Increasing magnitude of electrical potential along the renal distal tubule

FRED S. WRIGHT
Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510

THE RENAL DISTAL TUBULE is known to have a higher transepithelial potential difference than the proximal tubule. This potential has been found in past studies to be approximately 50 mV, lumen negative (3, 10, 20, 21, 23, 27, 20), and is thought to be an important determinant of the rates of transfer of sodium and potassium across the distal epithelium (10). Although it is known that early and late portions of the distal tubule differ with regard to concentrations of Na and K and transport rates for Na and K were not different in early and late portions of the tubule. The transepithelial PD increases along the length of the distal tubule. Diffusion potentials alone do not account for the observed PD.

In the course of an investigation of forces influencing distal potassium secretion (39), a difference between the transepithelial PD of early and late distal segments was observed (38). The present experiments were designed to explore further this difference between early and late parts of the distal tubule. Several methods, some that have not previously been employed, were used to identify length along the tubule and to confirm that the tip of the measuring electrode was in the tubule lumen. In addition, the finding that the PD is different in early and late portions of the distal tubule provided the opportunity to examine factors that might contribute to the generation of the potentials. Experiments were done to evaluate the importance of the ionic composition of luminal fluid and the passive permeability properties of the walls of the two segments.

METHODS AND MATERIALS

Male Long-Evans rats, maintained on laboratory chow (Purina), weighed 200–350 g. The animals were deprived of food 15 hr prior to the experiments but were allowed free access to water. They were anesthetized by intraperitoneal injection of Inactin (Promonta) 100 mg/kg body wt. A heated table was used to maintain body temperature at 38 C. Surgical preparation included tracheotomy, cannulation of the external jugular veins for infusions, and cannulation of a carotid artery for monitoring blood pressure. A solution of 0.15 M NaCl and 0.275 M mannitol was infused continuously at 0.08 ml/min throughout the experiment.

The left kidney was exposed by a flank incision and supported in a plastic holder in an agar medium (8). A solution of 2% agar in 0.15 M NaCl was liquified by boiling, cooled to 42 C, and then poured over the kidney. After it solidified, a small bit of the agar was lifted off the surface of the kidney leaving a depression that served to contain a layer of warmed saline that was slowly dripped onto the kidney. The kidney surface was illuminated by direct light at a low angle through a fiberoptic bundle and was viewed through a stereoscopic microscope at X100. The ureter was cannulated to assure free outflow of urine. The tip of the rat’s tail was amputated and the cut end was placed in a bath containing 0.15 M NaCl. This bath was connected by an agar-KCl bridge to a bath containing 3 M KCl. A reference Ag-AgCl electrode in this bath was connected to a common ground point.

Both single-barrel and double-barrel glass micropipettes, Ling-Gerard microelectrodes, were prepared for electrical measurements and had tips smaller than 1 mm. For most experiments the pipettes were filled by boiling at reduced pressure in 3 M KCl. In later experiments (Figs. 7 11) the pipettes were filled at low pressure in warm alcohol, the alcohol subsequently being replaced first with water and then with 3 M KCl (51). The resistance and potential were measured for each pipette tip before use. Tip resistances ranged from 10 to 30 megohms and tip potentials were usually less than –10 mV. Coupling resistances between the two sides of double barrel pipettes ranged from 0.1–0.2 megohm. The pipettes that were filled first with alcohol and water had tip potentials less than –2 mV. The pipettes were mounted in holders providing contact between the 3 M KCl
solution in the pipette and pellet electrodes composed of packed Ag and AgCl (26). The two barrels of double pipettes were connected separately to two Ag-AgCl electrodes. The electrodes were connected to separate amplifiers: a Keithley 602 electrometer and a W-P Instruments M-4 electrometer. Both sides of double pipettes could therefore be used to measure voltage, the output of the amplifiers being directed to a Brush 220 two-channel penwriting recorder. The W-P Instruments electrometer has a bridge circuit that was used to deliver pulses of current (7). Changes in transepithelial potential produced by the current pulses were recorded by the electrode connected to the Keithley electrometer in a manner similar to that described by Giebisch and Malnic (10).

**Determination of Distal Length**

Distal tubules were identified by observing the passage of lissamine green (Roboz, Washington, D. C.) through surface segments after injecting the dye either intravenously or directly into a surface segment of a proximal tubule. Following intravenous injection of 0.05 ml of 5% lissamine green, the dye passed through proximal convolutions and disappeared from surface tubules as it flowed through loops of Henle. When dye returned to surface tubules, colored fluid did not appear in all distal segments simultaneously. To determine whether shorter or longer transit times to specific distal segments are a reflection of length along the distal tubule—and not simply of different lengths of Henle's loops—experiments were done in which both the transit times for dye and the lengths of late casts were measured. Eleven rats were prepared as described above except the kidney was bathed with warmed mineral oil instead of saline. Transit time was taken as the time from first appearance of dye in surface capillaries to appearance time in a specific distal segment. The distal tubule was then filled with latex. Dissection of these latex casts permitted measurements of the length along the distal nephron. The results of these measurements in 20 tubules are in Fig. 1. Distal length is expressed as a fraction of the total length from the macula densa region to the point of confluence with another distal tubule. The initial 20% of this length is beneath the surface and is inaccessible. Over the range of transit times from 31 to 70 sec, corresponding distal lengths were found to range between 20 and 100%. Some of the variability in these measurements is due to the difficulty of microdissection. Since it is not always possible to see the glomerulus as the ascending limb of the distal tubule passes by it, the configuration of the tubule as it changes from its straight to its convoluted portion was also used to identify the macula densa region. It is probable, however, that a more important source of variability in the observed relation between transit time and length is due to different proximal flow rates among animals, and to differences among nephrons of the total length up to the beginning of the distal tubule. The correlation is significant (P < .005). The range of variation of the average length predicted from a measured transit time is indicated by the standard error of the line. Some caution must be exercised in using this relation to predict length from transit time. Although distal tubules frequently can be punctured at points up to 20% of total length, the average length at the earliest observed transit time in Fig. 1 is 39%. Nevertheless, it is clear that shorter transit times are observed in earlier portions of the distal convolution. Intravenous injection of dye was therefore used to determine length along the distal tubule either by noting the segments in which dye appeared first (“early” distal) and those segments from which dye disappeared last (“late” distal), or in other experiments by measuring the actual transit time to the punctured segment.

A second method of determining length along the distal tubule used injections of dye directly into proximal tubules. This method permitted identification of early and late segments in the same nephron. A small volume of a 2% solution of lissamine green was injected quickly from a micropipette with a tip diameter of 4 μm. The dye passed through later segments of the proximal tubule, disappeared beneath the surface and then appeared in the distal tubule of that nephron. When at least two distal convolutions could be seen, as was generally the case, it was possible to see whether the segments were separated widely enough to be in the early and late parts of the distal tubule. In a few cases both an early and late segment were on the surface and could be punctured; usually only one or the other was accessible although both could be seen.

