Analysis of electrolyte movement in thin Henle's loops of hamster papilla

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Marshall, Donald J., and Sidney Solomon. Analysis of electrolyte movement in thin Henle's loops of hamster papilla. Am. J. Physiol. 208(6): 1119-1128. 1965.—Saline solutions isolated between oil droplets within the lumen of thin limbs of Henle's loops remained at constant volume, while raffinose solutions underwent a continuous volume increase with time. After equilibrium, composition of the NaCl perfusates with respect to osmolality and Na and Cl concentrations became similar to that of vasa recta plasma. These results were identical for both ascending and descending limbs and suggest that neither segment transports salt actively. Descending limbs and vasa recta were isopotential under all conditions; the ascending limb during antidiuresis is 9 mv negative, the collecting ducts 17 mv negative. Ascending limb negativity was abolished by osmotic diuresis or stopped-flow microperfusion with saline. Measurement of ion concentrations showed that the ascending limb potential of antidiuresis is not a diffusion potential, and that its disappearance during osmotic diuresis is not due to the appearance of a diffusion potential of counterconvection. We suggest that the ascending limb potential is a streaming potential, but that the collecting duct potential reflects active ion transport.

The ascending limb of Henle's loops has two morphologically different segments, but most models of the renal countercurrent system are constructed on the assumptions that the entire limb is the site of active reabsorption of sodium chloride and restricted hydraulic conductivity (9, 12, 22, 27). The combination of these functional properties should lead to the removal from the tubular lumen of a hypertonic reabsorbate, rendering the tubular fluid hypotonic with respect to the adjacent interstitium. Since fluid at the beginning of the distal tubule is invariably more dilute than systemic blood (9), these assumptions are probably correct for the thick portion of the ascending limb in the outer medulla. Comparable information is not available about the function of thin ascending limbs in the inner medulla whose tubular cells lack cytological specializations usually associated with active transport (21).

Gottschalk and Mylle (9) found that fluid at the bend of the loop had the same osmolality as blood from adjacent vasa recta, indicating that the hydraulic conductivity of the thin descending limb is sufficient for water to remain near equilibrium as the tubular fluid moves through regions of continually changing osmolality. There is no evidence to indicate whether sodium chloride is actively transported, and since the fine structures of the descending and thin ascending limbs are so similar that they cannot be differentiated in electron micrographs (15), there are also reservations about the ability of the former to perform osmotic work.

In recent years, stopped-flow microperfusion techniques have been used for the quantitative evaluation of the transport capabilities of single renal tubules of Necturus (5, 24) and the rat (4). With this approach, flow of tubular fluid is obstructed with an inert oil and a test solution introduced into the lumen. This arrangement provides an oil-aqueous solution system arrayed longitudinally in the tubule so that material is free to exchange across the epithelial cells between the trapped column of solution and adjacent interstitium. The composition of the perfusion solution can be varied, and changes in volume and composition of the solution monitored as the system relaxes into a stationary state. This technique, in combination with microanalytical and electrical methods, has been used to study the...
duced by the administration of a saline or Ringer solution was added to normal saline through a jugular vein catheter at a rate of 0.25–0.50 ml/hr. At least 45 min were permitted to elapse between the beginning of the mannitol infusion and the start of the experimental procedure followed in the protocol.

All experiments were carried out on tubular structures visible on the surface of the exposed papilla, which was transilluminated with a quartz rod, or in later experiments, by a fiber optic-light guide. The illumination for photography was provided by a flash unit obtained from E. Leitz, Inc., which was arranged to transmit light through the same quartz rod used for standard lighting.

