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Electrolyte transport in kidney tubule cells

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Transtubular movement of Na and K takes place across an electrically negative cell compartment rich in K and poor in Na. Some properties of the luminal and peritubular cell boundaries with respect to ionic pump and leak characteristics are analysed.

Sodium enters the tubule cell from the lumen down an electrochemical potential gradient. Peritubular Na-extrusion takes place both by an ouabain-sensitive Na–K exchange pump and by an electrogenic ouabain-insensitive Na pump. Net Na transport can be uncoupled from peritubular K uptake. It is highly likely that peritubular K uptake is pH sensitive. Once sodium has been extruded into the peritubular infoldings net Na transepithelial-transport is further critically affected by physical factors regulating capillary uptake of interstitial fluid. Several lines of evidence indicate that a large, variable intercellular transport pathway is present at the proximal tubular level.

Tubular K secretion is controlled at the distal tubular level by: (1) the interplay of luminal and peritubular active K uptake into the tubule cell and (2) by a variable passive leak of K from cell into lumen across the partly depolarized luminal cell membrane. Changes in active peritubular K uptake regulate the size of a relatively small intracellular K transport pool and are critically involved in setting the rate of net tubular potassium secretion.

The conservation of all but a small fraction of the filtered fluid and electrolytes depends on the ability of the renal tubular epithelium to transfer large amounts of ions and water from the tubular lumen to the peritubular fluid compartment. In addition, some parts of the nephron are endowed with the capacity of secretory ion transfer, i.e. net addition of electrolytes to the tubular fluid.

From the functional point of view the nephron may be divided into several segments with distinctive and differing transport properties. The proximal tubular epithelium is characterized by the reabsorption of a large fraction of the glomerular filtrate against at best only small ionic concentration gradients. The reabsorption process takes place without generation of an osmotic concentration difference across the tubular epithelium (Walker, Bott, Oliver & MacDowell 1941; Giebisch & Windhager 1964). Along the loop of Henle, sodium and fluid transfer continues, but in contrast to the proximal tubular epithelium the water permeability declines along the ascending limb. Accordingly, the fluid entering the distal tubules is hypotonic. From this nephron site on, fluid tonicity is subject to regulation by the level of circulating antiduiretic hormone (Wirz 1956; Gottschalk & Mylle 1959). In the presence of this hormone, equilibration of the tubular contents with relative hypertonic extratubular environment leads to the progressive osmotic concentration of urine along the distal tubule and the collecting ducts. The distal tubular epithelium and the collecting ducts are characterized by a considerably more variable ionic transport pattern than the proximal tubular epithelium. Although the fraction of the glomerular filtrate reabsorbed at this site is small it is across the terminal nephron segment only that ionic concentration gradients of considerable magnitude are established. Normally, the concentration of sodium and chloride declines along the distal tubule, whereas the transport pattern of potassium is highly variable (Giebisch & Windhager 1964): massive addition of

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potassium ions by net secretion may lead to the excretion of potassium moieties greatly in excess of that filtered, whereas continued reabsorption of potassium along the distal tubule and the collecting ducts may reduce urinary excretion to less than 1 % of the filtered potassium (Malnic, Klose & Giebisch 1964; Giebisch 1969). Accordingly, the magnitude and direction of net potassium transfer are variable and can be shown to be dependent upon the metabolic demands.

In the following, two aspects of tubular electrolyte transport will be examined in some detail. First, evidence will be presented indicating that some important aspects of proximal transepithelial permeability to fluid and ions are determined by the properties of extracellular shunt pathways directly linking the luminal and peritubular fluid compartments. Secondly, some properties of tubular ion exchange processes will be considered. This latter analysis will centre on the role of sodium, potassium and hydrogen ions in tubular ion exchange processes. Recent experimental work has focused attention both on the mode of the interaction between the movement of these ion species across the luminal cell membrane of tubule cells as well as on relation between sodium extrusion and potassium uptake across the peritubular cell boundary of renal tubule cells.

Some properties of proximal tubular epithelium

The proximal tubular epithelium is the nephron site with the highest rates of transepithelial sodium and fluid transport. (Walker *et al.* 1941; Giebisch & Windhager 1964). Evidence in support of active sodium transport is available and consists, essentially, in the demonstration that net transport of sodium can be shown to proceed against an electrochemical potential difference (Giebisch & Windhager 1964).

A number of new aspects of the intraepithelial events of proximal tubular solute and water movement have recently been elucidated. They concern recognition of the fact that low resistance extracellular pathways are a functionally important element in the organization of this nephron segment. Several lines of experimental observations are pertinent.

(1) The transepithelial electrical resistance across the proximal tubular epithelium is unusually low, both when compared to the specific transepithelial resistance of other epithelial structures or when compared to the specific transepithelial resistance of more distally located segments of the nephron (Giebisch & Malnic 1969*a*; Burg, Isaacson, Grantham & Orloff 1968). Several investigators have measured the transepithelial specific resistance of proximal tubules in the amphibian of mammalian kidney. Whereas in the amphibian kidneys values of some 70 Ω cm^2 have been observed (Boulpaep 1970*a*, *b*), even lower values of some 5 Ω cm^2 have been found in the mammalian proximal tubule (Hegel, Frömter & Wick 1967; Windhager & Giebisch 1961; Boulpaep & Seely 1970*a*). These values are several orders of magnitude lower than those calculated on the assumption that the proximal tubular epithelium is lined with a membrane having resistance values of proximal tubular cells membranes (Windhager, Boulpaep & Giebisch 1967).

Figure 1 illustrates an experimental arrangement for transmembrane and transepithelial resistance measurements on tubular structures, and figure 2 shows data on intracellular voltage attenuation along a proximal tubule of *Necturus*. Both approaches make use of cable analysis and derive resistance values from the geometry of the tubular structures and the voltage attenuation subsequent to current injection over known distances both along tubular cells and the tubular lumen (Windhager & Giebisch 1961; Windhager *et al.* 1967). The main conclusion of these

studies was that the magnitude of the total transepithelial conductance greatly exceeded that to be expected on the basis of the resistive contributions of the bordering cell membranes. This observation argues strongly for the presence of a low resistance shunt path in parallel with the cell membrane resistances. It is relevant that the proximal tubular epithelium is characterized

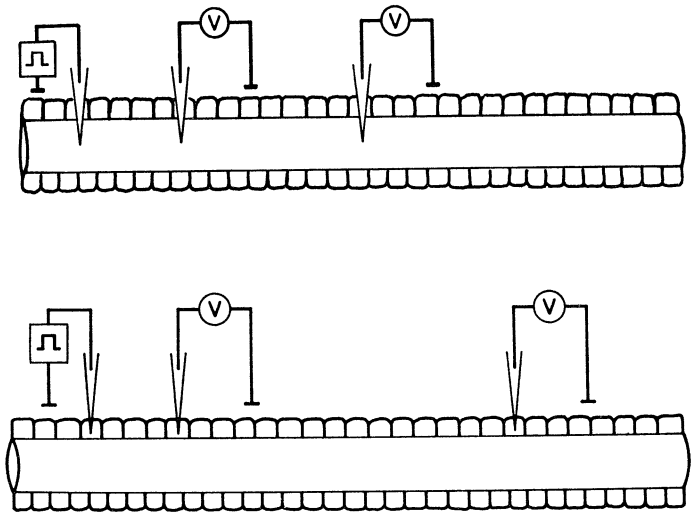

FIGURE 1. *Top*: method for the measurement of the transepithelial length constant. Representative values: *Necturus* proximal tubule, 492 μm; dog proximal tubule, 93 μm; rat proximal tubule, 50 to 100 μm; rat distal tubule, 300 μm; rabbit collecting tubule, <1000 μm. (Values from Boulpaep 1970*a*; Boulpaep & Seeley 1970*a*, *b*; Windhager & Giebisch 1961; Hegel, *et al.* 1967; Giebisch & Malnic 1969*a*; Burg, *et al.* 1968.) *Bottom:* method for the measurement of the intraepithelial length constant. (From Boulpaep 1970*a*; Windhager *et al.* 1967.)

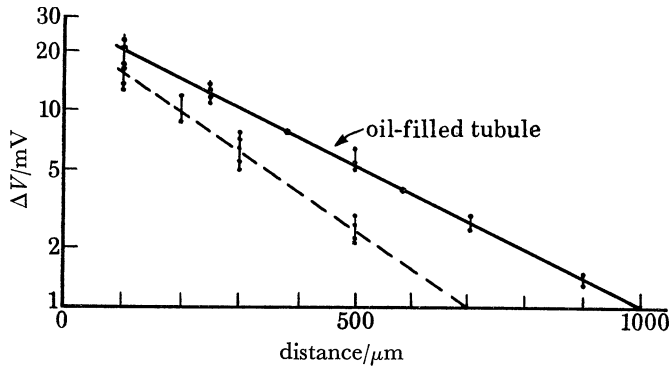

FIGURE 2. Results from the application of the method illustrated on the bottom of figure 1. Shown is the voltage attenuation to $1/e$ within the proximal tubular epithelium (*Necturus*) for an oil-filled tubule (continuous line), 300 μm, and for a tubule filled with Ringer's solution, 200 μm. The vertical bars indicate standard errors of the mean. (From Windhager *et al.* 1967.)

by exceedingly short junctional complexes (Farquhar & Palade 1963; Claude 1968). This area, as well as the intercellular fluid space, may determine the magnitude of the shunt conductance.