**Localization of Micropipette Tip**

Because the glass microelectrodes have tips with diameters that are less than 1 μm and are beyond the resolving power of the dissecting microscope, means other than visual observation are needed to verify that the potential being recorded is indeed that existing between tubule lumen and interstitium. Tip location was verified by four different methods in these experiments. Additional details of these methods are given in RESULTS but the procedures will be mentioned here briefly. In groups 1 and 1a the position of single-barrel micropipettes was checked by mechanical movements as described by Clapp et al. (9). In group 2, and in the additional experiments shown in Figs. 7-11, the tip

![FIG. 1. Relation of percent length along distal convolution measured from latex casts to transit time measured after injection of lissamine green. Correlation coefficient is 0.655. Linear regression of length on transit time is given by y = 10.1 + 1.58x. The broken lines indicate 1 σ of line.](attachment://image.png)
location of double-barrel electrodes was verified by passing current pulses through one barrel and recording the resulting voltage changes with the electrode connected to the other barrel (15). In group 3 an intraluminal location of the pipette tip was confirmed by injecting a 2% solution of lissamine green into a proximal segment while the transepithelial PD was being measured in a distal segment of the same nephron. In group 4 tip location was verified by perfusing the distal lumen with a solution low in sodium and potassium. The method of continuous perfusion was similar to that described by Giebisch et al. (11).

Solutions

Several solutions used for intraluminal microperfusion were prepared as follows: 1) 2% LG: 0.9 g/liter lissamine green in water; 2) choline Cl: 0.3 m choline chloride in water; 3) Tris-SO4: 0.225 m Tris (tris(hydroxymethyl)amino methane) plus 0.078 m H2SO4 in water plus 1:100 of solution no. 4; 4) 150 K: 0.075 m K2SO4 plus 0.020 m H2SO4 plus 0.075 m Tris in water; 5) 150 Na: 0.075 m Na2SO4 plus 0.020 m H2SO4 plus 0.075 m Tris in water; 6) a solution designated as equilibrium because it is designed to prevent water reabsorption while having normal concentrations of Na and K (18): 0.04 m NaCl, 0.01 m KCl, and 0.2 m raffinose. Lissamine green, 0.025%, was added to solutions 2-4 giving a final Na concentration of 1.1-2.0 mEq/liter. Lissamine green, 0.1%, was added to solution 5, increasing the Na concentration by 6 mEq/liter. Lissamine green into a proximal segment while the transverse pulse through one barrel and recording the result of the electrode connected to the distal segment. The potentials recorded were choline Cl, +3 mv; Tris-SO4, -11 mv; and 150 Na, -8 mv. These measured liquid junction potentials are in agreement with those calculated for these solutions by the Henderson equation and are small in comparison to the magnitude of the PD changes actually observed in perfusion experiments. Since the potentials measured in vivo are those present across an epithelial barrier, and not at a liquid junction, no correction is introduced in analysis of the data. The only correction that need be applied to these measurements would be changes in electrode tip potentials in the different solutions. These changes were less than 5 mv and were neglected.

RESULTS

Transepithelial Potential Differences

Measurement of the transepithelial PD in early and late surface segments of the distal tubule were made using several

<table>
<thead>
<tr>
<th>Table 1. Summary of measurements of transepithelial electrical potential difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>1a</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

Values are means ± SE. The number of tubules studied is given in parentheses. Different methods of confirming luminal localization of the electrode tip were used in different groups. The only correction that need be applied to these measurements would be changes in electrode tip potentials in the different solutions. These changes were less than 5 mv and were neglected.
different techniques both to determine the length along the distal tubule and to verify the placement of the electrode tip in the segment. The results grouped according to the method of confirming luminal localization of the electrode tip are summarized in Table 1.

In groups I and Ia early and late segments of the distal tubule were identified by intravenous injection of lissamine green. In group I early distal segments were chosen from those in which the dye appeared first, and late distal segments were chosen from those in which dye remained the longest. In group Ia the transit time of intravenously injected lissamine green to the segment was measured. Electrical measurements were made with single-barrel micropipettes and the placement of the tip was checked by mechanical displacement of the electrode (3). Stable potentials were recorded when the tip appeared to be in the lumen. Usually there was a transient large negative potential recorded when the initial impalement was made (Fig. 2). The PD then became stable at a smaller value. The tips could often be advanced down the axis of the tubule a distance of several diameters without a change in the PD. Displacements of the tip far enough to push the tip through the far wall of the tubule caused the PD to fall to near zero. The PD usually returned to its previous value when the tip was pulled back sufficiently to bring it into the lumen. Individual measurements using this method are shown in Fig. 3. The same method of electrode placement was used in group Ia and both sets of measurements are summarized in Table 1. The different method of determining length along the tubule in the measurements of group Ia is discussed below.

Simple mechanical displacements reduce some of the uncertainty of electrode localization but some high PD values might still be due to contact with cell interiors and some low values might be due to damage of the tubule wall providing a low-resistance shunt. Further experiments were done to verify tip localization by means independent of visual inspection and mechanical movements. In group 2 early and late distal segments were identified either as in group I (34 tubules in 4 rats) or by injection of 2 % LG into a proximal tubule and observing its passage through surface distal segments (37 tubules in 6 rats).

In five distal convolutions identified by proximal dye injection, the PD was measured in both early and late portions of the same tubule. Electrical measurements were made with double barrel micropipettes. Voltage was recorded simultaneously from each of the barrels at the time of impalement. When stable potentials were observed that were the same for both barrels, one of the electrodes was used to pass pulses (1/sec for a duration of 0.3 sec) of current (10^-8 A-5 x 10^-8 A) while the electrode connected to the other barrel recorded the transient changes in potential produced by the current pulses (Fig. 2). There was no evidence of rectification. Measurement of these voltage changes allowed calculation of the effective wall resistance at the point of current application. The effective resistance, $R_e$, for current flow from the pipette tip to ground (across the tubule wall) is given by $\Delta E/I$ where $\Delta E$ is the change in transepithelial PD and $I$ is the current applied at that point. Malnic and Giebisch (personal communication) have found that $R_e$ across the distal tubule wall of the rat averages $0.38 \times 10^6$ ohms and that $R_e$ across the peritubular cell membrane is approximately $1.8 \times 10^6$ ohms. This figure for the $R_e$ of the peritubular membrane is similar to the value of $2.6 \times 10^6$ ohms reported by Windhager et al. (35) and Boulpaep (1) for proximal cells of Necturus. The fivefold difference in resistance measured by intraluminal as opposed to intracellular electrodes was used to verify tip location. An $R_e$ of greater than $10^6$ ohms was arbitrarily chosen as indicating an intracellular location of the electrode tip. If an $R_e$ of greater than $10^6$ ohms was observed, advance or withdrawal of the pipette tip usually resulted in a sudden decrease in $R_e$; if $R_e$ remained above $10^6$ ohms, the potential measurement was disregarded. Individual values for PD measured in this way and mean values for $R_e$ in these tubules are shown in Fig. 3.

