Measurements of Electrical Potentials and Ion Fluxes on Single Renal Tubules

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Experimental technics are described which permit the measurements of stable electrical potential differences across the cell membrane of single renal tubule cells and across single tubules. The inside of the tubule lumen is normally found to be electrically negative to the outside, while the cell interior is found to be negative to both the peritubular fluid and the tubule lumen. Such electrical measurements, in conjunction with known concentration gradients and flux measurements of various ions across single renal tubules, permit some deductions to be made on the driving forces involved in the movement of various ion species across the luminal and peritubular cell membrane of renal tubule cells.

RENAL function studies have indicated that among biologic membranes involved in the transport of electrolytes, the renal tubules are exceedingly active in the net transfer of most ion species. However, in spite of the establishment of considerable concentration gradients across various tubular segments and the likelihood of different transfer rates of charged particles from the lumen to the peritubular fluid, factors favoring the creation of electrical potential differences, renal tubular structures have received relatively little attention as far as electrical phenomena are concerned. Also, little is known regarding the magnitude and the kinetics of bidirectional ion fluxes across renal tubules, although site and steepness of various ionic concentration gradients have been fairly well outlined by micropuncture studies on individual nephrons.

Some of these problems have recently been studied by a combination of microelectrode technics and the perfusion of single renal tubules with solutions of known composition containing various isotopes. Also, double-perfusion of an amphibian kidney with different electrolyte solutions has yielded a reasonably stable and viable preparation which permits one to study the effects of various ions on electrical potential gradients. This presentation will be concerned with observations made on single nephrons of Necturus, an amphibian in which the size of the nephron is adequate to permit perfusion of single proximal tubules and in which stable electrical potential differences can be recorded, not only across the tubular wall but also across the cell membrane of individual tubule cells.

Electrical Potential Measurements on Single Renal Tubules

I should like to discuss, first, electrical phenomena which we observed on single renal tubules. The purpose of this study was twofold: first, to investigate whether an electrical potential difference exists across the renal tubular wall and to what extent the maintenance of such a potential difference is dependent on the presence of various ion species. Our second aim was to measure transmembrane potentials of single renal tubule cells, and if possible, to evaluate permeability characteristics of the luminal and peritubular cell side from ionic concentration gradients and electrical potential measurements before and after specific changes of the ionic environment had been induced.
Figure 1 shows a schematic view of the site of the microelectrode tip in relation to renal tubular structures. When a glass microelectrode having a tip-diameter of less than 1 μm is lowered upon the kidney, a "transepithelial" potential difference (i.e., a transmembrane potential of a single tubule cell) can be recorded before the microelectrode gains access to the tubule lumen and records a "transtubular" potential difference. Note the site of the indifferent electrode. During most measurements, the indifferent electrode was located on the surface of the kidney, effectively grounding the extracellular fluid. In a number of experiments, however, we have chosen to place a Ringer-agar micropipet into the tubule lumen and record differentially between tubule lumen and cell interior. This specifically permits one to test the effect of changes in the composition of tubule fluid on the potential difference between tubule lumen and cell interior across the luminal cell membrane. Attention should be drawn to the possibility that part of a transtubular potential difference is shunted through the glomerulus, the nephrostome, or through lower parts of the nephron. Fortunately, direct evidence indicates that this does not occur to any significant extent. When a column of colored mineral oil is injected into the proximal tubule and split by deposition of a small amount of Ringer's fluid, the glomerulus, the neck, the proximal end, as well as the most distal part of the tubule, are filled with oil. The transtubular potential difference under these conditions is identical with that recorded across a nonperfused tubule.1-3

