Sodium and Water Transport in Kidney Proximal Tubular Cells

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Richards and his group showed, about 40 yr ago, that the blood is filtered in the glomerulus. The largest portion of the water and solutes of the filtered fluid is absorbed across the proximal tubular wall, toward the peritubular blood capillaries (see 17). Thus, proximal tubular absorption is a process of paramount importance in the maintenance of homeostasis (1, 16). I shall review here studies on the way in which this absorptive process is accomplished and refer to new experiments on the nature of the sodium pump in kidney proximal tubular cells.

ABSORPTION IN THE PROXIMAL TUBULE

The Nature of Water Absorption

Experiments were undertaken at the Biophysical Laboratory of the Harvard Medical School to investigate further the nature of the absorption across the proximal tubular wall. The following questions arose. Which was the *primum mobile* during absorption, the water or the solutes? If water moved first and the solutes followed, did the force driving the water originate in the osmotic pressure of the proteins that circulate in the peritubular capillaries, or did it originate in the tubular cells? On the other hand, if the solutes moved first, could the solute movement drive all the water?

To answer these questions, the stopped-flow microperfusion technique was developed (19). Single proximal tubules of *Necturus* kidney were perfused with fluids of known composition, for a measurement of the amount of fluid and solutes that moved across the proximal tubular wall under several experimental conditions (see 24). The relationships between water and solute fluxes (36) and between water and sodium fluxes were studied (15). It was found that (*a*) water flux was proportional to Na flux (Fig. 1); (*b*) the fluid moved isosmotically; (*c*) when a nonpermeant solute was used, the only solute that moved was Na (as salt, mostly as NaCl); (*d*) the intercept of the regression lines of Fig. 1 is practically 0; thus, no fluid was moved by forces other than those originating from the Na flux; and (*e*) it was calculated from the measured water permeability coefficient that the proteins could move only about 1 % of the amount normally absorbed by the proximal tubule (30). It was concluded that water movement was secondary to the movement of Na, and entirely dependent upon it (15, 30, 36). These observations have been extended to the mammalian kidney (see 11, 35; also 26, 29). Therefore, it was necessary to study the absorption of Na.

The Nature of Sodium Absorption

The electrical potential difference across the proximal tubular wall was measured with microelectrodes (5–7, 24, 33), making sure that the microelectrode tip was indeed in

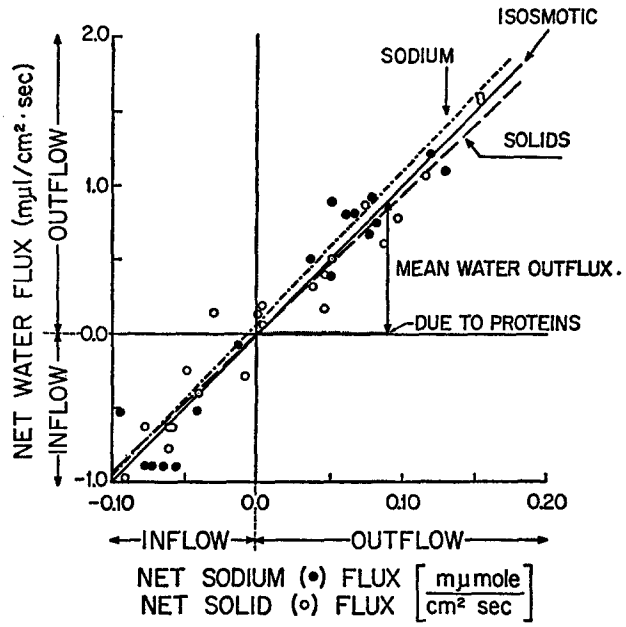

FIGURE 1. Net water flux as a function of net Na (solid circles) and net solid (open circles) flux across the wall of the proximal tubule of *Necturus* kidney. In these experiments there was no difference in water activity between tubular fluid and blood. The region to the upper right corresponds to movement of water, Na, and solids out of the tubule. The region to the lower left corresponds to movement of water, Na, and solids into the tubule. The solid line is that calculated for fluid moved isosmotically with the blood of the animal. The dashed lines were drawn according to the following equations: Net water flux = (10.1 ± 1.2) (net Na flux) + (0.061 ± 0.085) (mμl/cm²·sec), and Net water flux = (9.4 ± 0.4) (net solute flux) + (0.003 ± 0.03) (mμl/cm²·sec) (references 15, 29, 36). The mean water flux out of the proximal tubule under physiological conditions is shown. The contribution to the water flux of the osmotic pressure of the proteins that circulate in the peritubular capillaries is so small that it can barely be seen in this figure.

