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SOLUTE TRANSPORT ACROSS EPITHELIA: WHAT CAN WE LEARN FROM MICROPUNCTURE STUDIES ON KIDNEY TUBULES?

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INTRODUCTION

Epithelial transport covers an enormously wide field of research on tissues such as skin, intestine, salivary or sweat glands and kidney tubules which, on first view, seem to have little in common. However, despite the vast number of transport functions which these tissues perform, it appears that all operate on a relatively small number of general principles and it is my intention to describe some of those principles which we can discern. I will do so not by screening the literature for comparative aspects but by focussing mainly on one single epithelium, the rat kidney proximal tubule, and probing further and further into its properties.

Our interest in this epithelium was twofold: (1) we knew that the proximal tubule plays a paramount role in the absorption of the glomerular filtrate and hence in the maintenance of the water and electrolyte balance of the body – in man the proximal tubules absorb approximately 140 l. of tubular urine per day – and (2) we have found that, with respect to its transport functions, renal proximal tubule may serve as an ideal model tissue for a group of epithelia (comprising among others, small intestine, gall-bladder, and choroid plexus), as well as possibly some endothelia, to which the well known frog skin model of transepithelial transport cannot be applied. These epithelia we have classified (Frömter & Diamond, 1972) as 'leaky epithelia' in contrast to the frog skin type 'tight epithelia' which have different transport properties and serve different functions in the body. I will come back to the distinction between tight and leaky epithelia below.

A considerable disadvantage of the kidney tubules in transport studies is their small size. Rat proximal tubule has an outer diameter of $\sim 45\ \mu m$ and a lumen diameter of only $\sim 20\ \mu m$ (compare Fig. 1). The wall is formed of one layer of uniform cuboidal cells, with nuclei, vacuoles and a dense packing of mitochondria. The luminal cell membrane surface (brush border) and the basal cell membrane surface (basal labyrinth) are greatly amplified by microvilli or basal infoldings respectively. The gaps between neighbouring cells (lateral spaces) are closed near the luminal end by terminal bars (so-called tight junctions; see Fig. 9 below).

To study solute and water transport across such tiny structures as renal tubules requires appropriate micropuncture and microanalytical techniques. Such techniques were initially developed between 1920 and 1930 for work with the bigger tubules of frog and *Necturus* kidney (Richards, 1929) and since then have been more and more

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refined (Windhager, 1968; Ullrich, Frömter & Baumann, 1969; Giebisch, Boulpaep & Wright, 1972). The early micropuncture studies in kidney were mostly concerned with mapping of concentration profiles of individual substances along the nephron, thus investigating the question of *where* individual substances, after filtration in the glomerulum, are reabsorbed or secreted (in the proximal or distal tubule, in the loop of Henle or in the collecting duct). However, to study the question of *how* the transport mechanisms operate required more sophisticated techniques. Two different techniques were developed: one in which individual tubules are teased apart and perfused *in vitro* (Burg, Grantham, Abramow & Orloff, 1966); and another in which tubules are functionally isolated *in vivo* by appropriate luminal and peritubular

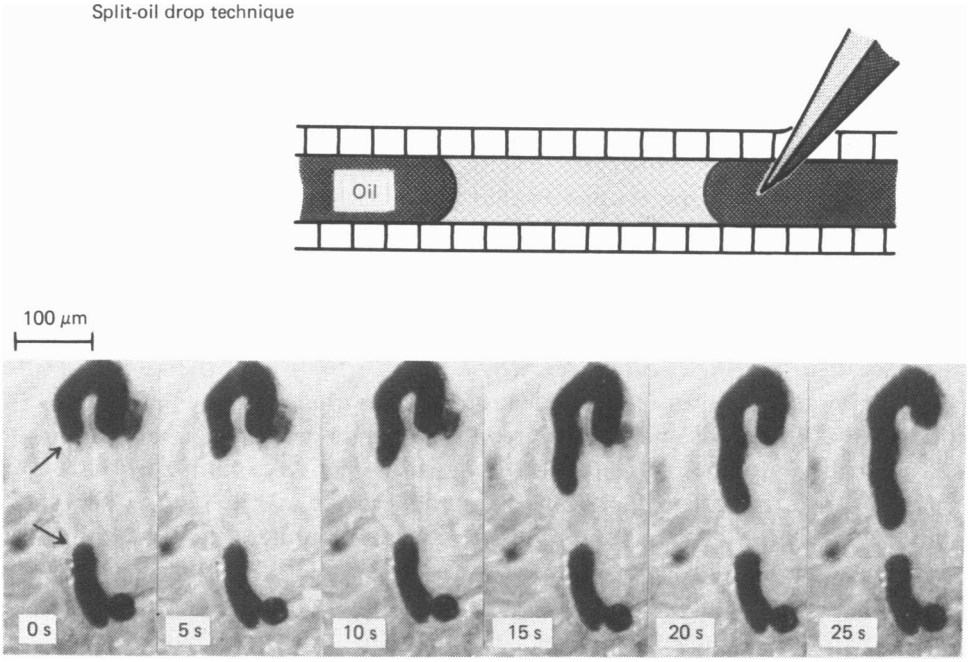

Fig. 1. Split oil-drop technique for determination of volume absorption from rat proximal tubule (Gertz, 1962). Upper panel: schematic view; lower panel: microphotographic pictures of the kidney surface during a split oil-drop experiment taken at 5 sec intervals as indicated on the bottom. The magnification is × 100. See calibration mark in upper left corner (courtesy of Ullrich, K. J. & Rumrich, G.).

perfusion techniques (perfusion of peritubular capillaries, Frömter, Müller & Knauf, 1969; Windhager, Lewy & Spitzer, 1969). Fig. 1 shows, as an example, the technique which we use to measure the rate of volume absorption from individual tubular loops. In an anaesthetized rat the kidney surface is exposed and a surface loop of the proximal convolution is punctured with a double-barrelled micropipette, the tip of which was cut at an outer diameter of 10 µm and was sharpened on a special grinder. By injecting a droplet of coloured castor oil from one barrel we fill the lumen of the loop with oil. Then a droplet of test fluid is injected from the other barrel of the pipette into the tubular lumen so that it splits the oil column and is isolated at either end from the tubular urine by oil. As the test fluid is progressively absorbed the droplet shrinks and the oil menisci approach each other. This process

is monitored by serial photographs (see Fig. 1, lower panel). Plotting the length of the fluid column versus time on semilogarithmic paper, one observes a straight line relationship (Fig. 2, open circles), from which the rate of fluid absorption can be worked out. This technique, which was initially devised by Gertz (1962), can now be extended by reaspirating the test fluid after given time intervals and analysing its composition to determine the fluxes of individual solutes. Moreover, it can be combined with perfusion of the peritubular capillaries with artificial solutions to determine the fluxes as function of the peritubular fluid composition; or can be supplemented by micro-electrode measurements to determine the electrical potential

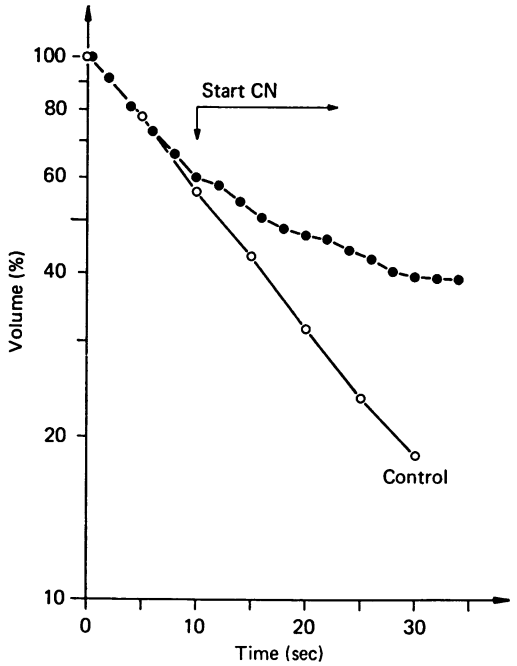

Fig. 2. Split oil-drop experiment. Abscissa: time in sec; ordinate: length of fluid column in per cent on logarithmic scale. Open circles: control data, filled circles: experiment in which the peritubular capillaries were perfused with cyanide Ringer solution as indicated (according to Györy & Kinne, 1971).

difference between lumen and interstitium or the electrical resistance of the tubular epithelium, the latter being achieved with the help of cable analysis. Generally speaking, we can say that these micropuncture and microperfusion techniques allow us to determine the fluxes and the respective driving forces for solutes and for water with considerable accuracy.

BLACK-BOX DESCRIPTION OF SOLUTE TRANSPORT ACROSS PROXIMAL TUBULAR EPITHELIUM

The first question which we want to consider is: which substances does the tubule transport and which forces drive the flows. Using the technique illustrated in Fig. 2, we can show that the tubule is able to absorb isotonic NaCl solution. Further inspection of this Figure reveals that the fluid absorption from the tubular lumen requires

metabolic energy. This is demonstrated by the curve with the black dots in Fig. 2 in which, following a period of normal absorption, the tubule was suddenly poisoned by perfusing the peritubular capillaries with Na^+ cyanide containing Ringer solution (Györy & Kinne, 1971). It can be seen that the rate of shrinking of the test droplet declines and that approximately 20 sec later the absorption comes virtually to a standstill. This experiment very clearly indicates that the absorption of fluid from the tubular lumen involves work performed by the tubular cells at the expense of metabolic energy, with hydrodynamic forces (hydrostatic or oncotic pressure differences between tubular lumen and interstitium) contributing only little, if anything, to the absorption process.

We are thus facing such problems as: how to describe metabolically driven fluxes properly, how to recognize them and distinguish them from other fluxes and how, eventually, to explain their physical nature. The only theoretical approach which is able to help us in this situation is the phenomenologic theory of membrane transport, based on irreversible thermodynamics (Kedem, 1961; Katchalsky & Curran, 1967; Sauer, 1973). Without going into details here (which would require an extra lecture series) I may briefly mention that the application of the phenomenologic approach essentially consists of establishing the relations between the observable net fluxes of solutes and water across a membrane and all external driving forces, such as chemical and/or electrical potential differences, and hydrostatic or osmotic pressure differences. All fluxes which can be accounted for by these external passive driving forces we define as passive; whereas all fluxes which are not driven by these forces (which means, for example, that they persist if all of the mentioned forces are nullified) are named active fluxes. These active fluxes are driven by metabolic energy, their conjugate driving force being the affinity of a chemical reaction, which proceeds inside the membrane. Our first task in analysing the transport properties of proximal tubular epithelium is, therefore, to determine which components undergo active transport and which are transported only passively. During this step of the analysis the epithelium is treated like a 'black box', of which only the input–output relations are determined. To arrive at a physical interpretation of individual transport mechanisms requires information from inside the 'black box', and such information will be discussed later.

Before presenting our data let us briefly look at other epithelia. The most intensively studied epithelium is frog skin which, like the kidney tubules, is capable of absorbing NaCl and water at the expense of metabolic energy. By 1951, it was shown by Ussing & Zehran that the frog skin transports only the Na^+ ions actively, chloride transport being passive. Since Ussing's technique of short-circuiting the skin and measuring the unidirectional fluxes with tracers was not applicable in rat tubules we had to devise alternative methods to determine the active and/or passive solute fluxes.

Qualitative analysis of active ion fluxes

In a first qualitative approach, we have measured the transepithelial potential difference in symmetrical Ringer solution. If one bathes frog skin on both surfaces with identical Ringer solution, avoids all pressure gradients and does not pass current across it, one finds that it generates a spontaneous transepithelial electrical

potential difference of about 50–100 mV, outside negative, blood side positive. This potential difference arises from the charge that is carried along with the actively transported Na^+ ions, as described by the following equation (Frömter, 1974):

$$\Delta\psi^{act} = -\frac{1}{g}\sum_{i=n}^{k} z_i F J_i^{act}, \tag{1}$$

where g is the membrane conductance, z_i and F are valence and Faraday number and J_i^{act} is the rate of active transport of ion i. $\Delta\psi^{act}$ will be named active transport potential. Using the technique of Fig. 3 to perfuse tubular lumen and peritubular capillaries with identical HCO_3^- Ringer solutions, we have in the first approach

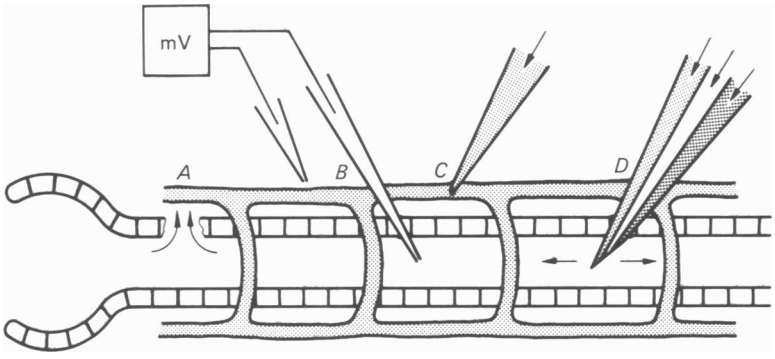

Fig. 3. Microperfusion technique for electrical potential measurements with complete replacement of luminal and peritubular fluid. *A*, drainage of glomerular filtrate. *B*, micro-electrodes, one remaining in the peritubular space, the other being placed into the tubular lumen. *C*, pipette for perfusing the peritubular capillaries. *D*, triple-barrelled luminal perfusion pipette (Frömter & Gessner, 1974 *b*).

determined whether or not the proximal tubules behave like the frog skin and generate an active transport potential. The answer is yes. At least in early loops, up to 1 mm distant from the glomerulum, we find a significant active transport potential. However, this potential is much smaller (in early loops it averages $+1{\cdot}0 \pm 0{\cdot}4$ mV, in late loops $+0{\cdot}2 \pm 0{\cdot}3$ mV) than that of frog skin and its polarity is opposite: the lumen is positive, the blood side is negative (Frömter & Gessner, 1974*b*). Does such a small potential difference possess any physical significance and, if so, why is the polarity opposite to that of frog skin? The significance of the potential difference has been demonstrated in two ways: (1) knowing the conductance of the tubular wall ($0{\cdot}2\ \Omega^{-1}\,cm^{-2}$, Hegel, Frömter & Wick, 1967) one can readily calculate that an active transport potential of ~ 1 mV reflects a four times larger active ion current than the much higher potential difference across frog skin and (2) when poisoning metabolism with cyanide, we find that the potential difference drops towards zero, as an active transport potential should, and even reappears if the cyanide solution is rapidly removed (Fig. 4).