Stopped-flow microperfusion was performed as described previously (4, 11, 14). Individual loops of Henle were punctured with two channel micropipettes. One channel contained oil colored with Sudan black, the second, the test solution. A small drop of oil was extruded from one channel of the pipette and was carried along the tubule by the flow of tubular flow, thus identifying the punctured segment as descending or ascending limb. The longitudinal flow of tubular fluid was then obstructed by extruding additional quantities of oil. The test solution was next introduced into the tubule and sealed off from the puncture site by injection of still more oil. Both ends of the aqueous column were always in view. In early experiments, lightweight mineral oil was used, but it could not be colored sufficiently to provide adequate contrast for photographic recording. For this reason, a thin watchmaker’s oil (obtained from Fabrik Hermann Koch, Hildesheim, West Germany) was used in subsequent experiments. Results were identical with the two oils, whether colored or uncolored, and half-volume reabsorption times of saline droplets in rat proximal tubules were the same with both oils as those reported by Gertz (4). The time between the initial injection of perfusion solution and final sealing with oil was 3–5 sec.

Volume changes of the perfusion solution were recorded by time-sequence photomicrography, as described by Gertz (4). A spring-wound 35-mm camera was mounted on one of the oculars of the stereoscopic microscope and activated by an automatic preset timing device. Determinations of fluid volume changes were made by projecting the film negative on a wall and measuring the distance between the two oil columns delimiting the perfused segment. The inner diameter of the perfused segment was the same as in the segments filled with oil, and the same as adjacent tubules which had not been punctured. In a few experiments, carbon particles were suspended in the perfusion solution to enhance visualization of the perfused segment. These experiments showed that the inner diameter of the perfused segment was the same as that of the oil-filled tubule.

In a separate series of stopped-flow microperfusion studies the fluid isolated between oil columns in loops of Henle was collected for analysis. Collection was made with an oil-filled single micropipette introduced into the perfused tubule. Four criteria were established to assure proper collection:

METHODS

Adult golden hamsters, chiefly males, were kept without food or water for at least 18 hr to insure hydropenia and also to deplete the intestine of its contents. They were anesthetized with Inaktin dissolved in saline, administered intraperitoneally in an initial dose of 160 mg/kg and supplemented as needed. The animals were placed on a heated animal table, the abdomen opened with a left parasagittal incision, and the abdominal contents retracted with a Lucite retainer mounted on the animal board. The left kidney was then decapsulated and immobilized in a Lucite cup. Perihilar fat was removed by blunt dissection, and the tip of the papilla exposed by excision of the renal pelvis, as described by Ullrich et al. (23). The kidney was bathed in warm mineral oil, except when electrical potentials were to be measured, in which case saline or Ringer solution was used. In some experiments osmotic diuresis was produced by the administration of a 20% solution of mannitol in normal saline through a jugular vein catheter at a rate of 0.25–0.50 ml/hr. At least 45 min were permitted

![Graph](https://via.placeholder.com/150)

**FIG. 1.** Relative volume changes of loop of Henle saline stopped-flow microperfusates as a function of time. $V/V_0$ is the ratio of volume at time $t$ to initial volume. Brackets delimit one standard error of the mean. The number of experiments is: a) ascending limbs antidiuresis, 14; b) descending limbs antidiuresis, 13; c) ascending limbs osmotic diuresis, 12; d) descending limbs osmotic diuresis, 7.

ability of the thin descending and ascending limbs to perform active transport of ions.
THIN LOOP ELECTROLYTE MOVEMENT

![Figure 2. Relative volume changes of loop of Henle raffinose stopped-flow microperfusates as a function of time. All measurements made in osmotic diuresis. \( V/V_0 \) is the ratio of volume at time \( t \) to initial volume. Brackets delimit one standard error of the mean. The number of experiments is: descending limb, 13; as cending limb, 12.]

1) The volume of the perfusion solution did not decrease after puncture of the tubule with the collection pipette, indicating that there were no leaks at the puncture site.

2) Small drops of oil extruded from the collection pipette were restricted to the lumen of the perfused tubule.

3) The column of perfusion solution decreased only when negative pressure was applied to the collection pipette.

4) With continued negative pressure the oil columns closed until the oil at the end of the perfused segment could be aspirated into the collection pipette.

