Mechanism of NaCl reabsorption by hamster thin ascending limbs of Henle’s loop

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MARSII, DONALD J., AND STANLEY P. AZEN. Mechanism of NaCl reabsorption by hamster thin ascending limbs of Henle’s loop. Am. J. Physiol. 228(1): 71-79. 1975.—Two models of hypertonic urine formation in the inner medulla were tested. The active model asserts that thin ascending limbs of Henle’s loop (ALH) reabsorb NaCl hypertonically by active transport; the passive model suggests the reabsorption is by diffusion down a concentration gradient. Using [Na+] in ascending vasa recta (AVR) as a measure of interstitial [Na+], we found no concentration difference between loop tubular fluid and AVR, when the comparison was made at the bend of the loop, or at an ALH sampling site 1 mm from the bend; the results were the same in antidiuresis and saline diuresis. In saline diuresis with flow of tubular fluid to the ALH slowed by simultaneous collection at the bend of the loop, or at an ALH sampling site 1 mm from the bend; the results were the same in antidiuresis and saline diuresis. In saline diuresis with flow of tubular fluid to the ALH slowed by simultaneous collection at the bend of the loop, or at an ALH sampling site 1 mm from the bend; the results were the same in antidiuresis and saline diuresis. 

countercurrent mechanism; active Na transport; hypertonic urine formation; NaCl permeability coefficient; urea permeation

HOW THIN LIMBS OF HENLE’S LOOP FUNCTION TO CONCENTRATE

The major question at issue is whether thin ascending limbs (ALH) participate actively or passively in a countercurrent system. ALH reabsorbs NaCl hypertonically (12), but either metabolism or a concentration gradient could supply the energy for this phenomenon. Attempts to demonstrate active transport with perfusion techniques have failed uniformly (4, 16, 17), and the evidence favoring active transport (5) is indirect. Nevertheless, the consensus has favored an active role for ALH because no plausible passive model has been offered. Recently, Stephenson (19, 20) and Kokko and Rector (10) put forth a consistent passive hypothesis for thin limbs. In their model, a concentration gradient between tubular and interstitial fluids drives hypertonic NaCl reabsorption from ALH, the concentration gradient is created by osmotic water flow from the descending limbs (DLH), and urea, which diffuses from the collecting ducts to the interstitial space, provides most of the force for this osmosis. In the model, urea can link the active work of the ascending limbs in the outer medulla to the passive structures of the inner medulla because the active multiplier of the outer zone raises collecting duct urea concentrations; the raised concentrations provide a diffusive urea source for the inner medulla. One-solute models (7, 14, 19) with an active outer and a passive inner medulla do not raise inner medullary tonicity because they lack a means for distributing the work of the active region. In the new passive model, a second solute, urea, distributes the work between the two regions.

The new model requires rather specific permeability properties of the thin limbs. DLH must be water permeable to permit osmotic equilibration, but they must be relatively urea and salt impermeable so that a urea concentration difference can provide an effective osmotic force; the osmotic water loss must also be able to raise tubular fluid NaCl concentration higher than in the adjacent interstitium without having the high NaCl concentration dissipated by diffusion. ALH must be NaCl permeable so that salt can leave when a concentration difference exists to drive it out, and water impermeable, so that the loss of NaCl by diffusion can leave the ALH tubular fluid hypotonic. These requirements differ substantially from those of the active countercurrent model. The active model is relatively indifferent to the mode of DLH equilibration and requires only that the ALH be salt and water impermeable so that active transport can lower the NaCl concentration below interstitial levels. Kokko and his colleagues (4, 8, 9) have dissected DLH and ALH from the kidneys of rabbits and perfused them while the tubular segments were immersed in an artificial bathing solution. This group found permeability results that appear compatible with the passive model. Morgan and Berliner (17) perfused DLH and ALH on the surface of rat inner medullas which had been excised and placed in an artificial bathing medium. Their results are consistent with the active model and differed in several important respects from Kokko’s measurements. Apart from a species difference, methodological differences between the two sets of measurements appear insubstantial, and the resultant conflict leaves no basis for choosing between the active and the passive models.

To resolve this question, we decided to measure the concentration differences between ALH tubular fluid and the adjacent interstitium. If the passive model is correct, tubular fluid NaCl concentration should exceed interstitial concentrations, while if the active mechanism is correct, the active transport mechanism might be able to lower tubular fluid NaCl concentration below interstitial levels. Interstitial fluid is not directly available, so we elected to sample ascending vasa recta (AVR) plasma, because our previous studies (15) led us to conclude that AVR con-
centrations more nearly approximate interstitial fluid concentra-
tions than do those in descending vasa recta.

METHODS

Experiments were performed on male golden hamsters, 60–90 g body wt, anesthetized with Inactin administered intraperitoneally, 0.2–0.5 mg/g body wt. The hamsters were placed on a heated table, and the left kidney was freed of peritoneal attachments and placed in a Plexiglas dish. The renal pelvis was excised to expose 2–2.5 mm of papilla, the kidney was covered with a flow of warmed mineral oil, and the papilla was illuminated with a fiberoptic bundle.

Of the 79 hamsters used in this study, 45 received intravenous infusions of isotonic NaCl to produce volume expansion. The infusions were administered with a syringe pump and delivered into a jugular vein through fine-gauge polyethylene tubing. At least 45 min elapsed between the start of the infusion and the beginning of the micropuncture collections. The infusions were begun with a priming load of 1.5 ml; the sustaining load was 0.4 ml/min. Since all hamsters that underwent saline diuresis received the infusion at this rate, the magnitude of the diuresis tended to vary inversely with body weight. In four hamsters, not otherwise studied, the mean femoral artery pressure (measured with a Statham P23Db transducer, a Statham SC 1100 amplifier, and a Gould recorder) increased from control levels of 100–110 mmHg to a mean peak of 156 mmHg (range 130–190 mmHg) 15 min after the start of the infusion; at the end of 1.5 h of continuous saline infusion, the mean arterial pressure had returned to 115 mmHg.