the tubular lumen during the measurements (25). It was found that Na can be absorbed against both a difference in electrical potential of some −20 mv (lumen negative) (24, 25) and a difference in concentration as large as 40 mM (15, 24, 36). The movement of Na against its electrochemical potential difference was described as an active process (15, 24, 36). This was confirmed by the action of inhibitors of active transport (18). Cl moved passively across the proximal tubular wall (10).

Permeability of the Proximal Tubular Cell Membrane

If Na were absorbed by active transport, and dragged Cl and water, the proximal tubular cell membrane would be more permeable to Cl than to Na, and it would discriminate sufficiently against Na and between Na and water. For a study of whether these requirements were satisfied, the permeability of the kidney cells to several non-lipid soluble nonelectrolyte molecules with a molecular radius between 1.5 and 6 A and to several electrolytes was estimated (31, 32).

TABLE I

PERMEABILITY COEFFICIENTS OF KIDNEY CELLS

Molecule	Molecular radius	Permeability coefficient*	
		Cell swelling	Isotope Influx
	A	(*cm/sec*) × 10^{-5}	
Water	1.5	43	
Urea	2.3	8.1	
Glycerol	2.9	2.9	
Erythritol	3.2	1.0	
Mannitol	4.0	0	
Sucrose	4.5	0	
Raffinose	6.0	0	
Potassium		11.5‡	10.9
Rubidium		3.1‡	2.4
Cesium		0.7‡	1.0
Sodium		0.4‡	0.9
Chloride			5.8

* Calculated from references 31 and 32, using equations 5 to 8 of reference 32, and a water osmotic permeability coefficient of 43 × 10^{-6} cm/sec taken from Fig. 4, reference 32.

‡ As chloride salts.

It may be seen in Table I that the cell membrane permeability varies critically with the size of the molecule. The cell membrane behaved as if it were perforated by pores with an equivalent radius of 5.6 A. Antidiuretic hormone increased the permeability of the kidney cells so that an equivalent pore radius of 6.5 A was required to fit the results (31). The permeability of the cell membrane to ions was K>Rb>Cs>Na, estimated from the ability of these ions to depolarize the cells, from their influx as isotopes, or from changes in cell volume (32). Therefore, the kidney cell membrane discriminates molecules critically by size: solutes from water, and solutes and electrolytes among themselves; the smaller the molecule, the more readily it penetrates the membrane. The permeability to Na is about 100 times smaller than that to water. The large discrimination of the membrane against Na explained the fact that water could move secondarily to the osmotic action of Na. Cl could follow Na transport since the permeability to Cl is about seven times larger than that to Na (32).

Location of the Na Active Transport Mechanism within the Cell

The cell membrane permeability, the ionic concentration, and electrical potential differences across the luminal and peritubular cell walls (24, 32), and the permeability of the luminal and peritubular walls to Na and K (3, 6–9), indicate that Na enters the

