Some electrical properties of distal tubular epithelium in the rat

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The amphibian and mammalian nephron has been the subject of electrophysiological studies which have been directed at elucidating three general problems. First, transepithelial potential differences and effective electrical resistances across the distal tubular wall were measured by microelectrode techniques. Specific membrane resistances were calculated according to cable analysis. A value in the order of 350 ohm cm² was obtained in control rats. Transepithelial resistance values across distal tubules were lower in animals given 5% NaHCO₃ or 0.15 m KCl while rats breathing 15% CO₂ had similar values to controls. Perfusion of distal tubules with solutions containing only K, Na, or Cl as the main permeant ion species indicates a progression of relative conductances in the order K > Na > Cl near neutral pH. Changes in pH ranging from 5.5 to 8.0 did not change the specific potassium conductance. From specific conductances and the electrochemical potential difference of potassium and chloride ions it was possible to evaluate whether the transepithelial electrical driving force is adequate to effect transtubular potassium and chloride movement passively. The transepithelial potassium conductance is high enough to allow passive entry into the lumen by the electrical potential difference, a finding which does not exclude participation of an active transport component. The transepithelial chloride conductance is too small to allow passive reabsorption at the observed transepithelial potential difference. This implies that distal chloride reabsorption may be partly active in nature.

The present investigation is concerned with a study of transepithelial distal tubular conductance properties and an evaluation of the relative importance of tubular sodium, potassium, and chloride currents. In addition, the influence of changes in pH and in the ionic content of the tubular fluid on the conductance properties of the distal tubular epithelium were investigated. Two approaches were used. First, an attempt was made to deduce relative transepithelial ionic conductances from the change in the electrical potential difference across the distal tubular epithelium subsequent to rapidly induced changes in the ionic content of the perfusion fluid with respect to potassium, sodium, and chloride. This constitutes an extension of previous studies from this laboratory in which some effects of ionic substitutions on the transepithelial potential difference across distal tubules was evaluated (22). A second approach consisted in the measurement of effective transepithelial resistances under conditions in which the composition of the tubular fluid was controlled with respect to its ionic composition. It was thus possible to obtain an estimate of specific transtubular conductances for the main intratubular ion species.

METHODS

Male Long-Evans rats weighing between 280-390 g, kept on a standard diet of Purina laboratory chow and having free access to water, were anaesthetized with Inactin (50-80 mg/kg) and prepared for micropuncture according to techniques previously described (37-39). Control animals and animals undergoing respiratory acidosis received an infusion of 5% mannitol in isosmotic saline at a rate of 0.1 ml/min. Respiratory acidosis was induced by breathing 15% CO₂-85% O₂. Metabolic alkalosis was achieved by
the infusion of 5% NaHCO$_3$ at a rate of 0.1 ml/min. Po-
tassium-loaded rats received a solution of 0.15 M KCl at 0.1
ml/min. At least 1 hr was permitted to elapse after initia-
tion of the different experimental conditions before begin-
ning the electrical measurements.

The composition of the tubular fluid was changed by
first puncturing individual distal tubules with double-
barrel micropipettes (29), observing the direction of flow,
and alternating perfusion of a tubular segment with two
solutions if the length of the downstream section was ade-
quate. One of the perfusion fluids (that with high ion con-
tent) was colored with lissamine green (0.04%) to facilitate
visibility of the perfused tubule. Tubular sites were localized
by perfusion with lissamine green colored solutions or by
the appearance time of lissamine green (48, 58). Perfusion
rate was controlled manually and exceeded the normal
distal tubular fluid rate greatly. Perfusion rate was of the
order of 60–180 nl/min, as checked by quantitative collec-
tions during six perfusion experiments. Tubules were not
blocked with oil during perfusion since the perfusion pres-
sure was kept sufficiently high to produce some backflow
upstream and a vigorous downstream flow as evidenced by
the dissolution of the luminal contents. To be sure that
the perfusion fluid was not contaminated by normal tubular
fluid or by ions entering via a transepithelial route, we col-
lected fluid downstream from the perfusion site. Analysis of
the collected perfusate showed that luminal concentrations
were kept within 3–5 mEq/liter (n = 6) of the initial per-
fusion fluid. As expected, larger deviations occurred when
perfusion fluids of low ionic content were used.

In a number of experiments the composition of both
intratubular and peritubular fluid was changed simul-
taneously. For this purpose a peritubular perfusion tech-
nique similar to that used by Spitzer and Windhager was
used (47). A capillary in close proximity to a distal tubule
was punctured with a micropipette and perfused with a
solution in which the ion species whose transepithelial
movement was punctured with a micropipette and perfused with a
solution so that the effective resistance was estimated from the current
voltage relationship at the site of tubular impalement. An
appropriate correction was made for interbarrel coupling
resistance.