After completion of the collection, the pipette was withdrawn from the tubule into the oil pool covering the papilla, and additional oil was drawn into the pipette to prevent evaporation from the orifice. As soon as possible after these procedures were completed, collection of the vasa recta specimen was begun using another oil-filled single micropipette. A drop of oil was extruded into an ascending vas, rectum, and the rate of collection adjusted to maintain a constant position of the oil drop. Unfortunately, it was not possible to randomize the order of collection of perfusate and blood specimen since the vessels bled vigorously after the pipette is withdrawn; the blood escaping from the puncture site quickly covers the surface of the papilla, obscuring vision and making further punctures difficult.

Specimens of normal tubular fluid were collected for analysis by standard micropuncture techniques. In these experiments, single-channel micropipettes filled with paraffin oil were introduced into the tubule lumen, a small droplet of oil extruded, and the collection rate again adjusted so as to maintain a constant position of this droplet distal to the collection site. Vasa recta specimens were also collected in these experiments.

The perfusate specimens and the corresponding vasa recta samples were analyzed for sodium or chloride concentrations, or for osmolality; sodium, potassium, or chloride concentrations were measured in normal tubular fluid collected during free flow, and in their matched plasmas. Sodium was measured with a Beckman DU flame spectrophotometer and an integration amplifier. Aliquots of standard solutions and specimens were taken up in an oil-filled siliconized Kimax glass volumetric pipette (16) and discharged into 5-pliter drops of doubly quartz-distilled water lying on a parafilm sheet. These drops were then aspirated into the total consumption burner of the DU and the photocurrent generated by each specimen integrated. The time-integral voltage was linearly proportional to the total quantity of sodium in the 5-pliter drop. Microperfusion specimens ranged in volume from 50 to 100 pliters; 10 replications of 80 pliters of a standard solution yielded a standard deviation of the method of ±0.8%. Larger volumes were available from free-flow collections. One to two pliters were used for these determinations. Kashgharian et al. (11) have reported the standard deviation of this method in this volume range to be ±2%. The same method was used to measure potassium concentrations, except that 5-pliter aliquots were used, the standard deviation of this method was found to be ±2%. Chloride concentrations were measured with the second potentiometric method of Ramsay et al. (17), and osmolality with the microcryoscopic method of Rama and Brown (16).

When either sodium, potassium, or chloride were to be measured the animal received heparin (12.5 IU) intraperitoneally 15 min prior to collection of vasa recta specimens. The blood was centrifuged and the cells discarded; the analyses were performed on the remaining plasma. Freezing-point measurements were made on whole blood. Specimen pairs were discarded when visual evidence of hemolysis could be detected in the plasma. All analyses were performed on the day of collection.

Electrical potential differences were measured with glass microelectrodes prepared and selected by methods described previously (13, 18). A length of polyethylene

<table>
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<th>TABLE I. Loops of Henle diameters</th>
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<td>Tubules used in compiling Fig. 1</td>
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<td>Tubules from which fluid reabsorption was observed</td>
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<tr>
<td>Data listed by Thurau and Henne (10)</td>
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<td>Data listed by Steinhausen (19)</td>
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<td>Values are means ± se. N = no. of experiments.</td>
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tubing filled with 3 M KCl agar served as the indifferent electrode. It connected the perirenal saline pool with a calomel half-cell. The glass microelectrode was filled with 3 M KCI and bridged with 3 M KCl agar to a second calomel half-cell; a high-impedance voltmeter was used to record potential differences. Electrode tips were localized in perfused tubular structures by the resistance step method of Eigler (3).

