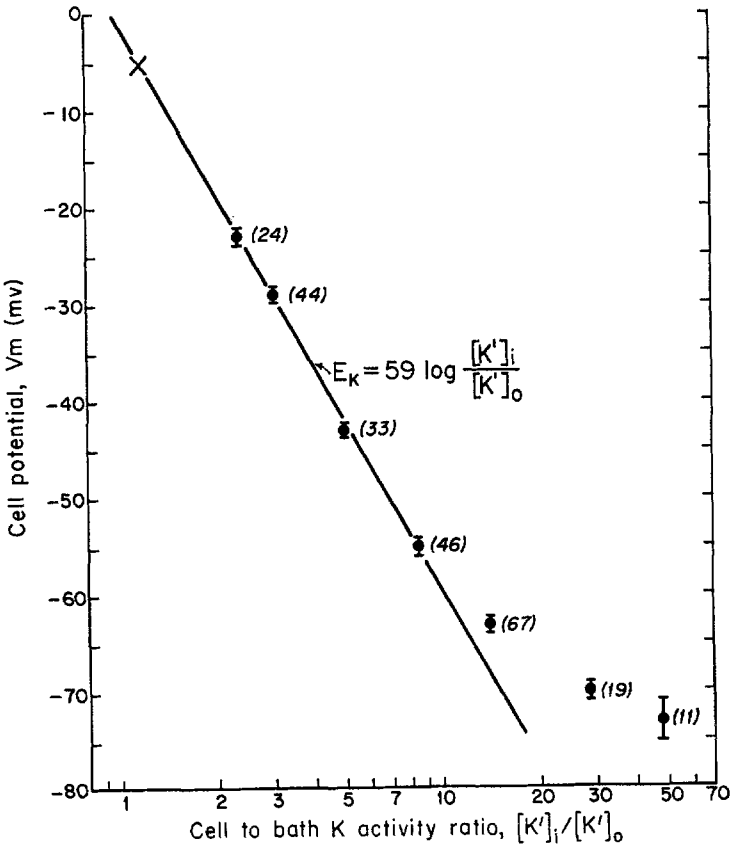

FIGURE 4. Membrane potential as a function of the cell to reimmersion medium K activity ratio. Number of impalements are given in parentheses. Vertical lines denote ± standard error. The continuous line has been drawn according to the Nernst equation and represents the K equilibrium potential, $E_K = -59 \log [K']_i/[K']_o$. The 3 experimental points to the right correspond to reimmersion medium K concentrations of 5.3, 2, and 1 mM. At these K concentrations the membrane potential is 7, 17, and 25 mv lower than E_K, respectively. It should be noted that these differences between V_m and E_K would be larger if concentration ratios were used instead of activity ratios. Activities were calculated using the activity coefficients for KCl in free solution (26).

not seem to be a very important factor in the present experiments. For instance, we have found that the membrane potential did not vary when the external Na concentration was reduced from 148 mmole/kg to 85 mmole/kg at an external K concentration of 1 mmole/kg. However, a definitive con-

clusion cannot be reached, because the good fit to the experimental data of Fig. 4 may be obtained by assuming that the permeability coefficient for Na is 0.01 times the permeability coefficient for K.

The Possibility That Some K Is Bound within the Cells

If the fraction of K bound within the cell were constant, it would become more noticeable at low cell K concentrations and one could qualitatively explain the results shown in Figs. 4 and 5. However, Fig. 5 shows that the difference between the estimated cell K concentrations and those calculated from the membrane potential increases at the lowest outside K activities.