Micropuncture collections were made at one or two sites in single loops of Henle. The bends of the loop are readily visible on the surface; they were punctured with sharpened micropipettes, 6 μm OD. ALH can be traced easily on the surface for only 100–200 μm. In this work we wished to sample ALH fluid at a site as far removed as possible from the bend so that transport operations of the epithelium could develop measurable concentration differences, both from tubular fluid at the bend of the loop, and also with respect to the adjacent interstitium. We therefore used a technique described previously (12) to identify ALH puncture sites about 1 mm from their respective hairpin turns. With this technique, the tubule was first punctured at the bend of the loop with a 6 μm OD pipette, and a droplet of mineral oil was expressed from the pipette into the tubular fluid stream. The position of the tubule was revealed as the fluid stream swept the oil droplet up the ALH toward the cortex. A second pipette, 8 μm OD, was used to puncture the tubule at a point as far removed as practical from the hairpin turn, usually 1 mm (range 750–1250 μm). An oil drop was expressed from the second pipette to verify the location of its tip and the ALH collection was begun. The collections lasted an average of 4.4 min in antidiuresis (range: 3–6) and 2.45 min in volume expansion (range: 1–4). Antidiuresis collections were spontaneous; saline diuresis collections required negative pressure to hold the oil drop. After the ALH collection was complete, a second collection was made at the bend of the loop with the first pipette which had remained in place during the ALH collection. These collections were rarely spontaneous because the narrower diameter of these pipettes made negative pressure necessary to hold the oil drop, but care was taken to prevent changes in tubule diameter, as judged by eye.

AVR were punctured immediately after tubular fluid collections were complete, within a radius of 50 μm from the loop collection sites. Collections were made into pipettes at a rate sufficient to maintain the erythrocyte velocity the same as in adjacent AVR, as judged by eye. When AVR collections are adjusted to hold an oil drop, erythrocyte velocity is invariably greatly increased. Blood collections required an average of 6.37 min to complete in antidiuresis (range: 3–15) and 4.48 min in saline diuresis (range: 2–11). The average elapsed time from the midpoint of the tubular fluid collection to the midpoint of the vasa recta collection was 11.6 min in antidiuresis (range: 6–20) and 6.95 min in saline diuresis (range: 4.5–15). In 14 volume-expanded animals, the papillas of which permitted micropuncture of more than a single loop of Henle, the Na+ concentration in tubular fluid taken from the bend of the loop declined 1.62 ± 0.84 meq/liter–mm−1, where the first specimen was collected at least 45 min after the start of the saline infusion and the second specimen was collected 15–30 min later. Measurements of changes in tubular fluid Na+ concentration with time in antidiuresis have been reported previously (12). Vasa recta collections were made into pipettes that were 10 μm OD and 6 μm ID. Despite the fact that the inner diameter of the pipette was less than the erythrocyte diameter, blood flowed spontaneously into the pipette and a slight opposing pressure usually had to be applied to maintain normal erythrocyte velocity in the sampled vessel. Hemolysis was negligible.

Plasma was separated from erythrocytes by centrifugation, as described previously (16). When volume flow rates were to be measured, tubular fluid collections were made over timed intervals; tubular fluid volumes were determined by measuring the length of the fluid occupied in a 100-μm ID constant-bore tubing.

Osmolality was measured with a Feltier junction nanoliter osmometer, Na+ concentration (in triplicate) with an ultramicro flame photometer (12), Cl− concentration (in duplicate) with the Ramsay method (16), and urea concentrations (in duplicate) with a microspectrophotometric method (13).

Results are expressed as means ± standard errors of the mean. Tests of statistical significance were made with the Student t test. A probability of the null hypothesis less than 5% was taken to indicate statistical significance.

RESULTS

Sodium measurements. The results of 15 paired measurements of Na+ concentration in tubular fluid at the hairpin turn and adjacent vasa recta plasma are shown in Fig. 1. The collections were made in antidiuresis from 11 hamsters. The mean tubular fluid Na+ concentration was 436.2 ± 35.1 meq/liter, the mean vasa recta plasma concentration was 696.8 ± 38.0 meq/liter, and the mean difference was 39.4 ± 26.9 meq/liter. This difference does not differ significantly from zero. Tubular fluid Na+ concentration was on average 7.4 ± 3.9% higher than vasa recta plasma. These
results confirm earlier reports of similar measurements (16). The plasma measurements were not corrected for the fraction of plasma volume occupied by proteins. In arterial plasma, this nonaqueous fraction is 0.05–0.06. The vasa recta plasma protein concentration is as high or higher than arterial plasma's (3, 22, 23), so that any correction would raise the sodium concentration in plasma water and reduce the already insignificant difference between tubular fluid and vasa recta plasma. These measurements do not support one of the passive models of ALH function (10). This model requires a NaCl concentration difference that drives salt from lumen to interstitium; the experiments do not reveal a difference. As we have noted (12), however, the loops penetrate an interstitium whose NaCl concentration varies with distance. The very fact of fluid flow in ALH through such an interstitium will continuously create a transmural concentration gradient favoring NaCl diffusion out of the loop. If ALH NaCl permeability is great enough to take advantage of the small transmural gradient created by flow through the corticomedullary interstitial gradient, the present results would still be consistent with the passive model.

