Urea Secretion by the Straight Segment of the Proximal Tubule

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ABSTRACT Studies utilizing in vitro microperfusion were designed to examine whether urea is actively or passively transported across superficial and juxtamedullary straight segments of rabbit proximal tubules. With perfusate and bath solutions containing 1 mM urea and electrolytes similar to normal plasma, the efflux (lumen-to-bath) isotopic permeability ($\times 10^{-5}$ cm s^{-1}) of superficial segments was 1.37±0.16 and of juxtamedullary segments was 2.14±0.20. In the same tubules, the influx (bath-to-lumen) isotopic permeability was 3.70±0.35 in superficial segments and 4.75±0.37 in juxtamedullary segments. Despite net water movement in the opposite direction (0.5 nl mm^{-1} min^{-1}), the influx rate was significantly higher than the efflux rate of urea in both groups. With a low perfusion rate (2 nl/min) and equivalent specific activities of [^{14}C]urea in bath and perfusate, the collected-to-perfused ratio of [^{14}C]urea, corrected for volume marker change, was 1.07±0.01 in superficial and 1.09±0.01 in juxtamedullary nephrons, thus indicating net secretion in both segments. In separate studies urea influx was inhibited by hypothermia (decrease from 37° to 28°C), by phloretin (0.1 mM in bath), by cyanide (1 mM), but not by probenecid (0.2 mM). In each case the inhibition was highly significant and reversible. These data suggest that urea is actively secreted by the straight segments of both the superficial and juxtamedullary proximal tubules. These segments may, therefore, contribute significantly to the high urea concentration found at the bend of Henle's loop by micropuncture.

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INTRODUCTION

The importance of urea in the formation of concentrated urine has been emphasized with the recent description of countercurrent multiplication models in which the inner medullary structures do not possess active transport mechanisms for NaCl (1, 2). These models require that for the kidney to form maximally concentrated urine a high concentration of urea must be present in the papillary interstitium. To maintain these high concentrations of papillary urea, it has been suggested that urea is "recycled" and thus trapped in the countercurrent flow systems known to exist in the renal medulla. In fact, previous micropuncture studies (3–7) have shown that the concentration of urea is higher in the fluid obtained from the bend of the loop of Henle than can reasonably be expected to exist at the end portions of the proximal convoluted tubule. These findings have been generally interpreted to indicate that urea recycles into the descending limb of Henle. A priori, these findings seem in variance with our demonstration that the descending limb of Henle is relatively impermeable to influx of urea (8). However, it must be remembered that a relatively long nephron segment, namely the straight portion of the proximal tubule (pars recta), is interposed between the proximal convoluted tubule and the descending limb of Henle. No previous studies have examined the mechanism of urea transport across this segment. Thus, the purposes of the present studies were to determine the passive permeability characteristics of the pars recta to urea and to examine whether urea is actively secreted or reabsorbed by the pars recta.

METHODS

All results were obtained by the previously reported technique, in which isolated segments of rabbit nephrons were perfused in vitro (9). In each case tubular segments were obtained from 1.5–2.5 kg female rabbits, fed a standard laboratory diet before guillotine decapitation. Straight seg-

ments of both superficial (SF) [1] and juxtamedullary (JM) proximal tubules were dissected and perfused by the techniques previously described (10). Similarly, the positive identification of the origin of the straight segment was accomplished by the methods utilized in our previous study (10). Straight segments of SF proximal tubules were obtained from the mid-cortex and juxtamedullary regions while straight segments of JM proximal tubules were obtained exclusively from the outer medulla.

Three different types of perfusates were used in these experiments: (*a*) isosmolal ultrafiltrate of rabbit serum; (*b*) solution A (Table I), designed to simulate normal ultrafiltrate of rabbit serum; and (*c*) equilibrium solution (Table I), designed to minimize transtubular fluid movement across SF proximal tubules. The choice of perfusate will be described in the Results section for each experimental protocol. Two different bathing media were used: commercially available rabbit serum or solution A (Table I) to which 5% vol/vol fetal calf serum was added. In addition, varying amounts of urea were added to these solutions as dictated by the specific protocol.

Net reabsorption of fluid (C), in nanoliters per millimeter-minute, was calculated by the same equations previously published (11):

$$C = \frac{V_i - V_o}{L} \tag{1}$$

where V_i is calculated by dividing the [^{125}I]iothalamate (Glofil-125, Abbott Laboratories, North Chicago, Ill.) counts per minute of the collected fluid by ^{125}I counts per minute per nanoliter of perfusion fluid and by the time of the collection period; V_o is obtained directly by a previously calibrated constant-volume pipette as originally described by Burg (12); L is the length of tubule in millimeters.