Figure 2 summarizes data obtained in anesthetized Necturus on the frequency distribution of renal tubular transepithelial potentials.1 Stable measurements can be maintained for as long as 45 minutes. Results obtained in animals with undisturbed renal circulation are compared with those having spontaneously sluggish renal circulation or complete renal vascular stasis. Also included are measurements on animals pretreated with various amounts of radiocholormerodrin (Neohydrin). Some of these animals had renal ischemia. Calculation of the mean of 113 observations gave a mean electrical potential difference across the membrane of single renal tubule cells of −72 mV (s.d. of the mean ± 7.2 mV). Significantly lower values were obtained in animals pretreated with chlormerodrin 24 to 48 hours prior to the experiment in doses effecting a
mercury content of the kidney of more than 200 \( \gamma/\text{Gm. kidney} \). Smaller amounts of mercury were less effective in reducing cellular transmembrane potentials. It is also apparent that impairment of the renal circulation depresses a fraction of the normal membrane potential (most potential measurements on ischemic kidneys yielded values ranging from \(-45\) to \(-55\) mV). Such a state of ischemia, lasting for even as long as 5 to 6 hours, does not lead to a progressive decline of renal tubular cell potentials. A phase of partial depolarization within the first 30 minutes is followed by stabilization of the potential at a lower level of about 65 to 70 per cent of the original values obtained before ischemia had begun. Additional observations on transeellular potentials of single tubule cells were obtained in the doubly perfused kidney of Necturus. This preparation was found to be valuable in testing the effects of changes in the composition of the medium surrounding the tubule cells on transeellular and transtubular potential differences. We have, in essence, followed the technics as originally described by Cullis and Hoeber's school, in which the aorta, anterior abdominal vein and postcaval vein were cannulated and perfused with oxygenated Ringer's solution. Effluent fluid from the postcaval vein was allowed to drain freely and, in some experiments, its electrolyte composition was analyzed for sodium, chloride and potassium. Either a bicarbonate- or phosphate-Ringer was used for control experiments. From a number of function studies it appears that this preparation is stable and viable for several hours.

The first modification of the perfusion fluid which was undertaken was to change the potassium concentration in the portal and
aortal perfusion systems. The results of measurements of peritubular transcellular potential differences of proximal tubule cells may be summarized by stating that above a concentration of 5 mEq./L., there is a straight-line relationship between transmembrane potential and the logarithm of external potassium concentration. A slope of 53 mV per tenfold change in potassium concentration is observed. Below an external potassium concentration of 5 mEq./L., the tranacellular potential difference increases, but deviates from the linear relationship which might be expected if the potential difference were solely due to the potassium concentration gradient across the cell membrane. We conclude from these results that the peritubular transmembrane potential difference of single proximal tubule cells is essentially like that to be expected from a potassium concentration cell. Stated differently, the fact that potassium ions depolarize renal tubule cells is indicative of considerable permeability of the peritubular, contraluminal cell side to potassium, permitting this cation to discharge the intracellular negativity when its concentration in the extracellular fluid is increased.

The fact that depolarization measured across the peritubular cell membrane occurs when the potassium concentration is increased does not permit one to decide directly to what degree the luminal side of the cell is permeable to potassium. Therefore, we have attempted to measure the effect of an increased potassium concentration on the electrical potential difference between lumen and cell interior in the following manner. A kidney of Necturus is perfused via aorta and portal system with fluid containing potassium at the normal level of 4.5 mEq./L. Next, a glomerulus is punctured and the adjoining single nephron perfused with a solution of high potassium concentration. This solution is colored by the addition of T 1824 (Evans blue) to give a sharp outline of the nephron and to indicate any excessive leakage of the perfusion fluid from puncture sites. A second micromanipulator is used to insert a fine-pointed glass capillary, filled with Ringer-agar, into the lumen of the perfused tubule, constituting the indifferent electrode. Finally, a third manipulator permits one to insert the recording microelectrode intracellularly. By this arrangement the effect of potassium ions on the electrical potential difference between tubule lumen and cell inside is measured directly. Expressed differently, it is possible to test whether or not potassium ions placed within the tubule lumen enter the cell at the luminal border and discharge its negativity. Although more data are needed, experiments performed in this way indicate that the renal tubule cell is permeable to potassium also at the luminal side, since increasing depolarization can be observed when the potassium content of the tubule lumen is raised in a stepwise fashion.