The results of 17 paired Na⁺ measurements in ALH tubular fluid and adjacent AVR plasma from a second group (13 animals) are shown in Fig. 2. These collections were also made in antidiuresis. The mean ALH tubular fluid Na⁺ concentration was 615.9 ± 30.1 meq/liter, while the mean AVR plasma concentration was 597.7 ± 24.3 meq/liter, and the mean difference between the two was 18.2 ± 23.8 meq/liter. The difference did not differ significantly from zero. The mean Na⁺ concentration in the bend of the same loops of Henle was 770.6 ± 52.6 meq/liter. Although the tubular fluid Na⁺ concentration fell 154.7 ± 30.0 meq/liter over about 1 mm of ALH, no Na⁺ concentration differences developed between tubular fluid and AVR. This result is clearly compatible with any passive ALH model that includes high-salt permeability, because one simple interpretation is that tubular NaCl remains very near equilibrium with AVR plasma. This result, however, cannot exclude an active transport hypothesis. Both interstitial and tubular fluid Na⁺ concentrations are less at the ALH sampling site than at the level of the loop's bend, but the velocity of an active transport mechanism might be inadequate to generate an axial gradient in tubular fluid greater than in the interstitium.

To eliminate the confounding effect the normal corticomedullary gradient introduces, we repeated these paired measurements in hamsters undergoing a brisk saline diuresis. The aim of these experiments was to reduce the corticomedullary gradient, so that an active transport mechanism, if it is present, could be freed to lower the tubular fluid Na⁺ concentration below interstitial levels. To judge how effectively the diuresis reduces the corticomedullary gradient, we made paired collections in AVR from eight hamsters. One sampling site was near the tip of the papilla, the second was in another AVR 1 mm nearer the cortex. The collections were made simultaneously in two separate but adjacent AVR to avoid the problem of bleeding that occurs after the pipette is withdrawn. Eleven paired measurements were made in this third group of hamsters; the mean corticomedullary Na⁺ gradient was 48.0 ± 18.7 meq·liter⁻¹·mm⁻¹. Over a similar distance in antidiuresis, the corticomedullary gradient in ALH tubular fluid was 154.7 meq/liter. Tubular fluid and AVR Na⁺ concentrations did not differ at either collection level so that the AVR gradient must have been of similar magnitude. Thus, the diuresis reduced the corticomedullary gradient to one-third its antidiuretic value.

Tubular fluid was also collected from the bend of Henle's loop in this third group of animals; the collection sites were immediately adjacent to the AVR sampled nearer the tip of the papilla. In these 11 paired collections performed in saline diuresis, tubular fluid at the hairpin turn had a Na⁺ concentration 20.0 ± 13.1 meq/liter higher than in adjacent AVR plasma. The difference was not significant. The average tubular fluid Na⁺ concentration in this group was 415.9 ± 33.6 meq/liter.

Figure 3 shows the results of 11 paired collections made at the ALH collection site in saline diuresis in a fourth group of animals (7 hamsters). The mean ALH Na⁺ concentration, 269.6 ± 13.8 meq/liter, was less than the mean AVR concentration, which was 276.6 ± 19.7 meq/liter, but the difference was not significant. In these measurements, tubular fluid Na⁺ concentration fell 20.8 ± 6.1
meq/liter during transit from the bend of the loop to the ALH sampling site. This fall was significant ($P < .005$) and indicates that Na$^+$ reabsorption continued in saline diuresis.

Despite the opportunity afforded by the reduction of the corticomedullary gradient, the ALH could not reduce tubular fluid Na$^+$ concentration below AVR levels. This result, however, still does not rule out an active transport mechanism, because the increased tubular fluid flow rate in saline diuresis complicates interpretation. This point is clarified by the following data. In 12 collections in a fifth group in antidiuresis (8 hamsters), tubular fluid flow rate at the bend of Henle's loop averaged $3.08 \pm .18$ nl/min. The mean Na$^+$ concentration in these collections was $85.19 \pm 70.8$ meq/liter, so that the Na$^+$ load delivered to the ALH was $2.6$ neq/min. If we assume that tubular fluid Na$^+$ concentration falls $155$ meq/liter between the hairpin turn and the ALH sampling site, as was the case in the collections shown in Fig. 2, and that there is no transmural water flux along the ALH (12), the net reabsorptive Na$^+$ flux was $4.76$ neq/min in ALH in antidiuresis. In 12 measurements made in saline diuresis in a sixth group (11 hamsters), the volume flow rate at the hairpin turn was $15.6 \pm 2.28$ nl/min. The mean Na$^+$ concentration at the bend of the loop was $352.9 \pm 16.7$ meq/liter, and the Na$^+$ load delivered to the ALH was $5.3$ neq/min. Saline diuresis increased tubular volume flow rate fivefold, so that Na$^+$ load doubled despite the reduced Na$^+$ concentrations. In these collections, tubular fluid Na$^+$ concentration fell $19.4 \pm 3.71$ meq/liter during passage of tubular fluid between the hairpin turn and the ALH sampling site. Again assuming no change in volume flow rate between the two sites, the reabsorptive flux, calculated for each individual experiment and averaged over the 12 experiments, was $297 \pm .068$ neq/min. In saline diuresis, the total reabsorptive flux was only 5.4% of the Na$^+$ load, so that the ALI might not be able to generate an observable transmural concentration difference, regardless of the mechanism causing the reabsorption.

