

# Sodium Chloride, Urea, and Water Transport in the Thin Ascending Limb of Henle

## GENERATION OF OSMOTIC GRADIENTS BY PASSIVE DIFFUSION OF SOLUTES

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**ABSTRACT** Studies were designed to examine whether the thin ascending limb of Henle (tALH) decreases its luminal solute concentration by an active or a passive transport process. In all experiments isolated segments of rabbit tALH were perfused *in vitro*. When tubules were perfused with solutions identical to the bath, active transport of NaCl was excluded by the following: (a) osmolality of the collected fluid remained unchanged and the same as the bath, (b) net water reabsorption could not be demonstrated, and (c) transtubular potential difference was zero. Isotopic permeability coefficients ( $\times 10^{-5}$  cm s<sup>-1</sup>) were calculated from the disappearance rate of the respective isotope added to the perfusate. These values indicate that tALH is moderately permeable to [<sup>14</sup>C]urea ( $6.97 \pm 1.95$ ) while having a higher permeability to <sup>22</sup>Na ( $25.5 \pm 1.8$ ) and <sup>36</sup>Cl ( $117 \pm 9.1$ ) than any other segment similarly studied. The influx (bath-to-lumen) isotopic permeabilities were not statistically different from the above efflux permeabilities. Osmotic water permeability was immeasurably small. When tALH were perfused with a 600 mosmol/liter solution predominantly of NaCl against a 600 mosmol/liter bath in which 50% of osmolality was NaCl and 50% urea (to simulate *in vivo* papillary interstitium), the collected fluid osmolality was decreased significantly below that of the bath (300 mosmol/liter/mm of tubule). The decrease in osmolality was due to greater efflux of NaCl as compared to influx of urea.

We conclude that active transport of salt by the tALH

was not detected by the experimental protocol of the current studies, and that the unique membrane characteristics of tALH allows for generation of osmotic gradients (lumen less concentrated than adjacent surroundings) on purely passive mechanisms when perfused with isosmolal salt solutions in a bath with appropriate salt and urea concentrations. These findings are consistent with the passive countercurrent model previously proposed from this laboratory.

## INTRODUCTION

The formation of concentrated urine by the renal countercurrent multiplication system (CCMS)<sup>1</sup> requires that at least one of the medullary structures has a capacity to transport salt in excess of water. Two observations suggest that the process of selective solute transport is localized in the ascending limb of Henle's loop. First, micropuncture studies of the early distal convoluted tubule show that the fluid emerging from the thick ascending limb of Henle has a very low concentration of NaCl, and it is hypertonic to plasma. Second, the micropuncture studies by Sakai, Tadokoro, and Yamaguchi (1) and by Jamison, Bennett, and Berliner (2) have shown that both the osmolarity and NaCl concentration in the thin ascending limb of Henle (tALH) is lower than the adjacent descending limb of Henle. The studies of Marsh (3) have also shown that the osmolality and the Na concentration in the tALH are lower than the

<sup>1</sup>Abbreviations used in this paper: ADH, antidiuretic hormone; CCMS, countercurrent multiplication system; CF, collected fluid; DLH, descending loop of Henle;  $L_p$ , hydraulic conductivity of water;  $P_{aw}$ , isotopic diffusional permeability of water; PF, perfusion fluid; tALH, thin ascending limb of Henle.

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osmolality and the Na concentration obtained by paired collection from the bend of the loop of the same nephron. Recently Rocha and Kokko (4) and Burg and Green (5) have shown that both the medullary and cortical portion of the thick ascending limb of Henle are water-impermeable and have the capacity to reabsorb salt by active chloride transport. In contrast, the transport characteristics of the tALH are less clear, and experimental attempts to demonstrate active salt transport out of the tALH have been uniformly unsuccessful.

Both Kokko and Rector (6) and Stephenson (7) have independently presented a new model of CCMS in inner medulla and papilla that may operate without active salt transport out of the tALH. In this model both the tALH and thin descending limb of Henle (DLH) act as passive equilibrating segments. According to the model as proposed by Kokko and Rector (6), the addition of salt in excess of water to the interstitial fluid and the subsequent formation of dilute intraluminal fluid by the tALH requires that this segment of the loop have the following unique permeability properties: impermeability to water; high permeability to NaCl; and moderate permeability to urea. This is in marked contrast to the permeability characteristics observed by Kokko (8, 9) for the DLH, which was found to be highly permeable to water and impermeable to salt (8) and urea (9).

The purpose of the present studies, therefore, was to evaluate the transport characteristics of the tALH. Isolated segments of rabbit tALH were studied with the *in vitro* perfusion technique. The following characteristics were examined: (a) presence or absence of active transport; (b) permeability to water, sodium, chloride, and urea; and (c) mechanism of formation of dilute intraluminal fluid. These studies showed that the tALH does not actively transport salt and is impermeable to water, highly permeable to sodium chloride, and moderately permeable to urea. In addition, it was shown that the tALH can produce hypotonic fluid by purely passive transport processes. These results, therefore, strongly support the passive diffusion model of CCMS recently proposed from this laboratory (6).

## METHODS

Segments of tALH were perfused *in vitro* by the same technique described previously for the thin DLH (8, 9). Female New Zealand rabbits weighing 1.5–2.5 kg were used in all experiments. All rabbits were fed a standard laboratory diet and had free access to water before guillotine decapitation. The kidney was quickly removed and cut into 1–2 mm slices. A segment of tALH was dissected out in a chilled dish of rabbit serum without the use of collagenase. The serum was kept at pH of 7.4 by continuous bubbling with 95% O<sub>2</sub>–5% CO<sub>2</sub>. Positive identification of the tALH (from the DLH) was accomplished by two criteria: first, only those thin segments were perfused in which the transition from thin to thick segment occurred at the junction of inner and outer medulla as contrasted to

the outer stripe of outer medulla in the case of DLH; second, the transition from tALH to thick medullary ascending limb is abrupt, as contrasted to the gradual change in pars recta epithelium to the DLH epithelium (Fig. 1). The dissection of the tALH in rabbit is much more difficult than the DLH because the tALH is so closely bathed in an extensive capillary network that is difficult to separate from the tALH without creating mechanical holes. In the initial experiments the possibility of a leak was checked by monitoring the appearance of the volume marker in the bath while in the later experiments the perfusate was colored with 0.2–0.5 mg ml<sup>-2</sup> FD&C green No. 3 (Keystone Aniline & Chemical Co., Chicago, Ill.) so as to visualize a potential leak immediately. Results with and without FD&C green gave identical results. Unless otherwise specified, tubules were perfused with an ultrafiltrate of rabbit serum made by pressure dialysis with PM-30 membranes (Amicon Corp., Lexington, Mass.). The tubular perfusion rate was controlled by varying the magnitude of the hydrostatic gradient connected to the end of the perfusion pipette.

