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Research Article

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Urea Transport in the Proximal Tubule and the Descending Limb of Henle

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ABSTRACT Urea transport in proximal convoluted tubule (PCT) and descending limb of Henle (DLH) was studied in perfused segments of rabbit nephrons in vitro.

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The reflection coefficient for urea, σ_{urea}, was calculated as the ratio of induced solution efflux when 95 mOsm/liter of urea was added to the bath, as compared to net fluid movement induced by addition to the bath of equivalent amount of raffinose. σ_{urea} in DLH is 0.95 ± 0.4 as compared to 0.91 ± 0.05 in PCT. σ_{urea} in DLH is approximately equal to σ_{Na}; however, σ_{urea} in PCT is higher than σ_{Na} (0.68).

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Several types of studies were conducted to examine the role of urea and urea plus sodium chloride in concentrating the fluid in the DLH. From the obtained results it was concluded that the intraluminal fluid of DLH is primarily concentrated by abstraction of water without significant net entry of solute. These results are discussed with respect to possible significance in the overall operation of the countercurrent system.

INTRODUCTION

The purpose of the present studies was to examine urea transport in the proximal convoluted tubule (PCT)[1] and the descending limb of Henle (DLH) by utilizing the direct technique of perfusing isolated segments of these nephrons in vitro. Three specific issues were examined: (*a*) whether urea transport in PCT is active (reabsorption or secretion) or passive; (*b*) a comparison of the phenomenological coefficients (passive permeability coefficient for urea and reflection coefficient for urea) for the PCT and DLH which govern passive transport in these segments; and (*c*) the role of urea in the osmotic equilibration of tubular fluid in the DLH.

METHODS

Isolated segments of proximal convoluted tubule and thin descending limb of Henle were perfused by techniques previously described (1, 2). In all experiments female New Zealand white rabbits weighing 1.5–2.5 kg were used. Rabbits were sacrificed by use of a guillotine after which a small slice of kidney was obtained as quickly as possible. This slice was transferred to a chilled dish of rabbit serum where a PCT or DLH was dissected free without use of collagenase or other enzymatic agents. Positive identification of DLH was ascertained by removal of most of the pars recta. After dissection the segments of nephrons were

[1] *Abbreviations used in this paper:* ALH, ascending limb of Henle; CF/PF, collected fluid/perfusion fluid; DLH, descending limb of Henle; P, permeability coefficient; PCT, proximal convoluted tubule; σ, reflection coefficient; TF/P, tubular fluid/plasma; UF, ultrafiltrate.

transferred into a perfusion dish of rabbit serum which was kept at 37°C and pH of 7.4 by continuous bubbling with 95% O_2–5% CO_2. The tubules were perfused using concentric glass pipettes and the exact same techniques outlined previously (2). The remainder of pars recta (approximately 100 μ) was always sucked into the holding pipette in such a manner that only the epithelium of DLH was exposed to the perfusate.

Albumin-^{125}I (E. R. Squibb & Sons, New York) which was used as the volume marker, was added in trace amounts to perfusion fluid in all studies. Perfusion rate, V_i, was calculated from the rate at which albumin-^{125}I was collected; total ^{125}I cpm in the collected fluid was divided by ^{125}I cpm/nl in the perfusion fluid and by the time of the collection period. The collection rate, V_o, was obtained directly by a calibrated constant bore collection pipette. Net fluid transport, C, was calculated as the difference in V_i and V_o, normalized to 1 mm by dividing by length of the nephron perfused, and therefore expressed in units of nl mm^{-1} min^{-1}. The length of the tubule is measured directly by micrometer eye piece.

In various experimental protocols small quantities of crystaline solid urea-^{14}C (New England Nuclear Corp., Boston, Mass.) were added to the perfusion fluid or to the bath. The chemical concentration of urea in these fluids ranged from 6.4 to 7.5 mM. As soon as the bath or collected fluid samples were obtained they were transferred to 1 cm^3 of water and immediately diluted by the addition of 10 cm^3 of liquid scintillation cocktail (1966 cm^3 Toluene, 980 cm^3 Triton X-100 [Packard Instrument Co., Inc., Downers Grove, Ill.] and 60 cm^3 of spectraflour PPO [Amersham/Searle Corp., Arlington Heights, Ill., 250 g PPO/1 liter Toluene]). If the addition of the cocktail was not performed immediately, there was a tendency for the ^{14}C counts to decrease with time which was thought to be secondary to bacterial breakdown of urea with subsequent liberation of ^{14}C-CO_2. Once the cocktail was added, the samples were counted for ^{125}I in Packard model 5000 gamma scintillation spectrometer at 17.5–75 kev, (Packard Instrument Co., Inc., Downers Grove, Ill.) and then transferred to Packard model 2420 liquid scintillation spectrometer for ^{14}C counting (7.5–152 kev). Using this combination of equipment, counting fluids, and window settings, the ^{125}I overlap to ^{14}C windows was approximately 7% and, accordingly, all ^{14}C counts were corrected by an exact overlap correction determined during each counting cycle. Overlap corrections were determined by counting separate pure ^{125}I samples both in the liquid scintillation spectrometer and the gamma counter. The overlap correction then is the ratio of counts obtained in the liquid scintillation spectrometer over those obtained in the gamma counter. In each case the overlap of ^{125}I counts to ^{14}C counts contributed less than 10% of the total counts obtained in ^{14}C windows and thus posed no statistical problem to analysis of ^{14}C counts.

