Function of the thick ascending limb of Henle’s loop

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BURG, MAURICE, AND NORDICA GREEN. Function of the thick ascending limb of Henle’s loop. Am. J. Physiol. 224(3): 659-668, 1973.—The thick ascending limb of Henle’s loop was dissected from rabbit kidneys and perfused in vitro. There was net NaCl absorption from the tubule lumen with resultant lowering of lumen NaCl concentration below that of the bath. Since the electrical PD was positive in the lumen, Cl is absorbed actively. Na absorption is largely passive as indicated by comparison of 1) Na flux ratio and PD (at fast perfusion rates); and 2) Na concentration ratio and PD (at slow perfusion rates). However, the possibility of some active Na transport is not ruled out. The PD results from Cl transport. It is eliminated reversibly by removal of Cl from bath and lumen (SO4 substitution) and by ouabain. The PD is increased by removal of Na from bath and lumen (choline substitution). Na permeability is greater than Cl permeability, as indicated by 1) the NaCl diffusion potential that is lumen positive when lumen NaCl concentration is low, and 2) radioisotope measurements of ion fluxes. Water permeability (Lw) is low, which combined with the NaCl transport accounts for the dilution of the urine in this segment.

NaCl transport; renal tubule; potential difference; electrical resistance; active chloride transport

THE THICK ASCENDING LIMB of Henle’s loop is the nephron segment in which lumen fluid is diluted by absorption of NaCl in excess of water. This transport property is important for both urinary dilution and concentration and accounts for a substantial fraction of the total NaCl reabsorption from the nephron. The thick ascending limb of Henle’s loop had not been previously studied directly since it is buried within the kidney substance and is inaccessible to micropuncture. Its function was deduced from the finding by micropuncture that the fluid in the lumen of the early distal convoluted tubule is dilute (33, 37). Changes in urine concentration and dilution measured in clearance studies have been used as an index of its function. These methods are indirect and do not permit a detailed analysis of the transport mechanisms in this segment.

We had previously dissected thick ascending limbs of Henle’s loops out of rabbit kidneys (7). We found that the nonperfused tubules survive in vitro, that they maintain a high cellular K content, that the cellular K exchanges rapidly with K radioisotope in the external solution, and that K content decreases when ouabain is added (7). In the present studies, isolated thick ascending limbs of Henle’s loop have been perfused in vitro in order to analyze directly the transepithelial NaCl and water transport in this nephron segment.

METHODS

The method employed in these studies has previously been described in detail (8) and is summarized below with additions and modifications.

Female rabbits weighing approximately 1.5 kg were decapitated. Fragments of the thick ascending limb of Henle’s loop were dissected and perfused in vitro under a variety of conditions that correspond to those previously used for studying other segments of the nephron (10, 16). For the majority of the studies, the tubules were dissected at room temperature in NaCl/HCO3 buffer (defined below) plus 5% v/v rabbit serum and the identical solution was used to bathe the tubules during perfusion. The addition of 5% v/v of rabbit serum was helpful since the tubules adhered less to instruments and surfaces in its presence. However, serum was not essential and when it was omitted from the bath during perfusion the tubules appeared to survive well. The tubules were generally 1–2 mm long, except for studies of electrical resistance, where shorter lengths were used.

The dissected tubules were transferred to a temperature-controlled chamber (37°C) and were observed through an inverted biological microscope at X25–400 magnification during perfusion. The micropipet systems used for perfusion and collection were modified (6) from those used earlier: 1) separate concentric outer pipets containing Sylgard 184 were used to insulate both ends of the tubule; 2) volumetric constriction pipets were used for all collections; 3) a concentric pipet within the perfusion pipet and extending to its tip was used to change lumen fluid during perfusion. The exchange of perfusion fluid was complete within a few seconds as judged by changes of resistance of the pipet tip when solutions of different resistivity were used and by measurements of radioisotopes in the collected perfusate.

The NaCl/HCO3 buffer (composition in mM: NaCl, 115; NaHCO3, 25; Na acetate, 10; KCl, 5; CaCl2, 1.0; MgSO4, 1.2; Na2HPO4, 1.0; glucose, 5.5) was equilibrated by bubbling with 5% CO2 + 95% O2, and was continuously bubbled with the same gas mixture in the dissecting dish or perfusion chamber. Rabbit serum, 5% v/v, was added to this buffer for use in the bath and dissecting dish, but not when it was used as a perfusion solution. The NaCl/HPO4 buffer (composition in mM: NaCl, 150; K2HPO4, 2.5; CaCl2, 1.0; MgSO4, 1.2) was titrated to pH 7.4 with HCl and was bubbled with 100% O2 while in the bath. This buffer was always used without added serum. In some experiments the NaCl concentration of either buffer was decreased (as indicated) for use as a perfusion solution.