The polarity of the active transport potential, on the other hand, indicates that in contrast to frog skin we are not dealing with active cation (say Na^+) absorption but with active anion absorption; or more exactly that *active anion absorption exceeds active cation absorption* (see eqn. (1)). This conclusion holds at least for the early part

of the proximal convoluted tubule, for which the significance of the small transport potential was established beyond doubt.

What are the actively transported anions and does the tubule transport cations (i.e. Na^+) actively or not? To distinguish between active Cl^- and active HCO_3^- absorption we did the following experiments. (1) We studied the effect of acetazolamide on the active transport potential. Inhibitors of carbonic anhydrase such as

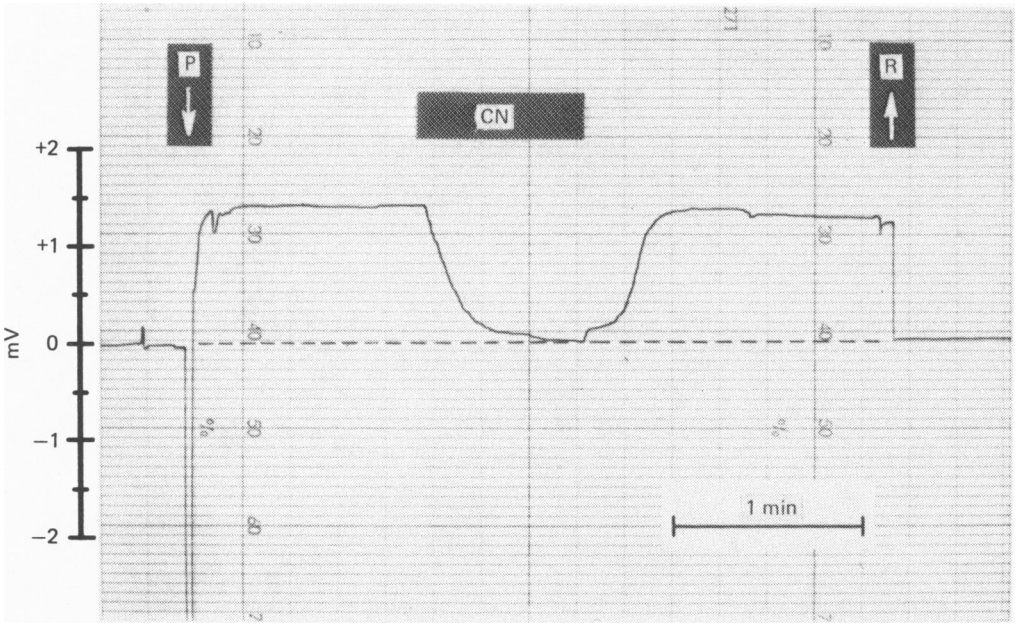

Fig. 4. Active transepithelial transport potential in early loop of rat proximal tubule. Effect of cyanide (CN). Trace record. Abscissa: time as indicated by bar; ordinate: potential difference in mV, upwards lumen positive. At P, the electrode was advanced into the tubular lumen and at R, it was retracted into the peritubular space. During the bar CN, the luminal perfusate was switched from HCO_3^- Ringer solution to a solution in which 7 m-mole/l. NaCl had been replaced by NaCN, but which was otherwise identical. Note that the active transport potential disappears reversibly, provided the exposure to cyanide is short (from Frömter & Gessner, 1974 *b*).

acetazolamide (Diamox^R) are known to reduce, quite specifically, proximal tubular bicarbonate absorption. As shown in Fig. 5 this drug reduced the active transport potential, which indicates a reduction of active ion current because the transepithelial conductance remained unchanged (Frömter, 1977). (2) We measured the active transport potential as a function of the ambient bicarbonate concentrations. In the absence of bicarbonate the active transport potential fell to 22 % of its control value and increasing HCO_3^- above normal increased the active transport potential (Frömter & Sato, 1976). These two experiments clearly suggest that the active transport potential is associated with the absorption of bicarbonate buffer. A third argument supporting this conclusion derived from the observation that early loops, which have a higher transport potential, also transport buffer more rapidly (Frömter & Gessner, 1974*a*; Ullrich, Rumrich & Baumann, 1975). The question of whether or not the tubule is capable of transporting also Na^+ ions actively could be answered by

stimulating Na^+ transport through addition of glucose and by applying ouabain, a specific inhibitor of active Na^+ transport. Such an experiment is shown in Fig. 6. During luminal perfusion with glucose containing Ringer solution the potential of the tubular lumen changes its polarity to lumen negative, which indicates according to eqn. (1) that active cation (i.e. Na^+) absorption now exceeds active bicarbonate buffer absorption. Furthermore, we notice that this effect is reduced during application of ouabain. Note also that the lumen-positive active transport potential does not collapse in the presence of ouabain, which indicates that the electrogenic part of active HCO_3^- absorption is independent of active Na^+ absorption (for details see Frömter & Gessner, 1975).

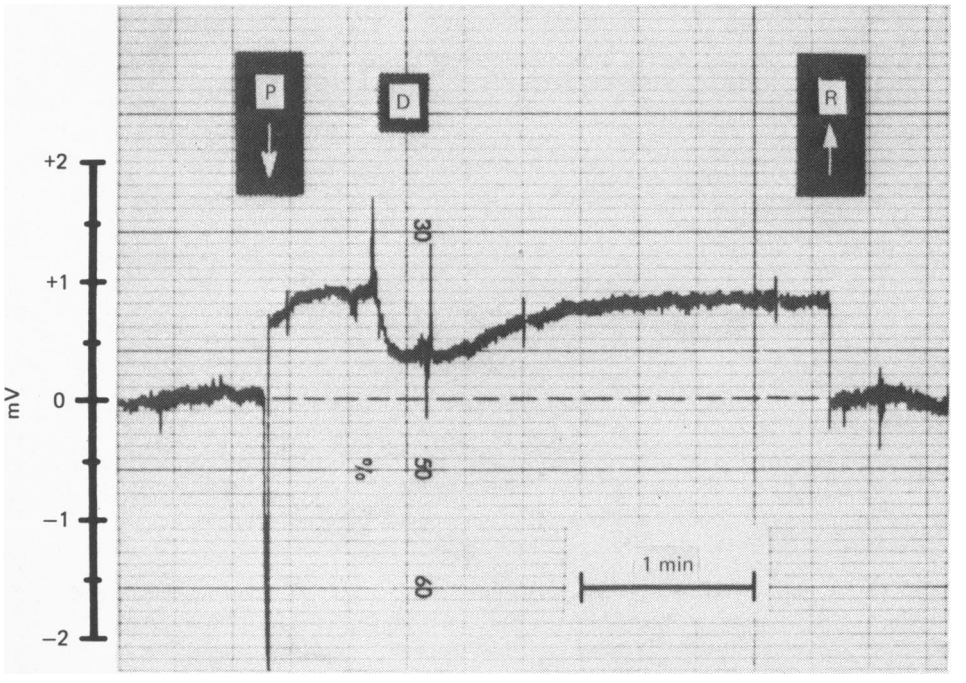

Fig. 5. Effect of acetazolamide on active transport potential. All details as in Fig. 4, except that during mark D the peritubular superfusion was changed from HCO_3^- Ringer solution to the same solution but containing in addition 10^{-3} mole/l. acetazolamide (Diamox^R). Note that the active transport potential declines but recovers when the acetazolamide is washed away (from Frömter & Sato, 1976).

Quantitative analysis of active and passive solute fluxes

Thus far I have presented evidence, admittedly indirect, from simple potential measurements which indicated (1) that the proximal tubular epithelium is capable of transporting bicarbonate buffer and Na^+ ions actively and (2) that both processes are to some extent independent of each other. More direct evidence which agreed with these results was obtained in a quantitative analysis of Na^+, Cl^- and HCO_3^- transport by rat proximal tubules which we undertook some years ago in collaboration with Ullrich and Sauer (Frömter, Rumrich & Ullrich, 1973). Neglecting ion–ion interaction, the net flux (J_i) of an ion (i) across a membrane can be described near

equilibrium by the following equation, which can be derived by application of the principles of irreversible thermodynamics (Sauer, 1973):

$$J_i = (1-\sigma_i)\bar{c}_i J_v + P_i\left(\Delta c_i + \bar{c}_i \frac{RT}{z_i F}\Delta\psi\right) + J_i^{act} \quad (i = 1 \ldots k). \tag{2}$$

In this equation σ_i and P_i are reflexion coefficient and permeability of ion i, J_v is the volume flow, Δc_i is the concentration difference of i between the external solutions, $\bar{c}_i$ is the mean (arithmetic) concentration of i in the external solutions, $\Delta\psi$ is the electrical potential difference measured with saturated KCl bridges, and R, T,

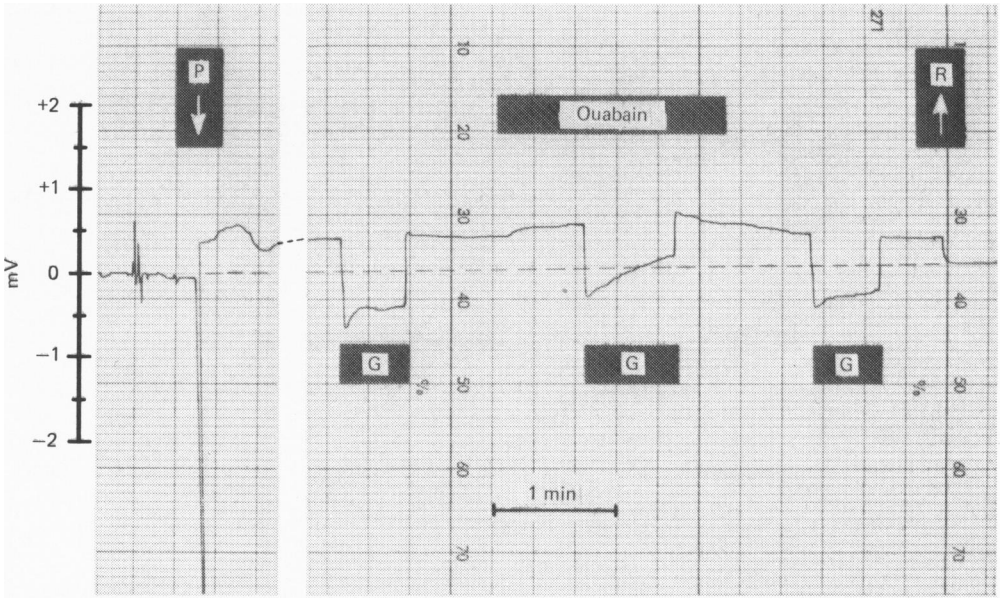

Fig. 6. Effect of ouabain on active transport potential. Details as in Fig. 4, except that during the marks G the luminal perfusate was changed from HCO_3^- Ringer solution to the same solution but containing in addition 5 m-mole/l. of D-glucose, which shifted the transport potential into lumen-negative direction (increase of active Na^+ absorption). During the mark ouabain, 18 m-mole/l. of ouabain was applied in the peritubular superfusion. Interference from streaming potentials was eliminated by appropriate additions of raffinose to the control solutions. Note that the lumen-positive transport potential does not collapse in the presence of ouabain although the glucose-induced additional active Na^+ transport is impaired (for further details see Frömter & Gessner, 1975).

z_i and F have their usual meaning. If we now do a split drop experiment as described in Figs. 1 and 2, but add some non-penetrating non-electrolyte to the luminal test fluid, the droplet will first shrink as in Figs. 1 and 2, but will then remain stationary, although in contra-distinction to Fig. 2 (filled circles) the metabolism is unimpaired. The development of this 'zero-net flux situation', which is sometimes also referred to as 'static head situation', can be explained as follows: during the initial reabsorption of some salt and water, the non-penetrating solutes are concentrated inside the lumen until they exert an osmotic effect which tends to keep water back in the lumen. Since the salt pumps continue to work, but the water cannot follow, the intratubular

salt concentration must decline until eventually salt starts to flow back from the peritubular space into the lumen; and zero net flux will be attained, when the passive backflux of salt equals the rate of active salt transport in the outward direction.