Active transport of urea was examined in experiments where tubules were perfused with an equilibrium solution (ultrafiltrate of rabbit serum diluted with isosmolal raffinose solution to a final [Na] of 125 mEq $liter^{-1}$ (3) in baths of regular rabbit serum. The purpose of this combination of perfusion fluid and bath was to keep net transport of fluid near zero. Equal amounts of urea-^{14}C were added per nanoliter of water in the perfusion fluid and the bath (35–40 cpm/nl). If, under these conditions, there is an increase in ^{14}C cpm/nl in collected fluid, this would then represent urea secretion, while a decrease in ^{14}C cpm/nl would indicate urea reabsorption. It is felt that this technique is more accurate than comparing unidirectional fluxes in separate families of tubules. In this set of experiments the perfusion rates were slower (3.1–8.7 nl min^{-1}) than normally used to allow for longer contact times in which differing bidirectional fluxes could be expressed.

Passive permeability coefficient for urea was estimated from the disappearance rate of ^{14}C from the perfusion fluid. Measurements were obtained under these conditions of zero net fluid movement: DLH was perfused with isosmolal ultrafiltrate of the same rabbit serum as the bath, while PCT was perfused with equilibrium solution. Passive permeability coefficient was estimated according to the following expression:

$$P_{urea} = \frac{V_i - V_0}{L}\left[\frac{\ln \frac{C_1^*}{C_0^*}}{\ln \frac{V_i}{V_0}} + 1\right] \quad (1)$$

where L = length of tubule, V_i = perfusion rate, V_o = collection rate, C_i^* = cpm/nl of ^{14}C in perfusion fluid, and C_o^* cpm/nl of ^{14}C in the collected fluid. This equation differs from the conventional equation (4):

$$P = \frac{V_i - V_0}{A}\left[\frac{\ln \frac{C_i^*}{C_0^*}}{\ln \frac{V_i}{V_0}} + 1\right] \quad (2)$$

where the symbolism is the same, except A = area of tubule. The purpose in changing to equation (1) instead of equation (2) is to express the permeability per unit length which can be measured much more accurately in the PCT than the inside diameter. In the case of the DLH the measurement of the inside diameter is as accurate as measuring the length; however, with respect to the PCT, it is difficult to decide which is the limiting membrane to diffusion. The units of the P_{urea} are square centimeters per second instead of the commonly accepted unit of permeability coefficient, centimeters per second.

The reflection coefficient for urea, σ_{urea}, may be defined as a deviation from the theoretical osmotic pressure expressed by (5):

$$\sigma_{urea} = \pi\text{observed}/\pi\text{theoretical} \quad (3)$$

where πobserved is the effective osmotic pressure exerted by a given concentration gradient of urea as compared to the theoretical osmotic pressure (πtheoretical) expected for that given urea gradient. If urea were completely permeant then it obviously could not exert any osmotic force and would have a $\sigma_{urea} = 0$, whereas if urea were completely impermeant its σ would be equal to 1. In these studies the σ_{urea} was determined by the "equal concentration" method (6) where σ_{urea} is equal to the ratio of the increment of net induced fluid movement caused by an osmotic gradient of urea, $\Delta Jv_{raffinose}$. Thus,

$$\sigma_{urea} = \frac{\Delta Jv_{urea}}{\Delta Jv_{raffinose}} \quad (4)$$

In each experiment a total of nine 10-min collections were made; three each of: (*a*) control; (*b*) when 95 mOsm/liter of urea was added to the bath; and (*c*) when 95 mOsm/liter of raffinose was added to the bath. The details

of the fluid constituents and the experimental protocol are covered more fully in the results section.

It must be recognized that measurements of reflection coefficients are only estimates of the true effective osmolality that a given molecule can generate. The reported reflection coefficients have an inherent approximate 5% error in addition to the uncertainties associated with the standard errors. The source of these errors have been fully discussed in our previous publications (2, 6). In the current studies we have compared a rate of water efflux secondary to osmotic gradients induced by equivalent concentrations of urea and raffinose. In the process of osmotic equilibration the net water movement is proportional to the perfusion rate. Thus, at perfusion rate of 10 nl/min osmotic equilibration is achieved by transport of approximately one-half as much fluid as at 20 nl/min. It would be ideal to perfuse tubules at exactly the same rate during the control and experimental conditions; however this is not possible due to technical difficulties. In these studies the mean perfusion rates are comparable during the various conditions; however, during any given experiment, there is some variation of perfusion rate between the periods, and accordingly, the net transport of fluid must be corrected proportionally by this difference (see Table III). An additional source of error, not pointed out before, can be developed if there is a significant movement of solute secondary to passive diffusion after generation of concentration gradients by osmotic movement of water. For example, when an osmotic gradient is imposed on a tubule by addition of urea to the bath, intraluminal fluid will be abstracted by osmotic mechanisms leaving the residual fluid with an NaCl concentration higher than the ambient bath. If the urea and NaCl permeabilities were significantly different, then this could be a source of error by allowing differential passive diffusion of solute with its iso-osmolar complement of water. However, the isotopic urea permeability is virtually identical to sodium permeability in the descending limb of Henle. With respect to the proximal convoluted tubule, the urea is less permeant than NaCl; however similar intraluminal Na concentration gradients will be generated by urea and raffinose when the latter are added by equivalent amounts to the bath. Thus contribution to the calculated σ_{urea} should be minimal. The purpose of this paragraph is to reemphasize that measurements of the reflection coefficients can be considered only approximate even though the latter are associated with small standard errors.