Perfusions were carried out either by gravity or with a
 Fluid absorption \( J_v \) was measured using inulin-\(^{34}\)C\(_2\) (New England Nuclear Corp.) as a volume marker:

\[
J_v = \frac{V_L}{L} \left( \frac{[\text{C}]_L}{[\text{C}]_0} - 1 \right)
\]

where \( V_L \) is the collection rate (volume of the constriction pipet \( \pm \) time for collection), \( L \) is the length of the tubule, and \([\text{C}]_L\) and \([\text{C}]_0\) are the concentrations of \(^{34}\)C in the collected and perfused fluid, respectively. \(^{34}\)C was also measured in the bath and the leak of fluid (and/or inulin) was found to be negligibly small (mean 0.02 nl mm\(^{-1}\) tubule length min\(^{-1}\)).

Electrical PD was measured between calomel cells connected to the fluid in the bath and perfusion pipet with bridges containing 0.16 NaCl agar (11). The PD was displayed on a Tektronix oscilloscope connected via a Bak ELSA-3 or ELSA-4 electrometer. In experiments testing the effects of different conditions the PD was first measured under a control condition (usually equal Na concentration in lumen and bath with flow in the lumen) and the control was repeated after testing each experimental variable. All experimental changes were immediately reversible except after the removal of ouabain which took longer (Fig. 6). As previously (18), electrical resistance was calculated by cable analysis using the voltage change at both ends of the resistance measurement, short lengths of tubule (150-400 \( \mu \)m) were used.

Ionic fluxes were measured with radioisotopes using \(^{36}\)Cl, \(^{22}\)Na, and \(^{3}H\) (ICN). For bidirectional Na fluxes there were two different protocols:

1) In the first, the perfusion rate was sufficiently rapid (15-50 nl min\(^{-1}\)) to minimize the decrease \( (< 10\% \) in the concentration of isotope in the fluid perfused through the lumen (Table 7, experiments 1-4). Lumen-to-bath (lb) and bath-to-lumen (bl) fluxes were measured separately in alternate sets of three periods, either lb-bl-bl (Table 7, experiments 1 and 3) or bl-bl-bl (Table 7, experiments 2 and 4). For lumen-to-bath flux \( J_{Na,lb} \), \(^{22}\)Na (50 \( \mu \)c/ml) was added to the perfusate, and the counts appearing in the bath were measured. Assuming that the electrical PD along the lumen is constant, the equation previously (16) used to calculate permeability of a nonelectrolyte with no net fluid absorption can be modified to calculate ion flux:

\[
J_{Na,lb} = \frac{V_{Na}}{L} \ln \left( \frac{[Na]_b}{[Na]_0} \right)
\]

where \( J_{Na,lb} \) is the flux from lumen-to-bath per unit tubule length of ion i for lumen concentration of i equal to \([i]_0\) (the concentration in the perfusate), V is the perfusion rate, L is the length of the tubule, \([C]_0\) is the concentration of the radioisotope of i perfused, and \([C]_L\) is the concentration of isotope collected from the lumen. This equation was modified for measuring \( J_{Na,bl} \) in the present experiments. Assuming that in a steady state the rate of appearance of isotope in the bath equals its rate of disappearance from the lumen,

\[
J_{Na,bl} = -\frac{V_{Na}}{L} \ln \left[ 1 - \frac{C_b}{C_0} \cdot \frac{t_0}{t_b} \right]
\]

where \( C_0 \) is the number of counts perfused in time \( t_0 \), \( C_b \) is the number of counts appearing in the simultaneous bath collection during time \( t_b \) and \([Na]_0 \) is the Na concentration in the perfusate. This equation was used since at fast perfusion rates \([C]_b \) approaches \([C]_0 \) and \([C]_b/[C]_L \) (equation 1) cannot be measured as accurately as can \( C_b \) and the other terms in equation 2.

2) In the second method, a slower flow rate was used (6-10 nl min\(^{-1}\)) and the bidirectional fluxes were measured simultaneously (Table 7, experiments 5-9). \(^{36}\)Cl (50 \( \mu \)c/ml) was perfused and \( J_{Na,lb} \) calculated using equation 1. \(^{36}\)Cl was present simultaneously in the bath and \( J_{Na,bl} \) was calculated using equation 3.

Chloride flux from bath to lumen, \( J_{Cl,bl} \), was measured by placing \(^{36}\)Cl (20 \( \mu \)c/ml) in the bath and measuring its appearance in the lumen at fast perfusion rates.

\[
J_{Cl,bl} = \frac{V_{[Cl]}[Cl]_L}{L[Cl]_b}
\]

where \([Cl]_L\) is the chloride concentration in the lumen. The use of this equation implies that there is no loss of \(^{36}\)Cl once it enters the tubule lumen. Since at the flow rates used (19-27 nl min\(^{-1}\)), the mean value of \([Cl]_L/[Cl]_b \) was 0.054, the loss of \(^{36}\)Cl from the lumen is negligibly small.