Although it seems doubtful that a low PD might be measured by error in a tubule with an $R_e$ similar to that in tubules with higher potentials, another approach to verifying location was sought. In six of the rats in group 2, those in which 2 % LG was injected into proximal tubules, it was found that if a small amount of dye was quickly injected into the proximal convolution of a nephron while the PD was being measured in the distal tubule, a transient hyperpolarization (ranging from 2-28 mv) was usually observed as the color wave passed the electrode. The maximum hyperpolarization was reached in a few seconds and the PD then returned to its previous value. If the electrode was not in the lumen as judged by the criteria of groups I and 2, no transient hyperpolarization was seen. Thus, in this third group of rats, proximal injection of 2 % LG served to identify early and late portions of a single distal tubule. The proximal...
The distal transepithelial PD depends on the presence of sodium or potassium in the lumen. Giebisch et al. showed that substitution of choline for Na and K by either choline or Tris-SO₄ produced similar changes in PD. The individual PD values measured when the tip localization was confirmed in this way and the mean depolarizations produced by the ionic substitutions are shown in Fig. 4. It is evident that the measurements of transepithelial PD in these four groups showed similar large differences between early and late distal segments. The early distal PD and the late distal PD, however, are nearly the same from group to group.

A modification of the method using intravenous injection of lissamine green was employed in an effort to examine the profile of the transepithelial potential along the length of the distal tubule. In the rats of group 1a, distal segments were identified by measuring the transit time of the dye. In every case both the appearance time of dye at the earliest distal segment in that region of the kidney surface (TT) and the appearance time of dye in the segment to be punctured (TT) were measured. Since the earliest distal appearance time varied in different kidneys, measurement of both times allows the transit time to the point of micropuncture to be expressed as a ratio of the time to the punctured tubule over the time to the earliest tubule (TT/TT). This ratio of transit times is taken as an index of distal length and provides some correction for differences in flow rate and proximal length. Potential difference was measured either with single-barrel electrodes, localized by mechanical displacement (70 tubules), or with double-barrel electrodes, localized by measurement of wall resistance (18 tubules). The

**Fig. 4.** Transepithelial PD in early and late distal tubules. In group 4 experiments (left), lissamine green injected into proximal tubule increased distal PD; average hyperpolarizations are shown. In group 4 experiments (right), replacement of luminal fluid with Na- and K-free solutions reduced the distal PD; average depolarizations are shown.

Although it is not certain why the dye produces this completely reversible transepithelial hyperpolarization, the effect might be due either to increased Na concentration in the lumen or to the large fuchsonimine anion itself (14). In either case, since there does not appear to be significant interaction between luminal and peritubular membranes (10), the hyperpolarization would indicate a luminal location of the electrode tip. In four tubules PD was measured in both early and late segments of the same tubule. The individual values for PD measured in this way and the average transient hyperpolarization in the early and late distal segments are shown in Fig. 4.

It has been shown that the distal transepithelial PD depends on the presence of sodium or potassium in the lumen (11). Giebisch et al. showed that substitution of choline for Na and K in the lumen causes the PD to fall to low values. In a fourth group of rats, ionic substitution of Na and K by choline or Tris was used to confirm the location of the electrode tip. Early and late distal segments were identified as in group 1. Double-barrel microelectrodes were then positioned in the distal tubule and then dye was injected a second time while the PD was being recorded. If there was no transient hyperpolarization as the green color wave passed, the electrode tip was regarded as outside the lumen and the PD measurement was discarded. In most of these tubules double-barrel electrodes were positioned initially by measurement of wall resistance, in a few cases a single-barrel electrode was positioned visually. The localization was then confirmed by proximal dye injection.

**Fig. 5.** Relation of distal transit time ratio, an index of distal length, to transepithelial PD. TT is transit time to site of puncture. TT is transit time after same dye injection to earliest appearing distal tubules in immediate vicinity.
two methods gave similar values and the results have been pooled. Figure 5 shows the potential measurements in this group plotted against the length along the distal tubule as indicated by the ratio of transit times. It appears that the PD is near zero in the earliest accessible segments of the distal convolution and that it increases to a maximum at a $TT/TT_0$ between 1.2 and 1.3. For inclusion in Table 1, early segments were taken as those with $TT/TT_0$, less than 1.25. The correlation shown between transit time and distal length in Fig. 1 permits the assignment of approximate values for length to these measurements of $TT/TT_0$. In Fig. 6, the data of Fig. 5 have been grouped arbitrarily by collecting the PD values in each 0.1 interval over the range of $TT/TT_0$. An average transit time was determined for the midpoint of each of these intervals from the average value of $TT_0$ in the 88 distal segments of $33.3 \pm 10.3$ sec. The length corresponding to each of these transit times was then determined from the relation described by the least-squares line in Fig. 1. The maximum PD appears to be reached at approximately half the distal length. The 95% confidence limits for the region of transition from low PD values in the first portion of the distal tubule to the maximum values in the last part locate it between 42 and 67% of distal length.

Relative Permeability for Sodium and Potassium

It has been proposed that the distal transepithelial PD is due to asymmetrical polarization of the peritubular and luminal cell membranes (10, 11). In this scheme a diffusion potential is postulated to exist across a nearly K-selective peritubular membrane. This potential approaches but does not equal the predicted K equilibrium potential because of a small Na permeability. A much smaller potential is maintained across a less selective luminal cell membrane. A higher relative conductance for sodium, $g_Na$, allows a greater contribution to the total membrane potential of the Na concentration difference between lumen and cell interior. In the normal tubule this significant $g_Na$, because of its inward-directed concentration gradient, acts to reduce the luminal membrane potential. Estimates of relative permeability have been made by observing instantaneous changes in membrane potential when ionic concentration differences across the membrane are suddenly altered (2, 16).

In order to estimate relative $g_Na$ and $g_K$ in the distal tubule, and to determine whether differences in $g_Na$ along the distal tubule can account for the different transepithelial PD in early and late distal segments, experiments were done with ionic substitutions while measuring PD and $R_o$. Early and late distal segments were identified as in group 1. Double-barrel microelectrodes were then positioned and luminal location was verified by measurement of wall resistance as in group 2. A double-barrel or triple-barrel micropipette was then placed in the earliest part of that segment. This pipette was used to perfuse the distal segment alternately with solutions having different Na and K concentrations.

In a first group of experiments, the effect on PD of substituting Na for K was tested (Fig. 7). The two perfusion solutions were 150 K and 150 Na. The transepithelial PD was continuously recorded in free-flow conditions and while the luminal contents were replaced by alternately perfusing with 150 K and 150 Na. When the perfusion solution was changed, the PD changed within a few seconds to a new

FIG. 7. Recordings of transepithelial PD during continuous micro-perfusion with solutions of K sulfate and Na sulfate. Superimposed on PD are brief hyperpolarizations caused by passing current, $5 \times 10^{-9}$ A, through second barrel of a double-lumen microelectrode.