Specific wall resistance of distal tubules was calculated
from effective resistance (single- or double-barrel microelec-
trodes) or the length constant $\lambda$ and tubular geometry
according to cable theory (12, 28, 30):

$$R_s = \pi^2 \cdot d^4 \cdot R_{at}/R_i \text{ or } R_s = 4R_i\lambda^2/d$$

where

- $R_s$ = specific wall resistance, ohm cm$^2$
- $R_i$ = internal resistivity, ohm cm
- $R_{at}$ = effective resistance, ohm
- $d$ = tubular diameter, measured by means of
- an ocular filament micrometer in perfused
- tubules
During free flow control conditions, $R_i$ was taken to be 100 ohm cm. This value is based on conductivity measurements of a solution made up to contain Na, K, and Cl at middistal concentrations (38, 39). The conductivity of the different perfusion solutions was measured with a Radiometer model CDM 2d conductivity meter. All transepithelial potential differences were corrected for junction potentials by measuring after each series of impalements the potential change between the reference electrode and the recording microelectrode when the latter was placed into the appropriate perfusion fluid.

Four methods were used to assure proper localization of the electrode tip within the distal tubular lumen. 1) The placement of the microelectrode tip was checked by mechanical displacement within the tubular lumen. Stability of potential measurements during axial displacement in the order of one tubular diameter was taken to confirm tubular location. The method has been described in detail by Clapp et al. (8) and by Wright (58). 2) Tubular localization was checked by the increase in transepithelial electrical resistance measured during the passage of oil injected upstream at the site of electrode impalement (27, 58). Although the method has been criticized when applied to localizing the electrode tip within proximal tubular structures (27), we have observed good correlation with other localization methods when applied to distal tubular measurements. 3) The effects of carrying out ionic substitutions can also be used to distinguish between luminal and cellular electrode penetrations since the potentials change in a different way depending whether one records across the tubular wall or across the peritubular cell membrane. The transepithelial potential difference across distal tubules increases with an elevation of the intratubular sodium and potassium concentrations (22, 49, 58). On the other hand, the potential difference across the peritubular cell membrane was found insensitive to abrupt changes in the luminal sodium and potassium concentration (see below). The peritubular potential difference is also sensitive to changes in the external potassium concentration, but an increase in the latter drastically lowers the transmembrane potential difference at this site. 4) Since the effective resistance of the distal tubular cell wall is some 5-10 times lower than that recorded across tubular cell membranes, this additional criterion can also be used to exclude erroneous recordings from intracellular sites.

The following approach was used to assess specific transepithelial conductances. It depended on an experimental estimate of: 1) the total electrical conductance due to a defined salt species, and 2) the transport numbers of individual ions. Specific partial conductances were obtained by multiplying the total salt conductance ($G$) with the transference number ($T$). Implicit in this approach is acceptance that diffusion potentials are the sole source of electrical potential differences and that there is no contribution of active transport to the observed potential difference.

Total salt conductances were obtained in a first step in which distal tubules were perfused with a series of solutions of the same salt at two different concentrations, 150 and 15 mEq/liter, isosmolality being maintained in the latter solution by the addition of mannitol. The difference between the conductances measured at the two different concentrations was considered to be due to the salt species under consideration, the participation of other ions to the total current remaining insignificant. It was thus possible to compare relative conductances of different salts. Since the transepithelial conductance of several epithelia is a linear function of salt concentration to which it is exposed (2, 44, 57), it seems reasonable to assume that the conductance changes due to the concentration change ($\Delta G$) between 15 and 150 mEq/liter would correspond to the conductance of the membrane if 135 mEq/liter would be present in the lumen.

The participation of individual ions to the total transepithelial current was evaluated by measuring the transference number of single ions from the voltage changes subsequent to the establishment of well-defined salt gradients across the epithelium (5, 14), treating the potential changes as junction or diffusion potentials. From the observed potential changes the relative mobilities or transference numbers, in particular the $T_{Na}/T_{Cl}$ ratio, was calculated according to:

$$E = 61(T_{Na} - T_{Cl}) \log \frac{[NaCl]}{[NaCl]_2}$$

where $E$ is the potential difference observed at defined salt concentration differences across the tubule, and $T_{Na} + T_{Cl}$ is unity. The considered concentration ratio $[NaCl]/[NaCl]_2$ is that maintained across the distal tubular wall. Individual partial ionic conductances were obtained by multiplying $G$·$T$. It should be noted that the ratio $T_{Na}/T_{Cl}$ may be related to relative permeability ratios $P_{Na}/P_{Cl}$ (5, 14).

RESULTS

Figure 1 and Tables 1 and 2 summarize results during perfusion of distal tubules with solutions containing different concentrations of sodium chloride or choline sulfate, potassium or sodium sulfate, and choline chloride, isosmolality being maintained by addition of mannitol. Each tubule was perfused with two different solutions containing the respective sodium, potassium, or choline salts at different concentrations (150 and 15 mEq/liter or 150.0 and 1.50 mEq/liter, respectively). During these perfusions and ionic substitutions, transepithelial potential measurements were carried out. Effective resistance was measured in most tubules simultaneously with the potential measurements.