A point of considerable additional interest in such studies was the finding that the voltage difference along the proximal tubule cell column declines logarithmically with distance. This observation clearly demonstrates significant intraepithelial current spread along low resistance coupling pathways between neighbouring cells (Windhager *et al.* 1967). Similar current spread between cells has been demonstrated in other epithelial structures (Loewenstein 1966). It is

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likely that zones of cell fusion (nexus) are the morphological correlates of the cell-to-cell junctions (Claude 1968).

(2) A comparison of the ionic selectivity of the luminal and peritubular cell membrane of single tubule cells indicates significant permeabilities of the peritubular cell membrane to potassium and chloride, whereas the luminal cell membrane, with the exception of some finite permeability to sodium ions, has very low potassium and chloride permeability (Boulpaep 1967). In contrast, the transepithelial conductance is entirely different, an observation in support of the notion that epithelial structures other than the cell membranes alone determine transepithelial conductance properties. Thus from the magnitude and direction of transepithelial diffusion potentials it can be shown (*a*) that the ranking within the separate cation or anion series agrees with that predicted from mobilities in free solution and (*b*) that anion movement compared to that of cationic species is relatively reduced (Boulpaep & Seely 1970*b*; Frömter *et al.* 1968). This latter finding is compatible with the thesis that there are negatively fixed charges within low resistance transepithelial fluid channels.

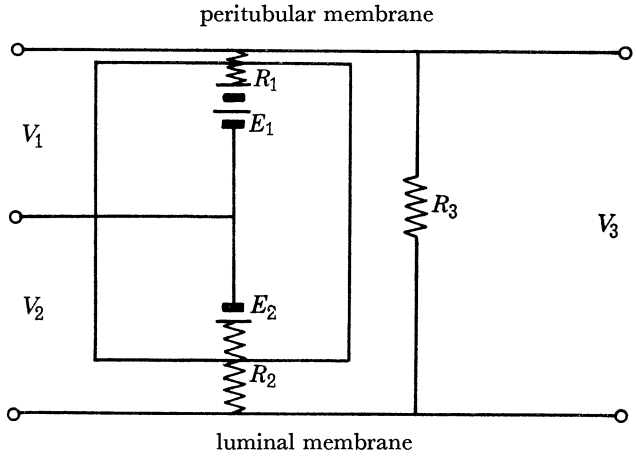

FIGURE 3. Equivalent circuit of a proximal tubule cell. V_1, peritubular membrane potential; E_1, peritubular e.m.f.; R_1, peritubular resistance; V_2, luminal membrane potential; E_2, luminal e.m.f.; R_2, luminal resistance; V_3, transepithelial potential, R_3; shunt or paracellular resistance. (From Boulpaep 1967.)

(3) A number of additional electrical phenomena also supports the idea that intercellular shunt pathways are present at the proximal tubular level. Coupling between electrical potential changes induced either at the peritubular of luminal cell membrane by appropriate ionic substitutions is best explained by electrotonic current spread along low-resistance intercellular fluid channels (Boulpaep 1967). Another pertinent observation is the presence of linear transepithelial current–voltage relationships, whereas investigations on single tubule cells show marked anomalous rectification (Boulpaep 1966).

Figure 3 summarizes some of the conductance properties discussed. An equivalent circuit of a single proximal tubule cell of *Necturus* is shown, and contains some of the better defined circuit elements. V_1 is the potential difference across the peritubular cell membrane. The concentration gradients of potassium, chloride and sodium represent pertinent electromotive forces, lumped as E_1, and specific ionic resistances are expressed as R_1. Concentration differences and relative conductances determine the contribution of an ion to a diffusion potential. Available evidence indicates that potassium, chloride and sodium ions participate in the generation of the peritubular potential difference of some 70 mV, cell negative (Giebisch 1961; Boulpaep 1967).

V_2 is the potential difference across the luminal cell membrane. Here, electrophysiological evidence implicates only sodium and chloride to generate diffusion potentials. V_3 is the total transepithelial potential difference and the resistor R_3 represents the shunt conductance discussed. It can be shown that V_2, the luminal potential step, is also a function of the peritubular potential difference and the magnitude of the shunt resistor.

The role of the described intercellular transport pathway has recently been explored in some detail under conditions of grossly altered proximal tubular net fluid and sodium transport (Boulpaep 1970*a*, *b*). It is well established that during extracellular volume expansion by saline, the rate of proximal tubular net sodium transport declines (Dirks, Cirksena & Berliner 1965; Cortney, Mylle, Lassiter & Gottschalk 1965). It appears that during such decrements of proximal tubular sodium reabsorption, increased back flux along the intercellular transport path renders less efficient the sodium transport system. This conclusion is based on the following experimental data (Boulpaep 1970*a*, *b*): (1) total transepithelial conductance can increase by a factor of 3.2 during extracellular volume expansion, at a time when cell membrane resistances remain unchanged, (2) the transepithelial permeability coefficient for NaCl similarly increases by a factor of 3.2, as measured by the influx of NaCl at a time when active transport is minimal, and (3) a significant increase of proximal tubular permeability to non-electrolytes such as raffinose is also oberved. These three lines of evidence provide strong evidence that enhanced passive backflux of sodium from peritubular fluid to the lumen is responsible for decreased net sodium reabsorption. Both the observed increase in transepithelial permeability to electrolytes at a time of unchanged cellular resistances as well as the observed increase in transepithelial permeability to non-electrolytes known to be excluded from the cell compartment points to an extracellular pathway as the route of increased ion and fluid leak. These observations are important in as much as they assign a crucial role to the extracellular shunt pathway in the regulation of net sodium transport. It is likely that the increased interstitial pressure associated with such conditions as extracellular volume expansion, increased renal venous pressure or increased arterial pressure may be causally linked to the described pheomenon of enhanced backflux of sodium chloride, since the tight junction represents an inherently leaky element in the organization of the proximal tubular epithelium.

Tubular ion exchange processes

It has been assumed for some time that a number of cations are involved in coupled ion exchange processes along the nephron. At the level of the proximal tubular epithelium sodium and hydrogen ions are thought to be exchanged across the luminal cell membrane. Whereas good evidence is available that hydrogen-ion movement across this cell site is active in nature (Rector, Carter & Seldin 1965), information with respect to the mode of sodium movement is insufficient to describe accurately whether active or passive transport takes place. The process of hydrogen-ion secretion leads to the titration of filtered bicarbonate, its reduction within the tubular fluid compartment and, in view of the isosmotic nature of proximal tubular fluid reabsorption, to a significant rise in proximal tubular chloride concentration (Walker *et al.* 1941; Malnic, Mello-Aires & Vieira 1970).

Previous views on distal tubular cation exchange may be summarized by stating that accumulation of potassium in the distal nephron subsequent to its complete proximal reabsorption was thought to be mediated by active secretion across the luminal cell boundary involving coupled

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carrier-mediated exchange with sodium as the tubular counter ion. Cellular hydrogen ions were also considered able to compete with potassium for exchange with sodium. Essentially, this thesis (Berliner 1961) was based on the observation that a reduction of urinary sodium excretion was frequently associated with a low rate of potassium excretion, implying a low sodium concentration at the distal tubular site of potassium secretion. Similarly, a low excretion rate of hydrogen ions during urinary alkalinization was thought to be coexistent with diminished distal tubular hydrogen ion secretion, thus permitting a larger than normal fraction of the postulated carrier to be available for potassium secretion.