The transtubular electrical potential difference (PD) was measured by techniques previously reported (10). Equivalent bridges of 300 mosmol/kg Ringer's solution in 4% agar were connected to the end of the perfusion pipette and the bath. The other ends of the bridges were submerged in saturated KCl solution that contained Beckman (Beckman Instruments, Inc., Fullerton, Calif.) calomel half-cells. Both ends of the tubule were sealed from electrical leaks by first coating and then placing Sylgard 184 (Dow Corning Corp., Midland, Mich.) in the tips of the holding pipettes.

The hydraulic conductivity of water ( $L_p$ ) was determined by measuring net fluid movement in response to an osmotic gradient. Both [<sup>125</sup>I]iothalamate (Glofil-125, Abbott Laboratories, North Chicago, Ill.) and [<sup>14</sup>C]inulin (New England Nuclear, Boston, Mass.) were used as volume markers. Neither of these were found to penetrate the tALH. After three 10-min control periods in which tubules were perfused with ultrafiltrate isosmolal to the bath, 314 ± 4.5 mosmol/kg, the osmolality of the bath was raised by addition of raffinose to 644 ± 6.5 mosmol/kg. 20 min were then allowed for equilibration, and three additional 10-min collections were made. In each case the osmolality of the perfusate, bath and collected fluid were measured by techniques previously described (8).  $L_p$  was then calculated by the following equation (8):

$$L_p = \frac{\Delta J_v}{\sqrt{(\pi_b - \pi_p)(\pi_b - \pi_c)}}, \quad (1)$$

where  $\Delta J_v$  is the increment in induced net water flux after raffinose was added to the bath,  $\pi_b$  is the osmolality of the bath,  $\pi_p$  is the osmolality of the perfusate, and  $\pi_c$  is the osmolality of the collected fluid.

The permeability coefficient, for Na, Cl, H<sub>2</sub>O, and urea were estimated by measuring the rate of appearance or disappearance from the luminal fluid of the respective isotope added either to the bath or to the perfusate. Either <sup>22</sup>Na (New England Nuclear) or <sup>24</sup>Na (ICN Corp., Chemical & Radioisotopes Div., Irvine, Calif.) were used for measurement of sodium permeability. In those experiments in which bidirectional fluxes were estimated simultaneously, <sup>22</sup>Na (20 μCi/ml) was added to the perfusate and <sup>24</sup>Na (10–20 μCi/ml) was added to the bath. The permeability coefficient for chloride was obtained by the use of <sup>36</sup>Cl (ICN