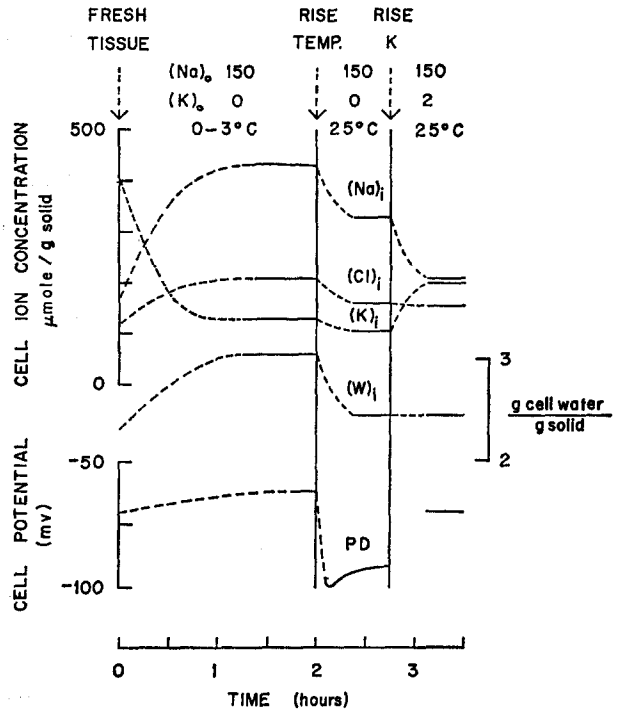

FIGURE 2. Experimental procedure to study the net Na efflux and its relationship to net K influx and to other ion movements. Cell Na, Cl, K, and water, and cell electrical potentials (*PD*) (27), are plotted as a function of time. Cells of kidney slices were loaded with Na and made to lose K by immersion in a chilled medium that contained 150 mM Na and no K. After 2 hr of immersion, the slices were reimmersed in a similar medium at 25°C. In the experiment shown here, the reimmersion medium was subsequently replaced by another containing 2 mM K. The continuous lines refer to balanced states (for details see reference 27).

cell, from the lumen, down its electrochemical potential gradient, and leaves the cell toward the peritubular space against its electrochemical potential gradient. Therefore, the Na pump could be located at the peritubular wall of the cell (6–8, 21, 24, 26, 32).

STUDIES ON THE SODIUM PUMP

The Extrusion of Na from Kidney Slices

To investigate further the characteristics of the Na pump, I used the outermost slices of the kidney of the guinea pig. They are convenient because the proximal tubules

occupy 97 % of the tissue volume (glomerulus 2 %, distal tubule 1 %), their cells can be subjected to large concentration changes, and their intact outer surface can be visualized under the microscope to measure the cell electrical potential with microelectrodes (27).

Fig. 2 illustrates the experimental procedure. Rewarming in a medium without K induces a net Na efflux accompanied by a net Cl efflux (see also 13). As the cell elec-

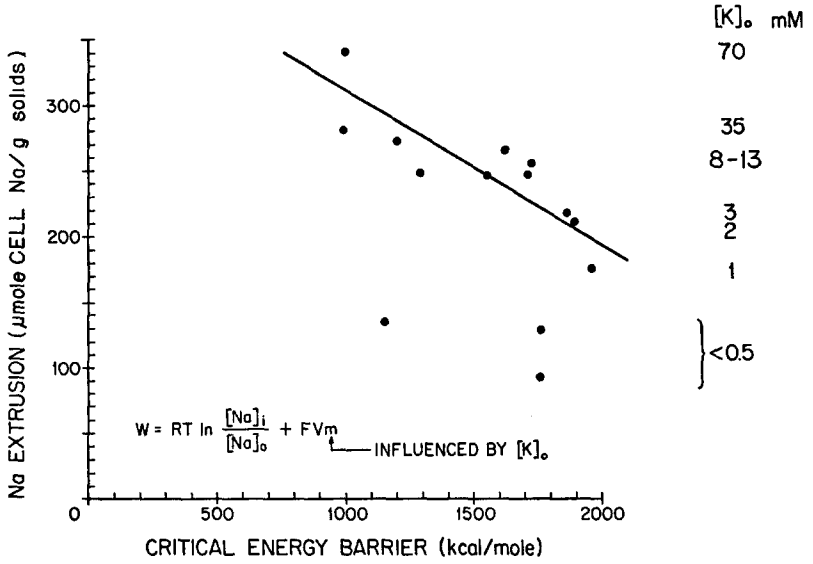

FIGURE 3. The Na extruded during reimmersion at 25°C until a balanced state was achieved is plotted as a function of the energy barrier that the pump overcomes to keep the cells in the balanced state, thus compensating the passive Na influx. This energy barrier is estimated as W, the work performed by the transport mechanism in the transference of a cation; R is the gas constant; T, the absolute temperature; F, Faraday's constant and Vm, the membrane potential. The reimmersion fluid K concentrations are shown at the right side of the figure, at the level of their corresponsing experimental points.