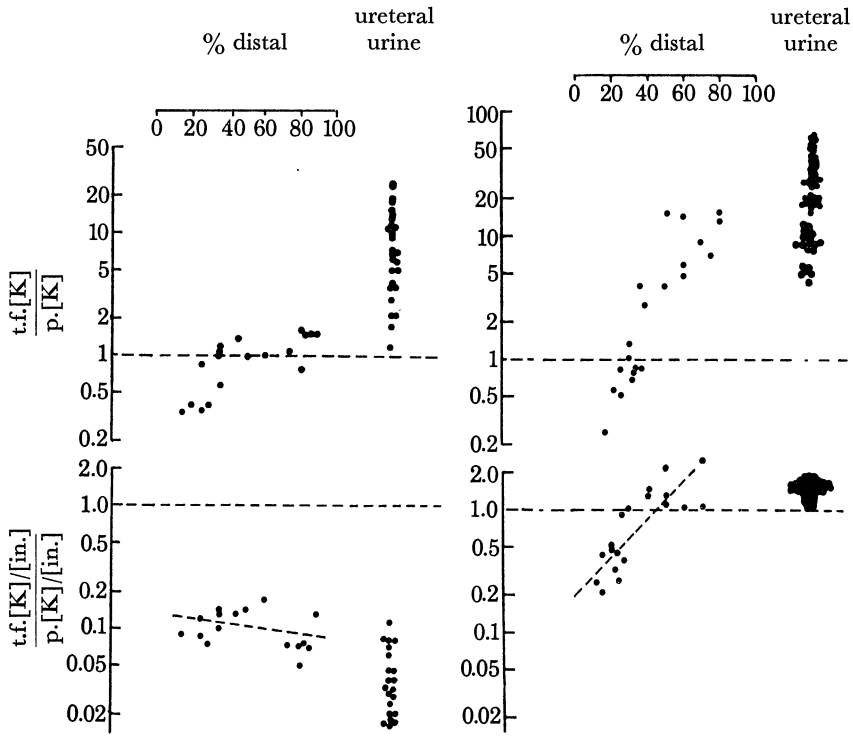

FIGURE 4. Summary of distal tubular potassium and potassium/inulin concentration ratios. Left: data from rats kept for several weeks on a low-potassium diet. Right: data from rats in which potassium excretion was maximally stimulated by pretreatment with a low sodium high-potassium diet and the infusion of potassium sulphate and dichlorphenamide. (From Malnic *et al.* 1964.)

With respect to the peritubular side of renal tubule cells it has become customary to place a sodium–potassium exchange pump at this cell site. This has, until recently, been more a matter of convenience but recent evidence has provided data in support of this thesis. However, it has also become clear that a single Na-K exchange mechanism is insufficient to account for both maintenance of constant cell volume and cell ion content and net sodium chloride and potassium transport.

Some aspects of these models of ionic interaction have recently been the subject of direct investigation. As a result of studies at the distal tubular level, it has become possible to answer at least partly the following questions: Is active, tightly coupled carrier-mediated ion transport involved in various tubular sodium–potassium exchange processes? Or is the observed cationic interaction of more unspecific nature? For instance, apparent cation exchange between lumen and cell could result from primary active cationic reabsorption, a low chloride or, more generally,

a low anion permeability of the tubular epithelium, and passive movement of another cation species in the opposite, i.e. secretory direction. In this case, the linkage between cationic reabsorption and secretion of another cation would not be provided for by carrier-mediated transport but by the electrical potential difference which results from different rates of reabsorptive cation and anion movement. Thus, whereas it is clear that some ion exchange must be involved whenever transepithelial sodium and chloride movement occurs at different rates, the fundamental nature of such ionic interchange may be different at various cell and nephron sites. Of obvious interest is the identification of the actively transported ion species and factors regulating their transfer rate.

In the following some relevant observations with respect to the role of potassium in tubular ion exchange processes along the distal nephron, particularly the interaction of potassium with sodium and hydrogen ion movement will be discussed. This topic has been investigated in our laboratory in collaboration with Dr G. Malnic, and by Grantham and his associates in the isolated cortical collecting tubule of the rabbit (Grantham, Burg & Orloff 1970).

In the rat, evidence obtained by micropuncture techniques shows that net secretion of potassium along the distal tubule accounts for most of the excreted potassium, confirming Berliner's thesis of an essentially distal secretory origin of urinary potassium (Berliner 1961). The excretion pattern of potassium in states of maximal potassium conservation and maximal potassium secretion is compared in figure 4. It is apparent that the potassium concentration along the distal tubule increases both during potassium depletion and potassium loading. However, whereas fluid abstraction more than accounts for the rise in potassium concentrations in rats kept on a low potassium diet, massive net addition of potassium along the distal tubule can be shown to occur regularly and account for at least some 90 % of the urinary potassium in animals in which stimulation of potassium secretion results in excretion rates equivalent to some 150 % of the filtered potassium load (Malnic *et al.* 1964). While the role of the collecting duct in secretion is small in most situations, fairly extensive net reabsorption may occur in either potassium or sodium depletion (Malnic, Klose & Giebisch 1966*a*; Diezi, Michoud, Aceves & Giebisch 1970). Extensive micropuncture studies indicate that in rodents and amphibians the distal tubule is the main control site of net potassium secretion (Malnic *et al.* 1964, 1966*a*; Giebisch, Klose & Malnic 1967; Giebisch & Malnic 1969*a*; Wiederholt *et al.* 1971).

Several attempts to demonstrate active secretory transport of potassium across the distal tubular epithelium as a whole were at first negative. In an extensive series of experiments the transepithelial potassium concentration difference was measured both in free-flow and stationary microperfusion experiments (Malnic, Klose & Giebisch 1966*b*) and compared with the electrical potential difference. Pertinent results are summarized in figure 5. It is apparent that the observed transepithelial concentration difference of potassium is consistently less, and in no instance exceeds the concentration difference expected from passive transepithelial distribution. Actually, the concentration difference of potassium in the tubule fluid is less than that required for electrochemical equilibrium, a finding implying two features of distal tubular potassium transport: (1) these observations are consistent with, but are not proof that potassium ions may enter the tubular lumen passively, and (2) that there is present at the distal tubular level a component of active potassium reabsorption lowering the potassium concentration below levels expected from electrochemical equilibrium. However, it should be realized that this approach does not include an analysis of the transport mode of potassium across the two individual cell boundaries of the tubule cell, but treats the cell as a single black box. Figure 6 shows a schematic

presentation of some pertinent properties of a single distal tubule cell. It was derived from the data just cited and from electrical potential measurements of single distal tubule cells (Sullivan 1968; Giebisch, Malnic, Klose & Windhager 1966). It is assumed that potassium entry across the luminal cell membrane is passive and opposed by active reabsorption. Supportive evidence for such a reabsorptive mechanism is: (1) The observation that net potassium reabsorption from

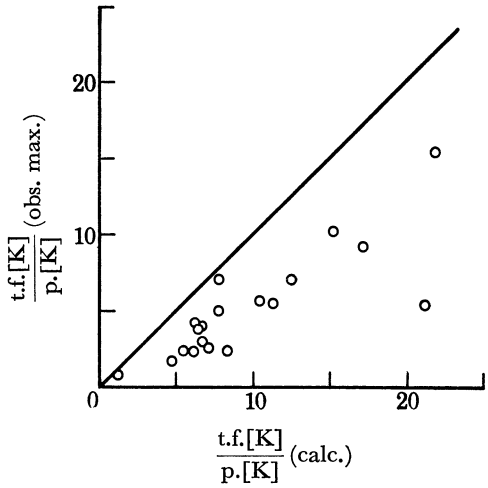

FIGURE 5. Summary of transtubular concentration ratios, calculated by the Nernst equation, across the distal tubule, compared to the observed maximal tubular fluid/plasma concentration ratios. Data from free-flow and stationary microperfusion experiments. (From Giebisch 1970.)

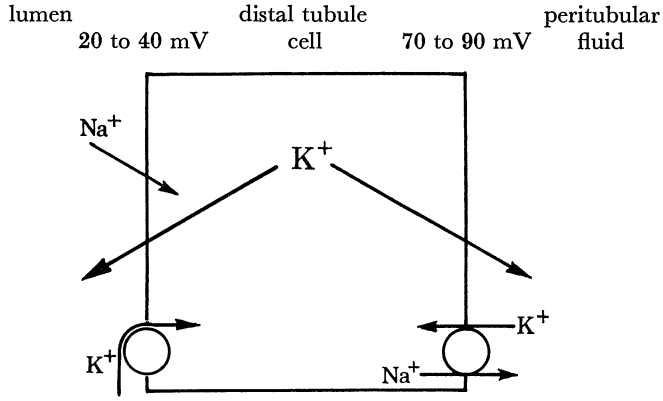

FIGURE 6. Schematic presentation of some properties of distal tubule cells. (From Giebisch, *et al.* 1967.)

the electrically negative lumen can occur in states of potassium depletion (and occurs regularly in the amphibian distal tubule). Such potassium movement is thus net movement against an electrochemical potential difference and, by the usual criteria, if independent, active in nature. (2) The observation that the luminal concentration of potassium is always lower than that expected from the transepithelial electrical potential difference (lumen negative). (3) The observation that the luminal potassium concentration increases subsequent to the administration of cardiac glycosides (Duarte, Chométy & Giebisch 1969, 1971; K. Strieder, R. Khuri & G. Giebisch, unpublished observations), compounds known to inhibit active potassium uptake into many cell systems (Glynn 1964).