To circumvent the complication caused by the high tubular fluid flow rates and still take advantage of the reduced corticomedullary gradient, we repeated the ALH/AVR comparison in saline diuresis in a seventh group (8 hamsters). In these animals, flow of tubular fluid to the ALH was reduced by sampling simultaneously into both tubule pipettes. In all the experiments reported above, no tubular fluid flowed into the pipette at the bend until the ALII collection had been completed. In this slow-flow series, the ALH collection pipette was opened to ambient pressure and the ALH collection proceeded spontaneously; the pressure in the pipette at the bend of the loop was controlled by syringe and adjusted to keep the flow in the ALH pipette constant, as judged by eye, while an oil drop blocked the tubule just distal to the ALH pipette. An AVR was sampled at the level of the ALH after the tubular fluid collection was complete. The results of 15 paired measurements are shown in Fig. 4. The mean ALII sodium concentration was $410.9 \pm 29.2$ meq/liter, the mean AVR Na$^+$ concentration was $459.7 \pm 30.45$ meq/liter, and the mean difference was $48.8 \pm 10.5$ meq/liter. This difference was highly significant ($P < .0005$). The mean ratio of concentrations, ALH/AVR, was $0.89 \pm 0.021$. The flow rate in these experiments was $1.92 \pm .31$ nl/min, one-eighth the normal value in saline diuresis and two-thirds the antidiuresis value. The Na$^+$ delivered to the ALH was $0.83$ neq/min; the computed reabsorptive flux was $0.094$ neq/min. This result would seem to demonstrate reabsorption of sodium, and presumably therefore of NaCl, against a significant concentration difference. Such contragradient transport is clearly compatible with the active transport model.

Imai and Kokko (4) recently observed that rabbit ALH, perfused in vitro, developed a positive electrical potential difference when the NaCl concentration of the perfusion solution exceeded that of the bath, a result they attribute to a higher tubular permeability for Cl$^-$ than for Na$^+$. Their finding raises the possibility that a concentration difference for Cl$^-$ between tubular and interstitial fluids could generate an electrical potential difference and provide a driving force for the reabsorption of Na$^+$ against a concentration gradient described in the previous paragraph. To evaluate this possibility, we measured Cl$^-$ concentration differences in an eighth group (7 hamsters) undergoing saline diuresis. In 14 paired measurements at the bend of Henle's loop, the tubular fluid [Cl$^-$] was $343.1 \pm .89 \pm 0.021$. The flow rate in these experiments was $1.92 \pm .31$ nl/min, one-eighth the normal value in saline diuresis and two-thirds the antidiuresis value. The Na$^+$ delivered to the ALH was $0.83$ neq/min; the computed reabsorptive flux was $0.094$ neq/min. This result would seem to demonstrate reabsorption of sodium, and presumably therefore of NaCl, against a significant concentration difference. Such contragradient transport is clearly compatible with the active transport model.

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17.7 meq/liter, the AVR concentration was 333.4 ± 18.5 meq/liter, and the mean difference was 9.7 ± 4.9 meq/liter. The difference was marginally significant (P < .05). Because of macroscopic electroneutrality, the observed Cl- gradient is too small by a factor of 5 to account for the generation of the Na+ concentration gradient that was observed in the slow-flow experiments.

**Urea.** Urea measurements were made only in antidiuresis. Results are summarized in Table 1. At the bend of Henle's loop, mean tubular fluid was 125 mM less than AVR concentration. The ratio of the two concentrations was 0.75 ± .046. This result is the same as reported previously (12). Since the tubular fluid Na+ concentration exceeds that in AVR plasma by only 30 meq/liter, the total osmolality predicted from the osmotic contributions of NaCl and urea should be lower in tubular fluid than in AVR. In 13 measurements, mean tubular fluid osmolality at the hairpin turn was 1,656.8 ± 71.5 mosmol/kg H2O and 1,744.2 ± 89.6 mosmol/kg H2O in their corresponding AVR samples. The difference was 67.5 ± 37.6 mosmol/kg H2O, AVR higher, and was marginally significant (P < .025). These data suggest that osmotic equilibration in DLH may not proceed to completion in antidiuresis.

Tubular fluid urea concentrations at the ALH site remained lower than AVR concentrations. The difference was less than at the bend, despite the fact that papilla urea concentrations were generally higher in this group of animals than in the hamsters from which the comparisons at the hairpin turn were made. The declining difference between tubular fluid and vasa recta plasma concentrations was accompanied by a 118.4 ± 26.1 mM increase of urea concentration between the bend of the loop and the ALH sampling site. This result shows that within the first 20% of the ALH, two-thirds or more of the urea concentration difference has been dissipated by an influx of urea.

**DISCUSSION**

The principal assumption needed to interpret our results is that measurements in AVR plasma give reliable estimates of interstitial fluid concentrations. The interstitium is inaccessible to direct measurement by methods currently available, although electron microprobe methods offer promise of resolving this problem (11). Until the various technical problems associated with microprobe analysis are solved, we have no other approach than to assume that capillaries are so permeable that capillary blood equilibrates with interstitial fluid, at least with respect to small solutes like NaCl and urea. End capillary blood is more likely to have equilibrated than capillary blood sampled randomly in the capillary bed; the optimal sampling site is therefore in venules. AVR are the venules of the medulla, but they traverse the corticomedullary gradient, and they will have higher concentrations of NaCl and urea than the interstitial fluid at all levels through which the vessels must flow to reach the cortex.