The radioisotopes were measured with either a Packard well scintillation counter \((^{22}\)Na, \(^{36}\)Na) or Packard liquid scintillation counter using Multisol fluid (Isolab Incorporated) \((^{3}H, ^{36}\)Cl, or \(^{14}\)C).

Results are given as means \pm standard errors (numbers of tubules studied).

RESULTS

Anatomy. The part of the thick ascending limb of Henle’s loop studied is indicated by the arrows in the diagram in Fig. 1. It extends from the outer medulla to the macula densa of cortical nephrons and was described by Sperber (31) as being thinner and more transparent than the part...
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PIG. 1. Diagram of rabbit nephrons (modified from (25)). Segment of thick ascending limb of Henle’s loop used in present studies is indicated by arrows.

of the thick ascending limb which precedes it (shown in black in the diagram in Fig. 1). This thinner segment runs through the cortex in bundles with the other straight segments (straight proximal tubule and cortical collecting tubule) but is easily distinguished from them by its small size when collapsed (see Fig. 5 in (8)). During perfusion (Fig. 2), the lumen diameter is similar to that of other nephron segments, but the cells are thin, accounting for the small diameter when collapsed. It is difficult to separate the thick ascending limbs from other segments (especially the cortical collecting tubules) without breaking the ascending limbs. Therefore, the perfused segments often had pieces of collecting tubule or other thick ascending limb adherent to them during perfusion.

**NaCl absorption.** Samples of tubule fluid have been obtained by micropuncture of various species (but not rabbits) from near the tip of Henle’s loop (14, 15) and from the distal convoluted tubule (35-37). Over this interval (which includes the portion of the thick ascending limb studied here) NaCl concentration and osmolality decrease from values much higher than the peripheral plasma to much lower, and it is generally accepted that the thick ascending limb must absorb NaCl from the tubule lumen without accompanying movement of water. Therefore, the initial experiments were designed to see if this transport system survives in the isolated tubules and to determine the optimal experimental conditions. Tubules were perfused at a slow rate (0.4-1.75 nl mm⁻¹ tubule length min⁻¹) with fluid of NaCl concentration similar to that in the bath and the Na concentration in the collected fluid was measured. This was uniformly decreased below that in the perfusate and bath (Fig. 3), confirming that the ascending limb reabsorbs Na in vitro as previously noted in vivo. No major difference was apparent between the different conditions of dissection and perfusion. The mean concentration of Na in the collected fluid was 96.4 mEq liter⁻¹ compared to 137-159 mEq liter⁻¹ perfused. The mean concentration of Na found by micropuncture in the distal convoluted tubule of other species is lower (35-60 mEq liter⁻¹ (1, 2, 24)). The tubule fragments used in the present studies, however, are less than the total length of the ascending limb, and it is conceivable that Na concentration might decrease further were the tubules longer (or perfused more slowly). Consistent with this possibility is the observation that Na concentration in collected fluid is correlated with perfusion rate per tubule length (Fig. 3). Cl concentration was measured in other experiments (Table 1) and decreased to a comparable extent indicating that NaCl is transported out of the tubule lumen, as in vivo.

**Water permeability.** For NaCl transport to result in a fall in concentration in the thick ascending limb of Henle’s loop, the tubule must be relatively impermeable to water. Water permeability (Lp) was calculated from net fluid absorption in the presence of an osmotic gradient (Table 2). With the lumen fluid dilute (180-185 mOsm kg⁻¹ less than the bath) fluid absorption was very low (0.13 nl mm⁻¹ min⁻¹). The Na concentration, and thus also the osmolality, of the tubule fluid changed very little when fluid with the identical

FIG. 1. Diagram of rabbit nephrons (modified from (25)). Segment of thick ascending limb of Henle’s loop used in present studies is indicated by arrows.

FIG. 2. Photomicrograph of a thick ascending limb of Henle’s loop during perfusion. Note that cells are extremely thin.

Electrical potential difference and resistance. An electrical PD is present across the epithelium of the thick ascending limb of Henle’s loop. When the tubule was undisturbed the PD remained stable for period of up to 4 hr. The PD is oriented lumen positive and with equal Na concentration of 150 mEq liter⁻¹ in the lumen and bath the PD is +3 to +9 mv (Tables 3, 4, and 6, and Fig. 1). In view of the orientation of the PD, Cl transport must be active since it opposes both the chemical and electrical gradients. Na transport, on the other hand, could be passive since the electrical gradient is favorable. Whether there is active as well as passive net Na transport will be considered in detail below.