An important question is the nature of the effect of changes in sodium metabolism upon potassium transport by the renal tubules. A low urinary sodium excretion frequently curtails potassium

excretion (see Kruhoffer 1960) and blocks the kaliuretic effect of adrenal steroids (Seldin, Welt & Cort 1956; Relman & Schwartz 1962; Howell & Davis, 1954; Malnic *et al.* 1966*a*). It had previously been assumed that in states in which the urinary sodium content had fallen to low levels (dietary sodium depletion, acute reduction in glomerular filtration rate) the sodium load to the distal tubule might also be diminished and lowered to such an extent as to provide an inadequate amount of sodium for exchange with potassium (Davidson, Levinsky & Berliner 1958; Berliner 1961). Direct studies on distal tubular sodium and potassium concentration

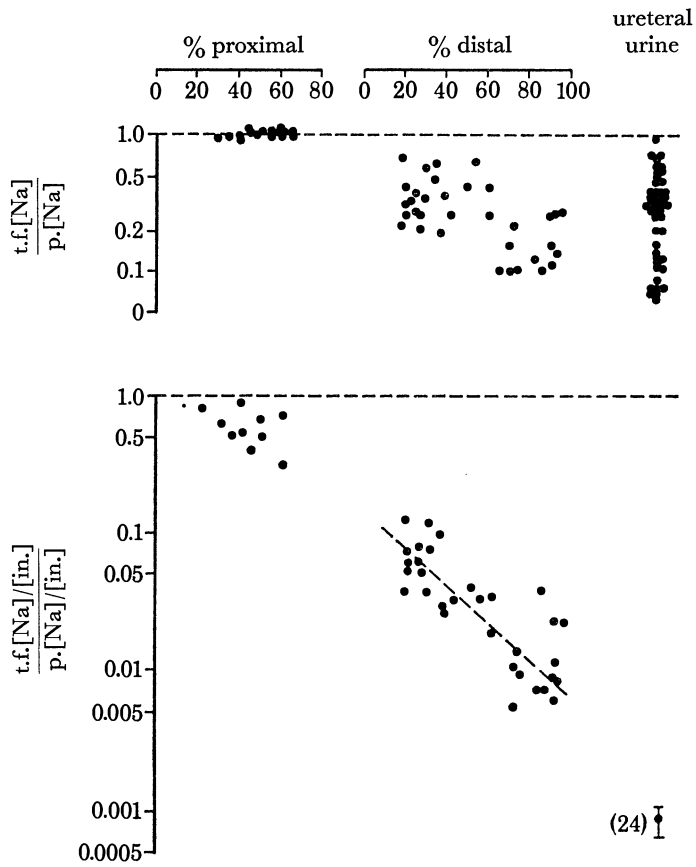

FIGURE 7. Summary of proximal and distal sodium and sodium/inulin tubular fluid to plasma concentration ratios. Data were obtained in rats on a normal sodium intake. (From Malnic, *et al.* 1966*a*).

profiles have not confirmed this notion. We have tested the thesis by measuring sodium concentrations and sodium reabsorption along distal tubules under conditions of low urinary sodium excretion rates. This was achieved by administration of a low-sodium diet or by acute clamping of the renal artery. Figures 7 and 8 provides data obtained in control and sodium-depleted animals. Figure 7 depicts the progress of concentration changes and of fractional reabsorption of sodium along superficial distal tubules of rats. It is apparent that the sodium concentration declines along the distal tubule and that an amount equivalent to some 8% of the filtered sodium load is normally reabsorbed along the distal tubule. Figure 8 summarizes data obtained in animals maintained on a low sodium intake for several weeks before the experiment. Shown are transepithelial sodium and potassium concentration ratios and the respective fractionals reabsorption and secretion rates. A low urinary potassium excretion rate (some 3% instead of a normal excretion rate of some 20%) is associated with a somewhat

reduced distal secretion rate (late distal fraction secretion of some 20 % instead of some 30 to 40 % in control animals) and marked reabsorption of potassium along the collecting ducts. Diezi and his associates (Diezi *et al.* 1970) have recently confirmed by micropuncture of terminal

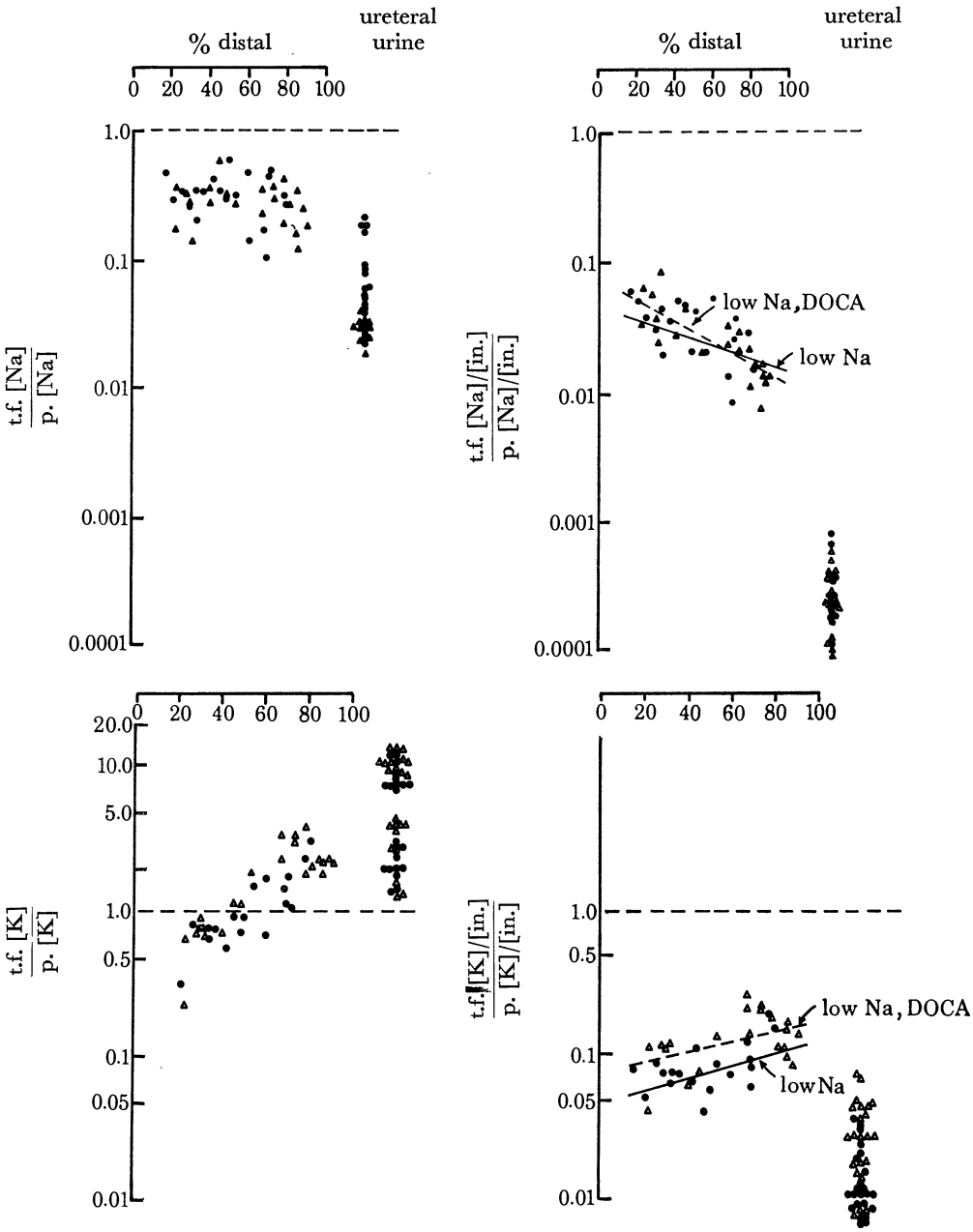

FIGURE 8. Summary of distal sodium and sodium/inulin tubular fluid to plasma concentration ratios (top) and potassium and potassium/inulin tubular fluid to plasma concentration ratios (bottom). Animals were kept on a low-sodium diet for several weeks before the experiment. DOCA-treated animals received DOCA at a dose of 1 mg/kg for two weeks. (From Malnic, Klose & Giebisch 1966*b*.)

collecting ducts that potassium ions can be actively reabsorbed along the collecting duct epithelium in low sodium animals. Inspection of distal sodium data shows that the delivery of sodium to the distal convoluted tubule is not limiting for potassium secretion. This is apparent

from the observation that the low urinary sodium excretion rate is not exclusively achieved by excessive reabsorption of sodium either prior to or along the distal tubule but is achieved largely by stimulation of sodium reabsorption along the collecting ducts. Although the amount of sodium entering the distal tubule was less than that in control animals, due to some enhancement of sodium reabsorption along the proximal nephron, the amount was still large enough to account for potassium secretion to proceed at a rate an order of magnitude in excess of that actually observed.