FIG. 8. Effect on transepithelial PD of substitution of Na and K sulfates for tubule fluid by continuous perfusion. Points are averages of 2–4 individual measurements as in Fig. 7 and lines connect measurements made in same tubule.
steady value as the previous luminal solution was washed out. Two to four such successive changes were made in each tubule. The results of these substitution experiments are summarized in Fig. 8. In 10 late distal segments replacement of 150 K by 150 Na was followed by a decline in transepithelial PD. The mean PD in late segments after both double-barrel pipettes were in place, but before perfusion, was $-43.0 \pm 4.9$ mV. Changing perfusion solutions from 150 K to 150 Na changed the mean PD from $-43.5 \pm 5.4$ to $-28.2 \pm 3.4$ ($P < .001$). Similarly, in eight early distal segments the mean PD from $-21.4 \pm 1.5$ to $-14.3 \pm 1.6$ ($P < .001$).

A second group of experiments tested the effect of PD on changing K concentration at a constant low Na concentration or of changing Na concentration at a constant low K concentration. The perfusion solutions were 150 K, Tris-SO$_4$ and 150 Na. Again, the PD was continuously recorded as the luminal solutions were repeatedly changed. Figure 9 shows the effect of changes in luminal K at constant Na and changes in Na at constant K. In 12 early distal segments, perfusion changed the PD from $-8.4 \pm 1.2$ in free flow to $-1.33 \pm 0.9$ after substitution with Tris-SO$_4$ and to $-22.8 \pm 2.7$ during perfusion with 150 K. In 17 late distal segments, the mean transepithelial PD was $-47.8 \pm 3.4$ mV in free-flow conditions. Perfusion of these segments with Tris-SO$_4$ reduced the PD to $-9.5 \pm 2.37$ and perfusion with 150 K returned the PD to $-48.5 \pm 3.18$. The pattern of potential changes was similar when luminal Na was changed at constant low K concentration. In six late distal segments and two early distal segments, a triple-barrel perfusion pipette was successfully positioned so that the PD could be continuously monitored in free flow and during alternating perfusions with 150 K, 150 Na, and Tris-SO$_4$. In four other late and six additional early segments, shown in Fig. 8, perfusion was accomplished with double-barrel pipettes. In early distal segments, the PD increased from $-4.5 \pm 1.5$ to $-14.3 \pm 1.2$ when the perfusion solution was changed from Tris-SO$_4$ to 150 Na. In late distal segments, the PD changed from $-9.2 \pm 2.8$ to $-28.2 \pm 3.4$. In these experiments $R_e$ was also recorded continuously as the perfusion solutions were changed. The values found for $R_e$ immediately after positioning the double-barrel electrode were similar to the measurements under comparable free-flow conditions in group 2 (Fig. 3). This decline in $R_e$ was probably due to electrical leakage caused by the second puncture. The variability in $R_e$ measurements is not, however, due entirely to leaks at the puncture site, since there was no clear correlation between PD and $R_e$ in either early or late segments. When the double-barrel perfusion pipette was then placed in the same tubule, the measured $R_e$ usually declined from 20–50%. When the lumen was

---

**FIG. 9.** Effect on transepithelial PD of increasing luminal concentrations of K (left) or Na (right) by continuous microperfusion.

**FIG. 10.** Effect on effective wall resistance of substitution of Na, K, and Tris-sulfate solutions for tubule fluid. Points are individual measurements in different distal tubules.

<table>
<thead>
<tr>
<th>Early Distal</th>
<th>Latex Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 K</td>
<td>150 K</td>
</tr>
<tr>
<td>1.5 K</td>
<td>150 K</td>
</tr>
<tr>
<td>219</td>
<td>119</td>
</tr>
<tr>
<td>90</td>
<td>33</td>
</tr>
<tr>
<td>274</td>
<td>136</td>
</tr>
<tr>
<td>59</td>
<td>15</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>101</td>
<td>30</td>
</tr>
<tr>
<td>126</td>
<td>102</td>
</tr>
<tr>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>Mean</td>
<td>-39.6</td>
</tr>
<tr>
<td>$+/-SE$</td>
<td>12.3</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Late Distal</th>
<th>Late Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 K</td>
<td>150 K</td>
</tr>
<tr>
<td>1.5 K</td>
<td>150 K</td>
</tr>
<tr>
<td>154</td>
<td>22</td>
</tr>
<tr>
<td>202</td>
<td>136</td>
</tr>
<tr>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>154</td>
<td>61</td>
</tr>
<tr>
<td>274</td>
<td>87</td>
</tr>
<tr>
<td>79</td>
<td>39</td>
</tr>
<tr>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>90</td>
<td>87</td>
</tr>
<tr>
<td>919</td>
<td>61</td>
</tr>
<tr>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>Mean</td>
<td>-56.2</td>
</tr>
<tr>
<td>$+/-SE$</td>
<td>13.9</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;.005</td>
</tr>
</tbody>
</table>

**TABLE 2.** Effect of increasing luminal [K] in distal tubule on specific wall resistance (ohm·cm$^2$)
When the luminal concentration of potassium was decreased 49% in early, and 62% in late, during these alternating perfusions are shown in Fig. 10.

Considering that current injected into the lumen flows longitudinally up and down the lumen as well as radially across the tubule wall, cable theory (3) allows calculation of the specific resistance, \( R_s \), as

\[
R_s = \frac{\pi^2 d^4 R_i^2}{R_t}
\]

where \( R_s \) is in ohm-cm², \( d \) is the tubule diameter in centimeters, \( R_t \) is the effective resistance in ultras and \( R_i \) is the resistivity of the fluid in the tubule lumen in ohm-cm. Since ions carry the current pulses across the wall, \( R_t \) is a measure of ionic conductance of the epithelium for the permeant ions in the lumen. Changes in \( R_t \) when the concentrations of K or Na in the lumen are changed should indicate relative changes in conductance of the epithelium for these ions. Sulfate and Tris were chosen for the substituting solutions because they are poorly permeant ions. \( R_s \) was measured for each of the solutions and \( d \) was taken as \( 3 \times 10^{-4} \) cm, thus allowing for distention of the tubule during continuous perfusion. When the luminal concentration of potassium was increased from 1.5 to 150 mEq/liter, the specific resistance of the tubule wall decreased 49% in early, and 62% in late, distal tubules (Table 2). \( R_s \) decreased in each of 12 early distal segments and in 16 of 17 late segments when luminal K was increased. Table 3 shows that similar decreases in \( R_s \) were seen in each of eight tubules when luminal sodium was increased from 1.5 to 150 mEq/liter at a constant low potassium concentration. \( R_s \) decreased 85% in early segments and 43% in late segments.

When the lumen was alternately perfused with the 150 K and 150 Na solutions, no change was seen in \( R_s \) (Table 4). The mean differences are not significantly different from zero.