There is an uncertainty of a few millivolts in the measurement of the PD in individual tubules. When the tubules were first entered a PD appeared. Advancing the perfusion pipet down the tubule lumen and simultaneously advancing the Sylgard seal around the tubule often resulted in a higher PD. The pipet was advanced until a stable PD was achieved and this PD usually remained constant. At times the PD declined spontaneously during changes of the bath but could usually be restored by adjusting the perfusion end of the tubule or by advancing the pipet a small distance further. In some tubules it was possible to test the uniformity of the lumen PD by advancing the perfusion pipet further down the lumen at the end of the experiment. The maximum additional distance advanced was 200–800 μ. Generally, there was no change or a small increase (<3 mv). The fact that the PD measured at the beginning of some tubules may be a few millivolts different from the mean PD down the length of the lumen, however, is a potential source of error which should be considered in evaluating studies where the mean lumen PD is important.

The transepithelial electrical resistance of the thick ascending limb is comparatively low. The value of 3,430–3,920 ohm cm (Table 3) is closer to that of isolated rabbit proximal convoluted tubule (917 ohm cm (22)) than of isolated rabbit cortical collecting tubule (138,000 ohm cm (18)). The space constant is short (10⁻⁹–12 p), indicating that the electrical resistance along the tubule lumen is high relative to that through the tubule wall. This provides an explanation for the observation that the PD measured at the beginning of some tubules may be a few millivolts different from the mean PD down the length of the lumen, however, is a potential source of error which should be considered in evaluating studies where the mean lumen PD is important.

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The transepithelial PD cannot be precisely evaluated without considering the liquid junction potential, which is the sum of the preexisting liquid junction potential and a diffusion potential across the tubule epithelium. A PD was +7 mV. With 50 mEq liter⁻¹ of NaCl in the lumen, the concentration was decreased (Fig. 4). With equal NaCl concentration in lumen and bath (150 mEq liter⁻¹), the mean PD increased. This increase is explained by the assumption that the PD resulting from active Cl transport is normally shunted by Na ions transported passively across the epithelium. When this shunt is diminished by replacement of Na by choline (which is presumably less permeant) there is less shunting and the PD increases.

The PD also increased when the perfusate NaCl concentration was decreased (Fig. 4). With equal NaCl concentration in lumen and bath (150 mEq liter⁻¹), the mean PD was +7 mV. With 50 mEq liter⁻¹ of NaCl in the lumen, the mean PD was +25 mV. There are at least two possible causes for the increase in PD: a liquid junctional potential and a diffusion potential across the tubule epithelium. A liquid junction potential exists between the perfusion solution and the bath when they differ in ionic composition. When the tubule is interposed, the PD which is measured is the sum of the preexisting liquid junction potential and actual transmural PD. The transepithelial PD cannot be precisely evaluated without considering the liquid junction potential which cannot be measured directly. The liquid junction PD can be calculated for simple solutions, using the modified Henderson equation (23):

\[ PD = \frac{\mu Cl - \mu Na}{\mu Cl + \mu Na} \cdot 60 \log \frac{[NaCl]_o}{[NaCl]_b} \]

where \( \mu Cl \) and \( \mu Na \) are the mobilities of the ions in free solution and [NaCl]₀ and [NaCl]ₜ are the concentrations in the perfusate and bath, respectively. (The actual liquid junction potential is less than that calculated for NaCl in this manner because of the presence of ions other than NaCl in equal concentration in the bath and perfusate.) By this calculation the liquid junction potential for 50 mEq liter⁻¹ of NaCl in the perfusate (which is the maximum potential expected in these studies) is -5 mV. Because the magnitude of the liquid junction potential is relatively small and is uncertain, we have not attempted to correct the data for it. Therefore, keep in mind that the actual transmural PD with dilute solutions in the lumen is a few millivolts less than that recorded. The second and more important cause of the increase in PD with dilute solutions in the lumen is the diffusion potential for NaCl across the epithelium. From the orientation of the change in PD (lumen more positive) it is evident that the tubule must be more permeable to Na than to Cl. All other things being equal, the ratio of the permeabilities can be estimated using equation 3 and substituting ion permeability across the membrane for mobility in free solution if it is assumed that the epithelium acts as a single uncharged membrane with equal partition coefficients for the ions. From Fig. 4 the change in PD with 50 mEq liter⁻¹ in the lumen is +18 mV of which approximately +5 mV is liquid junction potential. Assuming the difference of +13 mV is the diffusion potential, \( P_{Na}/P_{Cl} = 2.7 \). Evidently, this must be regarded as an approximation because of the number of assumptions made. Additional evidence for Na permeability exceeding that of Cl in the thick ascending limbs is contained in the radioisotope tracer studies considered below.