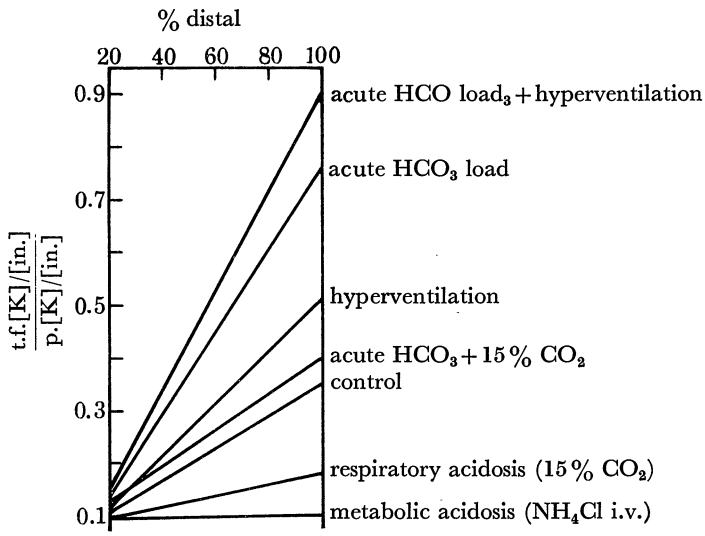

FIGURE 9. Summary of fractional distal tubular potassium secretion plotted as function of tubular length during control conditions and various acid-base disturbances. (From Giebisch & Malnic 1969*a*, *b*.)

Essentially similar findings are obtained when glomerular filtration rate is acutely reduced, a situation in which both urinary sodium and potassium excretion falls drastically. (Davidson *et al.* 1958; Landwehr, Schnermann, Klose & Giebisch 1968). It can be shown under these conditions, by puncturing individual distal tubules before and after acute reduction of the glomerular filtration rate by arterial clamping, that sodium delivery is not limiting for potassium secretion. Neither is the distal tubular sodium concentration nor the distal tubular sodium reabsorption drastically reduced. Again, enhanced reabsorption of sodium and potassium along the collecting ducts emerges as the key factor responsible for low sodium and potassium excretion. These experiments show that other mechanisms than limited distal sodium supply have to be sought and will be discussed subsequently, in order to explain the important interaction between sodium and potassium transport.

We have also investigated the relationship between distal tubular potassium secretion and hydrogen-ion secretion in a large number of acid-base disturbances (Giebisch & Malnic 1969*a*, *b*). Figure 9 provides pertinent observations on the range of distal tubular secretion rates during various types of acidotic and alkalotic conditions. It is clear that the distal tubule is responsible for the wide fluctuations in renal potassium excretion which accompany acid–base disorders. Induction of alkalosis stimulates, whereas acute acidosis, either of metabolic or of respiratory origin, supresses potassium secretion. The simultaneous measurement of potassium secretion and bicarbonate reabsorption allows a comparison to be made between the transport rates of these ions if the assumption is made that tubular bicarbonate reabsorption is accomplished by tubular hydrogen ion secretion (Giebisch & Malnic 1970; Rector *et al.* 1965). We feel that our

studies have not provided evidence supporting the notion that potassium and hydrogen-ion secretion are directly linked and vary reciprocally at the distal tubular level. Frequently, the rate of both potassium and hydrogen ion secretion varies in parallel (see table 1). We have also never obtained data indicating saturation of distal tubular hydrogen-ion secretion

TABLE 1. COMPARISON OF POTASSIUM SECRETION AND BICARBONATE REABSORPTION ALONG THE DISTAL TUBULE OF THE RAT. (From Giebisch & Malnic 1969*b*)

treatment	HCO_3 reabsorption	K secretion
	μmol/min kg ml g.f.r.	
control	2.28	1.30
15% CO_2 hyperventilation	1.11	1.12
$NaHCO_3$ met. acidosis	2.05	1.88
$NaHCO_3$ + hyperventilation	14.57	3.24
	0.40	0
	28.4	3.70

but consistently observed that hydrogen-ion secretion is proportional to the distal tubular bicarbonate (buffer) load. For instance, a marked decrease in distal tubular potassium secretion obtains in respiratory acidosis, irrespective of whether distal tubular hydrogen-ion secretion is lowered because of excessive bicarbonate reabsorption at a nephron site upstream of the distal tubule, or whether hydrogen-ion secretion is elevated, a situation which can be achieved by stimultaneous infusion of bicarbonate *and* by breathing 15% CO_2. Another relevant finding is that distal potassium secretion is significantly stimulated after administration of the carbonic anhydrase inhibitor diamox at a time when distal hydrogen-ion secretion is not diminished—as had previously been assumed, but actually enhanced due to the much larger bicarbonate load which enter the distal tubule due to significant proximal tubular blockage of dhyrogen-ion secretion and of bicarbonate reabsorption.

We feel that such observations are difficult to reconcile with the notion that potassium and hydrogen ions share a common transport pathway of secretion and that these ions compete directly for the same limited carrier-supply. Rather, our data are better explained by assigning an important role to cellular pH changes in regulating potassium secretion, independent of whether such cellular pH changes are associated with an increase in hydrogen-ion secretion rate or not. Additional evidence supports the interpretation that events not at the luminal cell border as had been previously assumed, but at the peritubular cell boundary, are of crucial importance in the regulation of transepithelial potassium movement. These may also be involved in the mediation of pH dependent changes in tubular potassium secretion.

Recently we have undertaken a series of tracer flux studies of distal tubular potassium transfer in amphibian and mammalian kidneys (Wiederholt *et al.* 1971; G. Malnic, Curran and G. Giebisch, unpublished observations). As shown in figure 10 we consider the tubular system reasonably well represented by a three-compartment arrangement: S_1 the lumen, S_2 the tubule cell and S_3 the infinite peritubular fluid pool. Information on rate constants and on the potassium transport pool can be obtained in either of the following ways: (1) by deposition of ^{42}K into the lumen and observation of the precise time course of disappearance of tracer from the lumen under conditions of zero net flux of potassium. (2) By adding ^{42}K to the peritubular compartment (perfusion of single peritubular capillaries) and observing, in a first step, tracer appearance in the lumen and, in a second step, tracer washout from cell compartment S_2 into

the lumen after abruptly discontinuing peritubular perfusion with ^{42}K. Both approaches provide enough information for solving the equations for rate constants and for the transport pool size.

The main conclusions which can be drawn from such studies are schematically summarized in figure 11. Two situations are shown: on top net reabsorption of potassium across the distal

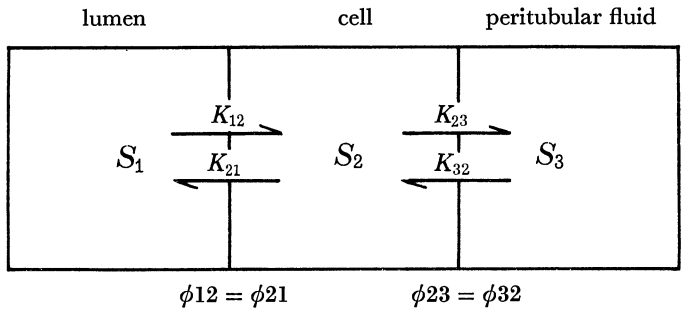

FIGURE 10. Schematic illustration of simple three-compartment system consisting of the tubular lumen, the cell compartment and the peritubular fluid compartment. S_1, S_2 and S_3 denote amount of solute in individual compartments, K_{12}, K_{21} and K_{23} and K_{32} are rate constants defining unidirectional solute movement across the luminal and peritubular cell membrane, respectively. The system is considered to be in the steady-state and net transport of potassium to be zero. (From Wiederholt, *et al.* 1971.)