Our recent study of vasa recta (15) provides a method for estimating just how different concentrations in AVR plasma and interstitial fluid may be, or even if descending vasa recta (DVR) would provide a better estimate than AVR. In that study, we reported several significant differences between DVR and AVR. Descending vessels were fewer in number, narrower, and had a lower Na+ permeability and a higher blood flow speed than their ascending counterparts. The product of the total surface area and permeability gives the solute conductance; the ascending conductance was found to be 4.2 times greater than descending conductance. The higher conductance of the AVR immediately suggests that they will have concentrations closer to interstitial fluid than will the DVR; the higher flow speed of the descending vessels can only accentuate this discrepancy. Equations 13, 14, and 15 of the mathematical model (15) can provide quantitative predictions of the concentration differences. Figure 5 shows Na+ concentration ratios (local concentration divided by arterial plasma concentration) in interstitial fluid, and in the plasma of DVR and AVR blood, as a function of distance along the corticomedullary axis. AVR concentration exceeds interstitial concentration; DVR concentration is lowest. At the part of the vascular bundle nearest the papilla, AVR concentration is only 0.5% greater than interstitial values, but DVR is 3% less than interstitium. The vascular bundles reaching the tip of the papilla are 8 mm long in the hamster (15); a sampling site 1 mm from the papilla tip is therefore at a fractional distance of 0.075 from the cortex. The curves of Fig. 5 show that at this distance, AVR concentration is 1% higher than interstitial concentration, while DVR concentration is 4% lower than the interstitium. We conclude

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**Table 1. Summary of urea measurements in antidiuresis**

<table>
<thead>
<tr>
<th>Tubular Fluid Collection Site</th>
<th>Urea Conc, mM</th>
<th>AVR Plasma Urea Conc, mM</th>
<th>Tubular Fluid:AVR Plasma Urea Conc</th>
<th>Difference, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bend of loop 17</td>
<td>360.6±30.4</td>
<td>485.3±32.3</td>
<td>-124.8±25.3</td>
<td>-7.3±0.5</td>
</tr>
<tr>
<td>Thin ascending limb 12</td>
<td>566.0±64.7</td>
<td>613.8±66.1</td>
<td>-47.8±21.8</td>
<td>-2.7±0.5</td>
</tr>
</tbody>
</table>

**FIG. 5.** Concentration profiles of Na+ in vasa recta and interstitium, computed from equations 13, 14, and 15 of ref. 15. Greek letters refer to parameters of equations; their values were taken from ref. 15. Beta is ratio of total AVR volume flow to total DVR volume flow; $k$ is product of DVR volume surface area times Na+ permeability, divided by DVR volume flow rate; $\xi$ is concentration of Na+ in fluid absorbed by capillaries from tubules; $\rho$ is total surface area times Na+ permeability of AVR, divided by total surface area times permeability of DVR.
forces acting on the anion and cation, and the opposite force does not require knowledge of the electrical potential difference, because the net force is given by the sum of the electrical terms to disappear entirely (6). With the vanishing of the electrical terms, and convective forces also not present (12), the driving force on the salt is determined solely by the concentration difference. If the electrical potential difference has the same sign in slow-flow saline diuresis as in antidiuresis, active transport of Na+ would be implied.

If the plasma Na+ concentration were corrected for the volume occupied by proteins, the gradient against which the transport occurred would be even greater than actually measured. A 6% correction applied to vasa recta samples in saline diuresis with normal flow of tubular fluid also leads to a finding of contragradient transport in NaCl. The electrical potential difference, which was found to be -10 mV, lumen negative, in antidiuresis (16, 24), has not been measured in saline diuresis. In the case of transport of a uniunivalent salt, however, evaluation of the net driving force does not require knowledge of the electrical potential difference, because the net force is given by the sum of the forces acting on the anion and cation, and the opposite signs of the respective valences cause the electrical potential terms to disappear entirely (6). With the vanishing of the electrical terms, and convective forces also not present (12), the driving force on the salt is determined solely by the concentration difference. If the electrical potential difference has the same sign in slow-flow saline diuresis as in antidiuresis, active transport of Na+ would be implied.

The evidence supporting active NaCl transport was obtained only after measures were taken to reduce tubular flow rates and corticomedullary interstitial gradients. Thus, it is necessary to ask whether the active transport flux can be made a quantitatively significant contribution to the reabsorption of NaCl in antidiuresis. We can provide a preliminary answer to this question by first estimating the NaCl permeability of ALH from measurements in saline diuresis and then estimating the contribution of passive processes to NaCl reabsorption in antidiuresis.

The equations, and the assumptions used to derive them, are given in APPENDIX, together with the numerical values of the variables obtained from measurements. The calculated value of the NaCl permeability of ALH is 10.8 ± 1.33 X 10^-4 cm/s, the statistical measure is the standard deviation, obtained by a Monte Carlo simulation as explained in APPENDIX. With this value, we can predict how much the ALH NaCl concentration would fall as tubular fluid moves from the bend of Henle’s loop toward the cortex in antidiuresis, under the assumption that active transport is zero. The predicted decline of concentration is 89.3 ± 74 (SD) meq/liter, while the measured decline was 154.7 meq/liter. As shown in APPENDIX, the measured concentration fall differs significantly from the calculated value (P < .0003). Since only 50% of the concentration dccrcasc is accounted for by passive processes, it seems a reasonable conjecture that active transport is responsible for the rest.

The results reported here are consistent with a significant participation of active transport in the observed reabsorption of NaCl. The reliability of the numerical estimates provided in the preceding paragraph depends not only on the accuracy of the values supplied from measurements, a factor that is open to statistical evaluation, but also on the validity of the magnitude of the concentration difference favoring passive diffusion. As noted below, the results of this study suggest that passive processes contribute significantly to net reabsorption of NaCl; minor flow reductions would not alter that conclusion.