RESULTS

Determination of volume changes in stopped-flow microperfusion experiments. To see whether the loops of Henle can actively transport sodium chloride, saline perfusion solutions of concentrations similar to those found in the adjacent interstitium were used. In nondiuretic hamsters, 450 mM NaCl was perfused; when the animal was undergoing mannitol diuresis, the solution was 225 mM NaCl. The volume changes of these isolated saline columns are summarized in Fig. 1, which shows that the perfusates remained at constant volume indefinitely. Although Fig. 1 gives the results only for the first 300 sec, perfusion columns were consistently seen not to change volume regardless of the length of the observation period the most extended experiment lasted 4 hr. The results from descending and ascending limbs were essentially identical. These results are independent of osmolality, since they are the same in nondiuretic and diuretic animals. The data may be contrasted with that from similar experiments on proximal and distal tubules, in which the volumes of isotonic saline perfusates decrease exponentially with time (4). The changes in volume seen in stopped-flow microperfusion experiments on proximal and distal tubules show that the tubular cells can perform net transtubular osmotic work; the absence of volume changes in similar experiments on loops of Henle raises the possibility that the cells in this region lack such a capability.

A strict interpretation of these results is impossible without additional information about the nature of water transfer across loop of Henle epithelia. Several authors suggest that various sections of the thin limbs are water impermeable (1, 9, 12, 22, 27). The results of the sodium chloride perfusion experiments might thus be due to an inability of water to move in response to a driving force. To test this question, perfusion fluids were used which contained only raffinose. By perfusing the lumen with sugar solutions, concentration gradients are established which favor the inward diffusion of other solutes present in the adjacent interstitial fluid. These experiments were carried out on hamsters undergoing a mannitol diuresis. This procedure reduces medullary osmolality to a predictable level (9) so that loops of Henle can be perfused with solutions of osmolality almost equal to that of the interstitial fluid. In these circumstances, the perfusate volume changes only because of water movement coupled to solute flow, and the resulting data can be analyzed by the methods used by Gertz in similar experiments on rat cortical tubules (4). The results of stopped-flow microperfusion experiments on thin ascending and descending limbs with 450 mM raffinose as the perfusate are presented in Fig. 2. In the immediate postinjection period, there is a distinct increase of the perfusate volume—apparently water can move across the thin-limb epithelia when driving forces are present. This volume increase continues during the entire observation period in contrast to results from similar experiments on rat cortical tubules where the inflow continued for only 50 and 150 sec in proximal and distal convolutes, respectively. The raffinose perfusion curves from ascending and descending thin limbs do not differ from each other.

While performing the perfusion experiments, both with sodium chloride and raffinose solutions, we tried to repeat measurements several times on the same tubule by injecting more oil, followed by a fresh column of perfusion solution which was then sealed from the puncture site with yet more oil. After the procedure was repeated two or three times it became apparent that the tubule was distended and the oil-water menisci were markedly deformed. These changes were associated with a decrease in volume of the perfusate. Further repetition of the procedure led to greater distention and deformation of the oil-water menisci and progressive reduction of half volume reabsorption times to 1 sec or less.

The extent of tubular distention is indicated in Table 1. Tubule diameters were measured from the oil-filled segments since the lateral borders of the aqueous columns cannot be clearly defined on the films. However, when viewed directly through the microscope both regions could be seen to have the same diameter in all experiments. The measurements of Thurau and Henne (20), and of Steinhausen (19), in both instances made on intact nonperfused tubules, are included for comparison.
TABLE 2. Analysis of relationship of NaCl stopped-flow microper fusates to adjacent vasa recta

<table>
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<tr>
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<th>Descending Limb</th>
<th>Ascending Limb</th>
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<tbody>
<tr>
<td>Osmolality</td>
<td>6 1.04±0.02</td>
<td>10 1.04±0.01</td>
</tr>
<tr>
<td>Sodium</td>
<td>7 1.02±0.03</td>
<td>7 1.03±0.04</td>
</tr>
<tr>
<td>Chloride</td>
<td>7 1.04±0.03</td>
<td>9 1.05±0.03</td>
</tr>
<tr>
<td>Potential diff.</td>
<td>8 -0.1±0.09</td>
<td>12 0.5±0.12</td>
</tr>
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Values are means ± SE. N = no. of experiments. Osmolality, Na, and Cl are expressed as TF/VR and electrical potential is given as perirenal bath minus TF.