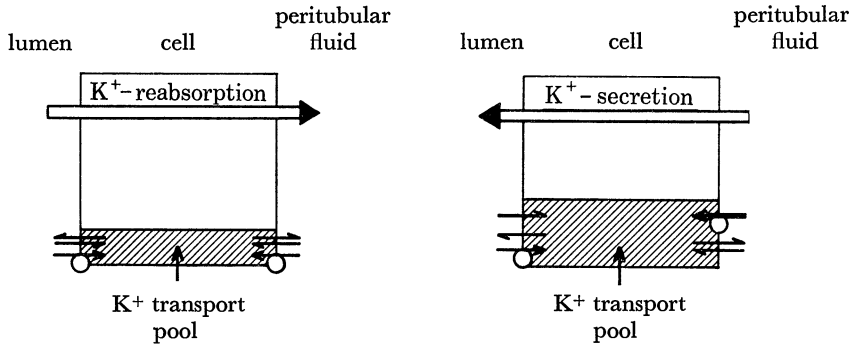

FIGURE 11. Cell schema depicting distal tubular potassium transfer across the luminal and peritubular cell membrane during the state of net reabsorption and net secretion of this ion. Note that the transport pool of potassium is larger as potassium secretion is stimulated. This is the consequence of increased potassium uptake across the peritubular cell membrane.

tubule, on the bottom net secretion. Two points are noteworthy. First, it is apparent that only part of the cellular potassium is involved in net potassium transport. Secondly, with transition from the state of net reabsorption to that of net secretion, i.e. with stimulation of secretory potassium transfer (induced either by potassium loading, administration of diamox or infusion of sodium bicarbonate) the potassium transport pool increases. Importantly, this increase is due solely to an increase in active potassium uptake across the peritubular cell border. Thus, we feel that peritubular active potassium uptake is an important control site responding both to an elevation of extracellular potassium levels and to changes in intracellular pH (stimulation of potassium uptake by alkalosis, suppression by acidosis). Peritubular potassium uptake controls the magnitude of the intracellular potassium pool and thus, presumably via concomitant concentration changes, the driving force acting on potassium as it moves across the luminal cell membrane. We have not obtained evidence that in the above described situations the luminal cell boundary affects potassium movement in any other way than by changes in the transmembrane potential difference.

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Figure 12 summarizes our views on distal tubular electrolyte transport. Rate of potassium accumulation is controlled by peritubular uptake. Luminally, potassium is reabsorbed by an ouabain-sensitive mechanism. Hydrogen ions can be secreted by an active mechanism but we do not believe that this mechanism is shared with that of potassium secretion. An important feature of the cell model is asymmetrical electrical polarization. An important element contributing to this functional property is the fact that the luminal cell membrane, in addition to a significant potassium permeability, also has a finite and important sodium permeability. Since potassium and sodium diffusion potentials are directed in opposite direction (cell K > luminal K, luminal Na > cell Na) the transmembrane potential difference across the luminal cell membrane is significantly less than that of the peritubular cell membrane which has been shown to behave almost like an ideal potassium electrode (Sullivan 1968; Giebisch *et al.* 1966; Wright 1970; Giebisch & Malnic 1969*a*).

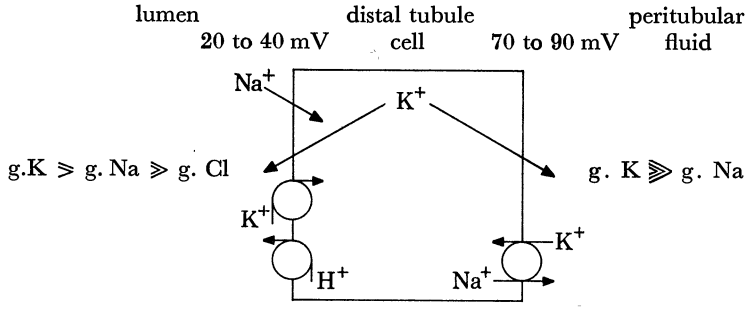

FIGURE 12. Schematic presentation of some properties of a single distal tubule cell. (From Giebisch 1969.)

The effects of sodium upon the potassium transport system could be mediated in the following way. Electrically, since it can be shown that intratubular negativity decreases with lowering of the luminal sodium concentration (Sullivan, 1968; Giebisch *et al.* 1966; Wright 1970). Furthermore, it has been shown that lowering of the sodium concentration also reduces the transepithelial potential difference across the collecting duct epithelium (Grantham *et al.* 1970). A reduction of tubular sodium concentration along the terminal nephron segment would reduce the electrical 'sink' into which potassium normally moves at the distal tubular level. It would also reduce the intratubular negativity within the collecting ducts where maintained negativity is necessary to prevent the escape of distally secreted potassium. This view is supported by our observation that potassium is lost from the collecting ducts at a time when the sodium concentration is dramatically lowered at this site.

A second mechanism by which sodium could affect potassium transport may be via changes in the sodium concentration in those distal tubule cells which are secreting potassium. A low sodium concentration, for instance during sodium depletion, could impede uptake of potassium across the peritubular cell membrane if the latter movement were coupled to sodium extrusion. Whittam & Willis (1963) have obtained evidence for such a type of inhibition of potassium uptake into tubule cells in kidney slices and in recent studies with Drs Whittembury and Sullivan (Whittembury, Sullivan & Giebisch 1970) we have confirmed the possibility of such an interaction. This mechanism, speculative at this time but open to investigation by the flux measurements alluded to previously, would be a revival of carrier-mediated potassium–sodium exchange situated at the peritubular cell membrane, instead of at the luminal cell membrane, as originally envisaged.

The discussion of tubular ion exchange processes should also include consideration of the recent work by Grantham *et al.* (1970) on isolated collecting tubules of rabbits. In this preparation potassium can be actively secreted in exchange for sodium.

Peritubular ion exchange

As pointed out before, the existence of a coupled sodium–potassium exchange mechanism for extrusion of sodium from the cell to the peritubular fluid space, was considered a likely mechanism that would explain how tubule cells absorb sodium from lumen to peritubular fluid compartment and maintain constant volume and ion composition (Whittembury, Sugino & Solomon 1961; Giebisch 1961). The existence of a coupled pump has been supported by the following lines of experimental evidence: (1) Sodium is expelled out of renal tubule cells against an energy barrier of about 8 kJ/mol (2 kcal/mol) (Whittembury 1965, 1968). (2) Sodium and potassium move in opposite directions across tubular cell membranes. Thus, in slices of renal tissue it can be shown that cell sodium decreases when cell potassium increases, and vice versa (Mudge 1951; Whittam & Davies 1953; Whittam & Willis 1963; Whittembury 1965). (3) Net sodium extrusion depends on the potassium concentration in the extracellular medium (Whittam & Willis 1963; Whittembury 1965; Whittembury & Proverbio 1970). (4) Potassium uptake depends on intracellular sodium concentration (Whittam & Willis, 1963; G. Whittembury, W. J. Sullivan & G. Giebisch 1970). (5) Ouabain inhibits extrusion of sodium in exchange for potassium (Whittam & Willis 1963; Whittembury 1968; Whittembury & Proverbio 1970). (6) Finally, ouabain's inhibition of sodium for potassium exchange is associated with ouabain's inhibitory action on a sodium–potassium stimulated ATPase (Whittam & Wheeler 1961; Whittam & Willis 1963; Proverbio, Robinson & Whittembury 1970).

However, several lines of experimental evidence indicate that this simple model is insufficient to explain some important aspects of renal electrolyte transport. For example: (1) Burg, Grollman & Orloff (1964), in studies on isolated tubules found a sodium to potassium coupling ratio inconsistent with a forced 1 to 1 exchange. (2) Torretti *et al.* (1970) observed that 50 % of the filtered sodium can still be reabsorbed despite complete inhibition of the sodium-potassium ATPase in ouabain-poisoned dog kidneys. (3) Maude (1970), in studies on perfused tubules of mammalian kidney slices, could not correlate changes in net sodium reabsorption and cell ion concentration and has proposed different mechanisms for transcellular and for homocellular sodium transport. (4) in agreement with observations of Kleinzeller & Knotkova (1964), Macknight (1968), Willis (1968), and Whittembury (1968) have found that kidney cells can extrude sodium with chloride even in the presence of ouabain doses that inhibit exchange of sodium for potassium. Whittembury (1968), Whittembury & Fishman (1969), and Whittembury & Proverbio (1970) have interpreted these findings as indicative of two modes of sodium extrusion from tubule cells.