This situation does not develop normally in the kidney because the water permeability is extremely high. If salt is absorbed in the absence of non-permeable solutes from the lumen, the development of minute degrees of luminal hypotonicity would immediately result in water efflux so that the luminal salt concentration cannot be effectively lowered below that in the peritubular space.

In this situation, with J_i and J_v being zero, we obtain from eqn. (2)

$$J_i^{act} = -P_i\left(\Delta c_i + \bar{c}_i \frac{RT}{z_i F}\Delta\psi\right) \quad (i = 1 \ldots k). \tag{3}$$

Using this equation we can thus determine the rates of active transport of each ion if we know the peritubular fluid composition and measure (1) the transepithelial concentration differences Δc_i which build up in the zero net flux situation, (2) the transepithelial electrical potential difference and (3) in the same condition, the passive ion permeabilities. Determination of the latter is possible by tracerflux measurements or by calculation from electrical measurements (transepithelial conductance and transference numbers).

This approach may not be applicable in all epithelia, particularly not in tight epithelia (see p. 14) because if the passive permeabilities are low, the attainment of the zero net flux situation may be associated with the development of steep transepithelial electrochemical potential gradients. In this situation, the near equilibrium condition required for the applicability of eqns. (2) and (3) may be violated: as, for example, if the rate of active transport at zero net flux differs from that in symmetrical Ringer solution. Furthermore, in both equations we assume that ion–ion interaction is negligible in the passive permeation pathway; this is justified in rat proximal tubule, as evidenced by the near-equality of tracer permeabilities and permeabilities calculated from electrical measurements (see Frömter *et al.* 1973), but need not hold in all epithelia.

TABLE 1. Active (J_i^{act}) and passive (J_i^{pass}) components of net Na^+, Cl^- and HCO_3^- absorption (J_i^{net}) from shrinking droplets in rat proximal tubule

	J_i^{act}	J_i^{net}	J_i^{pass}
Na^+	1·5	5·2	3·7
Cl^-	0·3	4·3	4·0
HCO_3^-	2·1	0·9	−1·2

All fluxes in 10^{-11} mole/sec.cm (length of tubule). Data from Frömter *et al.* 1973.

In column 1 of Table 1 are depicted the active transport rates for Na^+, Cl^- and HCO_3^- which we have determined from the data as analysed by eqn. (3). In agreement with the results from the study of active transport potentials, we find that Na^+ ions and HCO_3^- buffer are actively absorbed from the tubular lumen, while Cl^- ions do not seem to undergo active transport (the calculated active transport rate is so small that its significance cannot be assessed from the data). Furthermore, I have depicted in column 2 of Table 1 the steady-state net fluxes of Na^+, Cl^- and HCO_3^- which are observed in freely shrinking droplets formed of the same HCO_3^- Ringer solution but without addition of nonpenetrating electrolytes to the perfusion fluids. Comparing columns 1 and 2 we can conclude that (1) of the net Na^+ ion absorption from a freely shrinking droplet, only approximately one third is transported actively, the remaining

two thirds following passively (partially driven by a small potential difference of $\sim 1{\cdot}8$ mV lumen positive and partially driven by solvent drag, both driving forces developing in response to active HCO_3^- absorption; for details see Frömter *et al.* 1973). Note that this result contrasts with the situation in frog skin where, in isotonic Ringer solution, all net Na^+ absorption is active; (2) most, if not all, chloride absorption from freely shrinking droplets is passive (following either a concentration difference that develops in response to active HCO_3^- transport or being driven by water flow: for details refer to Frömter *et al.* 1973); (3) active HCO_3^- transport exceeds the rate of net bicarbonate transport in a freely shrinking droplet which indicates that HCO_3^- buffer is continuously leaking back passively.

TABLE 2. Actively and passively transported solutes as determined in stop flow experiments on rat proximal tubule by Ullrich and coworkers (Ullrich, 1973, 1976)

Active	Passive	Undetermined
Na^+	Cl^-	K^+
H^+/HCO_3^-	Urea	
Ca^{2+}		
$HPO_4^{2-}/H_2PO_4^-$		
D-glucose		
L-amino acids		
PAH		

In order to work under clearly definable conditions, the data of Table 1 were obtained during perfusion of the peritubular capillaries with artificial bicarbonate Ringer solution. It is possible, therefore, that these data are not completely representative of the tubular absorption in the normal kidney during free flow. However, the fact that the free flow transepithelial concentration and potential differences (Litchfield & Bott, 1962; Rector, Carter & Seldin, 1965; Vieira & Malnic, 1968; Barratt, Rector, Kokko & Seldin, 1974; Frömter & Gessner, 1974*a*; Seely & Chirito, 1975) resemble closely those which were observed in the split drop experiments of column 2 suggests that any possible differences are of minor importance. It may also be mentioned that the main conclusions concerning active Na^+ and bicarbonate absorption have been confirmed recently in other laboratories (Green & Giebisch, 1975; Neumann & Rector, 1976).

By extending the above approach, the presence of active transport has been documented for a great number of different substances including other ions as well as D-glucose and probably all naturally occurring L-amino acids (see Table 2). Since the active transport rate of almost all of these substances declined strongly when the ambient Na^+ concentration was reduced, it seemed likely that the active transport of most, if not all, of these substances was driven by local passive coupling to the active Na^+ flux (secondary active transport as defined in Frömter, 1978). I shall discuss this question in detail below.

LOCALIZATION OF ACTIVE AND PASSIVE TRANSPORT STEPS AND DISCUSSION OF POSSIBLE TRANSPORT MECHANISMS

To obtain information on transport properties of individual membranes in a composite system, one can either try to disrupt the system and study the properties of the individual elements separately (this has become possible recently through the development of appropriate separation and analysis techniques: investigation of membrane vesicles, Busse & Steinmaier, 1974; Aronson & Sacktor, 1974; Kinne, Murer, Kinne-Saffran, Thees & Sachs, 1975); or one can leave the system intact and try to obtain information from inside the cell by microscopic techniques. We have

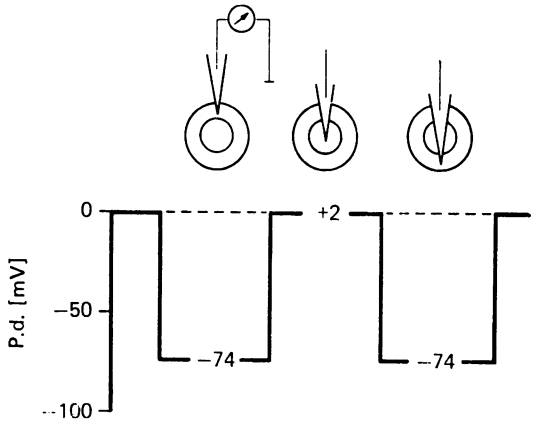

Fig. 7. Electrical potential profile across rat proximal tubule under free flow conditions (schematic). Abscissa: electrode advance as indicated diagrammatically in top; ordinate: potential difference in mV. Before entering the lumen the micro-electrode records the cell membrane potential, which is ~ 74 mV negative with respect to interstitium and ~ 76 mV negative with respect to the tubular lumen because the tubular lumen is ~ 2 mV positive under free flow conditions (intermediate and late proximal tubular loops). Data from Frömter *et al.* (1971) and Frömter & Gessner (1974 *a*).

followed the latter approach, using micro-electrodes to study the intracellular potential level and analysing cell potential changes in response to transepithelial currents or to changes of luminal or peritubular solution compositions. As depicted schematically in Fig. 7, when puncturing the tubules it is possible to insert a micro-electrode first into a tubular cell before it enters into the tubular lumen. Thus we can measure the cell membrane potentials. In good experiments, we find potentials of between 65 and 85 mV with mean values of about 74 mV, cytoplasm negative. With some skill and a lot of patience, it is possible to keep those potential measurements constant for up to 5 min or more, while the luminal or peritubular perfusate solutions are changed so that the effect of different ions or nonelectrolytes on the membrane potential can be investigated. From such experiments we are able (1) to deduce information on the partial ionic conductances (or respectively permeabilities) of the cell membranes (and of the paracellular shunt, see below), (2) to localize flux coupling mechanisms for Na^+ ions and various actively transported solutes and (3) to decide on the location of the active ion pumps.

The paracellular shunt path

Without going into details of the underlying electrical analysis here (published in preliminary form; Frömter, 1977) I want to present in Fig. 8 a major conclusion from our experiments designed to localize and quantify the resistance barriers of the proximal tubular epithelium for passive ion flow. Fig. 8 shows that the luminal cell membrane has a specific resistance of approximately 260 Ω cm^2 (all figures related to 1 cm^2 of tubular surface, rather than to the actual cell membrane area) while the peritubular cell membrane has a resistance of approximately 90 Ω cm^2. Furthermore, it shows that in parallel with the cell membranes, there exists a paracellular shunt path with a resistance of approximately 5 Ω cm^2 and hence about two orders of magnitude lower than the lumped resistances of the cell membranes.

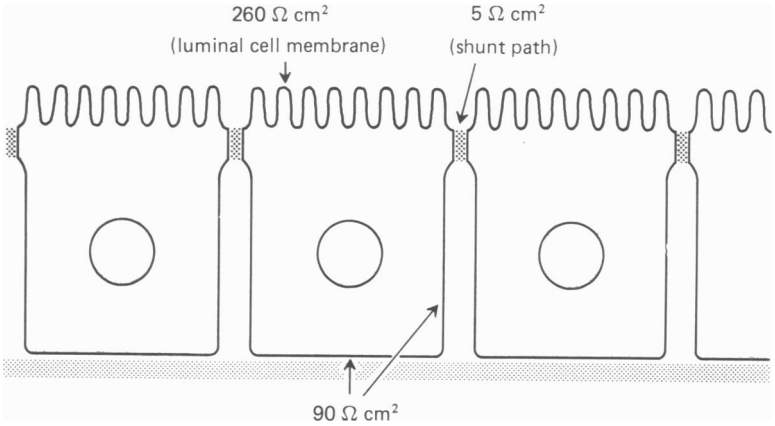

Fig. 8. Magnitude and distribution of resistance barriers in rat proximal tubular epithelium. All dimensions refer to 1 cm^2 of epithelial surface, not to the actual surface of the cell membranes or junctions (data of Frömter, 1977).

The finding of a low conductance shunt path across the epithelium raises a number of most interesting questions, such as: (1) where is the shunt located and does it represent a specific histological, structural element of the epithelium or a specific cell type; (2) what are the transport properties of the shunt path, are we dealing with a free solution shunt or does the shunt path possess specific transport mechanisms; (3) is the shunt unique for proximal tubular epithelia, or is it found in all epithelia; and (4) what is the physiological significance of a shunt (or what is the sense in having a big leak path in parallel with a pump system)?

With regard to the localization of the shunt path, we can infer from voltage scanning experiments on Necturus gall-bladder (Frömter, 1972), as well as from electronmicroscopic studies using La^{3+} as tracer ions (Tisher & Yarger, 1973) that the shunt path is situated in the terminal bars (or so-called 'tight junctions') by which neighbouring cells are attached to each other at their luminal end. It would appear, therefore, that the seal between the apposing cell membranes in the junction is not very tight so that small ions can leak from the lumen to the peritubular space without passing the cell membranes. A freeze-fracture cross-section of the terminal bars in rat proximal tubule is depicted in Fig. 9. Using normal transmission electron-