The mechanism by which fluid in the DLH osmotically equilibrates with hypertonic medullary interstitial fluid was evaluated under two sets of circumstances. In the first set, the effect of 112 mOsm liter^{-1} urea gradient was studied when the control conditions were obtained with both the bath and the ultrafiltrate at 298 mOsm liter^{-1}. In the second set, control bath and perfusion fluid had osmolalities of 605 mOsm liter^{-1}. These were prepared by addition of crystaline NaCl to commercially available rabbit serum until the final osmolality was 605. The perfusion solution was isosmolal ultrafiltrate of the hypernatremic serum. In this second set the effect of a 323 mOsm liter^{-1} gradient was studied during the experimental period by using a bath with an osmolality of 928 mOsm liter^{-1}. This osmotic gradient was achieved by addition of 204 mOsm liter^{-1} NaCl and 119 mOsm liter^{-1} urea to control bath of 605 mOsm liter^{-1}. In addition, crystaline urea-^{14}C (chemically trace amounts but achieving 10-min counts of 250–300 counts/nl) was added to the bath to estimate the amount of urea influx from the calculated specific activities. In each set of experiments the increase in osmolality of the perfusion fluid was compared with the increase in concentration of the albumin-^{125}I volume marker. The details of these experiments are covered in conjunction with the results.

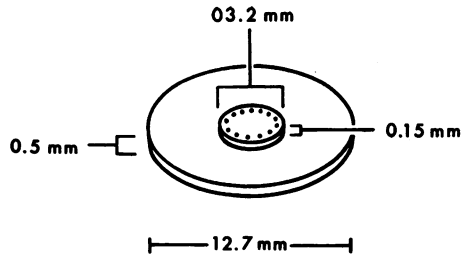

FIGURE 1 Diagram of the modified sample plate to Clifton nanoliter osmometer. Plate is made of a copper/brass alloy with dimensions as depicted. Note the circular arrangement of the sample holes, and the centrally depressed sleeve (0.15 mm) of the sample plate which insure equidistance of the samples from the cooling plate. The holes are 0.016 inches in diameter.

Tubular fluid and simultaneous bath osmolalities were measured by Clifton nanoliter osmometer (Clifton Technical Physics, New York) in which the sample plate had been modified to obtain more reproducible numbers. The modifications incorporated into the plate are relatively simple (see Fig. 1), but have provided for a greater degree of accuracy, ±2-5 mOsm liter^{-1} per single sample, than obtainable with the commercially available plate. The salient features are twofold: (*a*) the sample holes are all equidistant from the cooling plate, and (*b*) the samples, when placed in the middle of these holes, are in continuous line with the cooling waves generated by the dipole plate. Each osmolality reported is an average of three collected samples, and these in turn were usually run in duplicate. It generally takes 15 min to obtain 12 sample osmolalities after the samples have been loaded into the sample plate. Excellent agreement was consistently noted between same solutions analyzed by freezing 2-ml samples with a cryomatic osmometer, model 31CM (Advanced Instruments, Inc., Needham Heights, Mass.) as with the modified Clifton osmometer.

The commercially available human albumin-^{125}I was concentrated to contain approximately 300 μCi/cm^3 by the use of Amicon (Amicon Corp., Lexington, Mass.) pressure dialysis using PM-10 filters. Before use it was dialyzed in a cellophane bag against a Ringer's solution to minimize the free ^{125}I. The ultrafiltrate of rabbit serum was made by pressure dialysis using Amicon PM-30 membranes.

RESULTS

Active transport. Active transport out of the PCT was evaluated under conditions of zero net transport by perfusing slowly with equilibrium solution in a bath of rabbit serum. Equivalent counts of urea-^{14}C were added to the perfusion fluid and the bath. The concentration of ^{14}C was measured in the collecting fluid and compared to the mean concentrations in the perfusion fluid and the bath. The results are expressed in Table I. It is evident from these six consecutive experiments that active secretion or reabsorption of urea in the isolated perfused PCT

TABLE I

Evidence against Active Urea Transport by the Proximal Convoluted Tubule

	Length	Perfusion rate, V_i	Net reabsorption, C	Perfusion fluid urea-^{14}C, C^*_i	Bath urea-^{14}C, C^*_b	Collected fluid urea-^{14}C, C^*_o	Relative % changes in urea-^{14}C* $\frac{C^*_o - 0.5(C^*_i + C^*_b)}{0.5(C^*_i + C^*_b)} \times 100$
	μ	*nl min*$^{-1}$	*nl mm*$^{-1}$ *min*$^{-1}$	*counts nl*$^{-1}$	*counts nl*$^{-1}$	*counts nl*$^{-1}$	
	1420	8.66	−0.18	38.3	38.5	37.0	−3.7
	1440	6.92	−0.40	38.3	36.9	38.0	+1.1
	960	6.16	−0.33	35.3	38.1	36.2	−1.4
	1340	6.50	−0.06	35.3	38.0	36.0	−1.8
	1500	4.08	+0.06	38.2	39.4	39.4	+1.5
	1140	3.07	−0.05	38.2	40.0	44.7	+14.3
$\bar{X}$	1300	5.90	−0.16	37.3	38.5	38.6	1.7±2.4

In these experiments the perfusion fluid was equilibrium solution ([Na] = 125 mEq liter^{-1}) isosmotic to the bath of regular rabbit serum (306 mOsm liter^{-1}).