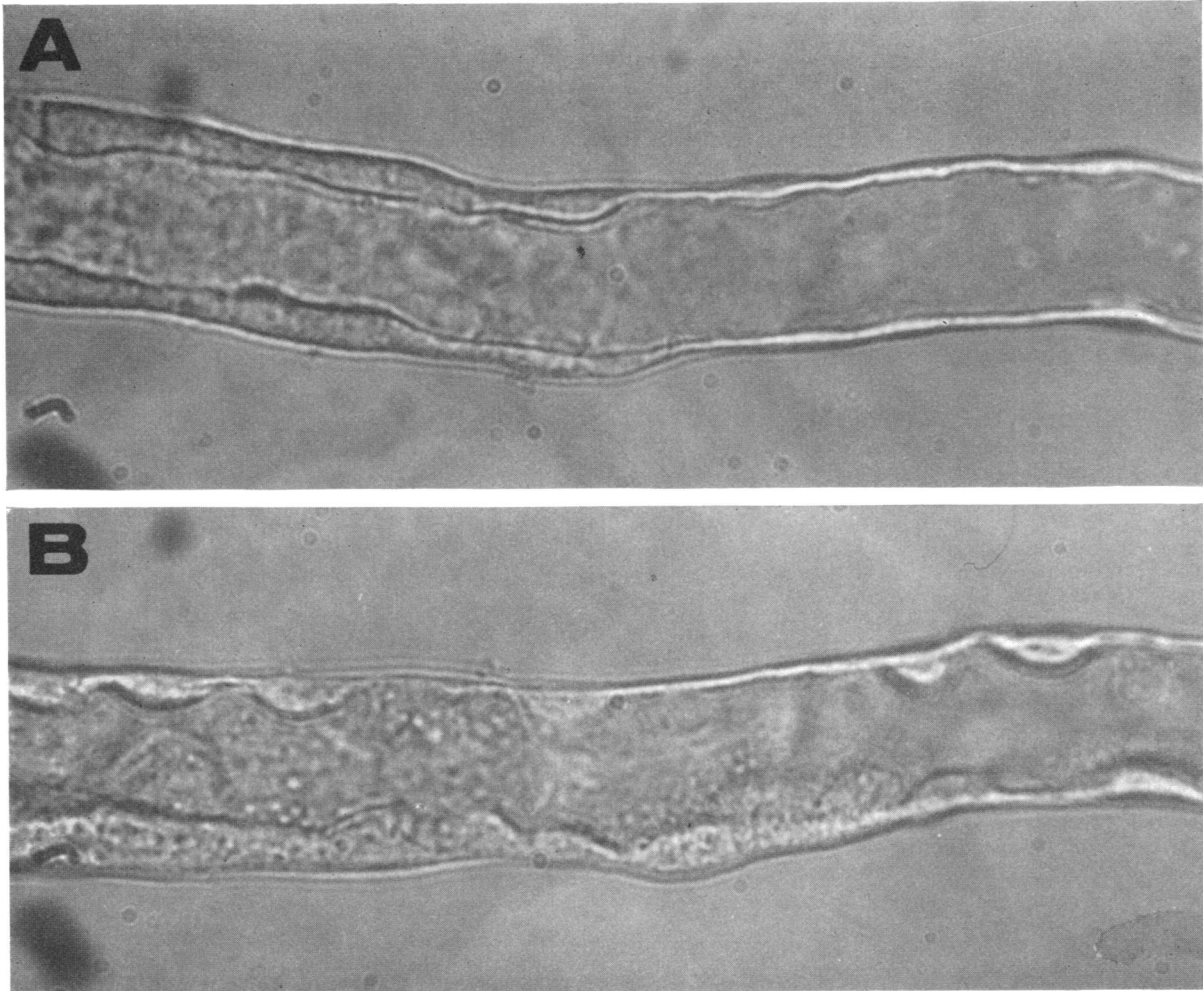


FIGURE 1 Microscopic magnification ( $\times 400$ ) of a perfused tALH (A), and thin DLH (B). Photographs taken from the transitional point of the thin-to-thick portion of the nephrons. Note that the tALH epithelium appears essentially the same as the DLH but gives an impression of having somewhat flatter epithelium. Also, it is clear that the transition from the tALH to the medullary thick ascending limb is quite abrupt, whereas the transition from pars recta to DLH is more gradual.

Corp.). When bidirectional fluxes of chloride were measured in the same tubule, successive determinations of each unidirectional flux was measured. In the initial three consecutive periods  $^{36}\text{Cl}$  ( $3 \mu\text{Ci/ml}$ ) was added to the bath, and its appearance rate into the collected fluid was monitored. The bath and the perfusate then were changed at least three times. The bath was made  $^{36}\text{Cl}$ -free, while  $^{36}\text{Cl}$  ( $3 \mu\text{Ci/ml}$ ) was added to the perfusate. 30 min was then allowed for re-equilibration, and then the outflux of  $^{36}\text{Cl}$  was measured. Bidirectional permeability and fluxes were similarly obtained for urea with [ $^{14}\text{C}$ ]urea ( $10 \mu\text{Ci/ml}$ , ICN Corp.). Diffusional permeability for water was measured from the disappearance rate of [ $^3\text{H}$ ]water ( $200 \mu\text{Ci/ml}$ , New England Nuclear) added to the perfusate. Unidirectional isotopic fluxes for respective substances were expressed in terms of apparent permeability coefficient (cm

$\text{s}^{-1}$ ) according to the following expression (11):

$$P_{ib} = \frac{V_i - V_o}{A} \left[ \frac{\ln C_i^*/C_o^*}{\ln V_i/V_o} + 1 \right], \quad (2)$$

where  $A$  is the area of the tubule calculated from the length of tubule and simultaneously measured inside diameter (the diameter can be measured within  $\pm 1 \mu\text{m}$  accuracy from photographs obtained at  $200\times$ );  $V_i$ , perfusion rate;  $V_o$ , collection rate;  $C_i^*$ , counts per minute per milliliter of isotope in the perfusate; and  $C_o^*$ , counts per minute per milliliter of isotope in collected fluid. In those experiments in which  $V_i = V_o$  exactly, then the permeabilities were calculated according to (11):

$$P_{ib} = \frac{V_i}{A} \ln \left[ \frac{C_i^*}{C_o^*} \right] \quad (3)$$

In those tubules in which the inward permeability ( $P_{bi}$ ,  $\text{cm s}^{-1}$ ) was measured either simultaneously or successively in the same tubules, the permeability coefficient was calculated by:<sup>2</sup>

$$P_{bi} = \frac{C_o^*}{C_b^*} \left[ \frac{P_{lb}}{1 - \exp(-AP_{lb}/\bar{V})} \right] \quad (4)$$

where  $C_o^*$ , counts per minute per milliliter of isotope in collected fluid;  $C_b^*$ , counts per minute per milliliter of isotope in the bath;  $A$ , area of tubule; and  $\bar{V}$ , the mean flow rate,  $(V_i + V_o)/2$ . In those experiments in which the permeability coefficient was measured from bath to lumen without the simultaneous determination of lumen to bath flux, the following equation was used:

$$P_{bi} = \frac{\bar{V}}{A} \ln \frac{C_b^*}{C_o^* - C_o} \quad (5)$$

The radioactivity of  $^{125}\text{I}$ ,  $^{22}\text{Na}$ , and  $^{24}\text{Na}$  were measured by a Packard model 3365 three-channel gamma spectrometer, while  $^3\text{H}$ ,  $^{14}\text{C}$ , and  $^{36}\text{Cl}$  radioactivity were measured with a Packard model 2420 liquid scintillation counter, (Packard Instrument Co., Inc., Downers Grove, Ill.). When  $^{24}\text{Na}$  was used in combination with other isotopes,

<sup>2</sup> Generally it is accepted that the two bidirectional permeability coefficients, lumen-to-bath ( $P_{lb}$ ) and bath-to-lumen ( $P_{bl}$ ), are equal. However, if there is a unidirectional force (such as active transport, for example) then the two apparent isotopic permeability coefficients are not the same. For this reason Eq 4 was derived, in which the bidirectional permeabilities can be measured and later compared for equality.