The main results and conclusions were: 1) confirming recent observations by Wright (58), the magnitude of the transepithelial potential difference across early distal tubules during free flow conditions was less than that across late distal tubules. The mean values of early distal (first half of distal tubular length) transepithelial potential differences was $-19.3 \pm 1.08$ mv (76 observations), whereas the corresponding late distal tubular value (second half of distal tubular length) was $-46.9 \pm 1.62$ mv (54 observations). These late distal values agree well with previous distal transepithelial potential differences reported by us (22) and others (8, 15, 33, 37-39, 55), an indication that most of these values were recorded from the easier accessible late
part of the distal convoluted tubule. 2) In agreement with previous observations (22) significant changes in the transepithelial potential difference on altering the intratubular concentration of sodium and potassium were observed. In all instances the potential difference became less negative on reduction of the intratubular sodium or potassium concentration. The mean slopes relating transepithelial potential differences to intratubular ion concentrations are graphically summarized in Fig. 1. It can be seen that the slopes are significant for concentration changes of sodium and potassium, whereas altering the concentration of chloride was found ineffective in inducing consistent changes in the transepithelial potential difference. These data show that sodium and potassium conductances must be considerably higher than those for sulfate since the lumen becomes progressively more negative on increasing salt concentrations in the lumen. On the other hand, the absence of significant potential changes on choline chloride con-

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### TABLE 1. Potential differences during distal perfusions with NaCl and choline SO₄ solutions

<table>
<thead>
<tr>
<th>Exptl Condition</th>
<th>Location</th>
<th>ΔV, mV</th>
<th>Rₑ, ohm cm²</th>
<th>Gₑ, mS cm²⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, free flow</td>
<td>E</td>
<td>100</td>
<td>0.495 ± 0.025 (69)</td>
<td>382.2 ± 2.62</td>
</tr>
<tr>
<td>K₂SO₄ perfusion, mEq/liter</td>
<td>L</td>
<td>100</td>
<td>0.411 ± 0.023 (54)</td>
<td>250.8 ± 3.58</td>
</tr>
<tr>
<td>Choline SO₄</td>
<td>150</td>
<td>0.599 ± 0.019 (57)</td>
<td>24.8 ± 4.03</td>
<td></td>
</tr>
<tr>
<td>Diff</td>
<td>150</td>
<td>0.198 ± 0.022 (32)</td>
<td>7.2 ± 1.39</td>
<td></td>
</tr>
<tr>
<td>Mobility (transference number) ratios in distal tubule</td>
<td>Tₛ⁻/Tᵢ</td>
<td>1.75</td>
<td>2.67</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 2. Transepithelial resistances of distal tubular wall during variations of tubular ion concentrations

<table>
<thead>
<tr>
<th>Exptl Condition</th>
<th>Location</th>
<th>ΔV, mV</th>
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<td></td>
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</tr>
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</table>

concentration changes indicates similar conductance properties for these ions. Some aspects of choline and sulfate permeabilities will be discussed below. Accepting the thesis that both choline and sulfate ions penetrate the luminal cell membrane less than potassium and sodium and that diffusion potentials contribute to the generation of the electrical potential difference across the luminal cell membrane of distal tubule cells (3, 22, 55), these findings indicate significantly greater permeability of the tubular wall to sodium and potassium than to chloride ions. 3) With respect to the effectiveness of potassium ions to change the transepithelial potential difference, no significant difference between early and late distal tubules was observed. However, a significant difference between early and late distal tubular effects of changing the sodium concentration was found. The mean potential change observed when luminal Na was changed from 1.5 to 150.0 mEq/liter was 34.5 ± 2.92 mv in the early distal tubule, against 53.6 ± 5.18 mv in the late distal segment. This difference is significant (P < 0.01). With solutions of 15.0 and 150.0 mEq/liter, the potential change in the early distal tubule was 11.6 ± 1.73 mv, and the change in late distal segments was 20.6 ± 3.99 mv. This difference is also significant (0.05 > P > 0.01). Thus, reducing the sodium concentration from 150 to 15 and 1.5 mEq/liter in the late distal tubule is more effective in changing the transtubular potential difference than it is in the early distal tubule. The values of the changes of the transepithelial potential difference for a potassium concentration change from 1.5 to 150 mEq/liter were 37.8 ± 3.93 mv for early distal tubules and 47.2 ± 4.81 mv for late distal tubules, a difference of borderline significance. For concentration changes from 15 to 150 mEq/liter a PD change of 26.4 ± 3.64 mv and 25.4 ± 2.43 mv was found for early and for late distal tubules, respectively. This difference between early and late distal tubular segments was not significant.

In order to compare the relative ionic conductances of sodium and chloride on the one hand and those of choline and sulfate on the other, distal tubules were perfused with solutions containing 150 and 15 mEq/liter NaCl and choline sulfate. The transepithelial potential changes observed on such ionic substitutions are summarized in Table 1. Several points should be noted. 1) It is apparent from the more negative transepithelial potential found on increasing the luminal sodium chloride concentration that the overall sodium mobility exceeds that of chloride. 2) Similar to results obtained during sodium sulfate perfusions, the potential changes after alterations in sodium chloride concentrations are significantly greater in late than in early distal tubules. 3) On perfusion with different concentrations of choline sulfate, the observed potential changes were much smaller than those found during sodium chloride perfusions. Since no consistent difference between early and late distal tubules was found, the values in Table 1 represent pooled values for this tubular segment. These findings indicate that the differences in choline and sulfate mobilities are not large. 4) From the observed potential changes the relative ionic mobilities (transference numbers) were calculated. Table 1 contains mobility (transference number) ratios for sodium chloride in early and late distal tubules. It is apparent that the late distal tubular epithelium is relatively more permeable to sodium than to chloride than the early distal tubule.