Figure 13 summarizes some relevant aspects of the experimental design (Whittembury 1965, 1968). Mammalian kidneys slices were used. Since tubular lumina are collapsed in this preparation ions should move predominantly between cell and peritubular spaces. Cells from kidney slices may be loaded with sodium by immersion in a chilled medium without potassium. Attention should be focused on ion and water movements induced by rewarming to 25 °C. The first important observation is that rewarming in a medium without potassium induces net sodium extrusion from cells accompanied by efflux of chloride and by volume loss but without

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potassium uptake. If potassium is added to the bath, one observes a further loss of sodium from the cells. However, now a reciprocal gain of potassium accompanies sodium extrusion. The second key observation is that 10^{-4} mol/l ouabain inhibits extrusion of sodium in exchange for potassium, but does not interfere with the extrusion of sodium with chloride (Kleinzeller &

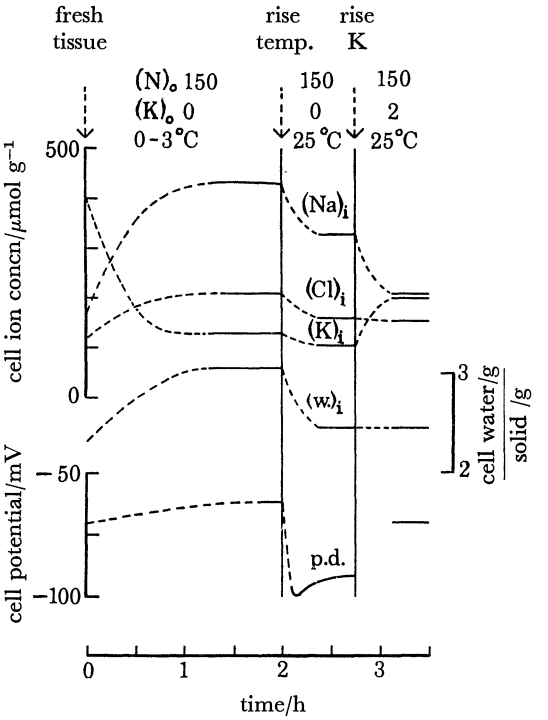

FIGURE 13. Net ion movement, fluid shifts and changes in membrane potential of cells from mammalian kidney slices. Slices were initially loaded with sodium and made to loose potassium by immersion in chilled medium that contained 150 mmol/l sodium and no potassium. After two hours of immersion the slices were reimmersed in a similar medium at 25 °C. The reimmersion medium was subsequently replaced by another containing 2 mmol/l K. The continued line refers to balanced states. (From Whittembury 1968.)

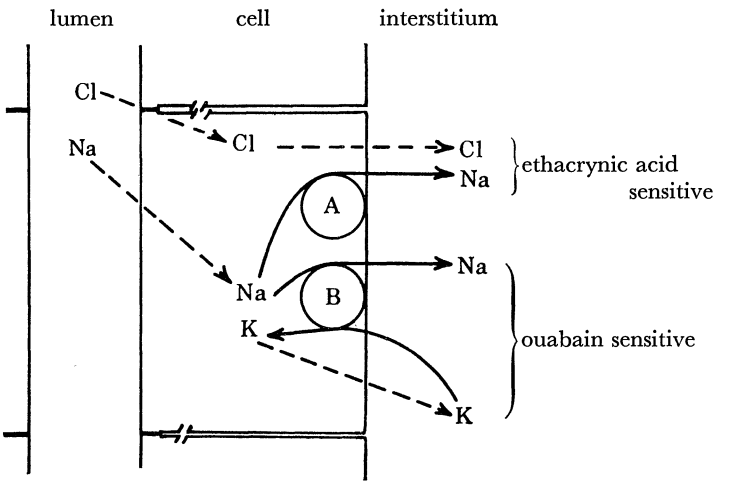

FIGURE 14. Schematic summary of the two modes of sodium extrusion. Sodium ions are extruded out of a common cell pool via either pump A or B. Before sodium extrusion, sodium ions would enter the cell, from the tubular lumen, down the electrochemical potential difference. (From Whittembury & Proverbio 1970.)

Knotkova 1964; Whittembury 1968; Willis, 1968). Even 10 mmol/l ouabain are ineffective (Whittembury & Proverbio 1970). The third important observation is that ethacrynic acid inhibits extrusion of sodium with chloride and water, but affects the extrusion of sodium in exchange for potassium to a very moderate extent only (Whittembury 1968; Whittembury & Proverbio 1970).

Thus two modes of sodium extrusion may be described (Whittembury 1968; Whittembury & Proverbio 1970). Figure 14 describes some pertinent properties. *Mode A* is extrusion of sodium with chloride and water. This type of ion and fluid movement is inhibited by cold, it is independent of the presence of potassium in the bathing medium, it is sensitive to ethacrynic acid but insensitive to ouabain. *Mode B* is extrusion of sodium in exchange for potassium. This type of ion movement may be observed to occur also in the cold. It is stimulated when the potassium concentration in the bathing medium is raised, it is sensitive to ouabain but quite insensitive to ethacrynic acid.

In order to explain these two modes of sodium extrusion on the basis of a single pump, a number of unusual and tenuous assumptions as to the site of action of the inhibitors, and as to the degree of their inhibition would be required. Thus, ethacrynic acid would have to block only the pathway for chloride movement and not the pump proper so that extrusion of sodium in exchange for potassium can persist in the presence of ethacrynic acid. Measurements of electrical potentials changes after ethacrynic acid rule out this possibility. If chloride movements were selectively inhibited by ethacrynic acid, sodium extrusion in the presence of ethacrynic acid should hyperpolarize the peritubular membrane. However, a depolarization is observed (Whittembury & Proverbio 1969). Furthermore, we would have to assume that ouabain would have to block potassium entry and not the pump proper, so that exchange of sodium for potassium would be inhibited but extrusion of sodium with chloride could perist. This assumption does not seem justified since ouabain also blocks the sodium–potassium stimulated ATPase in the kidney which is closely involved in active sodium extrusion in exchange for potassium (Charnock & Post 1963; Whittam & Wheeler 1961).

Sodium–potassium stimulated ATPase and sodium extrusion

A dual pump model raises some interesting questions concerning the source of energy for these pumps. According to the orthodox view a sodium–potassium stimulated ATPase should be of central importance. Thus, if the sodium–potassium ATPase were common to both pumps, inhibition of the ATPase should affect both pumps (Proverbio *et al.* 1970).

Proverbio *et al.* (1970) have attempted to correlate the inhibitory action of ethacrynic acid on mode A of sodium extrusion with the inhibitory action of ethacrynic acid on the sodium–potassium stimulated ATPase, and the inhibitory action of ouabain on mode B of sodium extrusion with the inhibitory action of ouabain on the sodium–potassium stimulated ATPase. For this purpose ion movements in kidney slices and ATPase activity of a microsomal fraction obtained from the same kidney slice preparation were studied. They found that the inhibitory action of ouabain on the sodium-potassium stimulated ATPase correlated well with its inhibition of the exchange of sodium for potassium (both being half inhibited with approximately 10^{-5} mmol/l ouabain and fully inhibited with 10^{-4} mol/l ouabain). In contrast, extrusion of sodium with chloride was independent of the action of ouabain on sodium for potassium exchange and on the ATPase. Importantly, the sodium–potassium stimulated ATPase was

found to be quite insensitive to ethacrynic acid. It was concluded that whereas pump B could be related to the ATPase system, pump A had a different energy supply. Additional experimental work is necessary to define its nature.

Electrogenic sodium extrusion

To investigate this problem Whittembury & Proverbio (1969) studied the time course of electrical cell polarization at the outset of rewarming since it can be shown that at this moment both sodium pumps are working at a maximal rate.

Figure 15 describes a representative experiment. The continuous line shows the results of experiments performed in a medium containing 16 mmol/l potassium. From a value of about -37 mV in the cold, the cell negativity reached, upon rewarming, about -76 mV and then returned to about -53 mV. It can be calculated that the hyperpolarization hump cannot be explained by assuming that the potential is solely generated by passive distribution of potassium across the cell membrane. It follows that other mechanisms than potassium diffusion contribute

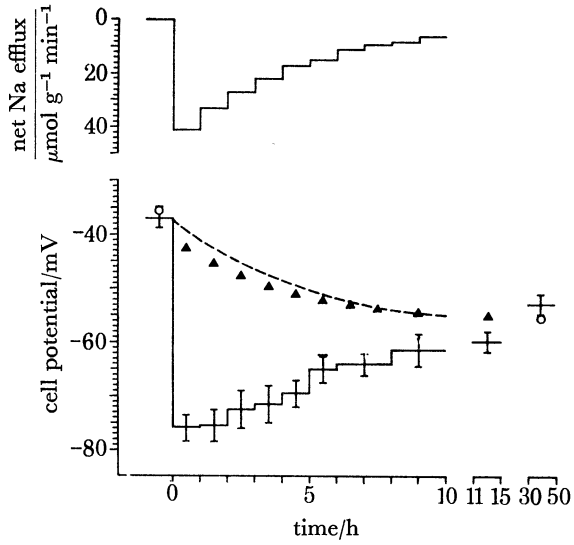

Figure 15. Time course of changes of sodium efflux and transmembrane potential after reimmersion of kidney slices into solutions containing 16 mmol/l potassium at 25 °C. For details, see text. ▲, values of calculated potassium diffusion potentials; ——, observed potential differences. Bars indicate mean values ± s.e. (From Whittembury & Proverbio 1969.)

to the hyperpolarization. Since the time course of the membrane potential change corresponds to the time course of net sodium efflux the transitory hyperpolarization was thought to be related to sodium efflux by assuming that a component of such sodium movement is electrogenic. Experiments with ouabain and with ethacrynic acid permit to assign to pump A an important electrogenic role. This would indicate that pump A can induce movement of chloride by a route different from that of the pump proper.