Permeability coefficient for urea (P_{urea}). Permeability coefficients for urea for lumen-to-bath (P^{lb}_{urea}) and for bath-to-lumen (P^{bl}_{urea}) were calculated from the rate of disappearance from or appearance into the luminal fluid of the [^{14}C]urea (10 μCi/ml, ICN Pharmaceuticals, Inc., Irvine, Calif.), added either to the perfusate or to the bath. In this series of experiments the perfusate was solution A while the bath was solution A plus 5% vol/vol fetal calf serum. In one series of studies, bidirectional permeability coefficients were measured on the same tubules by first adding the [^{14}C]urea to the bath and measuring the influx rate of [^{14}C]urea for three collection periods, followed by three collection periods after change of perfusate (by addition of [^{14}C]urea), and bath (by deletion of [^{14}C]urea), and then measuring the efflux rate of [^{14}C]urea. 30 min re-equilibration time was allowed between the changes of the solutions. The order of these studies was randomized to minimize systematic sources of error.

Unidirectional P^{lb}_{urea} was calculated by (13):

$$P^{lb}_{urea} = \frac{V_i - V_o}{A}\left[\frac{\ln C_i^*/C_o^*}{\ln V_i^*/V_o^*} + 1\right] \tag{2}$$

where A is the area of the tubule calculated from its length and an assumed inside diameter of 20 μm; V_i is perfusion rate; V_o is collection rate; C_i^*, counts per minute per nanoliter of isotope in the perfusate; and C_o^*, counts per minute per nanoliter of isotope in the collected fluid.

In those experiments in which $V_i = V_o$ exactly, the permeability coefficient was calculated according to (13):

$$P^{lb}_{urea} = \frac{V_i}{A} \ln \frac{C_i^*}{C_o^*} \tag{3}$$

In tubules in which P^{bl}_{urea} was determined together with P^{lb}_{urea}, P^{bl}_{urea} was calculated by (14),

$$P^{bl}_{urea} = \frac{C_o^*}{C_b^*}\left[\frac{P^{lb}_{urea}}{1 - \exp(-A P^{lb}_{urea}/V)}\right] \tag{4}$$

where C_o^* is counts per minute per nanoliter of isotope in collected fluid; C_b^* is counts per minute per nanoliter of isotope in the bath; A is area of tubule; and V is the mean flow rate, $(V_i + V_o)/2$.

In 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 (14):

$$P^{bl}_{urea} = \frac{V}{A} \ln \frac{C_b^*}{C_b^* - C_o^*} \tag{5}$$

The radioactivity of ^{125}I was measured by a Packard model 3365 three-channel gamma spectrometer while [^{14}C]-urea activity was measured with a Packard model 2420 liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

Reflection coefficient for urea. Reflection coefficient for urea, σ_{urea}, was measured in these experiments by the "equal concentration" method (15), where σ_{urea} is equal to the ratio of the increment of net fluid movement induced by an osmotic gradient of urea, ΔJv_{urea}, as compared to net fluid movement induced by an equivalent concentration gradient of nonpermeant compound, raffinose:

$$\sigma_{urea} = \frac{\Delta Jv_{urea}}{\Delta Jv_{raffinose}} \tag{6}$$

TABLE I

*Composition of Artificial Solutions Used**

	Solution A	Equilibrium solution
	mM	
NaCl	105	80
$NaHCO_3$	25	15
KCl	5	5
Na_2HPO_4	4	4
Na acetate	10	10
$CaCl_2$	1.8	1.8
$MgSO_4$	1	1
Glucose	8.3	8.3
Alanine	5	5
Urea	1	1
Raffinose	—	65.1
Osmolality*	297	297

* Gravimetrically measured. Osmolality measured by standard freezing point depression techniques.

[1] *Abbreviations used in this paper:* bl, bath-to-lumen; *C*, net reabsorption of fluid; J_v, net fluid reabsorption; JM, juxtamedullary; lb, lumen-to-bath; P_{urea}, permeability coefficient for urea; PD, transepithelial potential difference; Q_{10}, temperature coefficient of diffusion; σ_{urea}, reflection coefficient; SF, superficial; V_t, perfusion rate.