* The following is formula for per cent change in urea-^{14}C: $[(V_i = CL)(C^*_o) - (V_iC^*_i)/(V_iC^*_i)] \times 100$ where L is length of tubule. This formula would be applicable only if urea-^{14}C counts/nl were identical in the perfusion and bathing fluids.

could not be demonstrated under conditions of zero net fluid movement using perfusion rates ranging from 3.07 to 8.66 nl/min.

TABLE II

Passive Permeability for Urea in Proximal Convoluted Tubule and Descending Limb of Henle

	Length	Perfusion rate	Net reabsorption	^{14}C counts in	^{14}C counts out	Passive permeability
	μ	*nl min*$^{-1}$	*nl mm*$^{-1}$ *min*$^{-1}$	*counts/nl*	*counts/nl*	$\times 10^{-7}$ *cm*^{2} *sec*
PCT	1840	13.4	0.50	83.3	75.6	1.97
	900	9.5	0.27	83.3	65.0	4.76
	1080	10.6	0.27	83.3	67.7	3.77
	1360	11.1	0.19	66.5	56.7	3.00
	940	6.1	0.18	68.6	51.1	3.41
	1940	13.3	0.34	68.6	55.6	2.89
$\bar{X}$ (SE)	1343	10.7	0.29			3.30±0.35
DLH	2250	5.0	0.14	128.8	119.3	0.26
	1400	4.3	−0.22	128.8	105.4	0.42
	1500	5.1	−0.05	128.8	98.2	1.21
	2200	9.4	−0.01	967.5	790.3	1.06
	2150	9.5	0.11	315.0	218.2	2.37
	2350	9.4	−0.02	315.0	299.8	0.24
$\bar{X}$ (SE)	1975	7.1	−0.01			0.93±0.31

Proximal convoluted tubules were perfused with equilibrium solution, while the descending limb of Henle was perfused with ultrafiltrate of rabbit serum. Urea-^{14}C was added to the perfusion fluids. Bath in each case was commercially available rabbit serum and was isosmotic to the perfusion fluid. Counts/nl = ^{14}C counts per nanoliter counted for 10 min.

Passive permeability. Urea permeability was measured isotopically using either isosmolal ultrafiltrate (DLH) or equilibrium solution (PCT) as the perfusion fluid. In each case the bath was isosmolal rabbit serum. The results of these experiments are summarized in Table II and indicate a low passive permeability for urea in PCT ($3.30\pm0.35 \times 10^{-7}$ cm^2 sec^{-1}), about 2.5 times less than the previously determined Na permeability (6). The passive permeability coefficient for urea in the DLH ($0.93\pm0.31 \times 10^{-7}$ cm^2 sec^{-1}) indicates an even greater impermeability than in PCT. The above values were calculated on the basis of length. However, if equation 2 is used and the areas calculated from measured inside diameters in the case of DLH, and an assumed I.D. of 20 μ in case of PCT, then P_{urea} in DLH = $1.51\pm0.47 \times 10^{-5}$ cm sec^{-1}, and in PCT $P_{urea} = 5.3\pm0.6 \times 10^{-5}$ cm sec^{-1}.

Reflection coefficient. The reflection coefficient for urea, σ_{urea}, was measured in the DLH and the PCT by the "equal concentration" method in which the σ_{urea} is equal to the ratio of osmotic water movement induced by urea as compared to osmotic water movement induced by equivalent concentrations of an impermeant molecule, raffinose.

A single experiment involved studies of water movement under three different circumstances: rabbit serum alone, rabbit serum plus 95 mOsm liter^{-1} urea, and rabbit serum plus 95 mOsm liter^{-1} raffinose. In each case the perfusion fluid was kept unchanged and was the ultrafiltrate of the same rabbit serum as that used during control periods. The order of these studies was randomized. In each study three periods were obtained using different bathing fluids. A single complete experiment is given in Table III. Six such experiments were per-

formed, giving a total of 18 periods during each of three conditions. Tables IV and V summarize the means and the ranges of the results obtained. Using these values, the reflection coefficient for PCT is 0.91±0.05, while σ_{urea} in DLH is equal to 0.95±0.04.

Mechanism of osmotic equilibration in the DLH. The results of 12 experiments are summarized in Table VI. These studies were conducted under two different circumstances. In the first series of experiments control periods were obtained in which the bath and the perfusion fluid had the same osmolality of 298 mOsm liter^{-1}; in the subsequent experimental periods the osmolality of the bath was increased by the addition of 112 mOsm liter^{-1} of urea to a final osmolality of 410 mOsm liter^{-1}, while the perfusion fluid was left unchanged at 298 mOsm liter^{-1}. In a second series of studies the control periods were obtained with the bath and perfusion fluid at the same osmolality of 605 mOsm liter^{-1}. Subsequently, experimental periods involved an increase of the bath osmolality to 928 mOsm liter^{-1} by the addition of 204 mOsm liter^{-1} NaCl and 119 mOsm liter^{-1} urea. In both sets of experiments counts per nanoliter of volume marker and osmolalities of collected fluid were measured. The ratio of increase in concentration of volume marker is then compared to the increase in the ratio of osmolar changes. If there is significant influx of solute before osmotic equilibration by water efflux, then the osmolar ratio, (Osm) collected/(Osm) perfused, should increase more than the ratio of the volume marker, (cpm ^{126}I) collected/(cpm ^{125}I) perfusion. As noted in Table VI, there was a rise in the ratio of volume marker which was not statistically different from the rise in the ratio of the osmolalities indicating that all (within the technical limits) of the fluid concentration has occurred by abstraction of water without significant net entry of solute. In these studies short tubules were used with relatively rapid perfusion rates so that true estimation of urea influx could be obtained by the isotopic method. In the first set, there was an influx of 5.2±2.5 mM liter^{-1} of urea representing 6% of the total measured osmolal increase. In the second set, there was a mean influx of urea amounting to 7.8±2.3 mM liter^{-1} which represents 2.6% of the total 305 mOsm liter^{-1} increase noted. In each case the unaccounted increase in osmolality must be mainly secondary to concentration of intraluminal NaCl.