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Role of peritubular ion transport systems in transepithelial reabsorptive sodium movement

In order to study this problem Whittembury & Fishman (1969) used a kidney preparation in which, in contrast to the slice preparation, transepithelial sodium reabsorption was present. Transtubular reabsorption of sodium and chloride and intracellular ion concentrations could be studied in the perfused toad kidney, a stable preparations allowing extensive manipulation of the extracellular environment. The key conclusions from this study were: (1) Sodium reabsorption by the kidney tubule was inhibited by lowering the potassium concentration of the

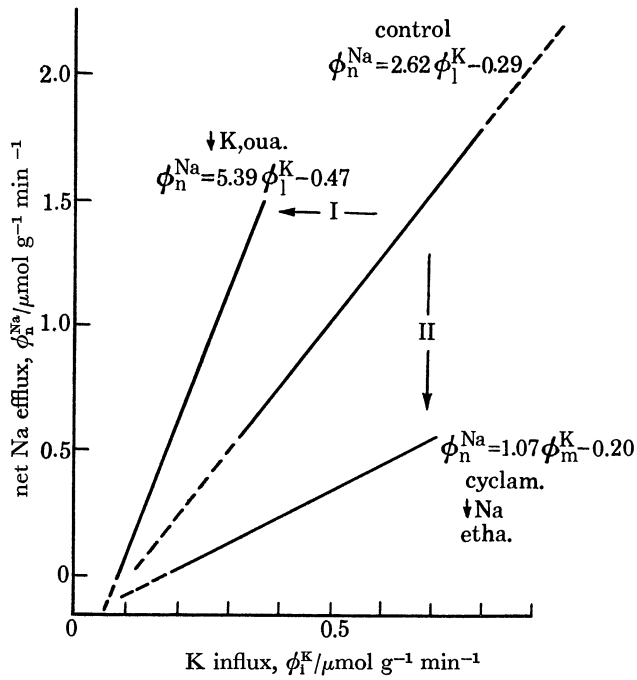

Figure 16. Relationship between net transepithelial sodium reabsorption and peritubular potassium uptake. The coupling ratio of 2.62 between the fluxes, observed in the controls can be changed to one of 5.39 with low K in the perfusion fluid or by addition of ouabain, or to one of 1.07 with perfusion fluids containing cyclamate instead of Cl and low Na or by addition of ethacrynic acid.

perfusion fluid. Concomitantly cell sodium rose and cell potassium diminished. (2) Ouabain inhibited net sodium reabsorption. Cells lost potassium. However, their sodium content remained well maintained, indicating continued sodium extrusion by another mechanism. (3) Ethacrynic acid also inhibited net sodium reabsorption. The cells gained sodium and chloride. However, their potassium content remained normal. Again, it is apparent that some other mechanism effecting potassium uptake was still working. Whittembury & Fishman's (1969) experiments support the dual pump hypothesis. Further, they indicate that not only in kidney slices, but also in the presence net sodium reabsorption are both pumps active and participate in net sodium transport across the epithelial wall of kidney tubules.

Recently, additional studies relating to this problem have been carried out (Whittembury, Sullivan & Giebisch 1970). Use was made of the doubly perfused *Necturus* kidney. We analysed the relationship between net sodium reabsorption across the tubular epithelium and the influx of potassium across the peritubular membrane of tubule cells. Figure 16 is a plot of net sodium

reabsorption as a function of the influx of potassium across the peritubular cell boundary The regression line in the middle corresponds to the spontaneous variation of sodium transport in controls. The regression line indicates that about 2.6 sodium ions are reabsorbed across the tubule per potassium ion that is taken back into the cell. Changes in sodium absorption are accompanied by parallel changes in potassium influx, keeping a ratio of 2.6. This could be due to the work of a single pump. However, the important results of these experiments are that we were able to modify this relationship in two different directions: (1) Potassium uptake was

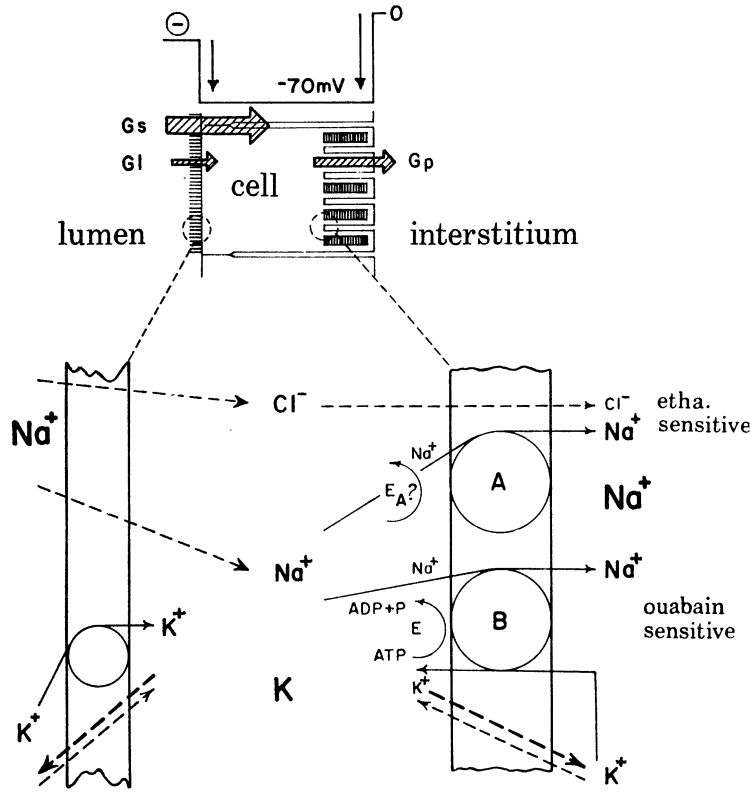

FIGURE 17. Schematic summary of some transport properties of renal tubule cells. The diagram includes properties common to proximal and distal cells. The general observation that cell sodium concentration is lower, and potassium concentration higher, than that of the surrounding fluid is shown by the size of the corresponding symbols. The electrical potential profile is shown at the top. The lumen is shown negative, its negativity increasing from proximal to early distal and to late distal convoluted tubules. The large well of cell negativity is also indicated. Sodium would enter the cell across the luminal membrane, down the electrochemical potential difference. It would be expelled from the cell out of a common cell pool, towards the peritubular space, (1) either by pump A, an electrogenic pump, that would drive chloride secondarily, or (2) by pump B, related to the sodium-potassium stimulated ATPase, that would exchange sodium for potassium. Potassium distribution across the luminal wall requires an active transport step (lumen towards cell) shown in the figure. Potassium enters the cells from the peritubular space driven by pump B, that would regulate cell potassium concentration. The latter should play a key role in potassium secretion by determining the amount of potassium that leaks into the tubular lumen. The observation that the proximal transtubular conductance is much bigger than the conductances of the luminal and peritubular cell membranes in series is shown at the top by appropriate arrows and stresses the importance of intercellular transport pathways. This intercellular pathway is probably much smaller or absent in the distal tubule.

markedly inhibited with only a small diminution of sodium reabsorption by diminishing the perfusate potassium concentration, or by adding ouabain. The sodium to potassium flux ratio rose to 5, which would indicate continued activity of pump A. (2) In contrast, sodium reabsorption could be inhibited with at best only a moderate inhibition of potassium uptake by using

the poorly permeant anion cyclamate instead of chloride in the perfusion fluid, or by using ethacrynic acid. The ratio between sodium and potassium fluxes then declined to values of about 1. Under these conditions it seems that the exchange pump predominates (Whittembury *et al.* 1970). These results are again best explained by two types of sodium extrusion mechanisms working in parallel.

Figure 17 schematically summarizes some relevant aspects of renal tubular electrolyte transport. The cell model incorporates pertinent properties of the luminal and peritubular cell membranes as well as the intercellular shunt path. Although there may be quantitative differences in cells of different tubule segments, some of the features shown are common to all tubule cells. Sodium ions enter the tubule cell from the lumen by movement down an electrochemical potential gradient. Evidence is also available that active potassium uptake lowers the luminal potassium concentration below that to be expected from passive distribution.

Sodium ions are expelled from kidney cells across the peritubular membrane by two pumps. Pump A is an electrogenic pump which extrudes sodium accompanied by chloride. It may be most important in cell volume regulation. Chloride ions are passively driven out of the cell by the electrical potential difference generated by the active sodium movement. Pump A and its energy source seem independent of the operation of the sodium–potassium stimulated ATPase. Pump B is an exchange mechanism, whereby sodium is interchanged with extracellular potassium. The energy responsible for its activity appears to come from the hydrolysis of ATP through the mediation of sodium–potassium stimulated ATPase. Both pumps seem to expel sodium out of a cell pool that may be common to both. This would account for the fact that both pumps are involved in net sodium reabsorption. Additional experiments are required to assess the relative role of each pump in net sodium reabsorption under a variety of conditions, and to locate them along the nephron.

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