These data show that nonreabsorption of saline columns is found in tubules of normal dimensions, but that reabsorption occurs from tubules distended to as much as twice their normal diameters. This apparent reabsorption could be induced more easily in descending than in ascending limbs, and was irreversible in both. The observations of tubular distention, deformation of oil-water menisci, and progressive increase of reabsorption rate with repetition suggest that the induced transmural volume flow is an artifact associated with elevated hydrostatic pressure within the tubules; when appropriate care was taken not to distend the tubules the results depicted in Figs. 1 and 2 were always obtained. The epithelial cells of thin limbs of Henle's loops are quite flat and almost devoid of infoldings on either side, so that the margin for distention of the tubule in this region is small, and the likelihood of damage correspondingly great.

Gottschalk has published preliminary reports of similar experiments using the stopped-flow microperfusion technique (6, 7). His observations on the ascending limb are the same as reported here: nonreabsorption of saline and a volume increase with raffinose solutions. He also records results similar to ours from raffinose perfusion of descending limbs, although no comment is made as to whether the volume increase continued indefinitely, as we observed, or reached a maximum. A discrepancy appears to exist concerning the behavior of saline perfusates in descending limbs, where Gottschalk reports reabsorption, in contrast to the data of Fig. 1. However, as noted in the preceding paragraph, we were able to reproduce Gottschalk's results, but only when the tubule had been distended to twice its normal diameter.

Osmotic and chemical gradients. Stopped-flow microperfusion experiments were carried out with 450 mM NaCl in loops of Henle in antidiuretic hamsters, care being taken to avoid distention of the tubules. After the perfusion fluid had been in the lumen for at least 2 min, the perfusate was collected, blood from an adjacent vas rectum was drawn, and the appropriate analyses were performed. Detailed results of these analyses are presented in Fig. 3 and summarized in Table 2. The osmolalities, sodium, and chloride concentrations are 2-5% greater in loop perfusates than in adjacent vasa recta blood. As noted below, there is no electrical potential difference across similarly perfused ascending and descending limbs. Thus, in the stationary state achieved in these experiments; i.e., when net fluxes are zero, the electrochemical potential gradients are: descending limb, Na - 1.3 mv, Cl +2.3 mv; ascending limb, Na - 1.9 mv, Cl +2.5 mv. Two factors are present in the experimental design which tend to cause the values of perfusate and vasa recta blood to differ. First, in all cases, blood was drawn after collection of perfusion solution; an average of 10 min elapsed between collections. Gottschalk and his colleagues (8) have noted that the osmolality of specimens collected from a given hamster papilla decreases with time. Our experiences are the same, and this time difference would have the effect of yielding values lower than those actually present at the time of the perfusion. Secondly, plasma electrolyte concentration has not been corrected for plasma water, since no reliable estimate of vasa recta plasma water exists. Although both factors introduce some uncertainty into the plasma values, the cumulative influence is small and would, in any case, have the effect of reducing the observed differences between perfusate and plasma. Thus, no significant osmotic pressure gradient could be detected in the stopped-flow microperfusion experiments with saline in either ascending or descending limb, so that the lack of water reabsorption can be attributed directly to the absence of a driving force. In addition, the electro-

![Figure 4](http://ajplegacy.physiology.org/Downloadedfromhttp://ajplegacy.physiology.org/July9,2017)
from the base of the papilla to the tip, it is evident that concentration of the medullary interstitium increases electrophoretically across epithelia is generally indicative of the operation of active ion transport. Hence, renal medulla require that two other possibilities be mechanisms, but the unique anatomical features of the nephron do not perform work to prevent these ions from relaxing to their minimal partial free energy gradient.