trical potential is negative as referred to the outside, the net Na efflux elicited by reimmersion is indeed an active process since it also occurs against its concentration gradient (27). Hereafter it will be called Na extrusion. When K is present in the reimmersion medium, there is further Na extrusion accompanied by net influx of K (27). Thus, there exists exhange of Na for K.

As an explanation of the existence of Na extrusion accompanied by Cl, and also of exchange of Na for K, two possibilities may be considered. On the one hand, there could be an electrogenic Na pump (27). Na extrusion would generate a potential, and K and Cl would move passively to maintain electroneutrality, since the cell membrane is most permeable to them (22, 27, 32). On the other hand, there could also exist a true exchange of Na for K at the pump.

The Possibility of Electrogenic Na Extrusion

If the influx of K originated from the activity of an electrogenic Na pump, $[K]_o$ should influence Na extrusion only by its ability to lower the energy barrier to Na extrusion (4). The energy barrier is made up of two terms: one depends on the Na concentration ratio between the cell and the outside, and the other depends on the cell electrical potential, which is influenced by $[K]_o$. In the experiments shown in Fig. 3, the energy

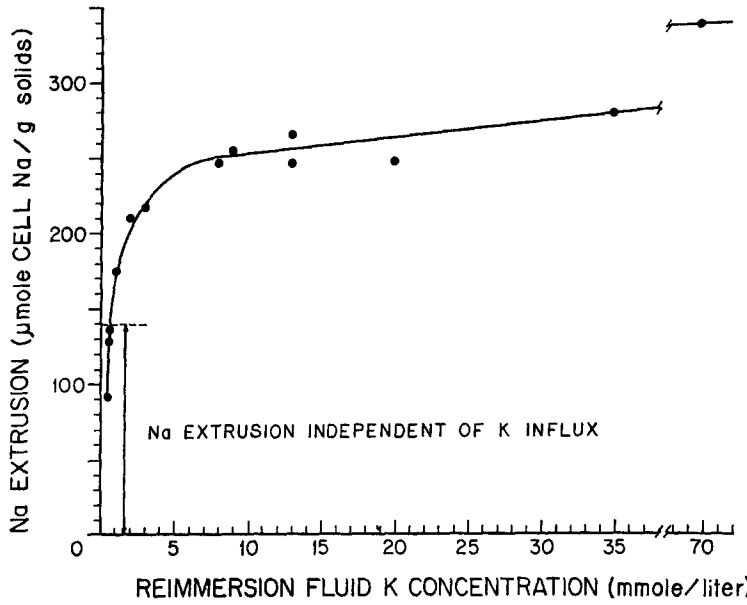

FIGURE 4. The Na extruded during reimmersion at 25°C until a balanced state was achieved is plotted as a function of the reimmersion fluid [K]. Kidney slices were reimmersed at 25°C in media with different $[K]_o$. During reimmersion in media without K, 100–140 μmoles cell Na per gram solids are extruded accompanied by Cl, without any K influx.

barrier was varied by changes in the outside Na concentration and by changes in the cell electrical potential, through changes in $[K]_o$. If $[K]_o$ acted only through the potential, more Na should be extruded as the energy barrier is made lower by increasing $[K]_o$ (27). The continuous line, drawn by eye, in Fig. 3 shows that this relationship indeed exists, as Smyth has also pointed out (20). The ion movements observed after reimmersion, the increase with reimmersion in the negativity of the cell electrical potential (Fig. 2), and the observation that Na efflux is faster than K influx immediately after reimmersion (27) are strong arguments favoring the possibility of electrogenic Na extrusion (27).