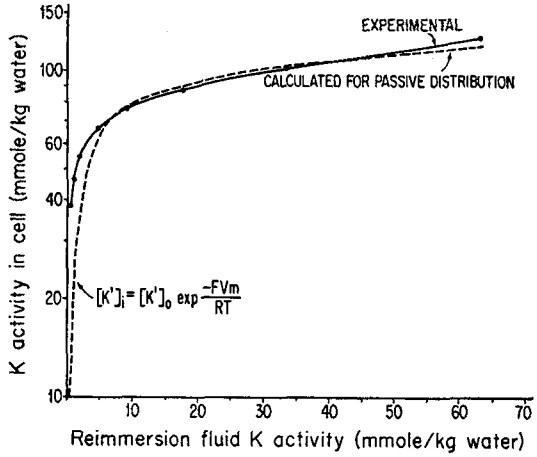

FIGURE 5. Cell K activities as a function of the K activities in the reimmersion medium, in the balanced state, replotted from Fig. 2. The dashed line corresponds to passive distribution, calculated from Equation 1 with data from Fig. 2 and Table III. K distributes passively except at low external K activities in which cell K activities, $(K')_i$, are higher than expected for a passive distribution. Consequently during reimmersion, the cells have gained more K than expected from the membrane potential. Activities were calculated using the activity coefficient for KCl in free solution (26).

Therefore, if this difference were due to K binding, one would have to admit that since the cells have gained K during reimmersion, a fraction of this K goes into the bound compartment, and that the concentration of bound potassium is inversely proportional to the cell K concentration.

Robinson (30) has concluded that 50 to 80 per cent of cell K is bound, as calculated from the Donnan ratio for Cl. This consideration would hold if Cl were passively distributed across the cell membrane. Bath to cell Cl concentration ratios between 1.5 and 4 have been reported (21, 31, 32, 11) and we have obtained in preliminary experiments ratios between 1.3 and 2 at an external K concentration of 5.3 mmole/kg. These ratios are significantly

lower than those expected from a passive distribution of Cl. Thus, ratios of about 10 are expected from the membrane potential, and of about 13 from the K ratio. Therefore, while K is nearly at equilibrium, Cl is far from it. Thus, the calculation of Donnan ratios from the Cl concentrations may not be justified for the slices.

Some evidence against K binding arises from a study of the influence of temperature changes on the membrane potential. An average of $-53 \pm$

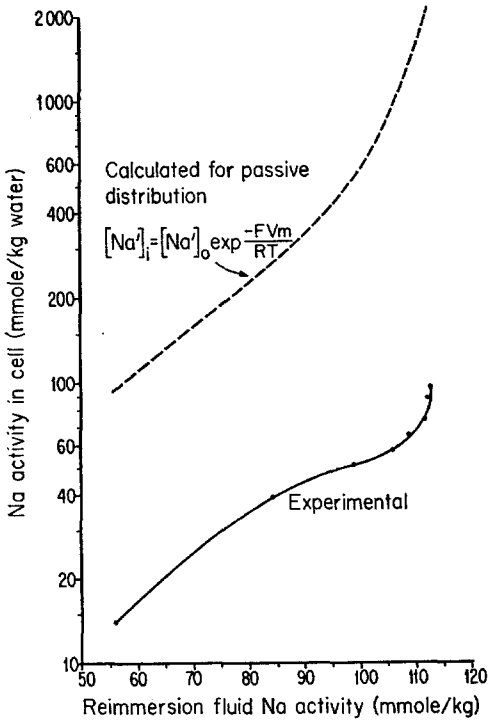

FIGURE 6. Cell Na activities as a function of the Na activities in the reimmersion medium, respectively, in the balanced state replotted from Fig. 2. The dashed line corresponds to passive distribution, calculated from Equation 1 with data from Fig. 2 and Table III. Cell Na activities, $(Na')_i$, are at least an order of magnitude lower than the calculated values. This is generally accepted to be secondary to active Na extrusion. Activities were calculated using the activity coefficients for NaCl in free solution (26).

1.3 mv (standard error, 13 measurements) was obtained for the membrane potential at 0°C, and one of -63 ± 0.8 mv at 25°C (Table III) using the reimmersion medium containing 145 (Na)/5 (K). From these figures, a $Q_{10} \sim 1.04$ may be calculated. A similar value is expected from RT/F. Mudge (33) and Whittam and Davies (34) have found that cell K is not homogeneous; about half of the K does not exchange in chilled slices, whereas practically all K exchanged at high temperatures. If their observation indicates binding of K at the low temperature, one would expect an influence of the temperature on the membrane potential larger than that described above.