When flow stopped in the tubule lumen, the PD rose over a period of approximately 20-40 sec to a mean value of +29 mV, independent of the concentration of NaCl originally in the lumen (Fig. 4). When flow started again,
the PD returned instantaneously to the prior value. The most likely cause of this increase in PD is that NaCl reabsorption continued during stop flow. Then, the resultant lowering of the lumen NaCl concentration caused a diffusion potential (and a liquid junction potential) to appear as above. If this reasoning is correct, the steady-state Na concentration in the lumens without flow is approximately 50 mEq liter⁻¹.

**Is there active Na transport?** Because the PD is positive in the lumen there must be active Cl and passive Na transport. Is there also active Na transport, and, if so, how much? Two methods were used in an attempt to answer this question: 1) comparison of the stop-flow PD to the measured steady-state lumens Na concentration, and 2) comparison of the free-flow PD to the Na flux ratio measured with radioisotopes.

As indicated above, the PD rises to a steady value when flow is stopped and the increase in PD is most likely due to lowering of the lumen NaCl concentration. If Na transport is entirely passive, the steady-state NaCl concentration in the lumen must relate to the steady-state (stop-flow) PD according to the Nernst equation. In order to measure the steady state Na concentration for a given tubule, the perfusate was diluted by removal of NaCl to levels approximating the expected steady-state Na concentration and the tubules were perfused at a slow rate (1-2 nl min⁻¹). There was little change in Na concentration in the collected fluid (Table 5, Fig. 5) compared to that observed when higher concentrations of NaCl were perfused (Fig. 1). This indicates that the concentrations of NaCl perfused are close to the steady-state value. When tubules were perfused with fluid containing 75-87 mEq liter⁻¹ of Na (Table 5 and Fig. 5), the Na concentration generally fell. When the perfusate contained 49-52 mEq liter⁻¹ of Na, the Na concentration increased. Therefore the steady-state lumen Na concentration is between these values. For each experiment the equilibrium PD (Eₙₐ) according to the Nernst equation for the ratio of Na concentration in the collected fluid [Na]ₗ to Na concentration in the bath [Na]ₐ is given. In general Eₙₐ was in good agreement with the stop-flow PD. The mean value of Eₙₐ (21.2 mv) is approximately equal to the mean value for the stop-flow PD (24.8 mv) (note that the difference is similar to the expected liquid junction potential). Therefore, there is no measurable active Na transport in these experiments.

An incidental finding was that the stop-flow PD appeared to be lower and steady-state Na concentration appeared to be higher in tubules with HCO₃-containing perfusate (experiments 1-4, Table 5), than with HCO₃-free perfusate (experiments 5-9, Table 5). The difference with regards to PD was confirmed in separate experiments (Table 6). When the same tubule was perfused with both HCO₃-containing and HCO₃-free solutions there was no difference in the free flow PD, but the stop-flow PD was greater without HCO₃ in the lumen (Table 6). In view of these results we suggest that the thick ascending limb of Henle’s loop absorbs NaHCO₃ poorly, if at all, and that this may account in part for the diminished urine concentration (32) and diluting (28) ability previously observed during metabolic alkalosis.

The second method used to test for active Na transport was a comparison of the Na flux ratio to the PD. According to the flux ratio equation (33), for passive monovalent ion transport:

$$J_{Na,lb} = \frac{[Na]_b}{[Na]_l} e^{m/PD}$$  \hspace{1cm} (6)

For equal concentrations of Na in the lumen and bath:

$$PD = -60 \log \left[ \frac{J_{Na,b1}}{J_{Na,b1}} \right]$$  \hspace{1cm} (7)

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**TABLE 5. Relation between PD and Na concentration in thick ascending limb of Henle’s loop**

<table>
<thead>
<tr>
<th>Exp No</th>
<th>Perfused Na mEq liter⁻¹</th>
<th>Collected Na mEq liter⁻¹</th>
<th>Bath Na mEq liter⁻¹</th>
<th>Flow PD mv</th>
<th>Stop Flow PD A/R</th>
<th>Eₙₐ B</th>
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<tbody>
<tr>
<td>1</td>
<td>79</td>
<td>63</td>
<td>153</td>
<td>16</td>
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<td>21</td>
<td>22</td>
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<td>60</td>
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<td>26</td>
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</tr>
<tr>
<td>Mean</td>
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<td>24.8</td>
<td>21.2</td>
<td>1.17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Perfusate was dilute NaCl/HCO₃ buffer (experiments 1-4) or dilute NaCl/HPO₃ buffer (experiments 5-9). Bath contained NaCl/HCO₃ buffer. ([Na] = 144-153 mEq liter⁻¹). Eₙₐ = 59 log [Na]ₗ/ [Na]ₐ, where [Na]ₗ and [Na]ₐ are the Na concentrations in the collected fluid and bath, respectively. Each Na measurement is the mean of three collections from a tubule. Each experiment was performed on a separate tubule.
Thick Ascending Limb of Henle’s Loop