The Existence of a True Na for K Exchange at the Pump

As shown in Fig. 2, addition of K to the reimmersion medium at 25°C, after rewarming in a medium without K, induces practically a one-to-one exchange of Na for K.

At 0°C some exchange of Na for K may also be observed although at a much slower rate (34; Whittembury and Proverbio, unpublished observations). Besides that which was mentioned in the last paragraph, there is something else in Fig. 3. It may be seen that at low external K concentrations, small increases in $[K]_o$ stimulate Na extrusion more than is expected from their ability to lower the energy barrier. Moreover, Fig.

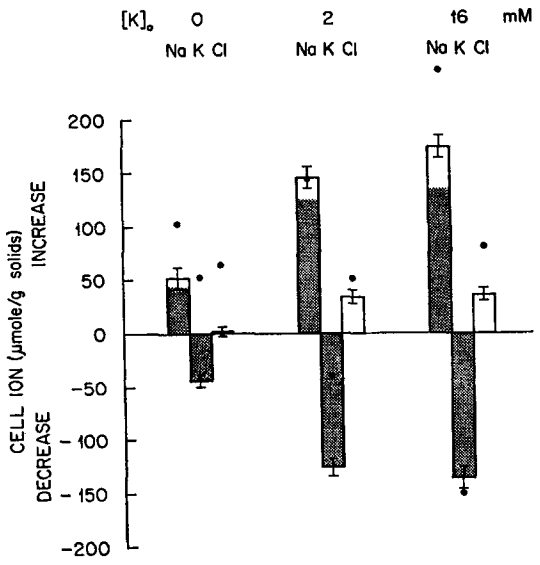

FIGURE 5. Action of 1 mM ouabain on cell Na, K, and Cl concentrations of kidney slices reimmersed at 25°C. Bars show differences between ouabain-treated slices and controls, at 0, 2, and 16 mM $[K]_o$, after 1 hr reimmersion. Bars show ouabain's inhibitiory action. Bars above 0 line show inhibition of net efflux; those below line, inhibition of net influx. Dots are relative ion concentrations before reimmersion. Shaded areas of Na bars show inhibition of Na extrusion in 1:1 ratio to inhibition of K uptake; light areas show inhibition of Na extrusion exceeding inhibited K uptake.

4 shows that although a significant amount of Na can be extruded without K uptake, Na extrusion depends markedly on the outside K concentration, so that small increases in $[K]_o$ induce great stimulation of Na extrusion. As a working hypothesis, we may call this a "specific" effect of K. It does not prove, but suggests, the existence of a true exchange of Na for K by the pump. This is further substantiated by the observation that K uptake practically stops in the absence of cell Na (23, 34).

The Effect of Ouabain

The existence of a true exchange of Na for K at the pump does not exclude the existence of an electrogenic pump, but would require that these two systems work separately. To investigate these possibilities further, F. Proverbio and I first tried ouabain. After immersion at 0°C, the slices were divided into two groups; one served as control for the other, which was treated with 1 mM ouabain. Both groups were reimmersed at 25°C in media without K, or with 2 mM $[K]_o$, or with 16 mM $[K]_o$. Fig. 5 shows

ouabain's inhibitory action on ion movements. The shaded areas are prominent. This indicates that ouabain inhibits mainly Na extrusion and K uptake. This effect depends on $[K]_o$ (28). Ouabain only slightly inhibits extrusion of Na accompanied by Cl efflux (light areas of Fig. 5), in agreement with observations of Kleinzeller (13, 14). The necessity for two pumps to explain that ouabain selectively inhibits the Na for K exchange, while the extrusion of Na accompanied by Cl has been left practically untouched, has already been proposed (28).