Figure 3 contains a summary of transmembrane potentials of single renal tubule cells as measured in nonperfused and in perfused kidneys of Necturus. On the left a summary is given of what has been discussed previously, demonstrating a normal mean value of −72 mV in the nonperfused kidney, and various degrees of depolarization effected by the administration of Neohydrin and the development of ischemia. On the right is a summary of our data obtained in the perfused Necturus kidney. So far, some 70 perfusions have been performed. The mean of 427 intracellular impalements gives an intracellular negativity of 64 mV. Again, the addition of HgCl₂ as labeled Neohydrin, to the perfusion fluid reduces the cell potential. We have also performed perfusions using a modified phosphate-Ringer, in which sodium was replaced by choline, and others in which sodium chloride was replaced by sucrose. Finally, we have substituted sulfate for chloride and observed a small degree of cell depolarization. However, considerably more depolarization was seen when sucrose was the main osmotic constituent, or in particular when choline chloride was used. The depression of transcellular potentials by the latter substitution to the degree observed is noteworthy and, particularly in the case of choline chloride, in contrast to observations made on nerve and muscle. Experiments performed by Maizels and Remington on
mammalian kidneys may offer an explanation. These authors observed considerable penetration of choline into renal cortical cells when slices were incubated in a low sodium–high choline chloride medium. It appears that choline penetrates kidney cells readily but cannot be extruded against a concentration gradient. These authors, as well as Whittam et al., described a loss of sodium and potassium from kidney slices under these conditions, since both are displaced in the intracellular fluid by choline. Since we have previously observed that the transmembrane potential of single tubule cells depends on the concentration gradient of potassium ions, a depolarization would be expected under conditions of perfusion in which most of the sodium ions have been replaced by choline.

Figure 4 gives a summary of our results on transtubular potential differences. On the left is the mean of 25 measurements in nonperfused kidneys. In all instances the tubule lumen was found to be negative with reference to the indifferent electrode, giving a mean of −20 mV (range: −8 to −30 mV). In contrast to the findings of Wilbrandt, we have never found the inside of more distal sections of the nephron to be electrically positive to the outside. So far we have done only a few measurements across the distal tubule, all of which were approximately 15 mV more negative (i.e., mean −35 mV) than the mean value obtained from proximal transtubular measurements.

Similar results on normal transtubular potential differences were obtained by a number of other authors. Of interest are the observations of Kennedy, Schatzmann et al., and of Whittembury, confirming our results obtained across normal tubules and showing that potential differences can be maintained across perfused single tubules of Necturus. S. Solomon reported values ranging from −19 to −39 mV across the proximal tubule in the rat, and significantly higher values interpreted to
have been measured across the distal tubule.\textsuperscript{14} Rector also reports similar values to those of S. Solomon for proximal transtubular potential differences.\textsuperscript{15}

From inspection of figure 4 it also can be seen that removal of sodium chloride in the perfusion fluid (perfusion with a buffered sucrose solution) drastically reduces the transtubular potential. Essentially the same effect is observed when choline chloride is used. The addition of Hg\textsubscript{2}O\textsubscript{3} as Neohydrin\textsuperscript{4} and of 2,4-dinitrophenol (2 \times 10\textsuperscript{4} M) to the perfusion fluid when single tubules are perfused\textsuperscript{5} is also effective in diminishing intratubular negativity. Sulfate-substitution results in enhancement of transtubular negativity.

An attempt will be made to present a concept of the origin of transecellular and transtubular potential differences. Our description of experimental results on a normal proximal tubule cell can be synthesized in terms of the diagram presented in figure 5. Certain key facts are significant in developing a theory of the origin of the observed electrical phenomena. First, the average transtubular potential difference is 21 mV, the tubule lumen being negative with respect to the peritubular fluid. The transecellular potential difference varies between 50 and 75 mV. In figure 5 the mean value of \(-64\) mV is indicated. There exists, therefore, a net potential difference between interior of cell and lumen of 43 mV. On the other hand, a potential difference of 64 mV is maintained across the peritubular cell border. Secondly, renal tubular cells have a high concentration of potassium and a low concentration of sodium. Under the conditions of our experiment, an approximate value of

\begin{align*}
\text{Figure 4} \\
\text{Summary of transtubular potential differences. The number of observations is indicated within the block diagrams.}^{1, 4} \text{ The value for DNP is that reported by Whittenbury}^{5} \text{ and Solomon.}^{26}
\end{align*}
90 mEq./L for potassium and 30 to 50 mEq./L for sodium may be assumed. Thirdly, evidence will be presented to indicate that the proximal tubule cells actively transport sodium ions from tubule lumen to peritubular fluid, while the transtubular movement of chloride constitutes a passive process.\(^{15}\) This is taken to indicate that the tubule cell must be permeable to these 2 ion species, a conclusion which is supported by a great number of observations made on kidney slices. By the same type of studies, considerable potassium permeability is also indicated.\(^{17-23}\)