**TABLE 6. Effect of lumen bicarbonate on PD**

<table>
<thead>
<tr>
<th>HCO₃ in Perfusate, mM</th>
<th>Flow</th>
<th>PD, mv</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp 1</td>
<td>Exp 2</td>
</tr>
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<td>0</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>0</td>
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<td>9</td>
</tr>
<tr>
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<td>12</td>
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</tr>
</tbody>
</table>

Bath was NaCl/HCO₃ buffer plus 5% rabbit serum. Perfusate was either the identical solution without rabbit serum, or was bicarbonate-free (NaCl/HPO₄) buffer. In the four tubules studied, the PD during flow was independent of the lumen bicarbonate concentration, but the stop-flow PD was higher when there was no bicarbonate in the lumen.

Na fluxes in the two directions were measured either alternately in the same tubule at fast flow rates or simultaneously at a slower rate (Table 7). The results of the two methods are in good agreement. The Na flux from bath to lumen was 23.9 × 10⁻¹² Eq cm⁻¹ sec⁻¹ and from lumen to bath it was 37.1 × 10⁻¹² (Table 7). This high bidirectional Na flux accounts for the relatively low electrical resistance. The mean PD, measured in these experiments was +6.1 mv, and the mean flux ratio was 0.65. If Na transport were passive, the flux ratio should have been 0.79 (substituting +6.1 mv in equation 8). The difference is statistically significant (P < .01). Therefore, by this criterion there may be active as well as passive Na transport, whereas none was apparent from the comparison of steady-state Na concentration and stop-flow PD given earlier.

Although the results using the two different methods to measure Na flux are in good agreement, neither method is ideal. In the first method the flow is fast enough to keep the composition of the lumen fluid uniform to within a few percent so that the NaCl concentration in the lumen is equal to that measured at the perfusion end. There was the disadvantage, however, that both JNa₁,lb and JNa₁,bl were found to decrease from one set of observations to the next in the same tubule (approximately 25% in 50 min). Therefore, to compare the fluxes a mean value had to be used for whichever flux was measured before and after the other. This procedure may conceivably have resulted in inaccuracy.

In the second method, the fluxes are measured simultaneously; however, the flow is slower and the decrease in lumen Na concentration due to net absorption could be as great as 10%. This might result in a greater PD in the tubule lumen at the collecting end, whereas constant PD is assumed in the derivation of the equations used (see Methods).

The mean net Na flux was 13 × 10⁻¹² Eq cm⁻¹ sec⁻¹ (Table 7). For lumen Na of 150 mEq liter⁻¹ this represents a Na clearance from the lumen of 0.52 nl mm⁻¹ min⁻¹, which is approximately 40% of the rate of net fluid (and isosmotic Na) absorption from the isolated rabbit proximal convoluted tubule (1.18 nl mm⁻¹ min⁻¹ (10)). This rate of Na absorption measured with radioisotopes is consistent with the results of the electrical and chemical measurements given earlier. In the electrical studies, when flow stopped, the PD increased gradually, reaching a new steady value within approximately 20–40 sec. This was ascribed to lowering of the lumen Na concentration. The volume of the tubule lumen for 20 µ diameter is 0.31 nl mm⁻¹ tubule length. Considering an absorptive Na clearance of 0.52 nl mm⁻¹ min, it is understandable that approximately 0.5 min should be required to lower the Na concentration to a steady value which is approximately 40% of the initial level.

In the chemical studies, lumen Na concentration was decreased by approximately one-third at a perfusion rate of 1 nl mm⁻¹ min⁻¹ (Fig. 1). This again is in reasonable agreement with the net absorptive Na clearance of 0.52 nl mm⁻¹ min⁻¹ measured with radioisotopes.

**Chloride flux.** The mean value of Cl flux from bath to lumen was 11.9 × 10⁻¹² Eq cm⁻¹ sec⁻¹ with Cl concentration of 118 mEq liter⁻¹ in the bath (Table 8). This is opposite in direction to the active Cl transport and is presumed to be passive.