The most likely mechanism of contragradient transport is active transport that derives its energy from metabolism. Results of this study cannot prove active transport, however, because the inevitable presence of urea in these fluids, even during the most brisk saline diuresis, introduces a possible other energy source that could drive NaCl against a concentration gradient without requiring the expenditure of metabolic energy in the ALH. Urea enters the lumen of ALH driven by a concentration difference. In a limited number of observations, we have found that this flux continues in saline diuresis although at substantially reduced concentrations. The dissipation of the urea gradient could drive NaCl against its gradient, provided that the membranes of the ALH permit an interaction between salt and urea as they flow past each other in opposite directions. We do not know of any experimental demonstration of such an interaction, and we have been unable to conceive of a mechanism by which it might occur. Therefore, it seems most likely that contragradient NaCl transport from ALH receives its energy from metabolism and so constitutes the active transport that has long been suspected in these regions of the nephron.

APPENDIX.

The delay between collection of the tubular fluid and vasa recta specimens also serves to bias the results against a finding of contragradient transport in saline diuresis. The delay averaged 7 min, tubular fluid Na+ declined an average of 1.6 mcg-liter^-1-min^-1; the tubular fluid concentration would therefore have been 11.2 mcg/liter lower at the time of the AVR collection than was actually measured. On the other hand, the presence of a pipette in the bend during collection from the ALH may have reduced volume flow rate, either by obstructing flow or by permitting a leak around the pipette in the bend. Although there were no gross discrepancies between the volumes collected at the ALH and at the bend, no attempt was made to exclude minor differences in flow rate. Since slowing flow did allow the tubule to reduce Na+ concentrations, this source of error, if it is present at all, would tend to underestimate
the assumptions we used to derive the expressions for the
estimates. Although these assumptions seem reasonable
first approximations, to the extent that they fail to de-
scribe reality they will introduce a source of error. Despite
this residual uncertainty, several conclusions do seem justi-
First, there is active transport of NaCl by thin ALH.
the process can lower tubular fluid NaCl concentrations
below interstitial values, and it might provide for an im-
portant fraction of the reabsorptive flux in antidiuresis.
Second, passive transport from ALH is not negligible; it
will have to be included in any quantitative modeling of
the countercurrent system. Third, the presence of active
transport in ALH removes some of the restrictions the
new models impose on the permeability properties of DI.H
(10, 19, 20). These models require the DLH to be relatively
impermeable to NaCl and urea. As we noted in intro-
duction, the direct evidence on this point is contradic-
tory. Previously, we have found a diffusive influx of urea into
DLH tubular fluid which increased the urea load 52%
in the terminal 1 mm of DLH (12). De Rouffignac and
Morel (2) have presented evidence that argues strongly in
favor of entry of NaCl into DLH tubular fluid in Psammomys
obesus. Their result implies a high NaCl permeability and
Acdxjq = --r' + p[c~(x> - G(x)] nx (1)
values, except when flow is slowed. With normal flow, the
reduce the tubular fluid concentration below interstitial
concentration is reduced less. The effect of increased flow
increased their reabsorption when flow rates increased. The
increased loads differs qualitatively from the behavior of C,(X) - C,(X) = -p -
question is whether the passive mechanism makes a quanti-
tative contribution to the operation of con-
centrating the urine.
Finally, it should be noted that the response of ALH to
increased loads differs qualitatively from the behavior of
thick ascending limbs. In this study, net NaCl reabsorption
decreased when flow rate was increased in saline diuresis;
Schnermann (18) found that thick ascending limbs in-
creased their reabsorption when flow rates increased. The
most plausible explanation for the result in thick limbs is
that with increasing flow rates, the active transport flux
becomes a smaller fraction of the load and the luminal
concentration is reduced less. The effect of increased flow
rates in thick limbs is thus to reduce the concentration
difference favoring passive movement of NaCl into the
lumen. In thin limbs, at least in the 1-mm segments we
have studied, active transport is not sufficiently intense to
reduce the tubular fluid concentration below interstitial
values, except when flow is slowed. With normal flow, the
passive flux component is in the reabsorptive direction,
and because saline diuresis reduces the corticomedullary
gradient, the effect would be to reduce the passive reab-
sorptive flux. In addition, the fall of tubular fluid NaCl
concentrations that saline diuresis produces may diminish
the active transport flux if the transport reaction is not
fully saturated at the concentrations found in this study.

APPENDIX
We wish to calculate the NaCl permeability of ALH from mea-
sured values of flow and concentration obtained in saline diuresis.
We make the following assumptions:
1) Transmural flow of water is negligibly small in all experiments,
as was found to be true in antidiuresis (12).
2) All the reabsorption in saline diuresis with normal volume flow
rates is by active transport.
The justification for this assumption is that the transmural con-
centration gradients are small and would be expected to give rise
to minimal diffusive movements under these circumstances.
3) The active transport rate found in free-flow saline diuresis is
the same as in slow-flow saline diuresis. The active transport mecha-
nism is either concentration dependent and operating at approximately
the same NaCl concentration in both groups of experiments or it is
saturated and therefore constant.
4) There is a backflux of NaCl in slow-flow experiments propor-
tional to the local transmural NaCl concentration difference.
We consider an element of volume in an ALH with radius r and
length 8. In the steady state, the change of NaCl mass in the
volume element is given by
\[ \Delta C(x) \frac{q}{dx} = -2\pi r [S + P[C(x) - C_0(x)]] \Delta x \]
where \(C(x)\) is the local concentration, \(q\) is the volume flow rate of
tubular fluid, \(S\) is the rate of active transport per unit of area, and
\(P\) is the permeability coefficient for NaCl. The subscript \(L\) refers
to lumen and subscript \(o\) refers to interstitium. Holding \(q\) constant
and taking the limit yields
\[ \frac{dC_L}{dx} = \frac{-2\pi r}{q} [S + P[C(L(x) - C_0(x))] \]
a nonhomogeneous ordinary linear differential equation.
The corticomedullary gradient in the interstitial space is a mono-
 tonic function of distance. To a reasonable first approximation, this
gradient can be assumed to be a constant over the sampling distance.
The known tubular fluid concentration at \(x = 0\) is the initial con-
tion, and equation 2 can be solved by standard analytic methods.
The solution, rearranged in terms of variables which were measured
experimentally, is:
\[ C_L(x) - C_0(x) = \frac{-S}{2\pi r q} \left[ \frac{C_0(x) - C_0(0)}{P} \right] \]
\[ + \left\{ \frac{[C_0(0) - C_0(x)] + \frac{S}{P} \left[ \frac{C_0(x) - C_0(0)}{2\pi r q} \right]}{2\pi r q} \right\} \cdot \exp \left[ \frac{-2\pi r x}{q} \right] \]