In the following, a hypothesis is presented which seems to be compatible with most observations. Let us consider the peritubular cell membrane first. The transtubular potential difference maintained across the peritubular cell membrane is close to that to be expected from the concentration gradient for potassium across this cell membrane. Accordingly, it behaves like a potassium diffusion potential. Additional and independent evidence that intracellular negativity is indeed closely related to the transtubular potassium gradient is given by the previously described variation of the peritubular cell-membrane potential as a function of the potassium concentration in the perfusion fluid. Taking a value of 90 mEq./L for cell water as found in kidney samples of perfused preparations, and 4.5 mEq./L for extracellular fluid, an average ratio of 20 obtains for \(K_t/K_o\). Using the Nernst equation, \(E = \frac{RT}{F} \ln \frac{K_t}{K_o}\), a ratio of 20 would correspond, at a temperature of 25 C, to a membrane potential of \(-77 \text{ mV}\). While we have observed values as high as this latter value, the majority of our measurements was lower (mean: \(-64 \text{ mV}\)). There is no information available at present to account for this difference. As in other cell systems where similar deviations are well known, the possibility of cell injury at the site of impalement, of active potassium uptake\(^{17}\) and of unequal activity coefficients\(^{9}\) cannot be excluded. However, it seems more likely that the potassium selectivity of the peritubular, contraluminal membrane of the tubule cell is incomplete in such a manner as to permit some sodium ions to leak into the cell interior along their concentration gradient, thus partly discharging its intracellular negativity.

The lower intracellular concentration of sodium and the finding that the proximal tubule normally pumps sodium out of the tubule lumen make it most reasonable to envisage the site of the active sodium extrusion mechanism at the peritubular cell border (see also \(^{20}\)). This would explain a compartment of low intracellular sodium concentration interposed between tubular and peritubular fluid phase, into which sodium could diffuse passively along an electrochemical gradient.

It is, at present, uncertain whether this sodium extrusion mechanism (carrier) shows linkage with potassium (sodium-potassium exchange) or whether it incorporates an exchange diffusion component (sodium-sodium exchange). The relatively high rate of sodium
efflux from the tubule lumen at the observed low net transfer rates,\textsuperscript{25, 26} makes the latter an attractive possibility.

Inspection of figure 5 indicates a potential difference of considerably smaller magnitude (43 mV) across the luminal cell membrane. Since the concentration of potassium is the same in tubular and peritubular fluid,\textsuperscript{24} the different electrical potential gradients cannot be caused by different concentration gradients of potassium ions across the respective cell membranes. Since we have seen that the presence of sodium appears to be essential for the creation of the different potential gradients across the luminal and peritubular cell membranes, it seems logical to attribute an important role to this ion. We favor the view that the luminal cell membrane has a higher permeability to sodium ions than the peritubular cell side. This would permit positively charged sodium ions to diffuse along their electrochemical gradient into the cell and to shunt partially the intracellular negativity at this site.\textsuperscript{*} Obviously, the effectiveness of this luminal depolarization also depends on the separation of sodium from its anions. If, for instance, chloride would enter the cell as readily as sodium, the effectiveness of the latter in reducing the transmembrane potential would be abolished.