### Table 3. Effect of increasing luminal [Na] in distal tubule on specific wall resistance (ohm-cm²)

<table>
<thead>
<tr>
<th></th>
<th>Early Distal</th>
<th>Late Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 Na</td>
<td>59 (10.0)</td>
<td>28 (10.7)</td>
</tr>
<tr>
<td>150 Na</td>
<td>9 (27)</td>
<td>92 (10.4)</td>
</tr>
<tr>
<td>ΔR_s</td>
<td>-50.5</td>
<td>-32.2</td>
</tr>
<tr>
<td>Mean</td>
<td>-30.5</td>
<td>-40.7</td>
</tr>
</tbody>
</table>

### Table 4. Effect of luminal [Na] and [K] in distal tubule on specific wall resistance (ohm-cm²)

<table>
<thead>
<tr>
<th></th>
<th>Early Distal</th>
<th>Late Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 K</td>
<td>30 (10.0)</td>
<td>102 (4.2)</td>
</tr>
<tr>
<td>150 Na</td>
<td>20 (11.2)</td>
<td>27 (11.5)</td>
</tr>
<tr>
<td>ΔR_s</td>
<td>-10.0</td>
<td>106 (4.2)</td>
</tr>
<tr>
<td>Mean</td>
<td>-3.7</td>
<td>2.1</td>
</tr>
</tbody>
</table>

In an additional group of animals experiments were done using the same methods for measuring PD as in the perfusion experiments. Instead of perfusing the lumen with solutions of varying Na and K concentration, however, an attempt was made to block the ends of the tubule segment with oil. Early distal segments were identified by intravenous injection of lissamine green. Double-barrel microelectrodes were positioned in the distal end of these segments and the location of the tips was checked by measurement of wall resistance. Double-barrel perfusion pipettes were then placed in the most proximal end of these segments. One barrel of this pipette was used to inject mineral oil into the tubule lumen. The PD and \( R_s \) were continuously recorded as the oil front was pushed slowly toward the microelectrode. At the moment that the oil column reached the electrode tip, a large increase in effective resistance was observed. This resistance change provided additional evidence confirming the luminal location of the tip. The equilibrium solution, containing 40 mM NaCl and 10 mM KCl and made isosmotic with raffinose, was then injected from the second barrel of the micropipette to push the oil column beyond the microelectrode. The PD and \( R_s \) measurements were then repeated as another oil column was pushed downstream toward the electrode tip. Figure 11 shows the results of such measurements of transepithelial PD in nine tubules in two rats. The PD measured when the proximal end of the distal segment was blocked with oil just before placement of an oil block immediately proximal to electrode.
TABLE 5. Summary of previous measurements of distal transepithelial potential difference

<table>
<thead>
<tr>
<th>Free-Flow Distal PD</th>
<th>Mean</th>
<th>Range</th>
<th>n</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-41.5</td>
<td>15-71</td>
<td>92</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>-60</td>
<td>30-105</td>
<td>98</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>-54.4</td>
<td>30-90</td>
<td>85</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>-37</td>
<td>10-54</td>
<td>41</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>-48.6</td>
<td>35-65</td>
<td>45</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>-47.6</td>
<td>31-63*</td>
<td>68</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>-49.0</td>
<td>30-68*</td>
<td>28</td>
<td>23</td>
</tr>
</tbody>
</table>
* Equals ± 2 SD.

DISCUSSION

In previous micropuncture investigations of the functional properties of the rat distal tubule, ionic concentrations, rates of ion transfer, and electrical potentials have been measured along the length of the distal convolution (12) and Table 5. When measurements of length have been reported, the total distal length has generally been taken as the distance from the macula densa region to the point of confluence with other distal tubules. This definition, which regards the straight portion of the distal tubule as part of the loop of Henle, is implied in the measurements of Walker et al. (34) and was explicitly stated by Gottschalk (13) and by Clapp and Robinson (4). It is known that potassium concentration increases and sodium concentration decreases along the length of the distal tubule (20, 21). Also, evidence indicates that the rate of sodium reabsorption is highest at the beginning, and the rate of potassium secretion is highest near the end of the distal convolution (12). In previous investigations of electrical properties, however, no difference in magnitude of the transepithelial electrical potential has been observed along the distal convolution. Previously reported values for distal PD are shown in Table 5. A wide range was found by all of the authors, but no correlation with length was evident.

The measurements of potential in the present investigation indicate that the PD is not constant along the length of the distal tubule. The PD was consistently found to be low at the beginning, and similar to the values in Table 5 near the end of the distal convolution (Table 1, Figs. 3 and 4). Transit times to the point of measurement of the PD were used as an index of distance along the tubule (Fig. 5). Since distal length is proportional to transit time (Fig. 1), the region of transition from lower to higher transepithelial potential may be located approximately. As shown in Fig. 6, estimates of length from measured transit times place the transitional region in the middle third of the distal convolution. The transepithelial potential is approximately −10 mV in the first third and rises to nearly −50 mV in the last portion. The present data estimate the limits of the boundary between these two segments but should not be interpreted as precisely defining its location.

Anatomical Considerations

The early part of the distal tubule differs from the late portion not only in the concentrations and rates of net transfer of sodium and potassium (12), but also in electrical potential. Some earlier anatomical observations take on added interest in the light of these differences in functional properties. In his work with microdissection of mammalian nephrons, Peter described morphological differences between the early and late parts of the distal convolution (25). He roughly divided what has more recently been defined as the distal convolution (13) into two segments of approximately equal length: a transition segment beginning at the point where the thick ascending limb passes its own glomerulus and a second segment that he called the initial collecting duct. He described the early part as appearing dark and blending without a sharply demarcated border into a more transparent part in which "crystals" become rarer. He noted that the later part had the same appearance as the collecting tubule.

A much more recent investigation using electron microscopy arrives at similar conclusions. Thoenes and Langer (32) have described fundamental structural differences between early and late parts of the distal convolution. The cells of the early distal tubule are characterized by extensive lateral and basal interdigitations suggesting extensive intercellular spaces, numerous large mitochondria, and few free ribosomes. These cells are similar to those of the thick ascending limb of Henle's loop. The late distal tubule, however, has two cell types (6, 32). They are differentiated by their electron density with light cells outnumbering dark cells by about 3:1. Both cell types have only basal interdigitations, suggesting a minor basal labyrinth, and have fewer and smaller mitochondria, and more free ribosomes and vesicles than the early distal cells. Thoenes, like Peter, describes the transition from the early to late epithelium as gradual and notes that the late cells are identical to those of the cortical collecting tubule.

Also relevant to this context are the observations of Steinhausen of the appearance of the surface tubules in the functioning rat kidney (29). He observed that intravenously injected lissamine green does not appear simultaneously in all distal tubules. He thus distinguished between early and late distal segments and reported two groups of transit times. It was not certain that these were early and late parts of the same nephrons, however, and he suggested that the different times might be due to different proximal lengths. The data in Fig. 1 indicate that distal length is one of the determinants of distal transit time. Also, in groups 2 and 3 after injection of lissamine green into a proximal segment, early and late segments of the same distal convolution could be identified on the surface and were punctured. Measurements in these tubules establish that the low and higher potentials observed in different nephrons do occur in early and late segments of the same distal tubule.

The observation of Peter that the late distal tubule appears more transparent than the early part provides a clue as to why the findings of this study differ from those in earlier reports. Distal segments previously were identified either after injections of indigo carmine (3, 27), or by the characteristic (to experienced observers) appearance of distal tubules when examined with a stereomicroscope and with favorable lighting conditions (18, 20, 21, 23). Thus, the tubules identified as distal were those that were more transparent and appeared to have thinner walls than...
adjacent proximal convolutions. These distal segments were probably in the late portion of the convolution. Indeed, in the course of the present study, an attempt was made to measure the PD in a blind fashion, that is before the approximate length had been revealed by dye injection. First, a distal segment was identified by its “distal looking” appearance, then the PD was measured, then lissamine green was injected, the segment was confirmed to be distal, and the length to puncture site was estimated. Nearly all of the tubules approached in this way were found to be late segments. So few early distal segments could be identified without the aid of injected lissamine green that this protocol was abandoned.