Table 2 summarizes values of effective and specific resistances during different experimental conditions. Specific resistance values were calculated from the effective resistance measured by double-barrel microelectrodes and tubular geometry. Inspection of Table 2 indicates that the specific wall resistance of distal tubules is dependent upon the absolute concentration values of sodium, potassium, and chloride in the lumen. In agreement with observations made on the small intestine (14) and the gallbladder (2, 57), the specific resistance generally increases with reduction of the ionic content of the perfusion fluid. Similar results on distal tubules with respect to sodium and potassium have also been obtained by Wright (55).

Table 3 provides a summary of the conductance changes based on data shown in Table 2. The differences between the transepithelial conductance measured during perfusions with 150 and 15 mEq/liter of the respective salt are listed. We have chosen to pool early and late distal values since the variability of the individual conductance measurements is fairly high (see Table 2). Mean conductance values are summarized in the last column of Table 3.

Table 4 shows the conductance values of individual ionic species calculated from the mean conductance changes (Table 3) and sodium and chloride mobilities (transference
The main conclusion of these perfusion experiments is that Table 5, C + P/C and L + P/L) were obtained under control free-flow conditions and during luminal perfusion by sulfate or choline ions. Similar results with respect to species. To obviate these difficulties, additional experiments were carried out in which the concentration of other species, not only potassium moving from lumen to the peritubular fluid compartment but chloride ions moving in the opposite direction, could, conceivably, contribute to the measured current. Similarly during application of inwardly directed current, sodium movement from peritubular to luminal fluid could complicate measurements of chloride conductances during perfusion of the lumen with solutions in which chloride ions are the main current-carrying ion species. To obviate these difficulties, additional experiments were carried out in which the concentration of other permeant ion species in the peritubular fluid was kept low during perfusion of the lumen with solutions of different potassium and chloride content, peritubular perfusions with solutions containing choline or sulfate in addition to the tested ion species were used.

The results of these experiments are shown in Table 5. The main conclusion of these perfusion experiments is that the transepithelial resistance is not significantly changed by peritubular perfusions containing either only 4 mEq/liter of potassium or, alternatively, only 100 mEq/liter chloride as in the permeant ion species. Accordingly, under experimental conditions in which only potassium and chloride ions represent the ion species under consideration, it is unlikely that changes in the peritubular ionic conductances significantly affect transepithelial current measurements. This could be due to a much higher conductance of the peritubular membrane compared to the luminal cell membrane such that the former contributes less to the total transmembrane resistance. Evidence obtained by others (E. L. Boulpaep, personal communications and (14)) supports the view that the luminal membrane resistance greatly exceeds that of the peritubular cell boundary. The observation in the present series of experiments that transtubular conductance measurements give results that are in general agreement with conductance data derived from electrical potential changes subsequent to luminal perfusions with solutions of variable ionic concentrations (22) are in support of this thesis.

In a second group of experiments transepithelial resistances were measured during free flow conditions in rats under noudiuretic conditions and during situations known to effect significant changes in distal tubular function. Also, different methods for measuring transepithelial resistance were compared. Table 6 summarizes pertinent results. In control animals, effective resistance values ranged between 0.208 and 0.383 megohm, corresponding to specific resistances of 112 and 369 ohm cm², respectively. The specific wall resistance under free flow conditions was also estimated from values of the tubular diameter (mean 31.3 μ) and the length constant λ. The mean value of the latter was 477 μ. Calculation of the specific resistance of the distal tubular wall yields a value of 377 ohm cm².1

1 Application of simple cable analysis seems justified in view of the satisfactory agreement between the measured internal resistivity (100 ohm cm) of the tubular fluid and the value of internal resistivity calculated from independent measurements of effective resistance and the length constant λ. Using a mean value of effective resistance of 383 megohm (double-barrel electrodes) and a mean value of 477 μ for λ, an Rτ value of 98.8 ohm cm² obtains.
than in control and other experimental groups despite the use of paired data. It should be noted that in the group receiving NaHCO₃ resistance values were generally lower than in control animals (bicarbonate-treated animals: P < 0.01; KCl-treated animals: P < 0.01). The rats breathing 15 % CO₂ was not significant.

In all three experimental groups, transepithelial potential difference, no significant change was observed when the lumen was alternatively perfused with solutions containing either 75 mEq/liter potassium at pH 8 or, alternatively, at pH 5.5, depending on whether bicarbonate or phosphate salts were used. Table 7 contains pertinent resistance values. The difference of resistance values between perfusions of pH 8 and 5.5 was not significant since identical tubular perfusion fluids were used. Also, specific resistance values measured were generally lower than values obtained during free flow conditions (see Table 7). Since the transepithelial conductance depends on the ionic content of the lumen—particularly that of potassium and sodium ions—and the potassium concentration is significantly lower under free flow conditions than during perfusion with 75 mEq/liter K⁺, it is to be expected that the conductance is higher during the latter experimental conditions.