Consider fluid flowing down a tube with a surface area of  $dx$ . Assume that the mean flow rate is constant and equal to  $\bar{V}$ . The change in luminal isotopic concentration,  $dC_x$ , may then be expressed as:

$$-V_x dC_x = P_{bl}C_b^* dx - P_{lb}C_x^* dx, \quad (F1)$$

where  $C_b^*$  represents concentration of the isotope in the bath (considered constant since its volume is infinite with respect to tubular volume) and  $C_x^*$  is the concentration of isotope entered lumen at any point,  $x$ . Eq. F1 may be rearranged as:

$$-\frac{1}{\bar{V}} dx = \frac{-dC_x}{P_{lb}C_x^* - P_{bl}C_b^*}. \quad (F2)$$

Since  $x$  varies from zero (at perfusion pipette) to  $A$  (end of tubule) and  $C_x$  from 0 to  $C_o^*$  (concentration of isotope in collected fluid), Eq. F2 may be integrated between these limits and rearranged as:

$$P_{bi} = \frac{C_o^*}{C_b^*} \left[ \frac{P_{lb}}{1 - \exp(-AP_{lb}/\bar{V})} \right] \quad (4)$$

If on the other hand, it is assumed that  $P_{lb} = P_{bl}$  in experiments in which the efflux permeability coefficient is not measured, then substituting this equality into Eq. F1 and integrating between the same limits as for Eq. 4 then Eq. F1 becomes:

$$P_{bi} = \frac{\bar{V}}{A} \ln \frac{C_b^*}{C_o^* - C_o} \quad (5)$$

$^{24}\text{Na}$  was counted immediately after samples were obtained while the other isotopes were measured 15 days after initial  $^{24}\text{Na}$  counting. After 15 days the  $^{24}\text{Na}$  had completely decayed so that its overlap into the spectral range of other isotopes was eliminated. Osmolality of rabbit serum, ultrafiltrate, and artificial solutions were determined by an osmometer (Advanced Instruments, Inc., Needham Heights, Mass.). Osmolality of collected fluid, perfusate, and bath were determined by a modified Clifton nanoliter osmometer (Clifton Technical Physics, Hartford, N. Y.) (8). Sodium concentration of rabbit serum, ultrafiltrate, and artificial solutions were determined by an IL flame photometer (Instrumentation Laboratory Inc., Lexington, Mass.), and that of collected samples and perfusate were determined by a helium glow photometer (American Instrument Co., Inc., Travenol Laboratories Inc., Silver Spring, Md.). The analysis of chloride concentrations in the collected fluid and perfusate was performed by the microelectrometric titration method of Ramsay, Brown, and Croghan (12).

The data of each tubule are obtained as a means of two or three collection periods. The results in turn are expressed as mean  $\pm$  SE of number of tubules ( $n$ ) studied. The statistics were calculated as either a paired or non-paired  $t$  test analysis.

## RESULTS

*Evidence against active transport of solutes.* Active transport of salt was evaluated in the tALH under two conditions: first, under conditions in which the perfusate was an isosmolal ultrafiltrate of regular rabbit serum, and second, hyperosmolar conditions in which the bath was regular rabbit serum to which was added 163 mM/liter NaCl, while the perfusate was an ultrafiltrate of this same hypernatremic serum. In each case the composition of the perfusate and both were identical to each other. The results of these two sets of experiments are summarized in Table I. In these two sets of experiments the mean perfusion rate was  $8.8 \pm 0.7$  nl/min with a range from 1.9 to 17.9 nl/min. The results of Table I were not dependent on the perfusion rate. In neither set of experiments was the transtubular PD statistically different from zero, nor was there any evidence of net transport of solute or water as noted from the lack of change in the volume marker or osmolar concentration ratios between the perfusion and collected fluids (CF and PF).

TABLE I  
*Evidence for the Absence of Active Transport in tALH*

Perfusate Bath	Ultrafiltrate (UF) Rabbit serum (RS)	UF + 163 mM NaCl RS + 163 mM NaCl
PD, $mV$	$0.00 \pm 0.14$ (18)	$-0.01 \pm 0.28$ (7)
(C/P) <sub>osm</sub>	$1.00 \pm 0.011$ (6)	$1.01 \pm 0.005$ (7)
(C/P) <sub>Na</sub>	$1.04 \pm 0.016$ (10)	$1.04 \pm 0.007$ (5)
(C/P) <sub>Cl</sub>	$1.04 \pm 0.035$ (5)	$1.02 \pm 0.011$ (7)
(C/P) <sub>gluofil</sub>	$0.97 \pm 0.076$ (17)	$0.99 \pm 0.005$ (7)

Results are mean  $\pm$  SE ( $n$ ). (C/P), collected fluid-to-perfusate ratio.

The (CF/PF) sodium tended to rise slightly but was statistically higher only under the hypernatremic conditions. The reason for this slight but significant rise in sodium concentration is not apparent, especially since (CF/PF) chloride concentrations did not vary. Collectively, these results would constitute evidence against active reabsorption of salt by the in vitro perfused tALH.

*Diffusional and osmotic water permeability.* Two types of experiments were performed to evaluate water permeability of the tALH. In the first set diffusional water permeability was measured by the disappearance rate of tritiated water, while in the second set the hydraulic conductivity of water was measured when osmotic gradients were imposed across the tALH.

The isotopic water permeability was measured in six tubules in which the perfusate was ultrafiltrate of same rabbit serum as bath. These experiments were performed either without antidiuretic hormone (ADH) in the bath or with either 0.2 or 2.0 mU ml<sup>-1</sup> ADH. These results are summarized in Table II and indicate that the tALH is as impermeable to diffusional water flow as is the cortical and outer medullary collecting duct in the absence of ADH (13, 14). After addition of 0.2 mU ml<sup>-1</sup> ADH

TABLE II  
*Diffusional Permeability for Tritiated Water with or without ADH*

Exp. no.	0*	P <sub>DW</sub> 0.2*	2.0*
	<i>10<sup>-6</sup> cm s<sup>-1</sup></i>		
1	32.2	36.3	39.5
2	42.3	44.6	45.8
3	39.3	43.1	38.9
4	52.5	55.3	52.6
5	36.9	56.5	53.1
6	68.4	80.6	70.7
$\bar{X}$	45.3	52.7	50.1
SE	±5.4	±6.4	±4.8

\* mU/ml ADH.

to the bath, there is a slight but a significant increase in the diffusional water permeability from 45.3±5.4 to 52.7±6.4 × 10<sup>-6</sup> cm s<sup>-1</sup> (*P* < 0.05). However, we do not believe this change to have any physiological significance, especially in view of the fact that increasing the ADH concentration in the bath ten times to 2.0 mU ml<sup>-1</sup> did