Luminal Membrane Potentials

The difference in transepithelial potential between early and late distal segments suggests that the potential across the luminal membrane is not the same for cells in the early and late parts of the convolution. If the cell interior is negative to both the interstitium and the lumen (12), then both the peritubular and luminal membranes are polarized with the magnitude of the luminal membrane potential being the difference between the peritubular membrane potential and the transepithelial potential. Furthermore, if the peritubular potential is approximately the same in early and late distal cells, the luminal membrane potential of early cells is about twice as large as that of late cells.

The peritubular membrane potential is measured when the pipette tip is inside the cell. Stable intracellular measurements are difficult in the rat because of the small size of the tubule cells (diam, 5–10 μm). Amphibian species have larger tubule cells and peritubular membrane potentials have been measured in both proximal and distal segments. In *Necturus* the proximal and distal peritubular membrane potential has been found to be approximately 65–70 mV (2, 9, 24, 37). In *Amphiuma* the peritubular membrane potential of distal cells has been found to be −72 mV (30). No similar measurements of stable potentials recorded with intracellular electrodes have been reported for the rat kidney. However, at the moment that a measuring pipette passes through the tubule wall, a transient negative potential, larger than the transepithelial PD, is frequently observed. Giebisch et al. (11) found these briefly recorded potentials averaged −75 mV and concluded that the cell ion concentrations are not changed immediately changes in the potential are observed when the concentration difference, driven by a concentration difference, is altered (11, 16). It is assumed that the cell ion concentrations are not changed immediately. Since the contribution of each of the normally present ions to the potential depends on its concentration and relative permeability, the relative permeabilities can be estimated by changing the concentration of one ion species while keeping the concentrations of the others constant (2, 16).

Comparison of the magnitudes of changes in PD per

10-fold change in sodium or potassium concentrations indicates that relative conductance of the luminal membrane for potassium $g_K$, exceeds the conductance for sodium, $g_{Na}$. When surface segments were alternately perfused with potassium sulfate or sodium sulfate while the transepithelial PD was being measured (Fig. 7), the potential was always higher when the luminal solution was 150 K than when it was replaced by 150 Na (Fig. 8). The higher transepithelial PD suggests that the 150 K solution was more effective than 150 Na in depolarizing the luminal membrane and thus that $g_K$ is greater than $g_{Na}$. Similar effects of ionic substitution on PD were seen in both early and late segments suggesting that the higher relative potassium conductance is present all along the distal convolution.

A quantitative estimate of these relative conductances was sought in the experiments summarized in Fig. 9 in which luminal K was changed at constant low Na, and luminal Na was changed at constant low K. It has been shown that replacement of both Na and K in the lumen by choline, a nonpermeant ion species, causes the PD to fall to low values (11). It is presumed that the fall in PD reflects hyperpolarization of the luminal membrane. In the present experiments, Tris-SO$_4$ was chosen for the substitutions because the buffering capacity of Tris allows preparation of the solution at neutral pH and because SO$_4$ is a poorly permeant ion. As shown in Fig. 9, changes in K concentration at constant Na and SO$_4$ concentrations produces quantitatively similar changes in transepithelial PD in both early and late distal segments. Changes in Na, as expected from the data shown in Fig. 8, resulted in smaller changes in PD. The slopes found for the relation between luminal Na or K concentration and transepithelial PD summarized in Table 7 show that changes in K concentration caused larger changes in PD than did changes in Na in both early and late distal segments. Furthermore, the partial conductance for sodium appears to bear a constant relation to that for potassium along the length of the tubule. The relative conductances of sodium to potassium, $g_{Na}/g_K$, calculated from the ratio of the transport numbers, are 0.59 in the early distal and 0.52 in the late distal tubule, values that are not different. That permeability properties are constant with length is also indicated by the equal hyperpolarizations seen in group 3 when the color wave passed the recording electrode (Fig. 4).

Another estimate of the ionic conductances is obtained from the measurements of electrical resistance. In the present study resistance measurements were made under two types of experimental conditions. These measurements allow estimates of relative changes in $R_e$, first, with length along the tubule and second, with changes in the ionic composition of the luminal fluid.

The first group of $R_e$ measurements are those made in free-flow conditions in the experiments of group 2. $R_e$ measurements made in early distal segments were compared with $R_e$ measured in late portions of distal tubules in the same rats. No difference was found between early and late segments. As discussed above, these measurements were selected by excluding a few values greater than one megohm from both the early and late groups. The use of this selection as a means of localization of the electrode tip may have biased the actual mean value for $R_e$ by the exclusion of some high values from both groups, but the selection does not alter the conclusion that there is no change in $R_e$ along the length of the distal tubule. The finding that $R_e$ is not different in early and late distal segments suggests that $R_e$ may also be constant along the length. This would certainly be true if there are no changes in $R_i$ and $d$ with length along the distal convolution. Although no measurements of $R_i$ and $d$ are available, if $R_i$ changed with length along the tubule it would be expected to increase (the sum of Na and K falls slightly) and if $d$ changed it would be expected to decrease (the late distal has a smaller volume flow and appears to be narrower). Since both of these postulated changes would tend to increase $R_e$, the only change in $R_e$ that could account for a constant measured $R_e$ in early and late segments would be a decrease with length. It can be concluded that $R_e$ is either constant or decreases along the distal convolution. The total ionic conductance, the reciprocal of $R_e$, is thus either constant or increases with length along the tubule.

The second group of $R_e$ measurements are those made during perfusion of the tubule lumen with solutions of high and low Na and K concentrations. Again, the experimental technique employed does not permit a statement regarding absolute values for $R_e$. Puncture of the tubule with the second double-barrel pipette, and the rapid perfusion of the lumen, resulted in lower values for $R_e$ than those in group 2 (Fig. 3). However, since $R_e$ was recorded continuously as the perfusion fluid was alternately changed either from 150 K to 150 Na or from 150 K or Na to 1.5 K and Na, the data are useful for estimating relative changes in $R_e$ under these conditions. When the luminal fluid was changed from 1.5 K to 150 K, $R_e$ and $R_z$ decreased in both early and late distal segments (Table 2). It appears that increasing the luminal K concentration increases the total conductance of the epithelium. Since in these perfusion solutions K was the only permeant ion, the increase in total conductance reflects an increase in $g_K$. Similar increases occurred in both early and late distal segments when luminal K was increased. Alternate perfusions with low and high Na concentrations caused comparable changes in $R_e$ (Table 3). In every tubule an increase in Na concentration when it was the only permeant ion present was associated with a decrease in $R_e$ and thus an increase in $gNa$. These changes suggest that $gK$ and $gNa$ both con-