TABLE III

Experimental Data of a Typical Single Study from which the Reflection Coefficient for Urea in the DLH was Determined

Bath	Collection period	Time of collection	Perfusion rate	Net transport
		min	*nl/min*	*nl/mm min*
Control	1	9.1	13.7	0.18
Rabbit	2	9.2	17.8	0.03
Serum	3	11.7	15.8	0.03
$\bar{X}$			15.8	0.08
Control plus	4	11.7	15.4	2.74
95 mOsm/liter	5	9.9	15.6	2.60
urea	6	11.1	16.0	2.95
$\bar{X}$			15.6	2.76
Control plus	7	10.0	17.5	3.21
95 mOsm/liter	8	11.7	15.3	2.77
raffinose	9	9.6	16.7	3.04
$\bar{X}$			16.5	3.00

$$\sigma_{urea} = \frac{J_{v\ urea}^{*}}{J_{v\ raffinose}^{\ddagger}} = \frac{2.68}{2.76} = 0.97$$

* $J_{v\ urea}$ = change in net transport of water from control as induced by 95 mOsm liter^{-1} urea.

‡ $J_{v\ raffinose}$ = change in net transport of water from control as induced by 95 mOsm liter^{-1} raffinose corrected to same perfusion rate when bath was made hyperosmolar by the addition of urea, and therefore is equal to: (15.6/16.5) (3.00) − 0.08 = 2.76 nl mm^{-1} min^{-1}.

TABLE IV

Reflection Coefficient for Urea in Proximal Convoluted Tubule

Perfusion fluid	Mean values for 18 periods in six experiments: Bath	Perfusion rate	Net reabsorption
	mOsm liter^{-1}	*nl min^{-1}*	*nl mm^{-1} min^{-1}*
Ultrafiltrate of rabbit serum	306 Rabbit serum	16.7 (15.0–19.0)	−0.11 (−0.31–+0.15)
diluted with isosmolal raffinose solution	306 Rabbit serum +95 Urea	16.9 (15.0–19.4)	2.17 (1.51–2.90)
	306 Rabbit serum +95 Raffinose	17.4 (15.4–20.0)	2.48 (1.88–3.73)
[Na] = 125 mEq liter^{-1}			

$$\sigma_{urea} = \frac{\Delta J_{v\ urea}}{\Delta J_{v\ raffinose}} = 0.91 \pm 0.05\ (n = 6).$$

TABLE V

Reflection Coefficient for Urea in Descending Limb of Henle

Perfusion fluid	Mean values for 18 periods in six experiments: Bath	Perfusion rate	Net reabsorption
	mOsm liter^{-1}	*nl min^{-1}*	*nl mm^{-1} min^{-1}*
Ultrafiltrate of rabbit serum (305 mOsm liter^{-1})	306 Rabbit serum	15.9 (14.3–17.9)	−0.01 (−0.25–+0.24)
	306 Rabbit serum +95 Urea	15.7 (13.7–18.3)	3.01 (2.74–4.34)
	306 Rabbit serum +95 Raffinose	15.9 (12.7–19.3)	3.22 (2.49–4.50)

$$\sigma_{urea} = \frac{\Delta J_{v\ urea}}{\Delta J_{v\ raffinose}} = 0.95 \pm 0.04\ (n = 6).$$

TABLE VI

The Relationship between Per Cent Increase in Osmolality of the Collected Fluid to Per Cent Reabsorption of Fluid when Bath is Made Hyperosmolar by: I. Addition of 112 mOsm liter^{-1} Urea, and II. Addition of 204 mOsm liter^{-1} NaCl and 119 mOsm liter^{-1} Urea

	Length, L	Perfusion rate, Vi	[^{125}I] in, A	[^{125}I] out, B	B/A	CF osmolality exp.	(CF/PF) osmolality	Urea influx
	μ	*nl mm^{-1} min^{-1}*	*counts nl^{-1}*	*counts nl^{-1}*		*mOsm liter^{-1}*		mM
I. Influence of increased urea in bath								
Control:	440	26.8	34.2	45.3	1.32	396	1.33	1.6
Bath: 298 mOsm/liter								
PF: 298 mOsm/liter	420	13.3	33.5	43.3	1.29	408	1.37	0.0
Exp.:								
Bath: 410 mOsm/liter	440	19.9	40.1	54.2	1.35	392	1.32	3.5
PF: 298 mOsm/liter								
	440	12.1	48.0	66.0	1.38	387	1.30	0.9
	260	35.3	35.5	43.1	1.21	368	1.23	17.9
	300	28.1	34.0	39.3	1.16	357	1.20	7.2
$\bar{X}$ (SE)	383	22.6	37.6	48.5	1.29±0.03	397±7	1.29±0.02	5.2±2.5
II. Influence of increased urea + NaCl in bath								
Control:								
Bath: 605 mOsm/liter	360	16.5	67.3	103.2	1.53	906	1.50	2.5
PF: 601 mOsm/liter								
Exp.:	440	17.3	67.9	106.3	1.57	926	1.53	14.6
Bath: 928 mOsm/liter								
PF: 605 mOsm/liter	440	17.3	53.1	80.6	1.52	922	1.52	1.3
	440	11.8	54.2	81.5	1.50	916	1.51	14.5
	380	23.9	54.6	79.9	1.46	920	1.52	10.6
	440	12.1	27.9	43.6	1.56	871	1.44	3.0
$\bar{X}$ (SE)	417	16.5	54.2	82.5	1.52±0.02	910±8	1.50±0.01	7.8±2.3