TABLE III  
*Effect of Imposed 300 mosmol/kg Gradient (Raffinose Added to Bath) on Net Fluid Transport and Collected Fluid Osmolality*

Exp. no.	Length $\mu\text{m}$	Perfusion rate ( <i>V</i> <sub>i</sub> ) <i>nl min<sup>-1</sup></i>	Net change in water flux ( $\Delta J_v$ ) <i>nl mm<sup>-1</sup> min<sup>-1</sup></i>	<i>PF</i> <sub>osm</sub>	<i>CF</i> <sub>osm</sub>	<i>B</i> <sub>osm</sub>	$\Delta CF_{osm} = (C-E)_{osm}$	Osmotic water permeability
								<i>10<sup>-6</sup> ml cm<sup>-2</sup> atm<sup>-1</sup> sec<sup>-1</sup></i>
1	300	C 1.91		305	316	300		
		E 3.23	-0.14	305	325	641	+9	-0.45
2	350	C 4.47		301	296	300		
		E 4.42	+0.03	301	315	674	+19	0.09
3	650	C 7.28		305	306	304		
		E 8.23	-0.11	305	303	629	-3	-0.34
4	300	C 6.00		325	314	304		
		E 5.49	+0.95	325	320	629	+6	3.23
5	400	C 9.10		322	317	308		
		E 5.72	+0.67	322	319	638	+2	2.19
6	450	C 7.15		324	330	330		
		E 7.75	+0.29	324	323	634	-7	0.87
Mean		C		314	313	308		
SE		E	+0.28 ±0.19	±4.5	±4.7 318 ±3.2	±4.6 644 ±6.5	+4.3* ±3.8	0.93* ±0.61

Abbreviations: *PF*<sub>osm</sub>, *CF*<sub>osm</sub>, and *B*<sub>osm</sub> are osmolality of perfusate, collected fluid, and bath, respectively. C denotes the control periods in which the tubules were perfused with isosmolal ultrafiltrate of same rabbit serum as used for bath. E denotes experimental periods in which the tubules were perfused with ultrafiltrate and bathed in rabbit serum to which raffinose was added.

\* Not significantly different from zero (*P* > 0.05).

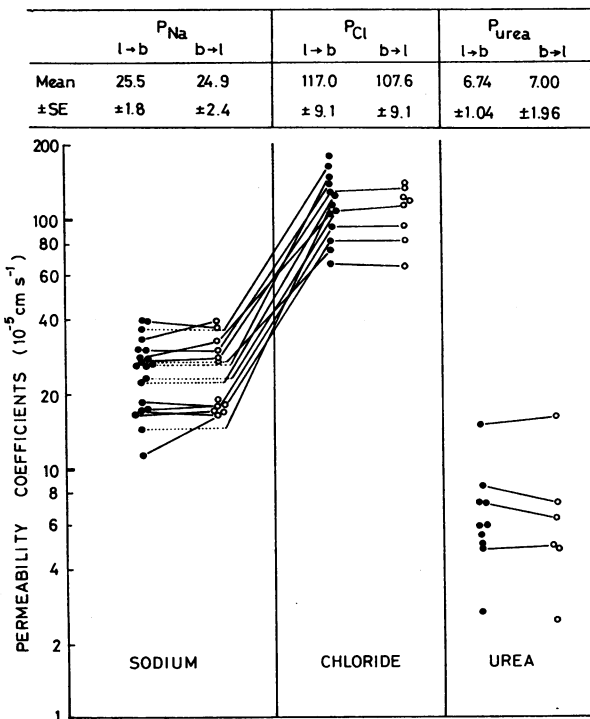


FIGURE 2 Diffusional permeability for sodium ( $^{22}Na$ ,  $^{24}Na$ ), chloride ( $^{36}Cl$ ), and urea ( $[^{14}C]urea$ ) in the tALH. The marks connected with solid lines indicate the data obtained from the same tubules. Each mark represents a mean of three samples. Closed circles denote lumen-to-bath permeability, and open ones bath-to-lumen. The scale of the permeability coefficients in the figure are shown as logarithmic scale because differences of permeability coefficients for each ion or molecule are too large to be depicted on a simple nonlogarithmic scale.

not increase the permeability more, nor was the isotopic diffusional permeability of water, ( $P_{aw}$ ) significantly different when 2.0 mU/ml ADH was present when compared to  $P_{aw}$  without ADH in the bath (paired  $t = 1.87$ ,  $0.1 < P < 0.2$ ).

The value for osmotic water permeability,  $L_p$ , is shown in Table III. It can be seen that at a mean perfusion rate of  $5.89 \text{ nl min}^{-1}$  there was only a small and statistically insignificant mean net change in water of  $+0.28 \pm 0.19 \text{ nl mm}^{-1} \text{ min}^{-1}$  when approximately 300 mosmol/kg raffinose was added to the bath. Also the associated increase in collected fluid osmolality was negligible,  $1.3 \pm 1.2\%$ . With Eq. 1 and the individual data from Table III, the mean calculated  $L_p$  was  $0.93 \pm 0.61 \times 10^{-6} \text{ ml cm}^{-2} \text{ s}^{-1} \text{ atm}^{-1}$ , which is not statistically different from zero, indicating that the tALH is virtually impermeable to osmotic flow of water.

*Isotopic permeability of Na, Cl, and urea.* The respective isotopic permeabilities for  $^{22}Na$ ,  $^{24}Na$ ,  $^{36}Cl$ , and

$[^{14}C]urea$  were measured in a total of 40 tubules perfused with an isosmolal ultrafiltrate of the same rabbit serum as the bath (approximate osmolalities of 300 mosmol/liter). The results of these experiments are summarized in Fig. 2 and Table IV. The calculated  $P_{Na}$  from lumen to bath was  $25.5 \pm 1.8 \times 10^{-5} \text{ cm s}^{-1}$  ( $n = 20$ ) while the calculated  $P_{Na}$  from bath to lumen was  $24.9 \pm 2.4 \times 10^{-5} \text{ cm s}^{-1}$  ( $n = 12$ ). These two values are identical when analyzed by nonpaired  $t$  test. In eight experiments these bidirectional permeability coefficients were examined simultaneously and indicated that the ratio of lumen/bath over bath/lumen was  $0.94 \pm 0.03$  ( $n = 10$ ), which was not statistically different from one by paired  $t$  test analysis. These values show that the tALH is highly permeable to sodium and, also, that in absence of sodium concentration gradients there is no net movement of sodium.