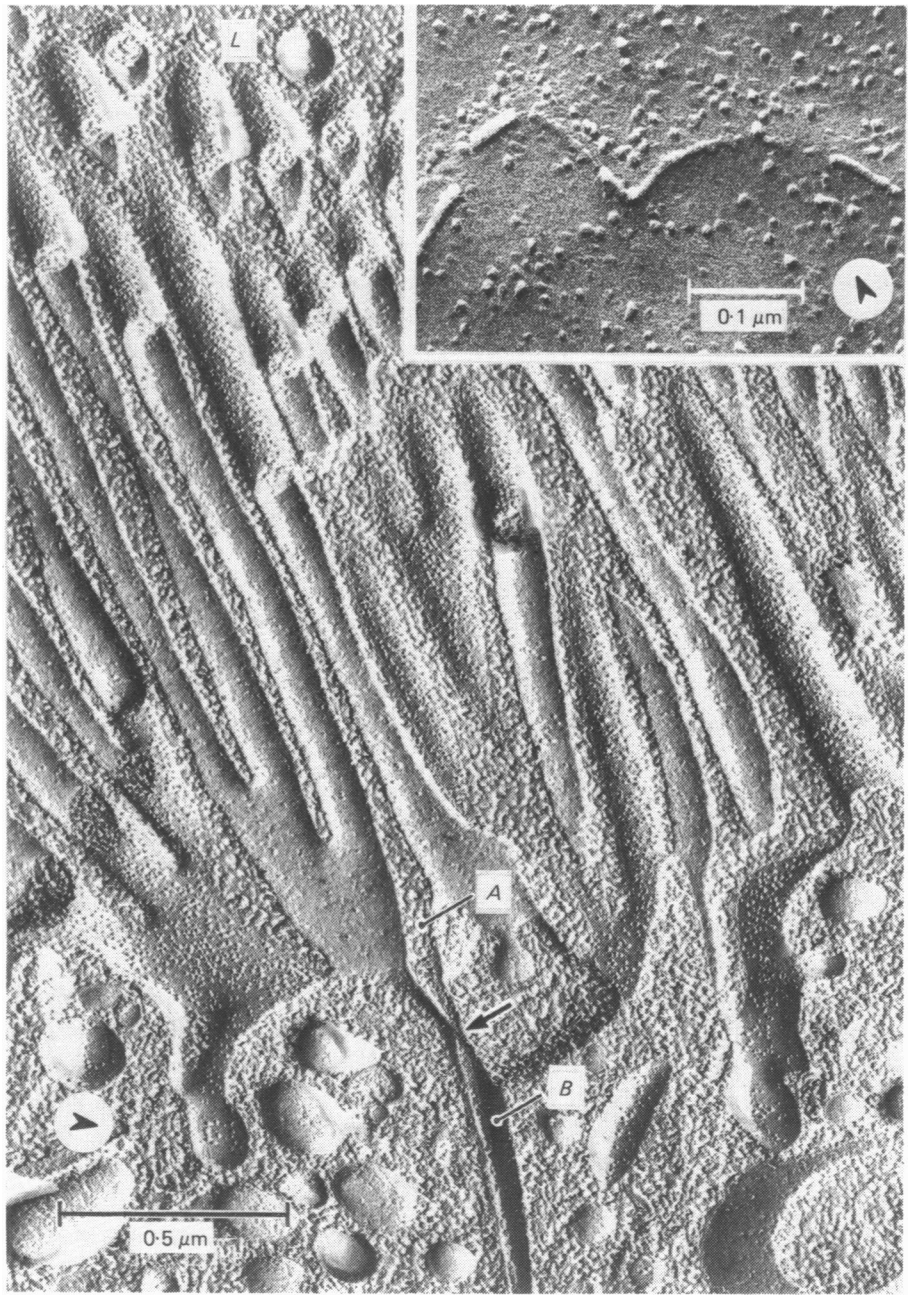

Fig. 9. Freeze-fracture picture of the terminal bar region of rat kidney proximal tubular cells. Depicted is the apical portion of two neighbouring cells which are attached to each other in the extremely shallow 'tight' junction (arrow). L, tubular lumen; A, luminal fluid compartment extending between the microvilli; B, lateral space providing free access to the peritubular surface. Magnification × 60 000. The inset shows the fracture face of the protoplasmic half membrane of a tubular cell in the region of the tight junction, which consists of a single ridge. Magnification × 150 000 (this figure was kindly provided by Dr W. Haase, Max-Planck-Institut für Biophysik, Frankfurt).

microscopy, most investigators (Farquhar & Palade, 1963; Tisher & Yarger, 1973) describe a very shallow zonula occludens within the terminal bar, in which the cell membranes of the neighbouring cells appear to 'fuse' over a length of approximately 20 nm. This observation contrasts with reports from other epithelia in which the zonula occludens appears to be much deeper (up to 200 nm) and in cross-section exhibits multiple points of fusion, corresponding to an attachment system of multiple ridges as seen in freeze fracture studies (Staehelin, Mukhergee & Williams, 1969). The question of whether the membrane fusion is complete, or whether there remain small waterfilled open slits between the hydrophobic leaflets of the apposing cell membranes, cannot be answered with assurance l y the present day electron-microscopic techniques; however, functional studies (Maude, 1970; Frömter, Müller & Wick, 1971; Boulpaep & Seely, 1971) indicate that the ion passage along the paracellular pathway resembles diffusion in aqueous solution (without being identical with it) rather than permeation across lipid bilayers or cell membranes in general.

Some years ago, we investigated the overall permeation properties of the tubular epithelium using electrophysiological techniques, i.e. measuring transepithelial membrane diffusion potentials and streaming potentials (Frömter *et al.* 1971). In view of the resistance distribution of Fig. 8, we can now interpret these data with confidence as representing almost exclusively the properties of the paracellular shunt path, in agreement with the tentative interpretation given previously. In Table 3, I have listed the main results of this study. It demonstrates that the permeation pathway across the tight junctions behaves like a simple, symmetric, passive membrane element which is not influenced by metabolic poisons. It has some properties of a fixed charge membrane (the surplus concentration of negative fixed charges is between 2 and 5 m-mole/l. of pore fluid) and exhibits some (albeit weak) ion membrane interaction, which suggests that it constitutes not simply a free solution shunt but imposes also small coulombic and/or geometric restraints on the migrating ions. Furthermore, there is no evidence for significant ion–ion interaction during the passage along the paracellular pathway.

The question of whether the terminal bars are leaky in all epithelia, or whether this feature is unique for proximal tubules, is closely related to the possible physiological significance of leaky junctions. Thus far we know that leaky junctions do also exist in renal proximal tubules of Newt and Necturus kidney (where the paracellular shunt was first discovered by Hoshi & Sakai (1967) and Windhager, Boulpaep & Giebisch (1967)); in gall-bladder (Frömter, 1972; Reuss & Finn, 1975); in small intestine (Frizzell & Schultz, 1972); and we suppose that they are present in the choroid plexus (Wright, 1972) and possibly also in endothelia such as corneal endothelium (Fischbarg & Lim, 1974). These tissues have in common that usually large quantities of isotonic fluid are transported between identical Ringer solutions with little selectivity; they all exhibit relatively low electrical resistances and show symmetrical potential responses to unilateral ion substitutions. On the other hand, there do exist epithelia in which the 'tight' junctions do indeed appear to be more or less tight. This has been demonstrated recently for amphibian and rabbit urinary bladder epithelia (Reuss & Finn, 1974; Lewis, Eaton & Diamond, 1976; Frömter & Gebler, 1977); and it may also be true for amphibian skin, salivary ducts and kidney collecting tubules. These 'tight' epithelia are characterized by their ability to build

up or maintain high electrochemical potential gradients for various ions, they have high transepithelial resistances of up to 70 kΩ cm², develop high active transport potentials in symmetrical Ringer solutions and exhibit an asymmetric potential response to unilateral ion substitutions. Comparing both classes of epithelia and the function that they fulfil in the body, it would appear therefore that leaky junctions were certainly detrimental for the function of a urinary bladder or a frog skin (since they would allow all concentration gradients to collapse) but that they are obviously advantageous for the function of isotonically transporting epithelia (in which no big gradients develop). In fact, by reducing the resistance to flow of passively transported

TABLE 3. Properties of the tight junctions in rat proximal tubule

I. Membrane–ion interaction: weak but present
Evidence:
(1) Permeability sequences: alkali cations follow seq. IV of Eisenman (1961); halide anions follow seq. III of Diamond & Wright (1969); bulky anions follow free solution sequence but the relative permeabilities decline more rapidly than the respective diffusion coefficients in free solution.
(2) Cation–anion discrimination: $t_{Na} > t_{Cl}$ (opposite to free solution behaviour) may partially arise from negative fixed charges (net concentration in the pore fluid: 5 m-mole/l.).
(3) Modifiers of membrane-ion interaction: Ca^{2+}, H^+, no direct effect of metabolic poisons.

II. Ion–ion interaction: weak or absent
Evidence:
No indication of coupled flows. Permeabilities calculated from tracer fluxes are nearly equal to those calculated from partial conductances (Frömter *et al.* 1973).

III. Ion–solute interaction: strong
Evidence:
Reflexion coefficients for Na^+ and Cl^- are significantly smaller than 1 (between 0·5 and 0·7) (Frömter *et al.* 1973).

ions (and other system components such as water) which must follow the actively transported ones, they can greatly facilitate the net flow of *salt* across a tissue. This view is in complete agreement with the data which we have presented above. In Table 1 we have shown that renal proximal tubule absorbs the major part of the Na^+ ions and practically all chloride passively and the data of Fig. 8 indicate that these passive fluxes proceed via the paracellular shunt (i.e. the leaky junctions in the terminal bars). It appears, therefore, that the leaky junctions facilitate – or 'amplify' (Diamond, 1974) – the net flow of salt across the tubular epithelium by allowing large passive ion fluxes to accompany the active ion transports. Since rat proximal tubule exhibits both active Na^+ and active HCO_3^- absorption, it is not surprising that we find two ions (Na^+ and Cl^-) to be passively transported along the junctions. In steadily shrinking droplets of HCO_3^- Ringer solution (as in the experiment of Figs. 1 and 2 or Table 1, column 2), the driving forces for the passive fluxes (which are eventually generated by the active transport processes) have been determined to be a transepithelial concentration difference and an effective osmotic concentration

(difference of reflexion coefficients between Cl^- and HCO_3^-) in the case of chloride; and a small electrical potential difference together with an effective osmotic concentration difference in the case of Na^+ ions (for details see Frömter *et al.* 1973; Frömter, 1974).

The cell membranes

While the passive transepithelial fluxes proceed along the tight junctions, the active fluxes must proceed through the cells. This means that two membranes must be passed in sequence and raises the possibility of an active transport step being located either in the luminal or in the peritubular cell membranes, or in both. In the following discussion of the active transport processes, we must therefore consider not only the possible transport mechanisms but first of all differentiate between luminal and peritubular location of the individual transport steps.

Active Na^+ transport

The localization of the active Na^+ transport step is rather straightforward. Knowing that the Na^+ concentration inside the cell is smaller than outside (Bauer, Bauer, Deneke & Thurau, 1977), the potential profile of Fig. 7 tells us that the Na^+ ions encounter a favourable electrochemical potential gradient at the brush-border membrane (which might drive Na^+ passively from the lumen into the cell) but an adverse electrochemical gradient at the peritubular cell membrane which must be overcome by means of active transport. Hence we conclude that a Na^+ pump must be located in the peritubular cell membrane. This conclusion is confirmed by biochemical studies in which a ouabain-inhibitable Na^+/K^+-stimulated ATPase was detected in microdissected basal portions of tubular cells (Schmidt & Dubach, 1971) and in purified peritubular cell membrane preparations (Heidrich, Kinne, Kinne-Saffran & Hannig, 1972). The properties of this pump are not yet very well defined; however, further electrophysiological observations indicate that we are dealing with a Na^+/K^+ exchange pump with an electrogenic transport component. It might thus resemble the pump in erythrocyte membranes which was found to transport in one cycle three Na^+ ions out of and two K^+ ions into the cell (Sen & Post, 1964). The presence of a Na^+/K^+ exchange pump mechanism is suggested by the biochemical characterization of the ATPase, as well as by the observation of a high K^+ permeability of the peritubular cell membrane in cell potential measurements (concentration step experiments with K^+ in the peritubular space; Frömter *et al.* 1971), which, as in nerve and muscle cell membranes, could provide the return path for recycling K^+ ions. The presence of an electrogenic transport component was concluded from the observation (Frömter & Gessner, 1975) of a rapid cell depolarization (half-time less than 2 sec) during rapid peritubular application of ouabain (sudden blockage of pump current). In addition the presence of a separate volume regulatory pump has been discussed by others (Whittembury, 1971).

The mechanisms by which the Na^+ ions enter the cell at the brush-border level will be discussed in greater detail later. It may suffice here to say that there exist various facilitated diffusion systems by which Na^+ ions are transferred in tight coupling with other solute species from lumen to cell. Concentration step experiments (Frömter, 1977) suggest that the brush-border permeability for Na^+ ions *per se* (without coupling to other solutes) is rather small.

Active transport of bicarbonate buffer

The localization of active and passive steps in the transport of HCO_3^- buffer across the cell is not as straightforward as in the case of Na^+, because the intracellular HCO_3^- concentration is not yet precisely known. A recent measurement with bicarbonate selective micro-electrodes indicates an intracellular HCO_3^- concentration of around 25 m-mole/l. (Khuri & Agulian, 1977). If this result is correct, it would appear that HCO_3^- buffer has to overcome an adverse electrochemical potential gradient at the luminal cell membrane but would be subject to a favourable gradient at the peritubular cell membrane. From this result, we would conclude that the buffer transport step from lumen to cell is active, while the transport step from cell to blood could be passive.

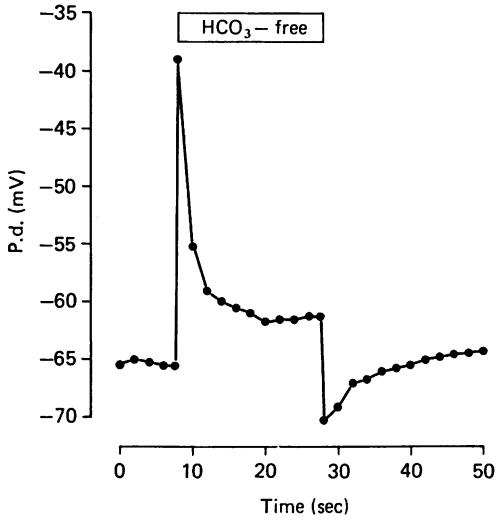

Fig. 10. Cell potential response to sudden removal of peritubular bicarbonate. Abscissa: time in sec; ordinate: cell potential in mV (note zero suppression). During the duration of the mark, HCO_3^--free, the peritubular capillaries were suddenly perfused with bicarbonate-free Ringer solution (from Frömter & Sato, 1976).