A number of observations are consistent with the proposed cell model. Thus at the luminal cell side the observed transeellular potential is further removed from the potassium equilibrium potential than that at the peritubular cell membrane, indicating a higher degree of leakiness of this cell side to sodium. Another pertinent observation is the previously mentioned finding that complete removal of sodium from the perfusion fluid results in reduced transtubular potential differences. It should also be recalled in this connection that the rapid loss of sodium from kidneys exposed to a low sodium medium is quite consistent with an unusually high degree of sodium permeability of kidney cells. It seems logical to locate the main site of this sodium leakage at the luminal cell membrane and not at the peritubular cell side where the observed potential difference deviates much less from the potassium equilibrium potential and hence precludes such a high degree of permeability to sodium.*

Essentially then, the luminal transmembrane potential would constitute a potassium diffusion potential similar to that at the peritubular cell side but significantly modified by an opposing sodium diffusion potential caused by a higher degree of sodium permeability at the luminal cell surface.

It should be stressed that the contribution of the potassium diffusion potential at the luminal cell membrane is envisaged to be greater than that of the sodium diffusion potential, since the cell inside is negative with respect to the tubule lumen. Two experimental findings are consistent with this view: first, the previously described sensitivity of the luminal potential difference to changes in intratubular potassium concentration, and second, the relative insensitivity of the same potential difference to variation in the sodium concentration within the tubule. Thus, Whittembury,\textsuperscript{3} in perfusion studies with choline chloride on single proximal tubules, showed

\*The observed reduction of transtubular and transeellular potential differences by mercurials is consistent with the observation made by others that mercurials inhibit proximal sodium chloride transport\textsuperscript{27} in vivo, and in vitro lead to sodium gain and potassium loss in renal tissue. Inhibition of the active sodium extrusion mechanism\textsuperscript{27-29} or increased passive sodium permeability\textsuperscript{30} would explain the loss of cell polarization. Again, reduced transfer rate of positive charge out of the tubule would depress intratubular negativity. Furthermore, the depression of water and solute transfer across the proximal tubule of Necturus by 2, 4-dinitrophenol\textsuperscript{31} and the simultaneous reduction of its transtubular potential difference\textsuperscript{32, 33} indicate the relationship between sodium movement and intratubular negativity.

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that significant reduction of the transtubular potential difference occurred only when the intratubular sodium concentration had reached a value of about 10 mEq./L. It should be pointed out that in terms of the total luminal potential difference, the degree of shunting accomplished by sodium is relatively small and that the potassium diffusion potential accordingly dominates in its role as determinant factor in the creation of the luminal potential difference.

Micropuncture studies performed by Bott indicate that in Necturus there is a net reabsorption of potassium across the proximal tubule amounting to some 30 per cent of the filtered quantity. This movement occurs against an electrical gradient of 20 mV and in all probability constitutes active transport. Since the peritubular potential difference is much closer to the equilibrium potential generated by the concentration gradient for this ion than the luminal potential difference, it follows that, as Whittembury has pointed out, the normal value for intracellular potassium is considerably in excess of that required for diffusional equilibrium across the luminal cell membrane. Stated differently, this smaller electrical gradient of 43 mV would be insufficient to prevent potassium leakage into the tubule lumen. A steady state can be maintained only if the deficiency in inward flux is compensated by an inward pumping mechanism which, for this purpose, must operate to give an inward potassium movement of the same magnitude as that due to an electrochemical potential of -20 mV.* Important considerations regarding the role of transcellular and transtubular potential differences, as far as the movement of hydrogen ions is concerned, have been presented by Pitts.

In summary, it can be stated that the proximal tubule cell appears to possess, in principle, membrane elements for ionic movements which are similar to those in nerve, muscle, and other cells effecting a net transport of ions. These include an active sodium extrusion mechanism (possibly coupled with potassium uptake) about which, as in other systems, little is known, a passive potassium-selective diffusion element, and a sodium-selective diffusion element. In nerve and muscle, the sodium-selective element is activated only for milliseconds. In the frog-skin, Koeffoed-Johnsen and Ussing showed that it is spatially completely separated from the potassium-permeable element. The proximal renal tubule cell seems to take an intermediate position in such a way that a potassium-selective diffusion element is dominant at both cell membranes, but the situation is modified by the presence of a significant sodium-selective diffusion element and possibly by active potassium uptake at that cell membrane which faces the tubule lumen.