For the sake of completeness, we introduce the following notation:
\[ Y_1 = Y_2 \]
\[ - S, measured in saline diuresis with normal flow, Y_2 = C_L(x) - C_0(x), Y_3 = C_L(x) - C_0(x), Y_4 = C_L(0) - C_0(0), \]
\[ Y_5 = 2\pi r q, Y_6, \ldots, Y_8 \] measured in saline diuresis with slow flow. The value of \(r\) was assumed to be 10 \(\mu\) cm (16). The means and standard
deviations are summarized in Table 2A. Since \(Y_3\) and \(Y_6\), as well as
\(Y_5\) and \(Y_8\), were not measured independently, it was necessary to
calculate the correlation between both pairs of parameters. The
resulting calculations yielded \(\text{Corr} \ (Y_3, Y_5) = -0.389,\) and \(\text{Corr} \ (Y_3, Y_6) = 0.585.\)

Since equation 3 cannot be solved explicitly for \(P\), it was necessary
to use numerical methods to solve for that quantity. Since it was also
of interest to estimate the standard deviation of \(P\), a Monte-Carlo
simulation was written for both purposes. In brief, the details of this
FORTRAN-IV program arc as follows: assuming that \(Y_1, Y_3, \ldots, Y_8\) have a multivariate normal distribution with means, standard
deviations, and correlations given above, \(n\) realizations \(Y_1, \ldots, Y_8\)
were generated using the IBM/SPS pseudo random number generator subroutine GAUSS. This n x 5 data matrix was then used as the input to the nonlinear least-squares program RMDX85 (1). The output of that program included the maximum likelihood estimate of P, as well as its asymptotic standard deviation. For the sample sizes considered, convergence occurred in seven steps. The resulting estimate of P was $10.8 \times 10^{-5}$ cm$^2$/s, for $n = 1,000$. Changing the sample size to $n = 100$ did not change these estimates, to a precision of five places.

Under the assumption that the active transport is zero, equation 3 

\[ \text{rate} \times S \times \text{permeability} \times \text{area} \]

can be arranged to solve for the concentration difference 

\[ [C_l(X) - C_o(X)] \]

in terms of variables that were measured in antidiuresis. The result is

\[ C_l(X) - C_o(X) = \frac{[C_l(X) - C_o(X)]}{2 \pi \sigma P \frac{1}{q}} \left( \frac{1}{2} \right) \exp \left[ - \frac{2 \pi \sigma P X}{q} \right] \]

We set $Z_1 = C_l(X) - C_o(X)$, $Z_2 = C_l(0) - C_o(0)$, $Z_3 = \lambda$, and $Z_4 = 2 \pi \sigma P / q$; all were measured in antidiuresis. Again, $\lambda$ is taken to be $10^4$ cm (16). The means and standard deviations are summarized in Table 2B. The correlation between the only two dependent variables, namely $Z_1$ and $Z_2$, was small enough to ignore for subsequent calculations. When the means of $Z_1$, $Z_2$, $Z_3$, and $Z_4$ (along with the mean value of $P$) were substituted into the right-hand side of equation 4, the mean decrease in tubular fluid Na$^+$ concentration was calculated as $\mu = -89.3$ meq/liter. To estimate the standard deviation of $[C_l(X) - C_o(X)]$, a second Monte Carlo simulation was written. In brief, the details of this FORTRAN IV program are as follows: assuming that $Z_1$, $Z_2$, $Z_3$, and $P$ are independent, normally distributed random variables with means and standard deviations given above, $n$ realizations of $Z_1$, $Z_2$, $Z_3$, and $P$ were randomly generated. For each realization, the quantity $[C_l(X) - C_o(X)]$ was calculated using equation 4. The standard deviation of the $n$ values of $[C_l(X) - C_o(X)]$ served as the desired estimate; convergence of this estimate was ascertained by increasing $n$. The resulting standard deviation was $\sigma = 74$ meq/liter.

Since the means and standard deviations of the variables $Z_1$, $Z_2$, $Z_3$, and $P$ result from very precise measurements, we assume that $\mu = -89.3$ meq/liter and $\sigma = 74$ meq/liter are the true mean and standard deviation of $[C_l(X) - C_o(X)]$, respectively. We now wish to test whether the mean of the measured concentration decline ($\bar{X} = -154.7$ meq/liter) is significantly different from $\mu$. Performing the usual $z$ test, we obtain

\[ z = \frac{154.7 - (-89.3)}{16} \]

which, tested against a one-sided alternative, is significant at $P < .0003$. Thus, we reject the null hypothesis that the calculated and measured concentration declines are the same and conclude that the mean concentration declines more than the value calculated on the assumption that active transport is zero.