This view agrees with conclusions that we had reached earlier from concentration step experiments with bicarbonate buffer, demonstrating that the peritubular cell membrane (but not the luminal cell membrane) had a high passive permeability for bicarbonate buffer. A trace record of such an experiment is depicted in Fig. 10. In this experiment, the peritubular capillaries were suddenly perfused with bicarbonate-free Ringer solution (sudden reduction of peritubular bicarbonate concentration at constant pH from its control value of 25 m-mole/l. to zero by means of replacing HCO_3^- by Cl^-, with identical results being obtained upon replacing HCO_3^- by SO_4^{2-} or by $H_2PO_4^{2-}/HPO_4^{2-}$). It can be seen that removing peritubular bicarbonate (but not luminal bicarbonate) leads to a sudden strong depolarization which is followed by a slower exponential-like return of the potential difference to near control values, while readmission of bicarbonate produces an almost mirror image-like effect. These results, which resemble the potential changes observed by Hodgkin & Horowicz

(1959) upon replacing the permeable chloride by the poorly penetrating sulphate ions in the extracellular space of frog muscle cells, indicate that the peritubular cell membrane is far more permeable to HCO_3^- than to Cl^-. Indeed, plotting the initial potential change versus the log of HCO_3^- concentration (Fig. 11), we observe a slope of 28 mV per decade, which after approximate correction for the effect of the closed-loop equivalent circuit of proximal tubular epithelium (see Figs. 8 and 12) yields a transference number for HCO_3^- buffer of the order of 0·62. The slow secondary potential changes in Fig. 10 we interpret, in analogy to the experiments of Hodgkin & Horowicz (1959) as reflecting the attainment of new intracellular steady-state concentrations following the increased outflux into the bicarbonate-free extracellular space.

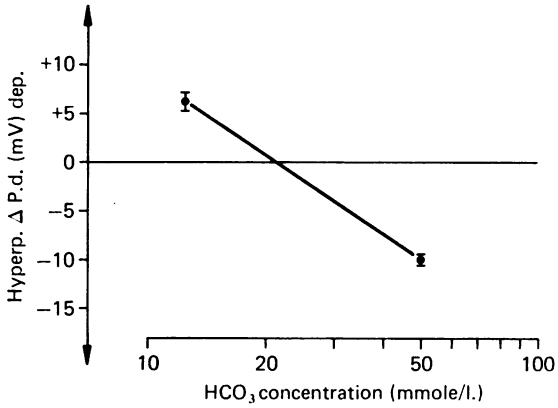

Fig. 11. Instantaneous cell potential deflexion as function of peritubular HCO_3^- concentration. Abscissa: HCO_3^- concentration of peritubular perfusate in logarithmic scale; ordinate: potential change in mV, depolarization upwards. Depicted are the initial potential changes observed in experiments similar to that in Fig. 10, except that the peritubular perfusate contained 12·5 or 50 m-mole/l. bicarbonate respectively (Frömter & Sato, 1976).

With regard to the mechanism of bicarbonate buffer transport, it is quite certain that – at least as far as the active transport step across the brush-border is concerned – the bicarbonate ions do not move as such, but are split into CO_2 and OH^-, with OH^- (or in opposite direction H^+) being the actively transported buffer component and CO_2 and/or H_2CO_3 diffusing passively. The earliest indication of such a mechanism derived from the observation that inhibitors of carbonic anhydrase, such as sulphanilamide, reduced bicarbonate absorption in the kidney possibly by reducing the enzymically catalysed generation of CO_2 from HCO_3^- (Pitts & Alexander, 1945). Further evidence supporting H^+ secretion as the active step in buffer transport was obtained by intratubular pH measurements, showing that during carbonic anhydrase inhibition, an acid disequilibrium pH developed in the tubular lumen (Rector, Carter & Seldin, 1965; Vieira & Malnic, 1968). This observation indicated that H^+ ions were secreted so rapidly into the lumen that the second step in the reaction

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

could not reach its equilibrium inside the tubule since the H^+ concentration measured in the tubule was higher than that calculated from the Henderson–Hasselbalch

equation for the prevailing HCO_3^- concentration, assuming tubular CO_2 pressure to equal arterial CO_2 (for more detailed discussion and alternative interpretations see Rector, 1973; Brodsky & Schilb, 1974; Malnic & Steinmetz, 1976). A third, completely independent, piece of evidence in support of H^+ secretion being the active step in buffer transport derives from the observation that chemically quite different buffers, provided they are lipid-soluble (such as glycodiazine, sulphamerazine or butyrate), can completely substitute for bicarbonate in yielding similar rates of net buffer transport and fluid absorption (Ullrich, Radtke & Rumrich, 1971) as well as generating the same active transport potentials (E. Frömter, unpublished).

The question of how the active H^+ secretion in the brush-border membrane is achieved is presently more difficult to answer. It seems as if there are two different systems working in parallel: (1) Several pieces of evidence point to the presence (at least in early loops) of primary-active H^+ ion secretion that is directly driven by metabolic energy ('H^+ pump'). The evidence favouring this mechanism comes from the active transport potential measurements described on p. 8; from the observation that the zero netflux concentration differences of buffers, unlike those of other substances (sugars, amino acids), are not dissipated when active Na^+ transport is blocked by ouabain (Ullrich, Capasso, Rumrich, Papavassiliou & Klöss, 1977); from bicarbonate flux measurements in isolated proximal tubules of rabbit, which were bathed in Na-free media (McKinney & Burg, 1977); and from the identification of a HCO_3^--stimulated ATPase in purified brush-border membrane fractions of rat kidney (Kinne-Saffran & Kinne, 1974*a*). (2) On the other hand, there exists in the brush-border membrane a Na^+/H^+ countertransport mechanism (Murer, Hopfer & Kinne, 1976), by means of which Na^+ ions, when entering along the electrochemical gradient from the lumen into the cell, expel simultaneously H^+ ions from the cell into the lumen. The energy for this 'uphill' movement of the H^+ ions from the electronegative cytoplasm of pH $\sim$ 7·4 (Struyvenberg, Morrison & Relman, 1968; Khuri & Agulian, 1977) into the tubular lumen of pH 6·8 (Gottschalk, Lassiter & Mylle, 1960; Rector *et al.* 1965; Vieira & Malnic, 1968) is derived from the energy stored in the Na^+ ion gradient across the brush-border membrane which in turn is due to the operation of the active Na^+ pump in the peritubular cell membrane.

With regard to the mechanism of the buffer exit from the cell into the interstitial space, which is thought to be passive, our knowledge is less well established. We know that the buffer can move independently (without coupling to other ions); and we know that the permeation appears to be affected by blockers of carbonic anhydrase (Frömter & Sato, 1976), as well as by the stilbene derivative SITS (I. Samaržija & E. Frömter, unpublished observations) which blocks anion permeation sites in other membranes (Cabantchik & Rothstein, 1972). However, whether the bicarbonate ions move as such or whether they are split into OH^- and CO_2 is not yet fully established.

Active transport of Ca^{2+} ions

Ca transport was not studied with electrophysiological techniques. However, micropuncture experiments utilizing the Cl^- distribution ratio between tubular lumen and peritubular fluid as indicator of the transepithelial potential difference have shown that a considerable part of the net Ca^{2+} absorption is active (Ullrich, Rumrich & Klöss, 1976); and biochemical experiments have shown a calcium-

stimulated ATPase to be present in the peritubular cell membrane (Kinne-Saffran & Kinne, 1974*b*). Furthermore, an ATP-driven Ca^{2+} uptake was observed in reversed cell membrane vesicles of the basolateral cell membrane together with a Na^+/Ca^{2+} exchange mechanism (Gmaj, Murer & Kinne, 1977). It would appear, therefore, that as in the case of H^+ transport (across the brush-border) there exist in the peritubular cell membrane two active calcium transport systems which operate in parallel: one driven by ATP directly and one driven by the Na^+ ion gradient (countertransport of Ca^{2+} from cell to peritubular space by Na^+ ions leaking back from the peritubular space into the cell).

Secondary active transport of various solutes

While active sodium transport is driven by a 'pump' mechanism utilizing energy from ATP directly and similar 'pumps' (ATPases) seem to exist for Ca^{2+} absorption and H^+ secretion/HCO_3^- absorption, there are numerous other substances for which transport was classified as active above (compare Table 2) but for which ATPases or comparable 'pump' devices have not been detected biochemically. This is actually not surprising, since we should note that in a black box approach, the classification, of a flux as active does *not* imply, of necessity, that this flux is driven in a sort of 'pump' directly by metabolic energy. Alternatively an apparently active flux of a component i, as determined in a black box approach, can be achieved by *local passive flux coupling* of the component i with another component j, which itself is undergoing active transport. In this case we speak of 'secondary active transport' (for definition see Frömter, 1978). The recognition of a flux as secondary-active is not simple and is ultimately only possible with information from inside the membrane system. Substitution experiments – investigating, for example, the flux of a component i in the presence or absence of a component j that undergoes primary active transport (such as sodium) – although suggestive – are never completely conclusive; nor are inhibitor experiments in which, for example, the flux behaviour of a substance i is studied while the active transport of other components j is selectively inhibited. The limitations of these approaches are discussed elsewhere (Frömter, 1978). In two examples described above, H^+ secretion and Ca^{2+} absorption, we have already seen that local passive flux coupling can contribute to the over-all active flux. In the following we will discuss the transport of further components, for which local passive coupling with actively transported Na^+ ions is the only means by which these substances are actively moved across the epithelium.

Sugar transport

While it has been known for many years that the kidney can absorb D-glucose against considerable urine-plasma concentration gradients, the localization along the nephron and the active nature of this transport was clearly established only some 10 years ago (Deetjen & Boylan, 1968; Loeschke, Baumann, Renschler & Ullrich, 1969). Since then, it has been observed that the active sugar transport depends on the presence of Na^+ in the test solutions (Ullrich, Rumrich & Klöss, 1974*a*) which suggested that this might be achieved by means of local passive coupling of glucose with the actively transported Na^+ ions, as postulated by Crane (1962) for glucose absorption from small intestine. Biochemical studies with vesicles from brush-border

cell membrane and from basolateral cell membranes (Aronson & Sacktor, 1974; Kinne *et al.* 1975), as well as electrophysiological studies (Maruyama & Hoshi, 1972; Frömter, 1977), have now confirmed this concept and have identified the individual transport steps. In the following I want to present some details of our electrophysiological analysis of sugar and amino acid transport.

The tubular absorption of D-glucose is associated with three major electrical events, which can be readily demonstrated in combined microperfusion and microelectrode measurements: (1) As shown in Fig. 12, right-hand side, stepping up

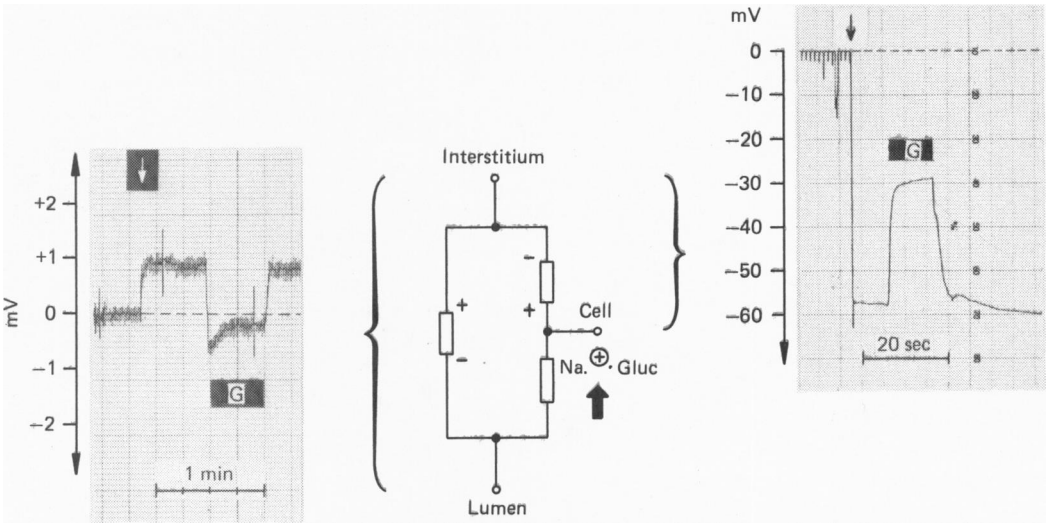

Fig. 12. Electrical response to luminal perfusion with glucose. Left panel: transepithelial potential; right panel: peritubular cell membrane potential. Abscissae: time as indicated; ordinates: potential difference in mV. During the mark G, the luminal perfusion was switched from HCO_3^- Ringer solution to the same solution but containing in addition 5 m-mole/l. of D-glucose. The circuit diagram in the centre illustrates that the polarity of the potential changes conforms to what could be expected if glucose induced a Na^+ ion current from lumen to cell.

suddenly the glucose concentration in the luminal perfusate (from zero to 5 m-mole/l.) leads to a sudden drop of the cell membrane potential (recorded across the peritubular cell membrane) from approximately 60 to 40 mV, which is complete within 200 msec or less (Frömter, 1977) and is followed by a slower phase of further depolarization by another ~5 mV until, approximately 20 sec later, a new steady potential level is obtained. (2) As shown on the left-hand side of Fig. 12, when recording transepithelially, we find that the lumen potential shifts suddenly by up to 2 mV in a lumen-negative direction. This potential shift occurs simultaneously with the rapid cell depolarization and is followed by a small recline of the potential, which exhibits the same time course as the secondary slow depolarization of the cell. (3) Not shown in Fig. 12 is a sudden drop of the luminal cell membrane resistance which occurs during the luminal exposure to glucose, all changes being completely reversible.