Ion Flux Measurements on Single Renal Tubules

The rest of this presentation will be devoted to a discussion of some experimental results obtained by microperfusion of single proximal tubules in Necturus. The method of microperfusion of single tubules was originally introduced by A. N. Richards and Walker. It has recently been modified, extended and successfully employed for the quantitative study of a number of problems of transtubular water and solute transfer by A. K. Solomon and his associates. Briefly, it consists of the deposition between oil of a small amount of fluid of known composition in a tubular segment, its withdrawal after a known time interval, and comparison with its original composition.

These methods have recently been employed to study some aspects of sodium and chloride transport across the proximal tubule. Several lines of evidence may be cited in favor of active proximal sodium transport in Necturus. First, net sodium movement occurs against an electrical potential gradient of some 20 mV.* Second, as Windhager et al. have shown, sodium movement also occurs against a sizable concentration gradient when a mixture of sodium chloride and manni-
Table 1

Flux Measurements and Flux Ratios of Chloride in the Perfused Proximal Tubule of Necturus

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>CP/IP</th>
<th>Inulin</th>
<th>CP/IP Cl⁻</th>
<th>Efflux (µEq. cm⁻² sec⁻¹)</th>
<th>Influx (µEq. cm⁻² sec⁻¹)</th>
<th>Net flux ratio (M₁/M₀)</th>
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<tr>
<td>1</td>
<td>1.18</td>
<td>0.164</td>
<td>370</td>
<td>338</td>
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<td>1.10</td>
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<tr>
<td>3</td>
<td>1.32</td>
<td>0.210</td>
<td>325</td>
<td>275</td>
<td>50</td>
<td>1.18</td>
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<td>415</td>
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<tr>
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<tr>
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<td>346</td>
<td>301</td>
<td>45</td>
<td>1.18 ± 0.1</td>
<td></td>
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</table>

*Flux measurements CP/IP refer to the ratio Cl⁻ inulin concentration in collected perfusate/Cl⁻ concentration in injected perfusate. The same applies for Cl⁻ CP/IP ratios.

It is deposited within the lumen of the proximal tubule. Finally, experiments carried out by Whittenbury and his associates have indicated such a permeability of the proximal tubule wall to water as to preclude the normally occurring colloid osmotic pressure difference from playing a significant role in proximal fluid reabsorption in Necturus.

As far as the reabsorptive chloride movement is concerned, it appears possible that it constitutes a passive process downhill along a transtubular electrical potential gradient. However, such downhill movement per se does not exclude the possibility of active chloride transport. A means of quantitating the relationship between electrochemical potential gradient and ion movement was given independently by Teorell and Ussing. The advantage of their approach consists in the fact that all driving forces acting upon an ion species under consideration can be measured and related to observed ion fluxes. In our specific instance of chloride transfer across the proximal tubule, we were particularly interested in whether the known electrical asymmetry could quantitatively account for the observed chloride fluxes, or whether an additional driving force had to be invoked.

In order to obtain direct information about bidirectional chloride movement, perfusion studies on single proximal tubules of Necturus were performed in collaboration with Dr. Windhager. Results are summarized in table 1. Net water movement across the proximal tubular wall was estimated by the use of C¹⁴ labeled inulin; the fact that all ratios exceed unity indicates significant fluid reabsorption. Chloride was added to the perfusion fluid and its radioactivity compared with that of the collected fluid (column 3). In a number of experiments, chemical chloride experiments were also done on the collected perfusate and the plasma, using Ramsay's second electrometric titration method. We did not find a significant difference of chloride concentrations between collected perfusate and plasma. For the calculation of chloride efflux we have used, in essence, an equation developed by Curran and Solomon. We have estimated the net movement of chloride from the chemical chloride concentration and the amount of fluid reabsorbed, the latter being measured by the inulin concentration ratios. Data on influx were derived from the measured efflux and net flux values.

The efflux values found averaged 346 µEq. cm⁻² sec⁻¹, those for influx 301 µEq. cm⁻² sec⁻¹. There is apparently a considerable variability between individual tubules, yet the ratio of efflux/influx is fairly constant. In a similar series of experiments, reported by Oken et al., a value of 266 µEq. cm⁻² sec⁻¹ was found for sodium efflux and 204 µEq. cm⁻² sec⁻¹ for sodium influx. Both these results indicate that net movement of chloride and sodium constitutes only a relatively small fraction of the total efflux. Stated differently, it means that proximal tubular rate of turnover for sodium and chloride is quite high.