**Table 7. Effect on transepithelial potential of changes in luminal potassium and sodium concentration**

<table>
<thead>
<tr>
<th></th>
<th>Early Distal</th>
<th>Late Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD slope, $\Delta[K]$</td>
<td>10.5</td>
<td>19.0</td>
</tr>
<tr>
<td>PD slope, $\Delta[Na]$</td>
<td>6.1</td>
<td>9.5</td>
</tr>
<tr>
<td>$T_k$</td>
<td>0.17</td>
<td>0.31</td>
</tr>
<tr>
<td>$T_{Na}$</td>
<td>0.10</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Average slopes of changes in transepithelial PD in millivolts per 10-fold change in [K] or [Na] are from data in Fig. 9. Assuming the potentials arise as the sum of diffusion potentials across the luminal and peritubular membranes, transport numbers ($T_k$, $T_{Na}$) are calculated as the ratio of the measured change in PD per 10-fold change in [K] or [Na] to the expected change in PD per 10-fold change in all permeant ions.
tribute to the total ionic conductance of the tubule wall. In the experiments in which 150 K and 150 Na perfusion fluids were alternated (Table 4), there was no change in \( R_i \). The relative conductances for K and Na can be determined from these data since \( g_{Na}/g_{K} \) is the reciprocal of the ratio of the resistances measured when one or the other of the ions was the only permeant species, \( R_K/R_{Na} \). Values for \( g_{Na}/g_{K} \) determined in this way arc 1.19 in the early distal and 0.97 in the late distal and are not different. These measurements thus do not indicate that \( g_{Na} \) differs from \( g_{K} \), but do confirm the conclusion from the PD measurements that conductances for Na and K do not increase with length of the distal convolution.

### Origin of Potentials

The magnitude of the luminal membrane PD of early distal cells appears to be approximately two times that of the luminal potential of late distal cells. Since the peritubular membrane PD is probably quite constant along the distal tubule, it is the decline in luminal membrane potential that is the source of the increasing magnitude of the transepithelial PD with length. In seeking to explain the origin of the distal transepithelial PD, one may consider whether any of the known functional differences between early and late portions of the distal tubule can account for the observed difference in luminal PD of early and late cells.

Concentrations of Na and K in luminal fluid. It is known that the concentration of Na declines and the concentration of K increases with length along the tubule. One may ask whether these changes in cation concentration can account for the observed changes in PD. Under conditions similar to those in the present experiments, Na has been found to average 55 mEq/liter in early segments and about 25 mEq/liter in late segments (21). Luminal K in the same experiments rose from 2 mEq/liter at 20\% of distal length to 12 mEq/liter at 80\% of distal length (20). Past discussions have considered the luminal membrane PD to arise as a diffusion potential, and have assumed that Na, K, and Cl are the only significantly permeant ions, and that the intracellular ionic concentrations and the relative ionic conductances are constant along the length of the tubule. If these assumptions are true, the known changes with length of luminal Na and K cannot explain a higher luminal PD in early distal cells. For this to be the case the sum of luminal Na and K would have to be lower in the early part of the distal convolution. The total cation concentration, however, appears to be highest in the early distal. Using the luminal and cellular concentration data in lines 6–9 of Table 8 and assuming \( g_{Na} \) equal to \( g_{K} \), calculation of luminal PD by the Goldman equation (16) gives values of 27 mv for early cells and 39 mv for late distal cells. The known concentrations of Na and K in the distal tubule, thus, might account for a slight decrease in transcellular PD with length but not for the large increase observed in the present experiments.

Relative permeability of Na and K. Previous work has not examined the relative permeability properties of early and late parts of the distal tubule. It might be asked whether changes in relative conductances for Na and K could account for the observed differences in PD along the tubule.

### Table 8. Summary of known quantities for potential, relative conductance, and concentration in distal tubule

<table>
<thead>
<tr>
<th>Source of Data</th>
<th>Early</th>
<th>Late</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Transepithelial PD</td>
<td>-10 mv</td>
<td>-50 mv</td>
<td>Tables 1, 5</td>
</tr>
<tr>
<td>2) Peritubular membrane PD</td>
<td>-60 mv</td>
<td>-75 mv</td>
<td>Table 4 and ref. 11</td>
</tr>
<tr>
<td>3) Luminal membrane PD</td>
<td>-30 mv</td>
<td>-25 mv</td>
<td>Difference between 1 and 2</td>
</tr>
<tr>
<td>4) ( g_{Na}/g_{K} ) (from PD)</td>
<td>0.39</td>
<td>0.32</td>
<td>Table 7</td>
</tr>
<tr>
<td>5) ( g_{Na}/g_{K} ) (from ( R_i ))</td>
<td>1.19</td>
<td>0.97</td>
<td>Table 4</td>
</tr>
<tr>
<td>6) Luminal Na</td>
<td>55 mEq/liter</td>
<td>25 mEq/liter</td>
<td>Ref. 21</td>
</tr>
<tr>
<td>7) Luminal K</td>
<td>2 mEq/liter</td>
<td>12 mEq/liter</td>
<td>Ref. 20</td>
</tr>
<tr>
<td>8) Cell Na</td>
<td>10 mEq/liter</td>
<td>10 mEq/liter</td>
<td>Ref. 11</td>
</tr>
<tr>
<td>9) Cell K</td>
<td>150 mEq/liter</td>
<td>150 mEq/liter</td>
<td>Refs. 18 and 29</td>
</tr>
<tr>
<td>10) Luminal Cl</td>
<td>28 mEq/liter</td>
<td>28 mEq/liter</td>
<td></td>
</tr>
<tr>
<td>11) Cell Cl</td>
<td>10 mEq/liter</td>
<td>10 mEq/liter</td>
<td>Ref. 11</td>
</tr>
</tbody>
</table>

It would be expected that a lower relative Na conductance would result in a higher membrane potential. Again, using the concentration data in lines 6–9 of Table 8, the Goldman equation can be used to calculate values for \( g_{Na}/g_{K} \) necessary to give the PD values in line 3 of Table 8. With these late distal concentration differences a diffusion potential of 25 mv requires \( g_{Na}/g_{K} \) to be 2.2. For the early distal luminal PD of 50 mv, the concentration differences shown require a \( g_{Na}/g_{K} \) of 0.4. Thus, for a change in permeability properties alone to explain the increasing transepithelial PD along the distal tubule, the relative conductance for Na would have to increase from half of the \( g_{K} \) in the early distal to greater than twice the \( g_{K} \) in the late distal. The estimates of conductance in these experiments (Table 8) exclude this possibility. Both methods of estimating \( g_{Na}/g_{K} \) show no difference between early and late distal segments.