**TABLE 7. Comparison of bidirectional Na fluxes and PD**

<table>
<thead>
<tr>
<th>Exp No.</th>
<th>Na Flux, pEq cm⁻¹ sec⁻¹</th>
<th>Flux Ratio B/A</th>
<th>PD, mv</th>
<th>Theoretical Ratio</th>
<th>Vr, nl mm⁻¹ min⁻¹</th>
<th>JNa net, Eq cm⁻¹ sec⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36.9 (6) 19.5 (3) .53</td>
<td>4</td>
<td>.86</td>
<td>27</td>
<td>17.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>32.7 (3) 24.3 (6) .74</td>
<td>8</td>
<td>.73</td>
<td>18</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>39.5 (6) 26.1 (3) .66</td>
<td>5</td>
<td>.02</td>
<td>24</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>35.7 (3) 24.2 (6) .68</td>
<td>7</td>
<td>.76</td>
<td>23</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>34.5 (3) 25.2 .75</td>
<td>5</td>
<td>.82</td>
<td>8</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>32.9 (3) 23.4 .72</td>
<td>4</td>
<td>.86</td>
<td>6</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>40.8 (3) 22.2 .54</td>
<td>4</td>
<td>.86</td>
<td>8</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>40.8 (2) 29.1 .71</td>
<td>9</td>
<td>.70</td>
<td>7</td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>39.9 (7) 21.3 .53</td>
<td>9</td>
<td>.70</td>
<td>8</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>37.1 23.9 .65 6.1 .79</td>
<td>13.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The flux ratio for passive Na transport (theoretical ratio) is calculated from the flux ratio equation (see Results). The Na concentration was identical in the perfused fluid and bath (mean 150 mEq liter⁻¹). The unidirectional fluxes were measured alternately in experiments 1-4, and simultaneously in experiments 5-9 (see METHODS). The mean difference between the observed ratios (B/A) and the theoretical ratios is 0.14 ± 0.04 (sem), P < .01. Each experiment was performed on a separate tubule.

**TABLE 8. Chloride flux across thick ascending limb of Henle’s loop**

<table>
<thead>
<tr>
<th>JCl, pEq cm⁻¹ sec⁻¹</th>
<th>PD, mv</th>
<th>Length, mm</th>
<th>Flow Rate, nl min⁻¹</th>
<th>No. of Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.0</td>
<td>5</td>
<td>1.75</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td>16.8</td>
<td>5</td>
<td>1.11</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>9.2</td>
<td>6</td>
<td>1.60</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>7.7</td>
<td>5</td>
<td>1.24</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>13.2</td>
<td>5</td>
<td>1.00</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>11.7</td>
<td>8</td>
<td>1.13</td>
<td>19</td>
<td>6</td>
</tr>
</tbody>
</table>

The chloride flux from bath to lumen (JCl,b) was measured by placing Cl in the bath and measuring its appearance in the lumen fluid. The bath contained NaCl/HCO₃ buffer ([Cl] = 118 mEq liter⁻¹) and the perfusate contained NaCl/HPO₄ buffer ([Cl] = 149 mEq liter⁻¹).
Cl was calculated from the PD, $J_{\text{Na},b}$, and $[i]_{b}$ using the constant field assumption (20). Permeability to Na is 1.8 $\times 10^{-7}$ cm$^2$ sec$^{-1}$ (referred to tubule length) or 2.8 $\times 10^{-5}$ cm sec$^{-1}$ (referred to lumen area for 20 $\mu$m diameter). Permeability to Cl is 0.89 $\times 10^{-7}$ cm$^2$ sec$^{-1}$ or 1.4 $\times 10^{-5}$ cm sec$^{-1}$. By this calculation Na permeability is 2 times that of chloride, which is in reasonable agreement with the ratio of 2.7 estimated earlier from the NaCl diffusion potential.

Effect of ouabain. Ouabain ($10^{-5}$ M added to the bath) caused the electrical PD to decrease reversibly (Fig. 6 and Table 9). The effect on Na absorption was tested in a single experiment using radioisotopes and one using chemical measurements: 1) net Na flux measured with radioisotopes was $-1.5 \times 10^{-12}$ Eq cm$^{-2}$ sec$^{-1}$ with $10^{-5}$ M ouabain compared to a mean value of $+13 \times 10^{-12}$ in controls (Table 7). 2) Na concentration in collected fluid was 148 mEq liter$^{-1}$, compared to 150 mEq liter$^{-1}$ in the perfusate with $10^{-5}$ M ouabain in the bath (flow rate 1.5 nl min$^{-1}$, mean tubule length 84 mEq liter$^{-1}$ during the control periods and increased to 146 mEq liter$^{-1}$ when low-$10^{-5}$ M ouabain was added to the bath (mean flow rate was 1.3 nl min$^{-1}$, mean tubule length 1.6 mm, and the concentration of Cl in the perfusate was 150 mEq liter$^{-1}$).