In several instances potential differences across the peritubular cell membrane of single distal tubule cells were recorded during the process of impaling the tubular lumen. A potential difference was considered likely to be recorded across the peritubular cell membrane if it was higher (more negative) than average transepithelial potential differences, if it was unstable on very slight advancement of the recording micropipette, and if, on further advancement of the tip, a lower but stable (intraluminal) potential was recorded. Two additional criteria support our view that these potential differences originated from single tubule cells. 1) In six cases it was possible to obtain an appropriate impedance measurement with use of double-barrel electrodes which made possible effective resistance measurements. Resistance values ranged from 1.08 to 2.66 megohms (mean 1.77 megohms). These values are almost one order of magnitude higher than resistance values recorded during luminal perfusion. Such high resistance values are typical for cellular membranes; similar values have been recorded from single proximal tubule cells in Necturus (3, 54). 2) The insensitivity of such presumed cell potentials to ionic substitutions within the lumen was striking. In 12 instances, changing the luminal perfusion fluid from one containing potassium or sodium at a concentration of 150 mEq/liter to one containing 1.50 mEq/liter elicited no significant change in the recorded potential differences. As has been pointed out before, similar substitutions produce profound changes when the potential difference is recorded between lumen and peritubular fluid. On the other hand, the cellular potential differences were sensitive if concentration changes with respect to potassium were induced peritubularly. A dramatic drop of the potential was observed when the peritubular potassium concentration was increased from 1.50 to 150 mEq/liter. A high sensitivity of the potential difference across the peritubular membrane of single proximal and distal tubule cells to changes in the external potassium concentration has been observed in Necturus (4, 17) and in Amphiuma (49) as well as in mammalian tubule.

### Table 6. Resistance measurements of distal tubular wall

<table>
<thead>
<tr>
<th>Exptl Condition</th>
<th>Rs, ohm cm²</th>
<th>Rs, mS/cm</th>
<th>Rs, ohm cm²</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, double-barrel electrode</td>
<td>100</td>
<td>0.383 ± 17.5</td>
<td>368.7 ± 30.8</td>
<td>99</td>
</tr>
<tr>
<td>Control, two single microelectrodes</td>
<td>100</td>
<td>0.208 ± 18.6</td>
<td>112.0 ± 25.1</td>
<td>25</td>
</tr>
<tr>
<td>Control, λ = 477 ± 53 μ</td>
<td>100</td>
<td>377.6 ± 84.8</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>NaHCO₃, 5%</td>
<td>60</td>
<td>0.164 ± 19.2</td>
<td>111.1 ± 30.5</td>
<td>12</td>
</tr>
<tr>
<td>KCl, 0.15 M</td>
<td>100</td>
<td>0.265 ± 17.8</td>
<td>177.4 ± 25.5</td>
<td>37</td>
</tr>
<tr>
<td>CO₂, 15%</td>
<td>100</td>
<td>0.367 ± 36.4</td>
<td>349.9 ± 74.3</td>
<td>21</td>
</tr>
</tbody>
</table>

Rs and Rs values are means ± SE. n = number of observations.

### Table 7. Specific wall resistances of distal tubular epithelium during luminal perfusion with 75 mEq/liter K⁺-solutions

<table>
<thead>
<tr>
<th>Exptl Condition</th>
<th>pH 5.5</th>
<th>pH 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs, ohm cm²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, outward current*</td>
<td>90.5 ± 21.5</td>
<td>91.2 ± 21.7</td>
</tr>
<tr>
<td>Control, inward current</td>
<td>117.4 ± 23.3</td>
<td>115.6 ± 21.0</td>
</tr>
<tr>
<td>NaHCO₃, 5%</td>
<td>63.8 ± 16.3</td>
<td>54.8 ± 13.6</td>
</tr>
<tr>
<td>KCl, 0.15 M</td>
<td>95.1 ± 16.0</td>
<td>84.6 ± 18.1</td>
</tr>
<tr>
<td>CO₂, 15%</td>
<td>98.3 ± 16.2</td>
<td>86.7 ± 15.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. n = number of observations. * From lumen to peritubular fluid.
cells (14) and strongly suggests that the electrode tip in the present experiments was indeed located within single distal tubule cells. Accepting the criteria outlined, a mean intracellular PD of $-71.1 \pm 1.38$ mV ($n = 57$) was obtained in control rats. Wright has reported similar values (58). In rats receiving 5% NaHCO$_3$ the measured potential difference was quite similar and averaged $-72.7 \pm 1.29$ mV ($n = 19$).

**Discussion**

A comparison of the transepithelial resistance values across the distal tubule of the rat indicates that this value (of the order of 350 ohm cm$^2$) is intermediate between resistance values reported for the proximal convoluted tubule and the collecting duct. Considerably lower specific resistance values ranging between 5 and 17 ohm cm$^2$ have been observed across the proximal tubule of the rat (11, 20) and the dog (5, 45). On the other hand, values in excess of 1,000 ohm cm$^2$ have been found across isolated cortical collecting ducts of the rabbit (6). Proximal transepithelial resistances are higher in amphibian tubular structures (4, 31, 54). Comparable distal tubular specific resistance values are not available in amphibian species.