[^{125}I] in, ^{125}I counts per 10′ nl^{-1} in perfusion fluid; [^{125}I] out, ^{125}I counts per 10′ nl^{-1} in collected fluid; Exp., experimental period; PF, perfusion fluid; CF, collected fluid.

DISCUSSION

It has generally been accepted that reabsorption of urea in PCT is a passive process secondary to reabsorption of salt and water. However, the finding (7–12) that urea concentration in cortical tissue is significantly higher than either plasma or tubular fluid has reopened the question as to whether or not an active transport process might be involved.

The isolated perfused PCT provides a means of examining whether there is active transport of urea. Several approaches could be used to examine this question. In one such procedure the bidirectional fluxes could be determined. An inequality in these parameters would suggest either active reabsorption or secretion, providing they do not parallel the net transport of fluid in an exact pattern. ^{14}C-labeled urea is the only isotopically available radionuclide of urea which is practical to analyze by the conventional techniques. Therefore, it becomes necesary to measure influx and efflux of urea in different groups of tubules. This approach is not desirable owing to the high scatter of data associated with microbiological techniques: up to a 25% difference could be lost in the variability of data. For this reason another approach was taken. In these experiments proximal convoluted tubules were perfused with equilibrium solution to insure that net transport of fluid was zero. In this way any urea transport by solvent drag is eliminated, thus making the interpretation of net urea movement straightforward. Urea-^{14}C was added in equal concentrations to the water of ultrafiltrate and bathing rabbit serum. Active transport of urea by the PCT could not be demonstrated under our specific protocol since the collected fluid urea-^{14}C concentration did not differ significantly from the perfusion or bathing fluid urea-^{14}C concentrations (Table I).

The failure to demonstrate active transport of urea in PCT indicates that net reabsorption in this segment must be mediated by passive processes. One possibility is that urea could be transported passively in association with bulk movement of fluid by net transport of sodium salts. Previously (13), it has been pointed out that transport by solvent drag can be estimated by $J_v\bar{C}$ (1-σ),

where J_v equals net transport of fluid, $\bar{C}$ is the mean concentration of solute in the membrane, and σ the reflection coefficient for the solute in question. Obviously, the greater the σ, the smaller the solvent drag term. The calculated σ_{urea} is significantly higher than σ_{NaCl} in the PCT (Table VII) and would indicate that only an insignificant amount of urea transport can be attributed to solvent drag.

Micropuncture studies have previously demonstrated that proximal tubular-fluid-to-plasma-urea concentrations are greater than one (12, 14–17) with most of the value ranging between 1.4 and 1.5. In the absence of active urea secretion this would imply that the PCT is relatively impermeable to urea and is consistent with the σ_{urea} of 0.91. Also, as was noted for PCT in Table VII, urea is approximately 2.5 times less permeable than the previously reported sodium permeability. These findings are consistent with the thesis that urea is concentrated in the PCT by abstraction of fluid. The generated concentration gradient for urea then provides a driving force for passive transport of urea down its chemical concentration gradient, accounting for the observed rates of net reabsorption.

A number of studies (7, 11, 12, 18–22) have shown that under certain conditions papillary tissue water concentration of urea is greater than in the final urine. This had lead to the postulation that the medullary collecting duct is capable of active transport of urea. More direct evidence in support of this view are the studies of Clapp (16) and Lassiter, Mylle, and Gottschalk (23) in which they noted net reabsorption of urea from collecting ducts against apparent concentration gradients. Urea which is transported out of the collecting duct then either reenters the countercurrent multiplication system by diffusion into the loops of Henle, or leaves the papilla in the vasa recta blood. If urea enters the loop then (TF/P) urea/(TF/P) inulin ratio would be higher in the early distal tubule than the late PCT. Conflicting data exist on this point in animals not undergoing saline diuresis (12, 14, 24); however, most of the observations suggest that recirculation of urea does exist, thus implying that either the descending or ascending thin limb of Henle should be relatively permeable to urea. In the present study, the role of the DLH was examined directly and noted to be as impermeable to urea as previously reported for Na (Table VII). This finding might lead one to the conclusion that only an insignificant amount of urea can diffuse into the DLH, and that the DLH is not involved in recycling of urea. However, as will be discussed later, the observed urea permeability, although very small, can in fact account for the magnitude of urea recycling observed by others.