Although all these considerations do not rule out the possibility that some K is bound within the cell, they suggest that this fraction should be at a concentration not very different from that of the free K within the cell.

The Possibility of Active K Uptake by the Cells

The cells from the slices have gained K from the bath, against its electrochemical potential gradient, when the bath K concentration was lower than 8 mmole/kg. Therefore, this uptake of K is active. Further evidence arises from experiments in which the time course of the membrane potential was followed after reimmersion, as shown in Fig. 7.

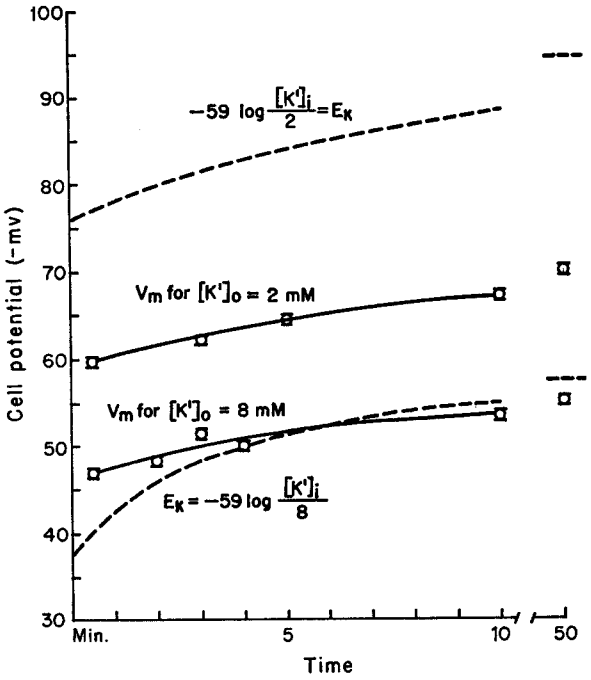

FIGURE 7. Time course of the membrane potential after reimmersion in media containing 142 (Na)/8 (K) and 148 (Na)/2 (K), at 25°C. Each point represents the mean ± standard error of 6 measurements performed in 6 slices, one in each, at the times noted. The dashed lines correspond to the K equilibrium potential calculated from the Nernst equation.

After immersion in the cold, some slices were set up in the chamber with medium containing 142 (Na)/8(K) and some with medium containing 148 (Na)/2 (K) at 25°C. Measurements of the membrane potential were started after 30 seconds of reimmersion. From K analysis of the tissue, the K equilibrium potentials were calculated. At the bottom of Fig. 7 are data corresponding to an external K activity of 8 mmole/kg. It may be seen that the membrane potential was more negative than the K equilibrium potential during the first minutes of reimmersion; later, the K equilibrium potential

approached the membrane potential, as would be expected for a passive behavior of K. The data at the top of Fig. 7 correspond to a K activity of 2 mmole/kg in the reimmersion medium; the K equilibrium potential was more negative than the membrane potential. Thus, at this external K activity the slices were taking up K against its electrochemical potential gradient.

From the rates of net movement obtained at the beginning of reimmersion (Table V) Q_{10} of 2.5 and 2.0 for net Na extrusion and net K entry, respectively, were obtained. Such Q_{10} values are not unique for active transport (35–36). However, in these experiments, the Q_{10} values became less than 1.1 for both ion movements, in similar media but containing 2,4-dinitrophenol.

TABLE IV
ENERGY BARRIER TO Na EXTRUSION

Reimmersion medium concentrations			
K	Na	Energy barrier*	Na extruded‡
mmole/kg	*mmole/kg*	*Kcal/mole*	*mmole/kg cell water*
0.3	148	1.76	0
0.3	85	1.15	20
2.0	148	1.89	50
8.0	142	1.71	65
13	148	1.61	73
13	85	1.20	80
20	130	1.29	75

* That must be overcome by the Na-extruding mechanism to maintain kidney cells in the balanced state.
‡ From the cell from the moment of reimmersion until the balanced state was achieved.