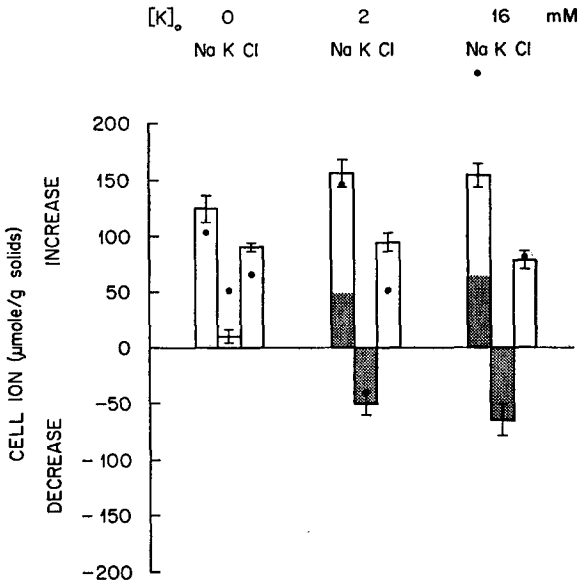

FIGURE 6. Action of 1 mM ethacrynic acid on cell Na, K, and Cl concentrations of kidney slices reimmersed at 25°C. As in Fig. 5, each bar indicates the difference in concentration between the slices treated with ethacrynic acid and the controls.

The Effect of Ethacrynic Acid

The use of ethacrynic acid was suggested to me by Dr. J. Hoffman. He also found it necessary to propose two pumps to explain his experimental findings in red blood cells (12). Fig. 6 shows ethacrynic acid's inhibitory action. Notice that this agent inhibits mainly extrusion of Na accompanied by Cl, and to a much lesser extent extrusion of Na accompanied by uptake of K, since the light areas in the figure are larger than the shaded ones. Other experiments showed that ouabain and ethacrynic acid added together have additive effects: they inhibit equally both a Na for K exchange and extrusion of Na accompanied by Cl.

The Source of Energy

Further experiments show that both modes of Na extrusion are inhibited by 2,4-dinitrophenol alone and also by gassing the preparation with N_2 instead of O_2, thus suggesting that generation of ATP is essential for both modes of Na extrusion. As has been discussed by Whittam and Willis (23) and Blond and Whittam (2), we found that transport and respiration seem to be closely linked. Thus, addition of ouabain

inhibits part of the respiration, and so does addition of ethacrynic acid (Fig. 7). This indicates that inhibition of Na extrusion in exchange for K, caused by ouabain, or inhibition of Na extrusion accompanied by Cl, caused by ethacrynic acid, is accompanied by inhibition of respiration. Respiration and transport seem to be linked in both directions, since stimulation of transport by addition of 2 mM K at 25°C to a K-free

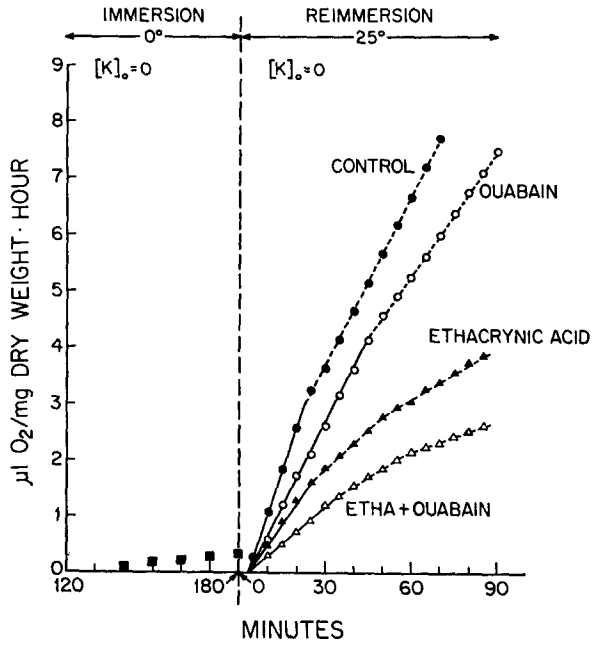

FIGURE 7. Effect of rewarming and of addition of ouabain and ethacrynic acid on respiration of kidney slices as measured in a Warburg apparatus. Oxygen consumption is very small during immersion in the cold (solid squares). Rewarming of the bath to 25°C, which was achieved within 1 min, greatly increases respiration. The rate of respiration is higher during the first 20 min of rewarming. Ouabain and ethacrynic acid each inhibited one part of the oxygen consumption.

medium also increases respiration (Whittembury and Proverbio, unpublished observations).