Electrotoneic spread of potential. In proximal tubules, current has been shown to spread longitudinally through the luminal fluid in a manner predicted by the postulates of cable theory (15, 17, 35). The cable properties of the distal tubule have not been studied as extensively as recent measurements of the length constant, \( \lambda \), in the distal tubule of rats indicate that \( \lambda \) is longer in the distal than in the proximal tubule; Malnic and Giebisch (personal communication) have found a \( \lambda \) of 477 \( \mu m \) in free-flow conditions in normal rats. This distance is approximately one fifth of the total length of the distal convolution. If the electrical properties of the distal tubule epithelium were constant over its entire length, a lower PD might be expected in the earliest portion of the distal tubule if it is connected to an epithelium, the ascending limb, that has similar resistance but that does not have a potential. Flow of current from a segment with an epithelium generating a potential along the conducting core of luminal fluid to a region not generating a potential would result in a lower transepithelial PD in the region of the point of transition. The extent of this region would depend on the length constant of the conducting core. Experimental reduction of this core conductivity...
might then be expected to increase the measured PD in the early distal segment.

Since the region of transition from low to high PD appears to be near the midpoint of the distal convolution it seems unlikely that the early distal epithelium can be generating a potential. Furthermore, the results of the experiments in which early distal segments were electrically isolated by oil blocks appear to exclude this possibility. As shown in Fig. 11, there was no tendency for the early distal transepithelial PD to be increased by an oil block separating the distal lumen from that of the ascending limb.

**Intercellular shunt pathway.** The low transepithelial PD in the early distal tubule suggests that the potential profile across the luminal and peritubular surfaces of these cells is similar to the profile described for proximal tubule cells (9). A low-resistance intercellular pathway has been identified in the proximal tubule of amphibia (17, 35). Boulpaep (2) has proposed that this parallel-shunt pathway plays a significant role in the origin of the low transepithelial PD across the proximal tubule. In this scheme, because the resistance of the intercellular shunt is much lower than the parallel resistance of the luminal membrane, the transepithelial PD is low and the luminal membrane PD is nearly the same as the PD generated by the peritubular membrane. The low early distal PD found in the present experiments might thus be due to a low-resistance shunt path that is present between early distal cells, but that does not exist in late portions of the distal tubule. Such a possibility is reasonable on anatomical grounds. The work of Thoenes and Langer (32) shows that early distal cells have extensive lateral intercellular spaces. The late distal epithelium, however, has tightly apposed lateral cell membranes and has an appearance similar to the collecting tubule. However, the measurements of electrical resistance summarized in Figs. 2 and 10, and in Tables 2-4, appear to rule out a significant difference of transepithelial resistance between early and late portions of the distal tubule. Measurements of effective resistance and the calculated specific wall resistances are not different in early and late distal segments.

**Electrogenic ion pump.** The simplifying assumption that the distal peritubular and luminal membrane potentials arise as diffusion potentials has been taken with caution by previous authors (11) but has fit with some experimental results. The peritubular PD approaches the potassium equilibrium potential and thus appears to have its origin in the concentration differences of K and Na maintained across a membrane more permeable to K than to Na. The transepithelial potentials and the estimates of relative conductances in the present experiments, however, do not support the hypothesis that passive movements of Na and K are the only factors contributing to the generation of the luminal membrane potentials. If the luminal membrane PD does decline along the distal convolution, as suggested by the finding of an increasing transepithelial PD, neither the known slight decline in the sum of Na and K, nor the constancy of the total wall resistance and of the relative conductances for Na and K, fit with the assumption that the luminal PD is a diffusion potential. The luminal membrane PD might not be in equilibrium with the observed Na and K if some electrogenic ion-transfer process contributes to the generation of this potential step. Reabsorptive movement of K and Cl and secretory transport of H are not definitely known to be electrically neutral and could move net charge.

A mechanism that transports K from the lumen against a steep chemical gradient has been identified in the distal tubule (22). It is presumed that this K-reabsorptive pump is located in the luminal membrane (10). If this active K reabsorption is not electrically completely coupled to anion reabsorption or to secretory cation transfer, it would be expected to depolarize the luminal PD. Thus, if distal K reabsorption were electrogenic it might contribute to the low luminal PD seen in late distal cells. Absence of such a K pump in the early distal tubule would then be associated with a somewhat higher luminal PD. As has been discussed above, however, even complete absence of active K absorption would not create the high luminal PD observed in early distal segments. Further, it seems unlikely that there might be active K reabsorption in the proximal nephron, the ascending limb and the late distal tubule but not in the early distal tubule.

Chloride is reabsorbed along the distal convolution but the mechanism involved in its transport has not been completely defined. There is agreement that distal luminal Cl is approximately one-third of plasma Cl (18, 23, 36). With these values for distal Cl and previous measurements of distal PD there has been no need to postulate the existence of an active Cl reabsorptive mechanism (18, 23). Thus, the minimum TF/P chloride concentration calculated from the Nernst equation for the average transepithelial PD observed in the late distal tubule (Tables 1 and 5) is 0.15. Except after sulfate infusion (27), measured TF/P chloride ratios have rarely been found below this value. The average Cl that is observed in the late distal tubule (Table 8) does not require the action of an active transport process (18, 23). Similarly, it can be calculated from the Cl-concentrations in lines 10 and 11 of Table 8 that passive forces can account for the distribution of Cl across the luminal membrane of late distal cells; the PD calculated for this concentration ratio is 27 mV which fits well with the value of 25 mV in line 3 of Table 8.

The lower transepithelial PD found in early distal segments (Table 1), however, does not fit with the assumption of a purely passive distribution of Cl. For a luminal membrane PD of 50 mV as in line 3 of Table 8, and if intracellular Cl is approximately the same in early- and late-distal cells, calculation of the expected early distal Cl from the Nernst equation yields a value of 66 mEq/liter. Since average values for early distal [Cl] are less than one-half of this calculated value (10, 23), it may be that Cl is actively reabsorbed in the early portion of the distal tubule. Thus, it is possible that there is active chloride reabsorption in the early distal tubule that is electrogenic and contributes to the higher luminal membrane potential. Absence of this Cl pump or a less active Cl pump in the late distal cells might account for their lower luminal membrane PD. A difficulty with this explanation is that, in the perfusion experiments with Na and K sulfate summarized in Fig. 8, luminal Cl was very low and Cl reabsorption must have been reduced. Nevertheless, the transepithelial PD was increased only slightly.
Hydrogen ion secretion might also be considered as possibly contributing to the distal PD. For an electrogenic hydrogen secretory pump to account for the difference between early and late distal PD it would have to be more active in or restricted to the early part of the distal convolution. Vieira and Malnic (33) found that distal pH was constant along the tubule and concluded that a limiting gradient for H secretion is reached early in the distal convolution. They did find additional acidification beyond the distal tubule but concluded that the collecting duct has a limited capacity for hydrogen ion secretion.

In short, there is no complete explanation to account for the origin of the distal transepithelial PD. It may be that both ionic diffusion and electrogenic ion transport contribute to the potentials. The present results are not consistent with the hypothesis that diffusion potentials are the sole source of the distal PD. And, although no quantitative information is available, it remains reasonable to speculate that the epithelium of the early distal tubule, which is like that of the ascending limb, has electrogenic transport mechanisms for Cl reabsorption and H secretion that may contribute to the PD of the luminal membrane. Cells in the late distal tubule might also possess active transport mechanisms for Cl and H that are equally powerful but that have different ionic coupling ratios thus accounting for the lower luminal PD.

REFERENCES