When there was flow of dilute lumen fluid, a positive PD was found in the presence of ouabain (Table 9). Contrary to the results with uninhibited tubules (Fig. 4), however, the PD decreased when flow stopped (Table 9). As proposed earlier, the relatively high PD with dilute fluid in the lumen is most likely a diffusion potential with Na permeability greater than Cl. This persists in the presence of ouabain. The increase in PD during stop flow in the uninhibited tubule was explained as due to a lowering of lumen NaCl concentration by continued transport. When flow stops in the ouabain-inhibited tubule which is perfused with a dilute solution, however, NaCl presumably diffuses into the lumen, increasing the NaCl concentration and causing the diffusion PD to disappear.

**DISCUSSION**

The most significant results of the present studies are that: 1) the PD is oriented lumen positive in the thick ascending limb of Henle's loop, and 2) NaCl transport consists of active chloride transport plus passive Na transport along the resulting electrical potential gradient. Although active Na transport is not completely excluded by the present studies, there is no very convincing evidence for it. Thus, at low flow rates the lumen Na concentration is lowered to the level predicted for passive Na transport from the measured PD. Also, the flux ratio for Na is close to that predicted for passive transport. Although the difference in flux ratio is statistically significant, it may be explained by small uncertainties in the estimation of the mean PD and the isotope fluxes (which are discussed above) rather than active Na transport. For the present, therefore, we prefer to consider that Na transport is largely and probably entirely passive in this segment. A major experimental problem is that the criteria used for evaluating active Na transport requires comparison of the PD measured at one (or possibly two) points in the tubule to transport integrated over the tubule length. In order to eliminate this problem it would be desirable to develop a method for accurately measuring both PD and transport at the same point in a tubule.

The electrical resistance across the thick ascending limb of Henle's loop is low, approaching the value previously found in the isolated perfused proximal convoluted tubule (22). Na conductance is high relative to that of Cl, as calculated both from the NaCl diffusion potential and the radioisotope permeabilities. This permeability arrangement is advantageous for efficient transport of NaCl. Thus, a relatively small PD from active chloride transport is sufficient to transport the Na passively because of the high Na conductance. The backflux of Cl is minimized both because the PD is low and because Cl permeability is low. The PD required to transport the Na passively can be calculated using the mean data from the present experiments:

$$PD = \frac{(J_{\text{Na} \text{,net}})(F)(R_T)}{\left(1 + \frac{g_{\text{Cl}}}{g_{\text{Na}}}ight)}$$

where $J_{\text{Na} \text{,net}}$ is $13 \times 10^{-12}$ Eq cm$^{-2}$ sec$^{-1}$ (Table 7); $F$, the Faraday constant, is 9.65 $\times 10^4$ coul Eq$^{-1}$; $R_T$ the electrical resistance, is 3,920 ohm cm (Table 3), and $g_{\text{Cl}}/g_{\text{Na}}$, the ratio of Cl-to-Na conductance, is approximately 0.45 (see results). Therefore,

$$PD = 8 \text{ mV}$$
which is close to the observed value of the PD and confirms that the observed PD is sufficient to account for much or all of the Na transport.

Na- and K-activated ATPase is found in high concentration in the renal outer medulla (4) and Schmidt and Dubach (29) localized it by direct analysis of microdissected tubule fragments to the medullary portion of the thick ascending limb of Henle's loop. The concentration of ATPase per dry weight in the cortical segment of the ascending limb was only one-half that in the medullary segment (29). Considering the extreme thinness of the cells in the cortical segment, tubule length rather than dry weight is probably a more appropriate standard for analysis, and on this basis the comparative amount of ATPase in the cortical segment of the thick ascending limb is even less. Further, it is not clear whether Schmidt and Dubach specifically tested the Na and K requirement of the ATPase or whether they tested only for ouabain inhibition. Therefore, it is questionable on the basis of this evidence whether the concentration of the Na- and K-activated ATPase is exceptionally high in the cortical thick ascending limb of Henle's loop. Its presence there was assumed to be associated with the large amount of active Na transport, which was believed to occur in the thick ascending limb (19).

There is a high K content in thick ascending limbs and the K turns over very rapidly (7). Possibly, any Na- and K-activated ATPase in thick ascending limbs is associated with this cellular K transport, rather than with trans epithelial NaCl transport. In the present studies, ouabain inhibited active Cl transport in the thick ascending limb of Henle's loop. Although cardiac glycosides were previously noted also to inhibit active Cl transport in frog skin (39) and stomach (12), their principal action is considered to be a specific inhibition of Na and K transport via their action on Na-K ATPase. It is difficult to reconcile inhibition of Cl transport with this theory. Either the action of ouabain is not specific to Na and K transport and it directly inhibits Cl transport as well or its effect on Cl transport is indirect, a secondary result, for example, of altered cellular K or Na transport. Such an indirect effect of cardiac glycosides was previously considered as a possible explanation for inhibition by the drugs of transport of other anions, namely, PAH in kidney slices (9) and iodide in the thyroid (38).

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