Our assumption that the active transport flux is equal to the net reabsorptive flux in saline diuresis is probably the least certain of the assumptions used in APPENDIX. Some fraction of the reabsorption could be due to passive mechanisms, and it is therefore necessary to evaluate the sensitivity of the various estimates to changes in $S$. The approach we adopted was to allow $S$ to vary in equation 3, while holding all other variables constant, and to solve for $P$. For this sensitivity analysis we used the average values listed in Table 2A; we could not repeat the Monte Carlo simulations for each value of $S$ because statistics are only available for the measurements that were actually performed. To find $P$ at each value of $S$, we varied $P$ between $5 \times 10^{-5}$ and $2.5 \times 10^{-4}$ cm$^2$/s, in increments of $5 \times 10^{-5}$ cm$^2$/s. The value of $P$ and $S$ were inserted into equation 5, and the value of $[C_l(X) - C_o(X)]$ was computed. The value of $P$ was then selected that minimized the square of the difference between the computed and the measured values of $[C_l(X) - C_o(X)]$. These values for $P$ were then inserted into equation 4, together with the average values listed in Table 2B, to compute the concentration decline in anti-diuresis attributable to passive diffusion. This procedure provides a less certain estimate of $P$ than the Monte Carlo simulation, but it does indicate the direction of the changes to be expected. The results are shown in Table 3; they indicate that $P$ increases with increasing $S$. To the extent that our assumption leads to an overestimate of $S$, it will also provide too high an estimate of $P$. This relationship is intuitively obvious, for the maintenance of a given transmural concentration difference at the ALH sampling site, the active transport flux and the passive leak flux must be approximately equal and oriented in opposite directions. If the active transport flux is decreased, the approximate equality of the fluxes can be maintained only by a reduction of the permeability. As expected, the passive flux in

### Table 2. Statistics for computation of permeability coefficients and tubular fluid Na$^+$ concentration decline in antidiuresis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean Value</th>
<th>Standard Deviation</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_1 = S$</td>
<td>$8.07 \times 10^{-9}$ eq/cm$^2$·s$^{-1}$</td>
<td>$8.27 \times 10^{-9}$ eq/cm$^2$·s$^{-1}$</td>
<td>12</td>
</tr>
<tr>
<td>$Y_2 = C_t(X) - C_o(X)$</td>
<td>$-4.8 \times 10^{-6}$ eq/cm$^2$</td>
<td>$4.72 \times 10^{-6}$ eq/cm$^2$</td>
<td>15</td>
</tr>
<tr>
<td>$Y_3 = C_t(0) - C_o(0)$</td>
<td>$-2.4 \times 10^{-6}$ eq/cm$^2$</td>
<td>$4.35 \times 10^{-6}$ eq/cm$^2$</td>
<td>11</td>
</tr>
<tr>
<td>$Y_4 = 2\pi \xi / q$</td>
<td>$2.60 \times 10^{-6}$ s·cm$^{-2}$</td>
<td>$1.37 \times 10^{-6}$ s·cm$^{-2}$</td>
<td>15</td>
</tr>
</tbody>
</table>

### Table 3. Sensitivity of computed ALH NaCl permeability coefficient and of computed NaCl reabsorption in antidiuresis to estimate of active transport flux

<table>
<thead>
<tr>
<th>Active Transport Rate, $S$, cm$^{-2}$</th>
<th>Computed NaCl Permeability, $P$, cm$^{-2}$·s$^{-1}$</th>
<th>Computed ALH Concentration Decline in Antidiuresis, meq/liter</th>
<th>Computed Fraction of ALH Concentration Decline Due to Passive Diffusion, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>1.35</td>
<td>19.1</td>
<td>12.3</td>
</tr>
<tr>
<td>4.0</td>
<td>2.90</td>
<td>33.5</td>
<td>21.7</td>
</tr>
<tr>
<td>4.5</td>
<td>4.20</td>
<td>45.7</td>
<td>29.5</td>
</tr>
<tr>
<td>5.0</td>
<td>5.45</td>
<td>56.1</td>
<td>36.3</td>
</tr>
<tr>
<td>5.5</td>
<td>6.00</td>
<td>63.0</td>
<td>42.0</td>
</tr>
<tr>
<td>6.0</td>
<td>7.80</td>
<td>79.7</td>
<td>47.0</td>
</tr>
<tr>
<td>6.5</td>
<td>8.95</td>
<td>79.6</td>
<td>51.5</td>
</tr>
<tr>
<td>7.0</td>
<td>10.1</td>
<td>85.9</td>
<td>55.5</td>
</tr>
<tr>
<td>7.5</td>
<td>11.2</td>
<td>91.3</td>
<td>59.0</td>
</tr>
<tr>
<td>8.0</td>
<td>12.3</td>
<td>96.2</td>
<td>62.2</td>
</tr>
<tr>
<td>8.5</td>
<td>13.4</td>
<td>100.7</td>
<td>65.1</td>
</tr>
<tr>
<td>9.0</td>
<td>14.5</td>
<td>104.6</td>
<td>67.6</td>
</tr>
<tr>
<td>9.5</td>
<td>15.3</td>
<td>108.2</td>
<td>69.9</td>
</tr>
<tr>
<td>10.0</td>
<td>16.6</td>
<td>111.6</td>
<td>72.1</td>
</tr>
</tbody>
</table>

# References

1. [IBM/SPS pseudo random number generator subroutine GAUSS](http://www.ibm.com/software/ibm/spss)
antidiuresis varies directly with the permeability. Thus, if our assumption about the magnitude of the active transport flux is overestimate, so also is our estimate of the contribution of passive processes to NaCl reabsorption in antidiuresis.

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