The reflection coefficient for urea was determined in order to examine whether urea can generate high effective osmotic pressure across the tubular epithelium. The high value of 0.95±0.04 indicates that urea, as well as sodium (Table VII), can abstract fluid out of the DLH by osmotic forces. A more direct measure of the role of urea in concentrating fluid in DLH was obtained from those experiments where it was shown that (CF/PF) osmolality increased to the same degree as did (CF/PF) ^{125}I, when the bath was made hyperosmolal by the addition of 112 mOsm liter^{-1} urea. A (CF/PF) osmolality ratio higher than (CF/PF) ^{125}I ratio would have indicated net entry of solute; however, the finding that these ratios increased by proportional amounts shows that the fluid in DLH is concentrated by primary abstraction of fluid. This is consistent with the high determined reflection coefficient for urea.

TABLE VII
Membrane Characteristics of Isolated Perfused Rabbit Nephrons

	Proximal convoluted tubule	Descending Limb of Henle
Net transport of fluid ($nl\ mm^{-1}\ min^{-1}$)	1.00	−0.07‡
Passive permeability for ^{22}Na ($cm^2\ sec^{-1} \times 10^{-7}$)	5.80*	1.00‡
Passive permeability for urea-^{14}C ($cm^2\ sec^{-1} \times 10^{-7}$)	3.30	0.93
Reflection coefficient for NaCl	0.68*	0.96‡
Reflection coefficient for urea	0.91	0.95
Osmotic water permeability ($ml\ cm^{-1}\ sec^{-1}\ atm^{-1} \times 10^{-6}$)	0.18*	1.45‡

Values summarized from previous work (6)* and (2).‡

It is difficult to extrapolate these findings directly to the in vivo situation that exists in the renal papilla because of the marked differences in the osmolality at which the system is operating. It is now well appreciated that the permeability characteristics of membranes may change under varying osmolar surroundings. This phenomena has been called nonlinear osmosis and indicates that a given osmolar gradient can induce varying net flows of fluid depending on the control ambient osmolar concentration. Thus a 100 mOsm liter^{-1} solute gradient may have a different effect on net water movement when imposed on 300 mOsm liter^{-1} bath as compared to 100 mOsm liter^{-1} gradient imposed on 600 mOsm liter^{-1} bath. Nonlinear osmosis has been noted for a variety of tissues (6, 25–29), including the renal proximal convoluted tubule (6). It is for this reason that the mechanism of fluid concentration secondary to osmotic gradient in the DLH was tested under two differing control osmotic conditions. In each case it was found that the fluid is primarily concentrated by abstraction of water. This does not necessarily indicate that the osmotic water permeability, σ_{urea}, P_{NaCl}, or P_{urea} remain unchanged. However,

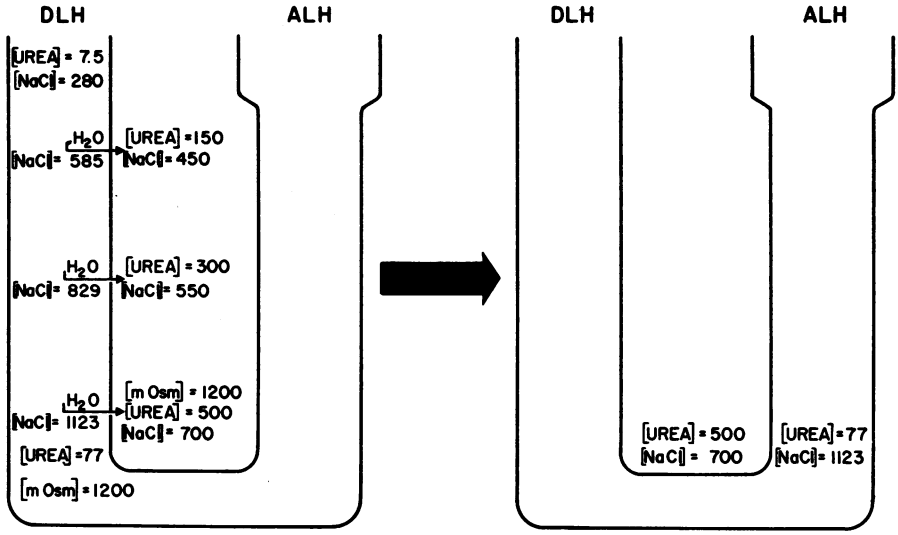

FIGURE 2 The role of urea in concentrating the fluid of the descending limb of Henle. Note that the sodium in the DLH is concentrated to higher values than the corresponding medullary tissue as a consequence of water abstraction by urea. Depicted concentrations are in mOsm/liter.

it does mean that if these parameters do indeed change, then these changes vary in such a manner as not to influence the conclusions as to the basic mechanism by which the osmotic equilibration takes place in the DLH.

These findings are in apparent conflict with the studies of Marsh (30) and deRouffignac and Morel (31) in which they show significant net entry of urea into the DLH. If, however, the mechanism of osmotic equilibration in the face of concentration gradients of solutes with σ of 0.95 is examined more precisely, it is apparent that our data are consistent with the findings of Marsh (30) and Lassiter, Gottschalk, and Mylle (14), as well as those of other investigators suggesting recycling of urea through the loop of Henle. The studies of Fordtran, Rector, Ewton, Soter, and Kinney (32) indicate that for a given membrane system osmotic equilibration always occurs at exactly the same rate, irrespective of the reflection coefficient of solute used to establish the osmotic gradient. The σ does not influence the rate of osmotic equilibration; however, the experimental evidence suggests that it does determine the relative proportion of water abstraction and solute addition. In the presented experiments which examined the mechanism of osmotic water equilibration (Table VI), it was demonstrated that practically all of the osmotic equilibration occurred by efflux of water. It was further shown that approximately 6% of total osmolal increase could be attributed to urea influx when bath osmolality was increased by addition of 112 mOsm/liter urea to the bath, while 2.6% of the total osmolal increase could be accounted for by influx of urea in those experiments in which bath osmolality was increased by addition of 119 mOsm/liter urea and 204 mOsm/liter NaCl to the bath. For illustrative purposes for the remainder of the discussion it will be assumed that osmotic equilibration occurs by 96% water efflux and by 4% solute (urea) entry.