As depicted in the equivalent circuit diagram of Fig. 12 the polarity of the potential changes conforms to what one would expect, if they were caused by a positive ion

current from lumen to cell (suggestive of Na^+ ion and glucose cotransport across the brush-border membrane) and subsequent propagation in the closed loop equivalent circuit of the epithelium. To test this hypothesis, we have measured the transepithelial and cell membrane potentials and their initial displacements in response to glucose as well as the transepithelial resistance and the voltage divider ratio (Frömter *et al.* 1971) before and during luminal glucose perfusion and have analysed the data according to the equivalent circuits of Figs. 13*A* and *B*. In these circuits R_a, R_b and R_s and $\Delta\psi_a^0$ and $\Delta\psi_b^0$ represent the resistances and zero current potentials of the luminal and peritubular cell membranes and of the shunt, respectively in the control state (the zero current potential of the shunt being negligible under symmetrical perfusion conditions) with the measured membrane potentials being (for derivation see Frömter & Gebler, 1977)

$$\Delta\psi_a = \frac{(R_b+R_s)\Delta\psi_a^0 - R_a\Delta\psi_b^0}{R_a+R_b+R_s},$$

$$\Delta\psi_b = \frac{(R_a+R_s)\Delta\psi_a^0 - R_b\Delta\psi_b^0}{R_a+R_b+R_s},$$

$$\Delta\psi_c = \frac{R_s(\Delta\psi_a^0+\Delta\psi_b^0)}{R_a+R_b+R_s}. \qquad (4)$$

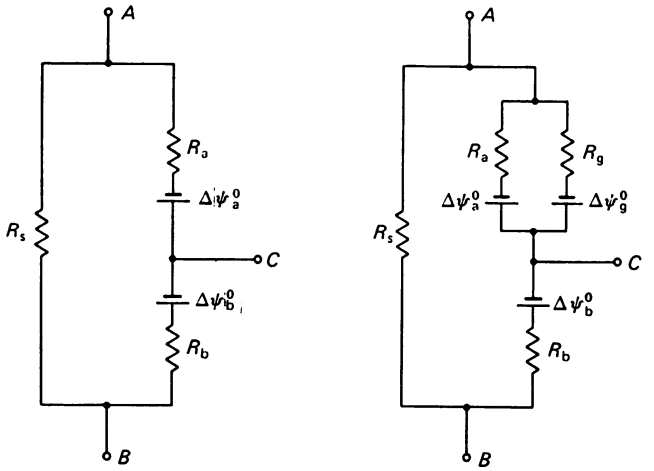

Fig. 13. Equivalent circuits to describe the glucose induced potential changes (initial rapid phase) quantitatively. *A*: lumen; *B*: interstitium; *C*: cell. For details see text.

If glucose enables Na^+ to flow into the cell via a common carrier, this process should be represented by an extra pathway R_g in the luminal cell membrane with the driving force $\Delta\psi_g^0$ (see Fig. 13*B*). Hence we obtain for the measured membrane potentials (initial phase)

$$\Delta\psi_a = \frac{(R_b+R_s)(R_g\Delta\psi_a^0+R_a\Delta\psi_g^0) - R_aR_g\Delta\psi_b^0}{R_aR_g+(R_a+R_g)(R_b+R_s)},$$

$$\Delta\psi_b = \frac{\{R_aR_g+(R_a+R_g)R_s\}\Delta\psi_b^0 - R_b(R_g\Delta\psi_a^0+R_a\Delta\psi_g^0)}{R_aR_g+(R_a+R_g)(R_b+R_s)},$$

$$\Delta\psi_s = \frac{R_s\{R_a\Delta\psi_g^0+R_g\Delta\psi_a^0+(R_a+R_g)\Delta\psi_b^0\}}{R_aR_g+(R_a+R_g)(R_b+R_s)}. \qquad (5)$$

From this analysis we have been able to conclude that the Na^+ ion current from lumen to cell accounts for $\geqslant 85\%$ of the initial potential change measured transepithelially (hence, knowing the transepithelial potential change and the transepithelial conductance, we can directly calculate the current flow associated with the Na^+-glucose cotransport across the brush-border), the remainder of the initial potential change reflecting a readjustment of the circular current flow from $\Delta\psi_a^o$ and $\Delta\psi_b^a$ in response to the resistance change in the closed loop equivalent circuit.

The present quantitative treatment is restricted to the rapid initial potential changes, because the slow secondary potential changes cannot be properly analysed with the presently available techniques. The secondary potential changes reflect the attainment of a new steady state with changing intracellular ion and solute concentrations which eventually lead to changes of $\Delta\psi_a^o$ and $\Delta\psi_b^o$, and possibly R_a and R_b.

Furthermore, we have been able to calculate R_g (for example, 3 m-mole/l. glucose lowers the resistance of the Na^+-glucose cotransport pathway from the assumed infinite value in glucose-free solution to $\sim 1200\ \Omega\ cm^2$ of epithelial surface); and we have been able to estimate the driving force $\Delta\psi_g^o$ behind the cotransport system, which under conditions approaching zero glucose concentration difference across the brush-border membrane yields a value that is compatible with estimates of the electrochemical potential difference for Na^+ ions across the brush-border membrane. Finally, a comparison of the active glucose transport as measured in rat kidney tubules (Loeschke & Baumann, 1969) with the glucose induced change of short circuit current (Frömter, 1974) suggests that each glucose molecule is transported with one ionic charge, and hence that the glucose-Na^+ coupling ratio of the cotransport system may be 1. This analysis was also supplemented by experiments in which the driving forces of the Na^+-glucose cotransport system were directly studied by looking at the dependence of the potential changes on the luminal Na^+ concentration and on the cell membrane potential and investigating the concentration dependence of the potential response to glucose and to other sugars, as well as to inhibition by phlorrhizin.

All data obtained thus far support the concept that the active transepithelial glucose flux is achieved by means of a passive Na^+-glucose flux coupling (cotransport) system situated in the brush-border membrane, which transfers glucose at the expense of energy derived from the Na^+ ion gradient across the brush-border into the cell. As a consequence, the intracellular glucose concentration will rise above the plasma level (in agreement with observations by Tune & Burg (1971)) until in the steady state, the passive glucose efflux to the peritubular space just equals the Na^+-coupled glucose uptake from the lumen. From experiments with membrane vesicles, we know that the glucose efflux from the cell proceeds via a facilitated diffusion system in the peritubular cell membrane (Kinne *et al.* 1975), which is similar to that in erythrozyte membranes but differs from the transport system in the luminal cell membrane by exhibiting no coupling with Na^+ ions and having a different selectivity pattern for hexoses.

Transport of amino acids

The tubular absorption of L-amino acids follows the same principles that were described above for D-glucose (Ullrich, Rumrich & Klöss, 1974*b*; Evers, Murer &

Kinne, 1976). All groups of amino acids studied thus far (neutral, basic and acidic) yield electrical phenomena qualitatively similar to those with D-glucose (see Fig. 14). This observation is particularly interesting in the case of the acidic amino acids aspartate and glutamate, which are negatively charged implying that more than one positive ion (possibly Na^+ plus H^+ or Na^+ plus K^+ rather than two Na^+ as indicated by our preliminary observations) must be transported together with one amino acid

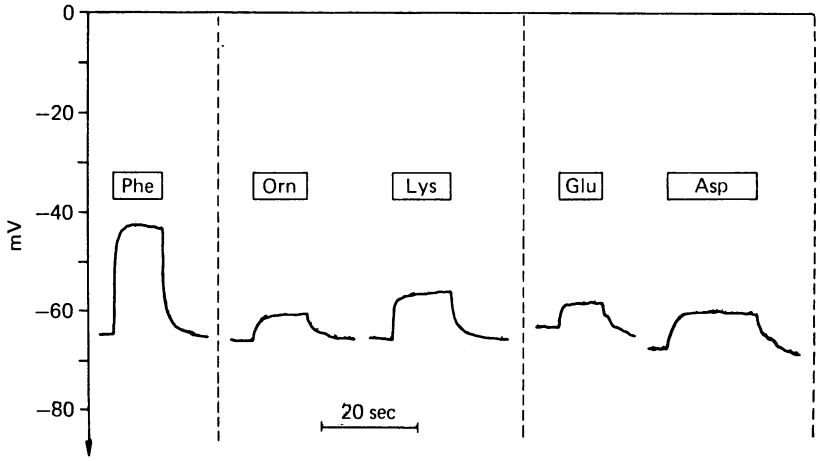

Fig. 14. Cell potential responses to luminal perfusion of various amino acids. Sections from trace records obtained in different rats. Abscissa: time as indicated by bar; ordinate: peritubular cell membrane potential in mV. The amino acids were applied during the respective marks in the lumen perfusate at a concentration of 5 m-mole/l. (I. Samaržija & E. Frömter, unpublished).

molecule in order to yield a net positive ion current from lumen to cell. Furthermore, the electrical potential response to all amino acids studied thus far depends on the presence of Na^+ ions in the tubular lumen (Fig. 15). These observations suggest that all amino acids (including the positively charged Arg^+, Orn^+ and Lys^+ which encounter a favourable electrical potential gradient across the brush-border membrane) are taken up from the tubular lumen into the cell by Na^+-dependent cotransport mechanisms situated in the brush-border. The question of how many different transport systems exist was also investigated with electrical techniques (I. Samaržija & E. Frömter, unpublished), and a brief summary of our results is presented in Table 4. Our conclusions agree largely with those derived from the investigation of hereditary amino acidurias in man (Scriver, Chesney & McInnes, 1976) and from recent work on brush-border membrane vesicles (Sacktor, 1977).

With regard to the exit step from the cell, our knowledge is less far advanced. While neither the neutral nor the basic amino acids produce conspicuous electrical potential effects when applied peritubularly, a clear-cut Na^+-dependent cell depolarization was observed during peritubular application of glutamate and aspartate. Its magnitude was similar to that observed during luminal application of the same amino acids. This observation suggests that glutamate and aspartate (but not glutamine and asparagine) are taken up by Na^+-dependent transport mechanisms from the peritubular surface into the cell. A peritubular uptake mechanism for these

amino acids was also previously postulated by Foulkes (1971) on the basis of kidney perfusion studies.

Phosphate transport

Inorganic phosphate is also actively reabsorbed in rat kidney proximal tubule in a Na^+-dependent fashion (Baumann, de Rouffignac, Roinel, Rumrich & Ullrich, 1975); and experiments on renal membrane vesicles have shown a sodium-phosphate

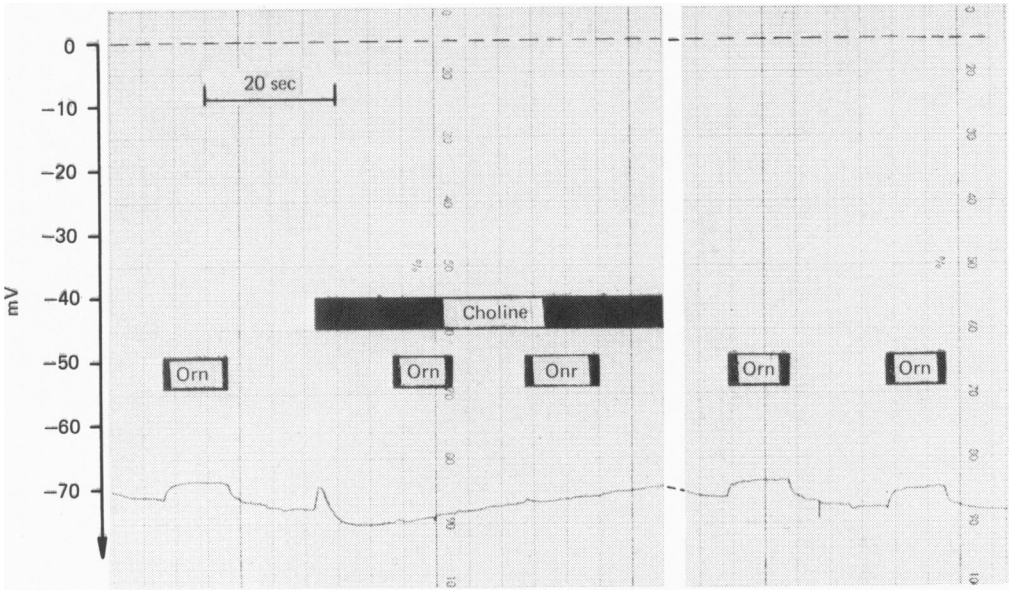

Fig. 15. Absence of the cell potential response to L-ornithine in the absence of Na^+. Details as in Fig. 14, except that the tubule was alternately perfused with one of the following four solutions: Na^+-HCO_3^- Ringer solution (not marked), Na^+-HCO_3^- Ringer solution containing 5 m-mole/l. ornithine (mark: ORN), choline-HCO_3^- Ringer solution (mark: choline) and choline-HCO_3^- Ringer solution, containing 5 m-mole/l. ornithine (mark: ORN during mark choline) (I. Samarzija & E. Frömter, unpublished).