Using a mean transtubular potential difference of 20 mV, as found by Schatzmann et al., by us, by Whittenbury, across normal and perfused proximal tubules of Necturus, a calculated flux ratio of 2.14 is obtained (fig. 6). The fact that the calculated flux ratio is greater than the observed one of 1.18 indicates that the usual driving force of 20 mV readily accounts for the measured flux.
asymmetry. We interpret this finding as evidence for passive chloride movement across the proximal tubule along the electrical potential gradient.

Since the observed flux asymmetry is smaller than that to be expected on the basis of a 20 mV potential difference, it seems possible that part of the observed chloride movement is due to exchange diffusion. Levi and Ussing\textsuperscript{40} first suggested such a mechanism, the essence of which is that part of an observed ion movement is independent of the electrical field and equal in both directions, thus not affecting net transport. This would essentially apply to that part of the chloride which moves in a bound, electrically uncharged form and is, consequently, not affected by the 20 mV driving potential. Similar situations for sodium and chloride appear to apply for a variety of biologic membranes, such as gastric mucosa,\textsuperscript{41,42} the large intestine of the frog,\textsuperscript{43} muscle,\textsuperscript{44} and erythrocyte.\textsuperscript{45}

A schematic representation of such a possible partition of chloride fluxes is shown in figure 7. The diagram also includes for comparison the values for bidirectional sodium fluxes as reported by Oken et al.\textsuperscript{25} A tubular negativity is indicated at the left. This 20 mV constitutes the driving force for that portion of free chloride movement which is passive and which is represented by the straight arrows in the lower part of the graph. Cooperstein and Hogben\textsuperscript{43} have given a similar schema for the large intestine of the frog, where flux discrepancies similar to those to be expected from the spontaneous potential and free chloride movement were observed. Following their calculation, our data would indicate that the larger part of the chloride movement occurs by this latter mode of transfer. This part of the chloride movement by exchange diffusion, being independent of the electrical potential, is visualized in the upper part of the diagram by the large circle, signifying no net movement. The data presented do not allow us to estimate the contribution of solvent drag on chloride movement. Additional data will be needed to settle this point which does not, however, materially affect the main conclusion of a passive nature of transtubular chloride movement.

In summary, experimental techniques for the measurement of stable electrical potential differences across single renal tubular cells

\[ \frac{M_o}{M_i} = \frac{C_i}{C_o} e^{\frac{ZFE}{RT}} \]

**Figure 6**
Summary of observed and calculated flux ratios assuming a transtubular potential difference of -20 mV. For further explanation, see text.

**Figure 7**
Mean of flux measurements across single proximal tubules of Necturus. The values for chloride\textsuperscript{16} are fractioned into a passive and exchange diffusion component. Values for sodium fluxes are those of Oken et al.\textsuperscript{25}
and across single tubules are described. They indicate that the inside of the tubule lumen is normally electrically negative to the outside and that the interior of the cell is negative with respect to both the peritubular fluid and the tubular lumen. It is suggested that these potential differences are created by the formation, within the cell, of an area rich in potassium and relatively low in sodium. An active sodium extrusion mechanism is located at the peritubular cell membrane, the latter being relatively less permeable to sodium than the cell membrane facing the tubule lumen. It is further suggested that the passive diffusion of sodium at a higher rate than chloride, and the active potassium uptake from the tubule lumen into the cell, partially shunt the potential difference across the cell and at the same time render the inside of the tubule electrically negative. Since the net reabsorptive movement of sodium across the proximal tubule occurs against an electrical gradient, it is an active process. The electrical gradient caused by this extrusion of positively charged sodium ions is oriented in such a way as to account quantitatively for passive reabsorptive movement of chloride out of the tubule. Perfusion studies of single proximal tubules demonstrate a high rate of turnover of both sodium and chloride, indicating that the net movement of these ions is only a relatively small fraction of the unidirectional fluxes.

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