Transepithelial resistance measurements are of interest in at least two ways. First, specific resistance measurements can give an estimate of the relative contribution of sodium, potassium, and chloride to the total conductance of the tubular wall. A second point of interest is that knowledge of the magnitude of the specific ionic conductance across the distal tubular wall permits a quantitative evaluation of passive transport mechanisms. Passive ion movement accounts for net reabsorption or secretion if the electrochemical potential gradient is favorable for passive ion movement and if the transepithelial specific ion conductance is large enough to allow for the net transfer of an ion at a rate at least equal to that observed. An evaluation concerning distal potassium and chloride transfer has been carried out and will be discussed below.

The experimental results presented indicate significant transepithelial conductances of the distal tubular wall of potassium and sodium but a smaller one of chloride. It has also been observed that the conductances measured at different concentrations for an ion species change markedly from high values at high luminal concentrations to low values at low ion concentrations. The observed relationship between intratubular ion concentration and transepithelial conductance is of special interest in the case of potassium and chloride since a component of passive ion transfer is known to be involved in their distal tubular transport mechanism.

Evidence is available indicating that a step of passive potassium movement across the luminal cell membrane into the tubular lumen is down an electrochemical potential gradient and sensitive to changes in the transepithelial potential difference (21, 37–40). Accordingly, distal transtubular potassium movement is likely to be affected by changes in potassium conductance of some component of the tubular wall. Concentration-dependent conductance changes may play a role in sustaining a high distal tubular potassium secretory rate such as observed during potassium, sodium, or bicarbonate loading (37, 38, 40).

Similar considerations may also apply to distal tubular chloride movement. Thus, at negative intratubular potential values it would be expected that the electrical potential difference across the distal tubule should be more effective in inducing passive chloride reabsorption during conditions of high tubular chloride content within the distal tubule. Such conditions are indeed frequently associated with enhanced distal tubular chloride reabsorption. A pertinent example is extracellular volume expansion by sodium chloride loading (36): the increased transepithelial conductance could initiate enhanced passive chloride transfer from lumen to peritubular fluid.

A comparison between the fraction of the total transepithelial current carried by an ion and its net movement provides information concerning the question whether passive ion transfer could account for the observed rate of net ion movement. The data obtained in the present study permit such an analysis with respect to distal tubular potassium and chloride transport.

With respect to potassium movement, net potassium secretion under control conditions can be calculated from published micropuncture data (38), taking a value of $4 \times 10^{-8}$ liter/min for single-nephron GFR, an early-distal TF/P inulin ratio of 4, and the early distal tubular potassium concentration to be 1.0 mEq/liter.

The amount of potassium leaving the distal tubule and entering the collecting duct can be similarly evaluated taking a late-distal inulin TF/P value of 20 and a late-distal tubular potassium concentration of 15 mEq/liter:

$$K_{exit} = \frac{4 \times 10^{-8}}{20} \text{ liter/min} \times 15.0 \text{ mEq/liter}$$

$$= 3 \times 10^{-9} \text{ mEq/min}$$

Accordingly, an amount equivalent to the difference between $K_{exit}$ and $K_{entry}$, i.e., $2.0 \times 10^{-8}$ mEq/min represents a mean rate of distal tubular potassium secretion under nondiuretic conditions.

The possible distal tubular secretion rate of potassium expected for passive potassium entry into the lumen can be calculated from electrical measurements:

$$J_K = \frac{\nabla_K}{R_K}$$

where $J_K$ is the passive potassium current, $\nabla_K$ is the mean electrochemical potential difference favoring potassium secretion, and $R_K$ is the specific transepithelial potassium resistance.

A value of 400 ohm cm$^2$ was obtained (see Table 4) for the specific potassium resistance, corresponding to intratubular potassium concentrations similar to those found under free flow conditions.

The mean electrochemical potential difference, $\nabla_K$, which drives potassium movement, is calculated from the
difference between $\bar{E}$, the mean distal tubular PD (lumen negative) favoring tubular potassium entry, and $E_K$, the mean chemical potential difference opposing potassium secretion (mean $[K^{+}]_{\text{tubule}} > \text{mean }[K^{+}]_{\text{plasma}}$) according to the following equation:

$$\bar{V}_K = E - E_K$$

where

$$E_K = \frac{RT}{F} \ln \frac{K_t}{K_p}$$

and $K_t$ = tubular potassium concentration and $K_p$ = plasma potassium concentration. Since the electrochemical potential of potassium is not a linear function of the potassium concentration, the mean $E_K$ ($E_K$) is the mean of the value of this function, calculated as the integral of $E_K$ from early- to late-distal tubule divided by the concentration difference between these sites.

$$E_K = \frac{1}{K_{te} - K_{tl}} \int_{K_{te}}^{K_{tl}} \frac{RT}{F} \ln \frac{K_t}{K_p} dK_t$$

A useful expression is

$$E_K = \frac{RT}{K_{te} - K_{tl}} \left[ K_{tl} \ln \frac{K_{tl}}{K_p} - (K_{tl} - K_{te}) - K_{te} \ln \frac{K_{te}}{K_p} \right]$$

Thus, $\bar{V}_K = 33.2 - 13.7 = 19.5$ mv. The value of 33.2 mv is the mean of early (-19.3 mv) and late (-47.2 mv) distal tubular potential values.