These observations support the existence of active transport processes for Na extrusion and for K uptake, which may be inhibited by dinitrophenol.

The Role of K in the Extrusion of Na by the Cells

The energy barrier that must be overcome by the Na-extruding mechanism in order to maintain the balanced state, thus compensating for the slow diffusion of Na inwards, may be calculated from Equation 2. From this equation it may be realized that a decrease in the external Na concentration will lower the energy barrier. Also, an increase in the K concentration in the reimmersion medium will lower the membrane potential, V_m. Hence, it will also lower the energy barrier through the term $V_m F$. Therefore, the amount of Na extruded from the moment of reimmersion until the balanced state has been achieved should depend on the direct influence of the external K concentration on the Na-extruding mechanism. Any indirect influence on the energy barrier due to lowering of V_m or of the external Na concentration has

already been included in Equation 2. The results of some representative calculations are shown in Table IV. It should be observed that Na extrusion was practically zero at a reimmersion medium K concentration of 0.3 mmole/kg (*cf.* references 32 and 37). Na extrusion increased with an increase in the external K, leveling off at a reimmersion medium K concentration of about 13 mmole/kg. Thus, Na extrusion depends on the external K concentration rather than on the energy barrier.

The Possibility of Coupling between Na Extrusion and K Uptake

The existence of some direct coupling between Na outflux and K influx requires that K in the bathing fluid be essential for Na extrusion and that cell Na be essential for K influx. This work shows that Na extrusion requires and

TABLE V

INFLUENCE OF TEMPERATURE ON THE NET Na OUTFLUX AND ON THE NET K INFLUX

In kidney cortex, slices reimmersed in the fluid containing 142 (Na)/8 (K).

Reimmersion time	Reimmersion temperature	Net Na outflux	Net K influx	Net Na outflux / Net K influx	Q_{10} of net Na outflux	Q_{10} of net K influx
min.	°C	*mmole / min. kg**	*mmole / min. kg**			
0–2	25	13	6.3	2.1	2.5	2.0
	15	5.1	3.2	1.6		
4–8	25	3.6	2.1	1.7		
	15	1.7	1.2	1.4		
8–10	25	0.8	1.8	0.4		
	15	0.9	1.0	0.9		

* Of cell water.

depends on the presence of K in the bathing fluid. Evidence that cell Na is necessary for K influx arises from experiments by Whittam and Willis (38). They found that the net influx of K stopped in Na-depleted cells and reappeared when Na was added to the immersion medium. Thus K influx depends on cell Na, this in turn being proportional to the external Na concentration (38, 39). However, the experiments that follow indicate that the ratio of fluxes varies.

Influence of the Temperature of Reimmersion on the Ion Movements

The slices were analyzed after reimmersion times of 0.5, 1, 2, 4, 8, and 10 minutes in media containing 142 (Na)/8 (K) at 25°C and at 15°C. Curves for K uptake and Na extrusion were obtained (Fig. 1) and tangents drawn to them. The rates of net ion movements were calculated for the first 2 minutes, for a period between 4 and 8 minutes, and for a period between 8 to 10 minutes of reimmersion. Table V shows that the ratios of net Na outflux to