**Electrical gradients.** When this study was begun, the electrical potential differences across nonperfused medullary tubules with an intact flow of tubular fluid had not been reported, and, in addition, these measurements were made for comparison with potential differences in perfused tubules. The results are presented as histograms in Figs. 4 and 5, and summarized in Table 3. Potential differences measured across the vasa recta and the descending limb of Henle’s loop were small and indicate that the two structures are isopotential; the mean value for thin ascending limbs was $-9 \text{ mV}$, lumen negative, while the collecting duct average was $-17 \text{ mV}$, lumen negative. These values are in close agreement with those reported recently by Windhager (25). During osmotic diuresis, the descending limb potential remained the same as in antidiuresis. In contrast, the luminal negativity of ascending limbs found in antidiuresis disappeared during mannitol diuresis.

Potentials were also measured in loops of Henle perfused in the same manner as for the experiments described above. The results of these measurements are also presented as histograms in Fig. 4. In stopped-flow microperfusion of ascending limbs with 450 mM NaCl, the trans-tubular potential approaches zero millivolt; the value for descending limbs remains at about zero millivolt. These values have been used to calculate the electrochemical potential gradients given above.

**Chemical gradients during free flow.** The demonstration of electrical potential differences across epithelia is generally indicative of the operation of active ion transport mechanisms, but the unique anatomical features of the renal medulla require that two other possibilities be given serious consideration. Since the sodium chloride concentration of the medullary interstitium increases from the base of the papilla to the tip, it is evident that fluid moving along the loop of Henle is continuously confronted with an interstitium different from that which it just left; thus, concentration differences across any given segment of the loop of Henle could arise because of incomplete equilibration, and produce a diffusion potential. Second, the medullary interstitial osmolality also increases from the base of the papilla to the tip. Hence, it is to be expected that flow of tubular fluid from one segment of the loop to the next will produce osmotic gradients which, if they cause transmural water flow, can generate streaming potentials. Of these two possibilities, the first at least is open to experimental investigation. When a membrane is highly perselective to a given monovalent ion, an electrical potential difference of 9 mV requires that at equilibrium a concentration ratio of approximately 1.4 be present as a lower limit. When the selectivity of the membrane is not perfect, the required ratio is greater. If ion concentration ratios of approximately this magnitude are found, the exact origin of the potential difference cannot be established without knowledge of relative permeabilities; however, the absence of such gradients would clearly establish that the observed potential is not a diffusion potential. Concentrations of sodium, potassium, and chloride were therefore measured in tubular fluid collected during free flow and compared with concentrations of the same ions in the adjacent vasa recta. Detailed results of these measurements are in Fig. 6, and the average ratios summarized in Table 3. The results indicate that none of the ratios found during antidiuresis is sufficiently close to 1.4 to require further consideration of the diffusion potential alternative. Further, similar ratios were found during osmotic diuresis, indicating that the disappearance of the ascending limb potential in this state cannot be attributed to the appearance, by whatever means, of a diffusion potential bucking the original potential difference. Because the results of the stopped-flow microperfusion experiments can be most consistently interpreted as indicating the absence of active salt-transport activity, the most likely explanation for the ascending limb potential difference is that it is a streaming potential. The disappearance of this potential during osmotic diuresis is consistent with such an interpretation since osmotic diuresis is known to obliterate the gradient of osmolality between the base of the papilla and its tip. This means that, during osmotic diuresis, fluid moving axially along the loop of Henle is confronted with an interstitium whose osmolality is relatively constant from one segment to the next; hence, reduced transmural water flow would be expected. In support of this notion, Thurau and Henne (20) have recently presented evidence indicating a large transmural flow of water into the thin ascending limbs in antidiuresis, but not during osmotic diuresis.