CONCLUSIONS

Fig. 8 summarizes our findings. We may describe two modes of Na extrusion. Mode A is accompanied mainly by Cl efflux. This net efflux of Na and Cl is accompanied by an important water movement. The movements of Cl and water are indicated by dashed lines to point out that they are passive and that they may occur through sites separate from those of the pump proper, possibly through pores at the membrane. Mode A is best shown by increasing the temperature from 0° to 25°C. Presumably this mode of Na extrusion stops in the cold. It is refractory to ouabain, and is sensitive to ethacrynic acid. It should be most important in the regulation of cell volume. It is tempting to suggest that this mode of Na extrusion should produce the largest net NaCl efflux out of the kidney proximal tubule (29).

Mode B is accompanied by uptake of K either by the pump proper or through a passive channel. The latter is possible since Na extrusion increases Vm at the outset of reimmersion (28). Mode B still works in the cold although at a much slower rate. It is best shown by addition of K outside. It is refractory to ethacrynic acid, and is sensitive to ouabain. Its function produces no volume change since it does not produce net solute flux. It should be most important in the regulation of the cell ion concentration. Israel (personal communication) has observations on the kidney in agreement with these two modes of Na extrusion.

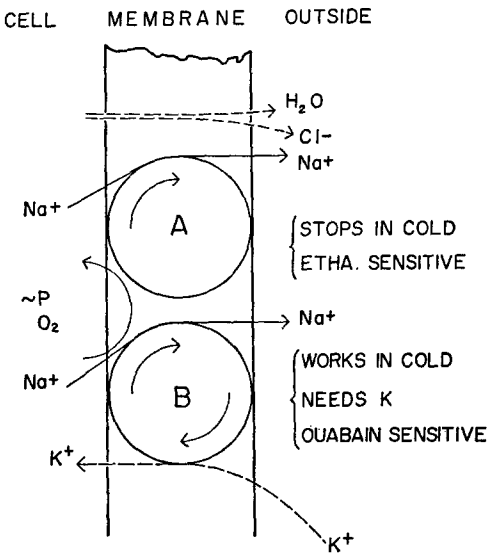

FIGURE 8. Schematic summary of the two modes of Na extrusion. For further explanations see text.

The arrow toward the inside of the membrane, which links the two modes of Na extrusion, indicates that both modes of Na extrusion stop in anoxia or when DNP is added; this indicates that energy comes in all probability from the splitting of ATP, as indicated by $\sim$ P, the high energy phosphate.

We have observed that respiration and transport are closely related (see 2, 23). Thus suppression of oxygen inhibits both modes of Na extrusion, and inhibition of either lowers respiration. Conversely, increase in transport increases respiration.

At present, it seems difficult to account for these results by the hypothesis of a single pump (29). If these two modes of Na extrusion are due to the work of two pumps, their action on passive ionic movements should be related. Thus, if pump A is electrogenic it could also induce some K uptake. Pump B, by the exchange of Na for K, would increase the cell K concentration and secondarily the cell electrical potential. This subsequently would induce some Cl efflux. This interdependence of the effects of the two pumps could explain why ouabain blocks some extrusion of Na accompanied by Cl (Fig. 5) and why ethacrynic acid blocks also some Na for K exchange (Fig. 6).

I gratefully acknowledge the collaboration of Mr. F. Proverbio in parts of this work, and I thank Doctors F. Herrera, L. Villegas, and R. Villegas for much criticism in the course of the work. I thank Miss M. Correa, Mr. J. Machin, Mr. L. Mateu, Mr. R. Pingarrón, and Mr. J. Mora for their assistance, and Dr. M. A. Rios, of Merck Sharp & Dohme, for the supply of ethacrynic acid.

Part of this research was supported by the United States Air Force under grant AF-AFOSR-620-66 monitored by the Air Force Office of Scientific Research of the Office of Aerospace Research.

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