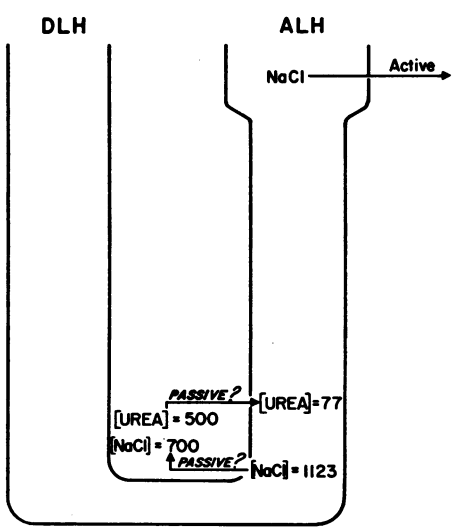

FIGURE 3 Hypothetical role of the thin ascending limb of Henle in the countercurrent multiplication system. The salient features are: (*a*) relative water impermeability, (*b*) passive influx of urea, and (*c*) passive outflux of sodium down its concentration gradient without active transport process.

If we further assume that the papillary interstitium had an osmolality of 1200 mOsm/liter (Fig. 2), with 500

mOsm/liter urea and 700 mOsm/liter NaCl, then isotonic fluid (300 mOsm/liter) entering the DLH would equilibrate to 96% of this value (1152 mOsm/liter) by water abstraction, and the remaining 48 mOsm liter^{-1} would be due to addition of solute, principally urea. If the fluid entering the DLH had a TF/P inulin of 2.0 and TR/P urea of 1.5, water abstraction would increase these ratios to 7.7 and 5.8, respectively. If the plasma urea concentration were 5 mM, water abstraction in the DLH would raise the urea concentration to 29 mM. In addition, the net entry of urea of 48 mOsm/liter would increase the total urea concentration to 77 mM giving a TF/P ratio of 15.4. The TF/P_{urea} : TF/P_{inulin} ratio at the tip therefore would be 1.99. This indicates that the quantity of urea present in loop fluid is 99% greater than was actually filtered and is in general agreement with the values reported by Lassiter et al. (14) and, more recently, by Roch-Ramel.[2]

Fig. 3 illustrates the mechanism by which urea generates a sodium concentration gradient in the DLH fluid. As the fluid passes from the cortico-medullary junction with an osmolality of 300 mOsm liter^{-1} to the papillary tip with an osmolality of 1200 mOsm liter^{-1}, it is continuously equilibrating with hypertonic medullary interstitium. If the medullary solute consisted entirely of sodium salts, then both the osmolality and the sodium concentration of loop fluid would remain identical to that of medullary interstitium. However, if urea comprises a larger fraction of medullary solute, then the osmolality of loop fluid and medulla would remain equal but the sodium concentration of loop fluid would be significantly higher than the medullary interstitium. In Fig. 3, this situation is illustrated by assuming that 500 mOsm/liter of the total papillary osmolality of 1200 mOsm/liter^{-1} is due to urea. The high papillary osmolality would abstract water out of the DLH until its osmolality would also equal 1200 mOsm liter^{-1}, but leaving the remaining fluid with a sodium chloride concentration of 1123 mOsm liter^{-1}. Thus, the papillary urea concentration of 500 mOsm liter^{-1} has allowed for a generation of a 423 mOsm liter^{-1} sodium chloride concentration gradient. The urea concentration will rise to 77 mOsm liter^{-1} by mechanisms described in the previous paragraph. The magnitude of the rise in intratubular Na concentration would be dependent on medullary osmolality and, to a lesser degree, the small amount of urea diffusing in. The generated Na concentration gradient would be a function of the fraction of urea which constitutes the total osmolality of the papillary interstitial fluid.

Fig. 3 demonstrates a hypothetical model involving the role of the thin ascending limb of Henle in the overall operation of the countercurrent multiplication system. If we postulate that the thin ascending limb of Henle has a higher Na permeability than the DLH, then it is possible to conceive how Na could diffuse down its concentration gradient out of the ALH without the necessity of postulating active transport of Na out of the ALH. For this model to be operative, it is necesary for the ALH to be less permeable to urea and more permeable to sodium than the DLH to the degree that more Na will be transported by passive diffusion than water by osmotic forces. By this mechanism the thin ALH fluid could be rendered hypo-osmolal to the adjacent interstitium by passive mechanisms. Using the data enclosed in this manuscript and the above-stated hypotheses concerning the thin ALH, a complete theoretical description of a new model for counter-current multiplication system has been developed in which the observed medullary concentration gradients can be generated by placing the energy-dependent transport process only in the thick ALH and allowing both the thin ALH and the DLH to operate as purely passive equilibrating segments.[3] This model would be consistent with those experiments (33–36) which have failed to demonstrate active transport of sodium by the thin ALH.

[2] Roch-Ramel, F. Personal communication.

[3] Kokko, J. P., and F. C. Rector. 1971. Countercurrent multiplication system without active transport in inner medulla—a new model. Submitted for publication.

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