**DISCUSSION**

When a sodium chloride solution is isolated between oil columns in thin loops of Henle, care being taken to avoid tubular distention, no volume change can be ob-
since this circumstance is possible only if work is done
electrochemical gradients were obliterated. Since this
equation are available to verify the integrity of tubules
served for prolonged periods of time. Since reabsorption
trained tubular active transport mechanisms are not
When stopped-flow microperfusion experiments are
osmotic gradient due to the difference in salt concentrations
due to transport the ion. Figure 3 and Table 3 show that in
stable state in which the net transtubular flux
not continue indefinitely; the inflow stops when
measured electrical potential difference is also zero,
equilibrium is achieved a subsequent volume decrease
solute concentration remains constant during this
absolute certainty, and no independent tests of tubular
was zero, since the osmotic gradient due to the
The results of electrochemical potential gradient mea-
served in regions of the nephron known to transport sodium
the presence of raffinose in the tubular lumen. In practice, once this maximum
achieved a subsequent volume decrease is seen due to the
infiltration process have no bearing on the interpretation
the perfused segment. The question as to why no initial
The effective osmotic work available from such a system
When stopped-flow microperfusion experiments are
content of the fluid columns can be regularly observed in
osmotic gradient when driving forces for such movement
system is present but is being short circuited by passive
results presented.

<table>
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<tr>
<th>TABLE 3. Summary of transmural potentials during free flow</th>
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<tr>
<td>Descending Limb</td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Antiduresis</td>
</tr>
<tr>
<td>-2.3±0.36</td>
</tr>
<tr>
<td>(25)</td>
</tr>
<tr>
<td>Osmotic diuresis</td>
</tr>
<tr>
<td>-0.83±0.72</td>
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<td>(6)</td>
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Values are means ± se. Number of measurements are given in parentheses. Values given as potential difference (in mv) between lumen of structure indicated and perirenal bath.

A summary of transmural potentials during free flow.
consistent with previous and independently established ideas of salt transport in these structures (4). The oils used in the present study gave the same half-value reabsorption times as measured by Gertz, and unless the thin limbs are specifically sensitive to some chemical property of the oils, there is no a priori reason to assign differing degrees of confidence to similar experiments performed on different regions of the nephron.

2) Damage from distention is excluded by the data in Table 1 by virtue of the fact that there was no demonstrable distention. In fact, evidence of “activity” could be elicited only when the tubules were distended far beyond the limits encountered in physiological circumstances, i.e., when the possibility of damage is greatest.

3) The absence of an electrical potential difference across perfused descending limbs is not necessarily indicative of damage produced by the perfusion, since the descending limb is normally isopotential with interstitium. Similarly, the disappearance of the thin ascending limb potential difference need not reflect tubular injury since it also disappears during mannitol diuresis. The disappearance of the potential is associated in both cases with a reduction of transmural water flow consistent with the streaming potential hypothesis discussed below.

4) In the proximal tubule mannitol diuresis is accompanied by a reduction of tubular fluid Na+ concentration to levels significantly lower than in the plasma (23). If there were active Na+ transport in the descending limb, a similar result should be obtained, but the data of Fig. 6 and Table 4 fail to reveal it. None of the ion concentrations were reduced to levels less than in the surrounding interstitium, and it can be concluded that the mechanisms in the proximal tubule that lower tubular fluid Na+ concentrations in mannitol diuresis have no analogues in the ascending limb. This conclusion is in accord with the interpretations placed on the results of stopped-flow microperfusion experiments. Specimens of tubular fluid were collected from the region of the bend of the loop, so that we have no data from which to apply a similar argument to the thin ascending limb.

5) As noted in the introduction, the fine structure of thin limbs is unlike that of other tubular segments in which active ion transport mechanisms are known to operate (15). Thin limb cells are flat and have few mitochondria, which are abundant in regions of the nephron that do transport actively. Those mitochondria that are present seem to be randomly oriented, in marked contrast to the regular arrays of these structures found among the basal invaginations of proximal and distal tubular cells. Large microbarrels are located between the cells, but whether these serve as extracellular shunts cannot be decided until more is known of the appearance of junctional complexes in these regions.

If there is active transport, solute and solvent should be driven from lumen to interstitium. Recently, Thurau and Henne (40) measured axial flow velocities in hamster loops of Henle. They showed that flow velocities in descending and ascending limbs are approximately equal, but since the ascending limb has the greater radius, the volume flow through it is larger; therefore, there must be a net flow of solvent from interstitium to lumen across the thin ascending limb. This result is expected if transmural flow is determined by osmotic gradients rather than active solute transport, since fluid in the ascending limb traverses regions of decreasing interstitial osmolality.