TABLE 4. Na^+-coupled transport systems for amino acids which have been identified by electrical measurements

A Luminal cell membrane:
1. Orn^+, Arg^+, Lys^+
2. Asp^-, Glu^-
3. Phe, Met, Gly, ProOH, CysSH, Pro, $GluNH_2$
4. Gly
5. ProOH, Pro

B Peritubular cell membrane:
Asp^-, Glu^-

Each system in order of decreasing maximal transport rate.

cotransport system to be present in brush-border membranes (Hoffmann, Thees & Kinne, 1976). Accordingly, the luminal application of phosphate led in our experiments to a cell depolarization similar to that induced by application of glucose or amino acids. However, the depolarization was only observed in low pH solutions

(pH 6·0) when phosphate is present in form of $H_2PO_4^-$ and not in high pH solutions (pH 8·2) when it is mainly present in form of HPO_4^{2-} (I. Samaržija & E. Frömter, unpublished). This pH dependence suggests that the cotransport system must be loaded with two Na^+ ions so that the coupled flow is electrogenic if phosphate bears a single charge, but is electroneutral if it bears two negative charges. Similar conclusions were reached in experiments with brush-border membrane vesicles (Hoffmann *et al.* 1976).

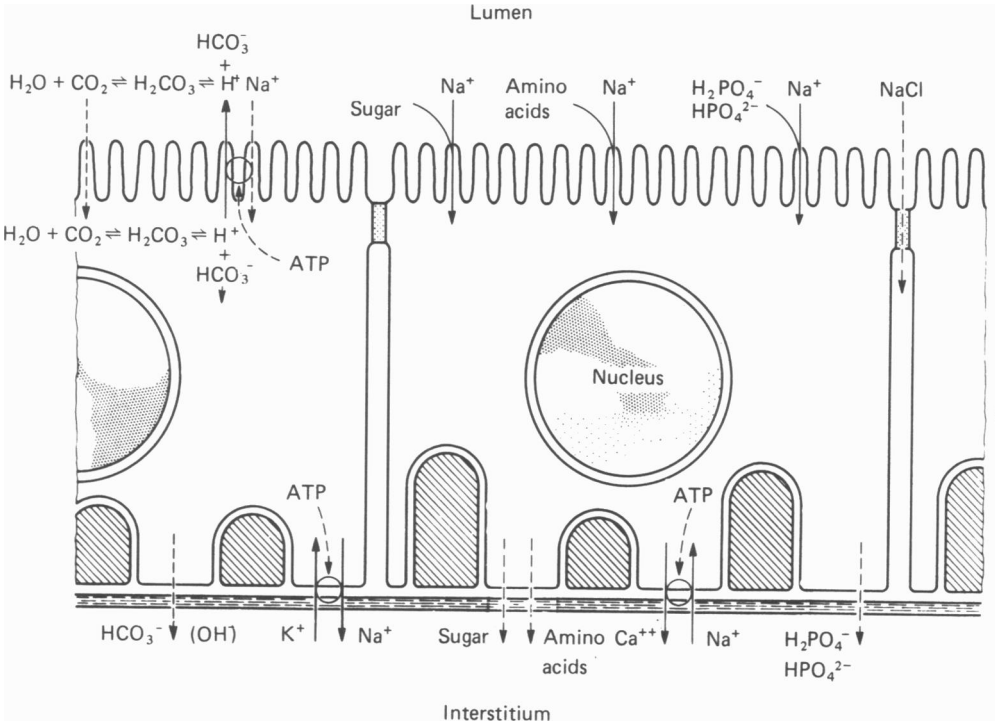

Fig. 16. Highly simplified schematic view of intra epithelial organization of solute transport processes in rat proximal tubular epithelium. Solid straight lines indicate active 'pump' fluxes, broken straight lines (– – –→) uncoupled passive fluxes, and fusing lines (⟶) cotransport systems.

CONCLUSIONS

In Fig. 16 I have tried to illustrate our conclusions with regard to the intra-epithelial localization of transport mechanisms in mammalian proximal tubule. It should be stressed, however, that this figure is by no means complete and contains only some of the information that is presently available. Thus, for the sake of simplicity all details of amino acid transport were left out and Ca^{2+} transport is considered only fragmentarily (see pp. 30 and 24). Represented are: (1) the paracellular shunt path, which handles the main part of the transepithelial fluxes that have been classified as passive; (2) the primary active transport mechanisms for Na^+ and H^+ (or respectively HCO_3^-) and Ca^{2+} in the peritubular and luminal cell membrane, respectively; (3) some of the flux coupling mechanisms for Na^+ and sugar, Na^+ and amino acids, Na^+ and H^+, Na^+ and $HPO_4^{2-}/H_2PO_4^-$ in the luminal

membrane (not shown is the flux coupling mechanism for Na^+ and glutamate (or aspartate) in the peritubular cell membrane); and (4) some facilitated diffusion mechanisms for uncoupled flow of HCO_3^- buffer, K^+, sugars, amino acids and phosphate in the peritubular cell membrane.

Even though we have arrived at some insight into the organization of tubular transport mechanisms, it should be mentioned that several important aspects were not covered. This is true for the over-all handling of K^+, aspects of which are unclear at present, as well as for the handling of Mg^{2+}. Furthermore, we know that tubules secrete weak acids (such as parahippurate or uric acid) and strong bases; however, the respective transport mechanisms are not yet defined. The same holds for the uptake of metabolites and of peptides and, most significantly, for the transport of water. The central question of whether a considerable part of the absorbed water flows across the paracellular shunt path, as suggested by the low reflexion coefficient of the epithelium for Na^+ and Cl^- (Frömter *et al.* 1973), cannot be answered with certainty at present. Nevertheless, I hope to have succeeded in conveying to you some of the knowledge which has been accumulated in the past years; and I hope, in particular, that I have succeeded in convincing you that results from micropuncture studies on kidney tubules are not only relevant for nephrologists but may yield important information on the organization and mechanisms of transepithelial transport in general.

REFERENCES

Aronson, P. S. & Sacktor, B. (1974). Transport of D-glucose by brush border membranes isolated from the renal cortex. *Biochim. biophys. Acta* **356**, 231–243.

Barratt, L. J., Rector Jr., F. C., Kokko, J. P. & Seldin, D. W. (1974). Factors governing the transepithelial potential difference across the proximal tubule of the rat kidney. *J. clin. Invest.* **53**, 454–464.

Bauer, R., Bauer, U., Deneke, H. & Thurau, K. (1977). Quantitative analysis of electrolytes in cells of rat kidney using the electron microprobe. *Pflügers Arch.* **368**, R 12.

Baumann, K., de Rouffignac, C., Roinel, N., Rumrich, G. & Ullrich, K. J. (1975). Renal phosphate transport: Inhomogeneity of local proximal transport rates and sodium dependence. *Pflügers Arch.* **356**, 287–297.

Boulpaep, E. L. & Seely, J. F. (1971). Electrophysiology of proximal and distal tubules in the autoperfused dog kidney. *Am. J. Physiol.* **221**, 1084–1096.

Brodsky, W. A. & Schilb, T. P. (1974). The means of distinguishing between hydrogen ion secretion and bicarbonate reabsorption: Theory and application to the reptilian bladder and mammalian kidney. In *Current Topics of Membranes and Transport*, vol. 5, pp. 161–224, ed. Bronner, F. & Kleinzeller, A. New York: Academic.

Burg, M., Grantham, J., Abramow, M. & Orloff, J. (1966). Preparation and study of fragments of single rabbit nephrons. *Am. J. Physiol.* **210**, 1293–1298.

Busse, D. & Steinmaier, G. (1974). Osmotically reactive plasma membrane vesicles prepared from rabbit kidney tubules by mild hypotonic lysis. *Biochim. biophys. Acta* **345**, 359–372.

Cabantchik, Z. I. & Rothstein, A. (1972). The nature of the membrane sites controlling anion permeability of human red blood cells as determined by studies with disulfonic stilbene derivatives. *J. Membrane Biol.* **10**, 311–330.

Crane, R. K. (1962). Hypothesis for mechanism of intestinal active transport of sugars. *Fedn Proc.* **21**, 891–895.

Deetjen, P. & Boylan, J. W. (1968). Glucose reabsorption in the rat kidney. Microperfusion studies. *Pflügers Arch.* **299**, 19–29.

Diamond, J. M. (1974). Tight and leaky junctions of epithelia. A perspective on kisses in the dark. *Fedn Proc.* **33**, 2220–2224.

Diamond, J. M. & Wright, E. M. (1969). Biological membranes: The physical basis of ion and nonelectrolyte selectivity. *A. Rev. Physiol.* **31**, 581–646.

EISENMAN, G. (1961). On the elementary atomic origin of equilibrium ionic specificity. In *Symposium on Membrane Transport and Metabolism*, pp. 163–179, ed. KLEINZELLER, A. & KOTYK, A. New York: Academic.

EVERS, J., MURER, H. & KINNE, R. (1976). Phenylalanine uptake in isolated renal plasma membrane vesicles. *Biochim. biophys. Acta* **426**, 598–615.

FARQUHAR, M. G. & PALADE, G. E. (1963). Junctional complexes in various epithelia. *J. cell Biol.* **17**, 375–412.

FISCHBARG, J. & LIM, J. J. (1974). Role of cations, anions and carbonic anhydrase in fluid transport across rabbit corneal endothelium. *J. Physiol.* **241**, 647–675.

FRIZZELL, R. & SCHULTZ, S. G. (1972). Ionic conductances of extracellular shunt pathway in rabbit ileum. *J. gen. Physiol.* **59**, 318–346.

FRÖMTER, E. (1972). The route of passive ion movement through the epithelium of Necturus gallbladder. *J. Membrane Biol.* **8**, 259–301.

FRÖMTER, E. (1974). Electrophysiology and isotonic fluid absorption of proximal tubules of mammalian kidney. In *Kidney and Urinary Tract Physiology, MTP Int. Rev. of Science*, Physiol. Series I, vol. 6, pp. 1–38, ed. THURAU, K. London: Butterworths. Baltimore: University Park Press.

FRÖMTER, E. (1977). Magnitude and significance of the paracellular shunt path in rat kidney proximal tubule. In *Intestinal Permeation*, pp. 166–178, ed. KRAMER, M. & LAUTERBACH, F. Amsterdam: Excerpta Medica.

FRÖMTER, E. (1978). Primary and secondary active transport mechanisms in rat renal proximal tubule. In *New Aspects on Renal Function*, pp. 27–36, ed. ULLRICH, K. J. & VOGEL, H. G. Amsterdam: Excerpta Medica.

FRÖMTER, E. & DIAMOND, J. M. (1972). Route of passive ion permeation in epithelia. *Nature, New Biol.* **235**, 9–13.

FRÖMTER, E. & GEBLER, B. (1977). Electrical properties of amphibian urinary epithelia. III. The cell membrane resistances and the effect of amiloride. *Pflügers Arch.* **371**, 99–108.

FRÖMTER, E. & GESSNER, K. (1974*a*). Free flow potential profile along rat kidney proximal tubule. *Pflügers Arch.* **351**, 69–83.

FRÖMTER, E. & GESSNER, K. (1974*b*). Active transport potentials, membrane diffusion potentials and streaming potential across rat kidney proximal tubular epithelium. *Pflügers Arch.* **351**, 85–98.

FRÖMTER, E. & GESSNER, K. (1975). Effect of inhibitors and diuretics on electrical potential differences in rat kidney proximal tubule. *Pflügers Arch.* **357**, 209–224.

FRÖMTER, E., MÜLLER, C. W. & KNAUF, H. (1969). Fixe negative Wandladungen im proximalen Konvolut der Rattenniere und ihre Beeinflussung durch Calciumionen. In *Aktuelle Probleme des Elektrolyt- und Wasserhaushalts, Nierenbiopsie*, pp. 61–64, ed. WATSCHINGER, B. Wien: Verlag der Wiener Med. Akademie.

FRÖMTER, E., MÜLLER, C. W. & WICK, T. (1971). Permeability properties of the proximal tubular epithelium of the rat kidney studied with electrophysiological methods. In *Electrophysiology of Epithelial Cells*, pp. 119–146, ed. GIEBISCH, G. Stuttgart, New York: F.K. Schattauer Verlag.