The calculated passive permeability coefficient for chloride,  $P_{Cl}$ , was extremely high. The  $P_{Cl}$  from lumen to bath was  $117.0 \pm 9.1 \times 10^{-5} \text{ cm s}^{-1}$  ( $n = 13$ ) and  $107.6 \pm 9.1 \times 10^{-5} \text{ cm s}^{-1}$  ( $n = 8$ ) from bath to lumen. In five experiments the bidirectional  $P_{Cl}$  was determined successively in the same tubules. The ratio of permeability from lumen-to-bath over bath-to-lumen was  $0.99 \pm 0.02$  ( $n = 5$ ). These results show that there is no net movement of chloride in the absence of a transepithelial concentration gradient of salt. Coupled with the finding of zero transtubular PD without salt gradients (Table I), this would constitute evidence against the existence of active transport processes for either Na or Cl in the isolated perfused tALH of rabbit.

The permeability coefficient for urea was determined

TABLE IV  
Diffusional Permeability of the tALH for  $[^{14}C]Urea$

Exp. no.	Perfusion rate	$P_{urea}$ <sub>l→b</sub>	Perfusion rate	$P_{urea}$ <sub>b→l</sub>	$P_{urea}$ <sub>l→b</sub> / $P_{urea}$ <sub>b→l</sub>
	$\text{nl min}^{-1}$	$10^{-5} \text{ cm s}^{-1}$	$\text{nl min}^{-1}$	$10^{-5} \text{ cm s}^{-1}$	
1	10.01	5.99			
2	5.38	5.38			
3	8.87	7.28			
4	10.23	2.66			
5	6.28	5.92			
6	6.73	4.99			
7	12.43	7.21	10.31	6.31	1.14
8	10.22	14.98	12.98	16.20	0.92
9	13.05	4.77	8.54	4.86	0.98
10	18.16	8.25	16.37	7.25	1.14
11			9.90	4.46	
12			8.67	2.72	
Mean	10.14	6.74	11.13	6.97	1.05
SE	±1.20	±1.04	±1.24	±1.95	±0.06

Abbreviations:  $P_{urea}$ <sub>l→b</sub> and  $P_{urea}$ <sub>b→l</sub> are permeability coefficients for urea from lumen to bath and bath to lumen, respectively.

TABLE V  
*Generation of Osmotic and Sodium Chloride Concentration Gradients by the tALH  
 by Passive Diffusion of Solutes*

Exp. no.	Length	$V_i$	PD	Osmolality					[Cl]			[Na]				
				PF	CF	B	B-CF	PF-CF	PF	CF	CF-PF	PF	CF	C-PF		
	$\mu\text{m}$	$\text{ml}/\text{min}$	$\text{mV}$	$\text{mosmol}/\text{kg water}$					$\text{meq}/\text{liter}$			$\text{meq}/\text{liter}$				
1	E <sub>1</sub>	430	14.9	+17.3	591	425	659	+234	-166							
2	E <sub>1</sub>	350	10.4	+15.0	598	479	634	+155	-119							
3	E <sub>1</sub>	470	10.9	+17.6	593	469	679	+210	-124	281	192	-89				
	E <sub>2</sub>	200	14.6		593	537	625	+88	-56	281	249	-32				
4	E <sub>1</sub>	450	10.5	+21.3	593	443	603	+160	-150	281	195	-86				
	E <sub>2</sub>	200	13.0		593	529	633	+104	-64	281	243	-38				
5	C	370	17.9	0.0	605	614	678	+64	+9	281	292	+11				
	E <sub>1</sub>	370	15.2	+18.3	605	484	659	+175	-121	281	196	-85				
	E <sub>2</sub>	180	12.6		605	555	627	+72	-50	281	251	-30				
6	C	420	13.7	0.0	605	613	616	+3	+8	281	283	+2				
	E <sub>1</sub>	420	13.2	+20.3	605	444	660	+216	-161	281	194	-87				
	E <sub>2</sub>	230	28.2		605	562	647	+85	-43	281	274	-7				
7	C	200	11.1	+1.2	615	624	662	+38	+9	283	296	+13	308	308	0	
	E <sub>1</sub>	200	13.6	+21.6	615	535	636	+101	-80	283	231	-52	308	280	-28	
8	C	300	4.6	-0.2	594	598	596	-2	+4	272	282	+10	308	321	+13	
	E <sub>1</sub>	300	2.9	+20.1	594	472	586	+114	-122	272	201	-71	308	262	-46	
	E <sub>2</sub>	200	3.3		594	535	594	+59	-59	272	233	-39	308	279	-29	
9	C	480	8.6	-1.4	604	610	634	+24	+6	274	279	+5	305	322	+17	
	E <sub>1</sub>	480	10.7	+18.3	604	515	593	+78	-89	274	224	-50	305	281	-24	
	E <sub>2</sub>	300	9.2		604	557	614	+57	-47	274	252	-22	305	304	-1	
10	C	400	10.3	+0.5	597	586	583	-3	-11	278	270	-8	308	322	+14	
	E <sub>1</sub>	400	6.4	+19.2	597	499	639	+140	-98	278	214	-64	308	262	-46	
	E <sub>2</sub>	150	7.7		597	551	613	+62	-46	278	250	-28	308	289	-19	
11	C	250	11.4	-1.2	614	611	632	+21	-3	277	286	+9	305	314	+9	
	E <sub>1</sub>	250	12.2	+17.3	614	539	594	+55	-75	277	232	-45	305	274	-31	
12	E <sub>1</sub>	460	5.8	+16.3	624	499	593	+94	-125							
	E <sub>2</sub>	260	5.8		624	541	629	+88	-83							