Before we can be sure that active salt transport does not occur, it is necessary to account for the potential difference across thin ascending limbs, since, as is well known, transepithelial active salt transport is almost invariably associated with electrical polarization. Windhager’s recently published studies with metabolic inhibitors (25) would seem to support the hypothesis that the ascending limb potential is due to active ionic transport. He used a continuously running perfusion and found that with 1.8% saline alone as the test solution, the potential normally present in free flow was generated across the ascending limbs; when potassium cyanide and iodoacetate were included in concentrations of 3 mM each, the potential disappeared. One of us (unpublished observations) has recently confirmed this observation; however, when the concentrations of the inhibitors were increased 10-fold, it was found that the lumen of the ascending limb became positive with respect to the interstitium by about 15 mV. In these experiments, cyanide and iodoacetate are present only within the tubular lumen. Large concentration gradients for each exist and the diffusion potentials that result would tend to make the lumen more positive. Thus it seems that the disappearance of the potential during
perfusion with the inhibitors at a concentration of 3 mM is due to the presence of a diffusion potential of equal magnitude, but opposite polarity to that normally present, rather than to an inhibition of a metabolic process responsible for the generation of the potential.

Two alternative sources of the ascending limb potential have been considered. One of these, the diffusion potential hypothesis, can be excluded by a consideration of the results of measurements of tubular fluid to vasa recta concentration ratios. As discussed above, the observed ratios for sodium, chloride (which are in reasonable agreement with Windhager's results (25)), and potassium are not sufficiently different from unity to generate a diffusion potential of the observed magnitude. The proposal that the intralumenal negativity of the ascending limb potential seems to us the most plausible and consistent explanation. Streaming potentials occur when hydrostatic or osmotic flow of an electrolyte solution is produced across a membrane with unequal anionic and cationic permeabilities, and have been described in other epithelial systems (2). It is clearly not possible to give a rigid proof of this hypothesis from the available data. The lumen of the ascending limb is negative in antidiuresis, when, as shown by Thurau and Hennic (20), water is flowing from interstitium to lumen, and it is isopotential with the interstitium during osmotic diuresis when the water flow is markedly reduced. The observed polarity of potential and flow requires that the anion permeability be greatest. In recent studies (unpublished observations) the effect of ionic substitutions on the ascending limb potential in continuous-perfusion experiments has been measured, and the relative order of permeabilities were Cl > K > Na. Thus, at present it can be said that the available data are consistent with the streaming potential hypothesis, and that no contradictory evidence seems to exist.

Recently Gottschalk et al. (8) have shown in a conclusive fashion that the osmolality of loop tubular fluid increases as the fluid flows down the descending limb in the inner medulla. If there is no active transport in the thin ascending limb, some other mechanism must produce these concentration changes. The results of these studies do not in themselves lead to the formulation of a new model, but do suggest additional lines of investigation. Although our data do not substantiate the assumption of thin ascending limb active salt transport, they do reveal in the electrical potential difference measurements a functional differentiation between descending and ascending limbs which is inherent in the notion that the loops of Henle participate in renal countercurrent function. The present data do not permit meaningful speculation about the significance of this difference, except to state that some knowledge of membrane properties is needed before the precise function of the thin limbs can be defined.

Finally, it may be useful to reconsider the role of active salt reabsorption from the collecting ducts. Hilger et al. (10) have shown that sodium chloride is reabsorbed from the lumen of these structures in the inner medulla. The finding of luminal negativity in this and in Windhager's (25) study, along with the fact that collecting duct sodium concentrations are invariably lower than those of the adjacent interstitium, indicates that sodium at least is reabsorbed against an electrochemical gradient. The collecting ducts appear to be the only tubular segments in the inner medulla capable of performing osmotic work, but a consistent model relating this work to the generation of medullary hyperosmolality has not yet been given.

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REFERENCES