From the calculated mean electrochemical potential difference of 19.5 mv and the specific potassium resistance of 400 ohm cm², and transforming potassium current into ion flow (distal tubular surface area $1.32 \times 10^{-8}$ cm², assuming a tubular diameter of 25 µ and a tubular length of 1.7 mm which corresponds to 85% of the distal tubule), one obtains a potassium influx into the tubule of $3.55 \times 10^{-8}$ mEq/min. This value is considerably higher than the observed secretory rate of $2.0 \times 10^{-8}$ mEq/min and indicates that the existing favorable electrochemical potential difference could drive potassium passively from the peritubular space into the distal tubular lumen. It should be emphasized that this observation does not exclude an active transport component to participate in distal tubular potassium transfer.

Applying considerations of this kind of chloride, a mean electrochemical potential gradient (assuming an early distal concentration of 60 mEq/liter and a late value of 20 mEq/liter (40)) of 8.6 mv favorable for CI reabsorption can be calculated. A value of 1,123 ohm cm² was used for specific chloride resistance (see Table 4). These values yield a passive chloride outflow of $0.64 \times 10^{-8}$ mEq/min per tubule. The actual amount of CI reabsorbed per tubule, however, is of the order of $56 \times 10^{-8}$ mEq/min per tubule. Thus, despite the existence of a favorable electrochemical potential gradient, this is not a driving force sufficient to account for the observed distal tubular chloride reabsorption. The observed discrepancy appears to be of sufficient magnitude to be of significance. Therefore, in this case an active reabsorptive transport would have to be invoked. These conclusions are consistent with experimental work suggesting that chloride ions may not solely distribute themselves passively across the various segments of the renal tubule (23, 24, 37, 42, 58).

The finding of a decreased transepithelial resistance of distal tubules of rats undergoing KC1 or Na2CO3 diuresis is of significance. The increased potassium secretion which is observed under these conditions may be due, in part, to the greater ease with which potassium ions will be driven into the distal tubular lumen by the transepithelial electrical potential difference.

The influence of changes in hydrogen ion concentration or that of sodium, potassium, and chloride on electrical membrane properties has been studied in a variety of preparations. Of interest are studies in frog muscle showing that a decrease in external pH leads to a fall in chloride (26, 32) and sodium conductance (34, 43). With respect to potassium a dependence of its conductance on the direction of its chemical potential difference has been reported in frog muscle (2, 29). In frog skin, an increase in external sodium concentration decreases the passive permeability for sodium (7) and the chloride permeability has been found reduced in the same preparation in the absence of external sodium (35).

It was considered possible that acid-base-induced changes in distal tubular potassium secretion could be mediated by passive permeability changes of distal tubular cell membranes. Thus, increased secretion could have been evoked by an increased permeability of the luminal cell membrane of distal tubule cells and the enhanced passive leakage of potassium ions into the tubular lumen. Experimental evidence obtained in gallbladder epithelium (56) and in muscle fibers (26, 32, 43) indicates that changes in the hydrogen ion concentration have significant effects on passive ionic conductances. In the present study we were unable to detect significant changes in the transepithelial conductances or potential differences when pH was changed within the lumen from 3.5 to 8.0. It is possible that, as was observed in the case of the rabbit gallbladder (56) and of muscle fibers (26), the isoelectric point of the luminal membrane might be relatively low. Wright and Diamond (56) have found in the gallbladder that conductance changes observed on changing pH could be explained by assuming
fixed charges in this membrane or in the interspace region which were negative at physiological pH values. This would explain the observation of relatively higher cation than anion permeability. These investigators also observed that on reduction of the pH below 3 (the isoelectric point of this membrane) positive charges would predominate and result in the chloride conductance exceeding that of cations. If the isoelectric point of the fixed charges of distal tubular membranes would similarly lie outside the physiological pH range, no significant alterations in membrane resistance would be expected during pH changes in the range of 5.5–8.0. It is of interest that the relative conductances for ions in the gallbladder epithelium are qualitatively similar to our data for the distal tubule, being in the order K > Na > Cl. In view of these negative findings concerning the pH-sensitivity of the distal tubular potassium conduction, it is unlikely that the very extensive change in the rate of distal tubular potassium secretion which is observed during different acid-base conditions of the rat (40) is mediated by pH-related alterations of the passive potassium permeability of distal tubular cell membranes.

The present data confirm previous conclusions that the magnitude of the distal transepithelial potential difference depends on the concentration of sodium and potassium ions in the tubular lumen (22). In an extensive microperefusion study of distal rat tubules these conclusions were recently confirmed by Wright (58). The observation that a sudden reduction of the sodium and potassium concentration in the distal tubular lumen is associated with a significant reduction of the transepithelial potential difference is consistent with the view that diffusion potentials contribute to the generation of the electrical potential difference across the distal tubule (3, 20, 22, 55). According to our view the distal tubule cell is asymmetrically polarized because the potential difference across the luminal is different from that of the peritubular cell membrane. Across the peritubular membrane, potassium ions are the main ions contributing to the diffusion potential, rendering the cell inside electrically negative by some 70 mV. Other cations contribute much less to passive ion current at this site.