$V_i$ , perfusion rate; B, bath; C, control period in which the perfusate and bath are identical in composition without NaCl concentration gradient; and E represents a bath containing approximately 300 mosmol/kg urea. E<sub>1</sub> and E<sub>2</sub> have the same bath composition, but E<sub>2</sub> utilizes shorter segments of same tubule as in periods designated E<sub>1</sub>.

like that for <sup>36</sup>Cl. In 10 experiments,  $P_{\text{urea}}$  from lumen to bath was  $6.74 \pm 1.04 \times 10^{-5} \text{ cm s}^{-1}$ . In six experiments,  $P_{\text{urea}}$  from bath to lumen was  $6.97 \pm 1.95 \times 10^{-5} \text{ cm s}^{-1}$ . In four of these experiments bidirectional permeabilities were performed successively in the same tubule. The ratio of the bidirectional fluxes ranged from 0.9 to 1.14 with a mean value of  $1.05 \pm 0.06$ . These experiments suggest that active transport processes for urea does not exist under our given experimental conditions and furthermore show that permeability of the tALH to urea is significantly less than to either sodium or chloride.

*Generation of osmotic gradients by passive diffusion of solutes.* Because we were unable to demonstrate any evidence of active salt transport by the tALH, the next series of experiments were specifically designed to determine whether this segment of the nephron might generate a relatively hypotonic intraluminal fluid by passive processes when exposed to conditions simulating

the intact renal medulla. In all experiments the tubules were perfused with an ultrafiltrate of rabbit serum that had been made hypertonic by addition of 163 mM/liter NaCl. In the control periods the bath was made similarly hypertonic by addition of the same amount of NaCl. Thus, in these periods the bath and the perfusate were similar in osmolality and composition (Table V). In experimental periods the tubules were bathed in rabbit serum made equally hypertonic by addition of approximately 300 mM/liter urea. Thus, in the experimental period the bath osmolality was made up of approximately 50% of urea and 50% salt to simulate the in vivo papillary concentrations. Tubules were studied at two lengths. After initial readings, part of the tubule was drawn into the collection pipette by suction, and studies were then repeated. In each collection period of each tubule, the osmolality, sodium, and chloride concentrations were determined in the bath, the perfusate,

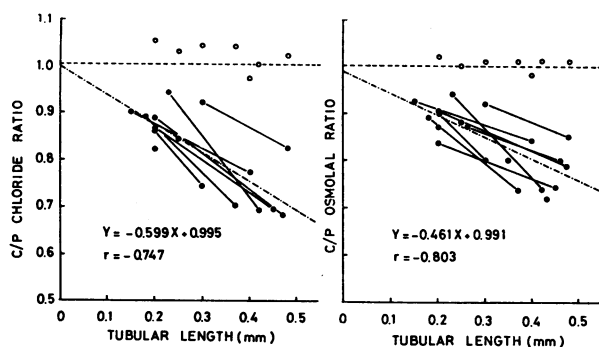


FIGURE 3 Generation of chloride and osmotic gradients in the tALH by passive diffusion of solutes. Perfusate in each case is ultrafiltrate plus 300 mosmol/kg NaCl. Bath was either identical to the perfusate (open circles) or isomolar serum to which 300 mosmol/kg urea was added (closed circles). On left panel is plotted the collected-to-perfusate (C/P) chloride concentration while on the right panel is the collected-to-perfusate osmolal ratio. Lines connecting two points are from same tubules studied at two different lengths.

and collected fluid. The results of these experiments are summarized in Table V and Fig. 3. During the control period in which the composition of the perfusate and bath were identical there was no change in the osmolality of the collected perfusate. Thus, the tALH is not capable of generating relatively hypoosmolar solution when the salt concentration on the two sides of the membrane is identical. In contrast, during the experimental periods when the bath osmolality consisted of 50% and 50% urea, the perfusate became relatively hypoosmolar to the bath, with osmotic gradients as great as 234 mosmol/kg being generated. As shown in the right panel of Fig. 3 the magnitude of the osmotic gradient was dependent on the length of tubule perfused. The osmotic gradient is somewhat smaller (slope of  $-0.461$ ) than the generated fall for Na and Cl (slope of  $-0.599$ ),

as shown in the left panel of Fig. 3, indicating that there must have been some influx of urea.

## DISCUSSION

The tALH plays an integral role in the overall operation of the CCMS. Previously it has been hypothesized that the tALH has the capacity for active outward transport of NaCl and, therefore, that it participates in the generation of a hypertonic medullary interstitium. The data of Jamison et al. (2), showing that osmolality and Na concentration in tALH are less than at a similar level in DLH, has been interpreted as evidence for an active transport process existing with the tALH. However, since these two segments are not in contact with one another (15), the concentration difference observed by Jamison et al. (2) does not mean that NaCl is transported out of the tALH against a concentration gradient. Moreover, previous direct attempts by split droplet (16, 17) or microperfusion techniques (18, 19) have not been able to demonstrate the existence of active transport out of the segment. Because of the controversy that exists concerning the transport properties of the tALH, studies were designed that could directly examine the various membrane characteristics that govern salt, water, and urea transport out of the *in vitro* perfused rabbit tALH.

In the present studies it was shown that the tALH does not have the capacity of active outward transport of NaCl. The evidence for this is as follows: first, it was found that there was no net fluid efflux from the tubules when perfused with solutions identical to the bath; second, the transtubular PD was zero; and third, the collected fluid osmolality remained unchanged (Table I). These findings would be in agreement with the earlier *in vivo* studies that have not demonstrated active transport of fluid out of the tALH (16).