net K influx varied. Thus, at the beginning of reimmersion more Na was extruded than K entered the cells. After this, both fluxes became equal, and later K influx was larger. The ratio of fluxes appeared as 1 to 1 (28, 40, 41) only as an over-all result of reimmersion. Leaf (42) has pointed out that this 1 to 1 coupling was only apparent, and that expressed on a tissue water basis, a simple addition or subtraction of an isotonic solution of a sodium salt to or from a tissue will produce reciprocal changes in tissue potassium without any potassium necessarily being lost or gained by the tissue. It is important to recalculate some of our results on the basis of dry weight. At the end of immersion, the average values were 18 gm solids/100 gm tissue (0.32 kg cell solids/kg cell water), 40 mmole K/kg cell water, 138 mmole Na/kg cell water. When a balanced state was achieved after immersion in a medium containing 142 (Na)/8 (K), the average values were 22 gm solids/100 gm tissue (0.42 kg cell solids/kg cell water), 93 mmole K/kg cell water, 85 mmole Na/kg cell water. During reimmersion the cells lost 0.75 kg water, lost 230 mmole Na, and gained 95 mmole K, per kg of cell solids. The ratio of net Na outflux to net K influx, expressed per kilo cell solids per minute, is larger than one for the first 2 minutes of reimmersion, and smaller than one for the period between 8 and 10 minutes of reimmersion. Therefore, our results could be taken as evidence that the coupling mentioned in the last paragraph, between Na extrusion and K uptake is not 1 to 1, but varies and that this coupling may be loose.

The real rates of Na extrusion and of K uptake by the pump have in parallel passive Na and K movements which obscure them (43). By assuming that the passive movements change (as a function of time or of the ion concentration), the existence of a unique coupled pump, not necessarily of the 1 to 1 type, can still be maintained because changes in the passive movements would influence the net fluxes which would appear as variable.

Na outflux to K influx ratios greater than 1 have been reported (44, 45). A coupled pump which transfers Na ion faster than K ion would also be electrogenic and explain some of the observations that are discussed below. It would be difficult to conceive such a pump in which a net uptake of K continues at a rate higher than that of Na extrusion (Table V), if one takes into account that the cell permeability to K is much larger than that to Na.

The Possibility of an Electrogenic Pump

Na extrusion by an electrogenic pump (46–50) would make the membrane potential more negative than the K equilibrium potential. This would result in an influx of K up to the point where the K equilibrium potential equals the membrane potential, as may be seen at the bottom of Fig. 7. This type of pump would explain why the cell K concentration was higher, in slices incubated in Na-free sucrose-Ringer's solution, than in those incubated in

Na-free choline-Ringer's solution, although the cell Na concentration was the same under both conditions (51). It would also explain why the membrane potential was more negative in sucrose than in choline (29). The entry of choline (52, 11) would make the membrane potential less negative and, secondarily, would lower the cell K concentration. However, this type of pump could not explain the findings of Whittam and Willis (38), where it was noticed that K influx stops in Na-depleted cells. Therefore, some coupling between Na extrusion and K uptake, however loose it may be, is required.

The cells of chilled slices gain Cl (40, 1, 12, 6) and lose it on reimmersion at higher temperatures. An electrogenic Na pump could also result in a passive Cl outflux, the Cl leaving the cell with the Na as an ion pair. Preliminary experiments in this laboratory indicate that the cells do lose Cl down its electrochemical potential gradient after reimmersion. Thus, diffusion of Cl inwards is not the source of the hyperpolarization shown at the bottom of Fig. 7. On the contrary, this passive net Cl outflux would have the effect of reducing the potential generated by the Na pump. Therefore, it would act in part against the possibility that K is dragged in by the Na pump. In these circumstances, despite a high degree of passive permeability to K, some parallel active uptake of this ion, although not evident at K activities higher than 8 mmole/kg, might be needed. The latter is clearly required in reimmersion media with a K concentration less than 8 mmole/kg (top of Fig. 7). The high Q_{10} obtained for K influx supports its existence.

Variations of thirty- to one hundred-fold in H ion concentration produce only slight changes in the Na and K concentration of mammalian kidney slices (1, 2, 30) and no changes in the peritubular face potential or in the transtubular potential difference in the *Necturus* kidney (53). Therefore, the possibility that H ions are directly related to the Na pump seems remote.

In summary, the active Na outflux would be divisible into one fraction coupled to K influx and another that leaves the cell with Cl. The largest fraction of K influx is purely passive. Besides, there seem to be one fraction actively transported and one coupled to the Na outflux, which become especially noticeable with low external K concentrations. At the present time, it is difficult to provide a simple picture and to ascertain whether, at all times, all mechanisms operate or whether some of them predominate under certain experimental conditions.

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