FRÖMTER, E., RUMRICH, G. & ULLRICH, K. J. (1973). Phenomenologic description of Na^+, Cl^- and HCO_3^- absorption from proximal tubules of the rat kidney. *Pflügers Arch.* **343**, 189–220.

FRÖMTER, E. & SATO, K. (1976). Electrical events in active H^+/HCO_3^- transport across rat kidney proximal tubular epithelium. In *Gastric Hydrogen Ion Secretion*, pp. 382–403, ed. KASBEKAR, D. K., SACHS, G. & REHM, W. S. New York: Dekker.

FOULKES, E. C. (1971). Effects of heavy metals on renal aspartate transport, and the nature of solute movement in kidney cortex slices. *Biochim. biophys. Acta* **241**, 815–822.

GERTZ, K. H. (1962). Transtubuläre Natriumchloridflüsse und Permeabilität für Nichtelektrolyte im proximalen und distalen Konvolut der Rattenniere. *Pflügers Arch. ges. Physiol.* **276**, 336–356.

GIEBISCH, G., BOULPAEP, E. L. & WRIGHT, F. S. (1972). Renal micropuncture techniques a symposium. *Yale J. Biol. Med.* **45**, 187–190.

GMAJ, P., MURER, H. & KINNE, R. (1977). Calcium binding and transport by brush border and basal lateral membrane vesicles of renal cortex. *Pflügers Arch.* **368**, R 21.

GOTTSCHALK, C. W., LASSITER, W. E. & MYLLE, M. (1960). Localization of urine acidification in the mammalian kidney. *Am. J. Physiol.* **198**, 581–585.

GREEN, R. & GIEBISCH, G. (1975). Ionic requirements of proximal tubular sodium transport. 1. Bicarbonate and chloride. *Am. J. Physiol.* **229**, 1205–1215.

GYÖRY, A. Z. & KINNE, R. (1971). Energy source for transepithelial sodium transport in rat renal proximal tubules. *Pflügers Arch.* **327**, 234–260.

HEGEL, U., FRÖMTER, E. & WICK, T. (1967). Der elektrische Wandwiderstand des proximalen Konvolutes der Rattenniere. *Pflügers Arch. ges. Physiol.* **294**, 274–290.

HEIDRICH, H. G., KINNE, R., KINNE-SAFFRAN, E. & HANNIG, K. (1972). The polarity of the proximal tubule cell in rat kidney. *J. cell Biol.* **54**, 232–245.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol.* **148**, 127–160.

HOFFMANN, N., THEES, M. & KINNE, R. (1976). Phosphate transport by isolated renal brush-border vesicles. *Pflügers Arch.* **362**, 147–156.

HOSHI, T. & SAKAI, F. (1967). A comparison of the electrical resistances of the surface cell membrane and cellular wall in the proximal tubule of the Newt kidney. *Jap. J. Physiol.* **17**, 627–637.

KATCHALSKY, A. & CURRAN, P. F. (1967). *Nonequilibrium Thermodynamics in Biophysics.* Cambridge, Mass.: Harvard University Press.

KEDEM, O. (1961). Criteria of active transport. *In Membrane Transport and Metabolism*, ed. KLEINZELLER, A. & KOTYK, A., pp. 87–93. New York: Academic.

KHURI, R. N. & AGULIAN, S. K. (1977). Intracellular bicarbonate and pH of single cells of proximal tubule of rat kidney. Proc. IUPS Paris, vol. 8, Abstr. no. 1128.

KINNE, R., MURER, H., KINNE-SAFFRAN, E., THEES, M. & SACHS, G. (1975). Sugar transport by renal plasma membrane vesicles: characterization of the systems in the brush border microvilli and basal lateral plasma membranes. *J. Membrane Biol.* **21**, 375–395.

KINNE-SAFFRAN, E. & KINNE, R. (1974*a*). Presence of a bicarbonate stimulated ATPase in the brushborder microvillus membranes of the proximal tubule. *Proc. Soc. exp. Biol. Med.* **146**, 751–753.

KINNE-SAFFRAN, E. & KINNE, R. (1974*b*). Localization of calcium stimulated ATPase in the basolateral plasma membranes of the proximal tubule of rat kidney cortex. *J. Membrane Biol.* **17**, 263–274.

LEWIS, S. A., EATON, D. C. & DIAMOND, J. M. (1976). The mechanism of Na^+ transport by rabbit urinary bladder. *J. Membrane Biol.* **28**, 41–70.

LITCHFIELD, J. G. & BOTT, P. A. (1962). Micropuncture study of renal excretion of water, K, Na and Cl in the rat. *Am. J. Physiol.* **203**, 667–670.

LOESCHKE, K. & BAUMANN, K. (1969). Kinetische Studien der D-Glucoseresorption im proximalen Konvolut der Rattenniere. *Pflügers Arch.* **305**, 139–154.

LOESCHKE, K., BAUMANN, K., RENSCHLER, R. & ULLRICH, K. J. (1969). Differenzierung zwischen aktiver und passiver Komponente des D-Glukosetransports am proximalen Konvolut der Rattenniere. *Pflügers Arch.* **305**, 118–138.

MCKINNEY, T. D. & BURG, M. B. (1977). Bicarbonate and fluid absorption by renal proximal straight tubules. *Kidney int.* **12**, 1–8.

MALNIC, G. & STEINMETZ, P. R. (1976). Transport processes in urinary acidification. *Kidney int.* **9**, 172–188.

MARUYAMA, T. & HOSHI, T. (1972). The effect of D-glucose on the electrical potential profile across the proximal tubule of Newt kidney. *Biochim. biophys. Acta* **282**, 214–225.

MAUDE, D. L. (1970). Mechanism of salt transport and some permeability properties of rat proximal tubule. *Am. J. Physiol.* **218**, 1590–1595.

MURER, H., HOPFER, U. & KINNE, R. (1976). Sodium/proton antiport in brush-border membrane vesicles isolated from rat small intestine and rat kidney. *Biochem. J.* **154**, 597–604.

NEUMANN, K. H. & RECTOR Jr., F. C. (1976). Mechanism of NaCl and water reabsorption in the proximal convoluted tubule of rat kidney. *J. clin. Invest.* **58**, 1110–1118.

PITTS, R. F. & ALEXANDER, R. S. (1945). The nature of the renal tubular mechanism for acidifying the urine. *Am. J. Physiol.* **144**, 239–254.

RECTOR, F. C. (1973). Acidification of urine. In *Handbook of Physiology*, sect. 8, Renal Physiology, ed. ORLOFF, J. & BERLINER, R. L., pp. 431–454. Washington: Amer. Physiol. Soc.

RECTOR, F. C., CARTER, N. W. & SELDIN, D. W. (1965). The mechanism of bicarbonate reabsorption in the proximal and distal tubules of the kidney. *J. clin. Invest.* **44**, 278–290.

REUSS, L. & FINN, A. L. (1974). Passive electrical properties of toad urinary bladder epithelium. *J. gen. Physiol.* **64**, 1–25.

REUSS, L. & FINN, A. L. (1975). Electrical properties of the cellular transepithelial pathway in Necturus gallbladder. I. Circuit analysis and steady state effects of mucosal solution ionic substitutions. *J. Membrane Biol.* **25**, 115–139.

RICHARDS, A. (1929). Methods and results of direct investigations of the function of the kidney. Beaumont Foundation Lectures, Wayne County Med. Soc. Baltimore: Williams & Wilkins. Cited by SMITH, H. W. (1964). In *The Kidney*. New York: Oxford University Press.

SACKTOR, B. (1977). Transport in membrane vesicles isolated from the mammalian kidney and intestine. In *Current Topics in Bioenergetics*, vol. 6, pp. 39–81. New York: Academic.

SAUER, F. (1973). Non-equilibrium thermodynamics of kidney tubule transport. In *Handbook of Physiology*, sect. 8, Renal Physiology, ed. BERLINER, R. W. & ORLOFF, J., pp. 399–414. Washington: Amer. Physiol. Soc.

SCHMIDT, U. & DUBACH, U. C. (1971). Na-K-stimulated adenosinetriphosphatase: Intracellular localization within the proximal tubule of the rat nephron. *Pflügers Arch.* **330**, 265–270.

SCRIVER, C. R., CHESNEY, R. W. & MCINNES, R. R. (1976). Genetic aspects of renal tubular transport: Diversity and topology of carriers. *Kidney int.* **9**, 149–171.

SEELY, J. F. & CHIRITO, E. (1975). Studies of the electrical potential difference in rat proximal tubule. *Am. J. Physiol.* **229**, 72–80.

SEN, A. K. & POST, R. L. (1964). Stoichiometry and localization of ATP dependent Na and K transport in the erythrocyte. *J. biol. Chem.* **239**, 345–352.

STAEHELIN, L. A., MUKHERGEE, T. M. & WILLIAMS, A. W. (1969). Freeze etch appearance of the tight junctions in the epithelium of small and large intestine of mice. *Protoplasma* **67**, 165–184.

STRUYVENBERG, A., MORRISON, R. B. & RELMAN, A. S. (1968). Acid-base behaviour of separated canine renal tubule cells. *Am. J. Physiol.* **214**, 1155–1162.

TISHER, C. C. & YARGER, W. E. (1973). Lanthanum permeability of the tight junction (zonula occludens) in the renal tubule of the rat. *Kidney int.* **3**, 238–250.

TUNE, B. M. & BURG, M. B. (1971). Glucose transport by proximal renal tubule. *Am. J. Physiol.* **221**, 580–585.

ULLRICH, K. J. (1973). Permeability characteristics of the mammalian nephron. In *Handbook of Physiology*, sect. 8, Renal Physiology, ed. ORLOFF, J. & BERLINER, R. W., pp. 377–398. Washington: Amer. Physiol. Soc.

ULLRICH, K. J. (1976). Renal tubular mechanisms of organic solute transport. *Kidney int.* **9**, 134–148.

ULLRICH, K. J., CAPASSO, G., RUMRICH, G., PAPAVASSILIOU, F. & KLÖSS, S. (1977). Coupling between proximal tubular transport processes. Studies with ouabain, SITS and HCO_3^--free solutions. *Pflügers Arch.* **368**, 245–252.

ULLRICH, K. J., FRÖMTER, E. & BAUMANN, K. (1969). Micropuncture and microanalysis in kidney physiology. In *Laboratory Techniques in Membrane Biophysics*, ed. PASSOW, H. & STÄMPFLI, R., pp. 106–129. Berlin: Springer.

ULLRICH, K. J., RADTKE, H. W. & RUMRICH, G. (1971). The role of bicarbonate and other buffers on isotonic fluid absorption in the proximal convolution of the rat kidney. *Pflügers. Arch.* **330**, 149–161.

ULLRICH, K. J., RUMRICH, G. & BAUMANN, K. (1975). Renal proximal tubular buffer (glycodiazine) transport. *Pflügers Arch.* **357**, 149–163.

ULLRICH, K. J., RUMRICH, G. & KLÖSS, S. (1974*a*). Specificity and sodium dependence of the active sugar transport in the proximal convolution of the rat kidney. *Pflügers Arch.* **351**, 35–48.

ULLRICH, K. J., RUMRICH, G. & KLÖSS, S. (1974*b*). Sodium dependence of the aminoacid transport in the proximal convolution of the rat kidney. *Pflügers Arch.* **351**, 49–60.

ULLRICH, K. J., RUMRICH, G. & KLÖSS, S. (1976). Active Ca^{2+} reabsorption in the proximal tubule of the rat kidney. Dependence on sodium – and buffer transport. *Pflügers Arch.* **364**, 223–228.

USSING, H. H. & ZEHRAN, K. (1951). Active transport of sodium as the source of electric current in the short circuited isolated frog skin epithelium. *Acta physiol. scand.* **23**, 110–127.

VIEIRA, F. L. & MALNIC, G. (1968). Hydrogen ion secretion by rat renal cortical tubules as studied by an antimony microelectrode. *Am. J. Physiol.* **214**, 710–718.

WHITTEMBURY, G. (1971). Relationship between sodium extrusion and electrical potentials in kidney cells. In *Electrophysiology of Epithelial Cells*, ed. GIEBISCH, G., pp. 153-178. Stuttgart: Schattauer.

WINDHAGER, E. E. (1968). *Micropuncture Techniques and Nephron Function*. London: Butterworths.

WINDHAGER, E. E., BOULPAEP, E. L. & GIEBISCH, G. (1967). Electrophysiological studies on single nephrons. *Proc. 3rd Int. Congr. Nephrol., Washington, 1966*, vol. 1, pp. 35–47. Basel, New York: Karger.

WINDHAGER, E. E., LEWY, J. E. & SPITZER, A. (1969). Peritubuläre Kontrolle der Flüssigkeits-Resorption im proximalen Tubules. In *Aktuelle Probleme des Elektrolyt- und Wasserhaushaltes, Nierenbiopsie*, ed. WATSCHINGER, B., pp. 25–34. Wien: Verlag der Wiener Med. Akademie.

WRIGHT, E. (1972). Mechanism of ion transport across the choroid plexus. *J. Physiol.* **226**, 545–571.