The passive permeability characteristics of the tALH were uniquely different from all other nephron seg-

TABLE VI  
Permeability Coefficients of Various Nephron Segments to Water, Sodium, Chloride, and Urea in Rabbit

	$L_p$	$P_{dw}$	$P_{Na}$	$P_{Cl}$	$P_{urea}$
	$ml\ cm^{-2}\ s^{-1}\ atm^{-1}$				
Proximal convoluted tubule	29-63 (20)		9.3 (20)	3.8 (21)	5.3 (9)
DLH	171 (8)	446.0*	1.61 (8)		1.5 (9)
tALH	0.93	45.3	25.5	117.0	6.7
Thick ascending limb of Henle	0.12 (4)		6.27 (4)	1.06 (4)	
Cortical collecting tubule					
without ADH	0.44 (14)	40.1 (13)	0.06 (22)		0.16 (13)
with ADH	13.6 (14)	77.2 (13)			0.15 (13)

Numbers in parenthesis indicate the reference cited.

\* Unpublished data of Imai and Kokko. ( $466.0 \pm 113.5$ , at perfusion rate of  $16.1 \pm 3.2$  nl/min,  $n = 6$ )  $L_p$ , osmotic water permeability; P, isotopic diffusional permeability of water (dw), sodium (Na), chloride (Cl), and urea.



ments similarly studied. Table VI summarizes the comparative permeability characteristics of tALH with respect to other *in vitro* perfused rabbit nephron segments. It is of interest that the tALH is relatively impermeable to osmotic flow of water although the epithelium of tALH cannot be differentiated from the DLH, (which has a high water permeability, [Table VI]) at 400 $\times$  magnification while being perfused *in vitro* (Fig. 1). It is this water impermeability that allows for the maintenance of generated osmotic gradients. Morgan and Berliner (18), working with excised rat papillae, and Sakai, Tadokoro, and Teraoka (19) using micropuncture techniques in the hamster, have also found the tALH diffusional water permeability to be low. Of special interest is the finding of high sodium and chloride permeability in face of the impermeability to osmotic flow of water. The salt and urea permeabilities are higher than any other segment thus far studied, though urea permeability is significantly less than that of salt (Table VI). Our high permeability coefficients for salt are not consistent with the earlier findings of Morgan and Berliner (18), who found very little sodium to move across the tALH membrane when a concentration gradient of NaCl was imposed across it. This may reflect species differences between rat and rabbit, although other unidentified sources of discrepancy in the results are more likely. With respect to the hamster, we calculated a  $P_{Na} = 38 \times 10^{-5} \text{ cm s}^{-1}$ , assuming a perfusion rate of 30 nl mm $^{-1}$  and an inside diameter of 20  $\mu\text{M}$ , by using the disappearance rate of  $^{22}\text{Na}$  from the tALH that Sakai et al. (19) have published. This latter  $P_{Na}$  would reflect a high sodium permeability and would be quite similar to our *in vitro* rabbit results summarized in Table VI.

The failure to demonstrate active outward transport of salt by the tALH raises the question as to the mechanism by which this segment of the nephron can reabsorb salt in excess of water, dilute its intraluminal fluid, and contribute to the papillary interstitial hypertonicity. Kokko and Rector (6), however, have previously pointed out that active outward transport of salt was not necessary provided and that tALH had precisely those permeability properties demonstrated in the present studies; namely, water impermeability, high salt permeability, and moderate urea permeability. According to their model the predominantly saline fluid entering the DLH equilibrates with the hypertonic medullary interstitial fluid primarily by water abstraction. Although the fluid at the bend of the loop would be iso-osmotic with adjacent interstitial fluid, its NaCl would be higher and urea concentration lower than the interstitial fluid. Thus as fluid moves up the tALH there would be a large outward concentration gradient with diffusion of NaCl and inward diffusion of urea. With the above list of permeability properties (Table VI) the outward

diffusion of NaCl should be greater than inward diffusion of urea, thereby generating a relatively hypo-osmolar intraluminal fluid. In the present studies, experiments were designed to test this prediction. When segments of tALH were perfused with ultrafiltrate made hypertonic by addition of NaCl and bathed in a bath made equally hypertonic by addition of urea, the outward diffusion of NaCl greatly exceeded the inward diffusion of urea (Fig. 3) and consequently large osmotic gradients were generated with intraluminal fluid being as great as 234 mosmol/kg, hypoosmolar with respect to bath (Table V). These large osmotic gradients could be maintained because of the virtual total osmotic impermeability of this segment to water. Thus under conditions simulating the intact renal medulla, in which the solute concentration in the renal papilla consists of 50% urea and 50% NaCl, the tALH can produce a relatively hypo-osmolar intraluminal fluid by the purely passive downward diffusion of NaCl.

In the above studies the perfusate was made hypertonic by addition of NaCl because in our earlier studies we found that *in vitro* perfused DLH of rabbit osmotically equilibrated principally by water abstraction rather than urea entry, and it was thus predicted that *in vivo* the fluid leaving the DLH and entering the tALH would have a high NaCl concentration (9). However, such large NaCl concentration gradients between the tALH and other medullary structures have not been observed in *in vivo* micropuncture studies (16, 23). The difficulty in interpreting such micropuncture studies, however, arises from the inability to sample the interstitial fluid that the tALH is equilibrating and from the uncertainty as to which of the other medullary structures best reflects the composition of the interstitial pool. If during fast flow the vasa recta do not completely equilibrate with each other, as suggested by Jamison (24), then the descending vasa recta would underestimate, while the ascending vasa recta would overestimate the salt concentration in the interstitial fluid with which the tALH is equilibrating. However, since the tALH is surrounded by capillaries of the descending vasa recta (15), it would be predicted that the descending vasa recta would more accurately reflect the pool with which the tALH is equilibrating than the ascending vasa recta. In evaluating future micropuncture studies designed to test the validity of passive model of CCMS proposed by Kokko and Rector (6), these complex anatomical interrelationships become critical in valid interpretation of the data. Although the NaCl concentration gradients are clearly much higher than those noted *in vivo* in the current studies, they clearly represent directional changes that may occur. Whether or not the smaller concentration gradient that exists *in vivo* can account for the observed rates of dilution utilizing the present measured

permeability coefficients is a complex kinetic question that will require a careful computer analysis of the entire system.

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