An electrical potential difference of −25 to −50 mV is maintained across the luminal cell membrane. The variation of the extent of this relative depolarization with tubular length results in a larger transepithelial potential difference across the second half of the distal tubule (58). The luminal cell membrane appears to be less selective in discriminating passive ion movement. In the first place there is a significant potassium conductance which should make the cell interior electrically negative with respect to the tubular lumen ([K+]lumen > [K+]lumen). In addition, the sodium conductance across the luminal cell membrane is higher than across the peritubular cell membrane. This allows for a greater contribution of sodium ions to the total membrane current. In view of the direction of the concentration difference between lumen and cell interior ([Na+]lumen > [Na+]lumens) under normal free flow conditions (22) a significant sodium permeability is expected to lower the electrical potential difference across the luminal cell membrane. This effect may become even more pronounced when, at very low intratubular sodium concentrations, the direction of the sodium concentration gradient may even reverse ([Na+]lumen > [Na+]lumens).

The results of the present study are in general agreement with the role assigned to various ion species in the generation of the transepithelial potential difference. However, it is clear that a number of assumptions are made using the present approach to evaluate tubular membrane permeabilities. These deserve comment. 1) It is assumed that diffusion potentials participate in the electrical potential differences measured. Supportive evidence that the electrical potential changes after sudden luminal alterations in sodium and potassium concentration involve alterations in the magnitude of diffusion potentials is provided by the observation that transepithelial resistance changes similarly reflect very significant contributions of these ion species to transepithelial currents. 2) It is assumed that the transepithelial electrical potential changes subsequent to luminal ion substitutions are against significant changes in ionic cell composition. With respect to the luminal membrane being involved in the potential changes observed on luminal ion substitutions the observation that the peritubular potential difference underwent only minor changes on luminal alterations in the ion content is pertinent. This finding strongly suggests that the potential change recorded across the distal tubular epithelium reflects changes in the potential difference across the luminal cell membrane. However, in view of the technical difficulties involved in recording stable cell potentials this conclusion is based on only a relatively small number of successful experiments. Also, the data summarized in Table 5 indicate that transepithelial conductances are primarily determined by changes in the conductance of the luminal cell membrane. This conclusion is based on the finding that peritubular perfusions with different concentrations of permeant ion species other than potassium do not noticeably affect transtubular conductances. 3) In the present treatment the existence of electrogenic pumps, i.e., a direct contribution of active transport to the membrane potential and the possible effect of ionic concentration changes on their activity was disregarded. This may be a simplification but there is presently no evidence in support of a direct contribution of any pump mechanism to the generation of distal tubular potential differences.

The present study permits a number of general conclusions. The distal tubular epithelium has a higher cation than
anion permeability and the transepithelial potassium conductance greatly exceeds that of other ion species. Both choline and sulfate permeate the distal tubules and cannot be considered impermeant ion species. In the interpretation of transepithelial potential changes subsequent to a variety of ionic substitutions, the finite conductances of these ion species must be taken into account.

Evidence presented in this study also supports our previous view (22) that in the generation of distal transepithelial potential differences permeability differences between the peritubular and luminal cell membrane play an important role. An essential feature is the larger permeability of the luminal cell boundary to sodium ions and its relatively low chloride conductance. The latter is significant in at least two ways. First, a low chloride conductance makes it highly unlikely that distal tubular chloride reabsorption solely results from passive movement driven by the electrical potential difference, a finding which again raises the possibility of active chloride reabsorption. Second, the low chloride permeability is of significance with respect to the distal tubular secretory mechanism of potassium ions. Were it not for a low distal tubular chloride conductance, potassium movement from the peritubular to the luminal fluid could be coupled to chloride secretion rather than to sodium reabsorption. However, it should be emphasized that a significant albeit small chloride conductance makes it possible that part of the potassium moiety entering the distal tubule is accompanied by chloride ions. Absence of net chloride secretion across the distal tubule is not a cogent argument against potassium chloride secretion since this fraction of the unidirectional secretory movement of chloride could be matched by a higher reabsorptive chloride flux resulting in net reabsorption. Additional experiments will be necessary to obtain quantitative information on the relative role of sodium reabsorption and chloride secretion in the overall process of distal tubular potassium secretion.

In contrast to the proximal tubular epithelium, which is relatively unselective with respect to cationic and anionic permeation (43), the distal tubule as a whole shows considerably greater ionic discrimination. Thus, recent evidence by Boulpaep and Seely (5) shows that the proximal tubular potassium permeability is only 2.2 times larger than that of choline, and that of sodium exceeds that of chloride by a factor of only 1.38. Much larger values (>8) are obtained in the distal tubule when measured in the range of normal potential values. The fact that both the total transepithelial resistance and ionic selectivity are higher in the distal than the proximal tubule suggests that nondiscriminative leak paths determine to a lesser degree the overall transepithelial permeability properties across the distal nephron.

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