TABLE II

Unidirectional Permeability Coefficients for [^{14}C]Urea, P_{urea}, across SF and JM Segments of Proximal Straight Tubules

Origin of segment		N	Length	V_t	J_v	P_{urea}
			mm	*nl min^{-1}*	*nl min^{-1} mm^{-1}*	*10^{-5} cm s^{-1}*
Superficial	bl	18	1.52	19.32	0.51	3.70*
			±0.07	±0.76	±0.04	±0.33
	lb	13	1.58	14.53	0.43	1.37
			±0.14	±1.34	±0.03	±0.16
Juxtamedullary	bl	20	1.05	17.14	0.59	4.75*
			±0.23	±1.05	±0.10	±0.37
	lb	15	1.36	11.44	0.27	2.14
			±0.08	±0.75	±0.05	±0.20

Perfusate was solution A (Table I); bath was solution A plus 5% vol/vol calf serum; n = number of tubules.

* $P < 0.001$ (bl vs. lb).

In each experiment a total of nine collections were made, three each of: (*a*) control perfusate; (*b*) when 50 mosmol/liter of urea was added to the bath; and (*c*) when 50 mosmol/liter of raffinose was added to the bath. The order of addition to the bath of urea or raffinose was randomized. Perfusate in each experiment was solution A (Table I).

Transtubular potential difference (PD). Transtubular PD was measured by techniques previously reported (16). In summary, Beckman (Beckman Instruments, Inc., Fullerton, Calif.) calomel half-cells were connected to the bath and the perfusion pipette via juxtaposed bridges of 300 mosmol/kg Ringer's solution in 4% agarose. The PD was measured by Keithley model 602 electrometer (Keithley Instruments, Inc., Cleveland, Ohio) and recorded on a Rikadenki model B-261 multipen recorder (Rikadenki Kogyo Co. Ltd., Tokyo, Japan).

Transport inhibitors. Various concentrations of three different transport inhibitors were used: probenecid (Merck Sharp & Dohme, Div. of Merck Co., Inc., West Point, Pa.), sodium cyanide (J. T. Baker Chemical Co., Phillipsburg, N. J.) and phloretin (ICN K&K Laboratories Inc., Plainview, N. Y.).

The data of each tubule were obtained as the mean of three or four collection periods. The results are expressed as mean±SE of number of tubules studied.

RESULTS

The results of the initial series of studies in which solution A was used as perfusate and in which the unidirectional urea permeabilities were measured are summarized in Table II. The bath-to-lumen urea permeability, $3.70\pm0.33 \times 10^{-5}$ cm/s ($n = 18$) across the SF segment of the proximal tubules is significantly higher ($P < 0.001$) than the lumen-to-bath permeability, $1.37 \pm 0.16 \times 10^{-5}$ cm/s ($n = 13$). Similarly, the bath-to-lumen permeability of $4.75\pm0.37 \times 10^{-5}$ cm/s ($n = 20$) (of the JM segments) is higher than the lumen-to-bath permeability, $2.14\pm0.20 \times 10^{-5}$ cm/s ($n = 15$).

The above data, though performed on a large number of tubules, might be criticized for two reasons: the permeability coefficients were measured under conditions in which net fluid movement was not zero; and significant differences exist between permeability coefficients obtained from separate tubules. In view of this, the experiments depicted in Table III were performed. In the top panel both the bath-to-lumen and lumen-to-bath permeability coefficients were determined in the same segment sequentially with solution A as the perfusate. In agreement with the previous studies, described in Table II, the results of Table III indicate that influx is greater than efflux of urea.

To examine the bidirectional permeabilities in the absence of net water flow, equilibrium solution (Table I) was developed for the SF tubules. Net fluid reabsorption was not statistically different from zero with equilibrium solution as the perfusate. Again, the influx of urea was approximately twice as great as the efflux of urea (Table III).

Similarly, the influx of urea in JM tubules was approximately twice as great as the efflux of urea when these two parameters were measured sequentially in the same tubules. The results of these experiments are summarized in Table III when solution A was used as perfusate. For reasons that are not clear, we have been unable to develop an "equilibrium" solution for JM tubules where the net reabsorption of fluid is zero. Consequently, we have not measured the bidirectional urea fluxes in JM tubules when net reabsorption was zero.

Net secretion. The possibility of net secretion of urea into the lumen of SF and JM straight segments of proximal tubules was evaluated with solution A as the perfusate and similar solutions with added 5% vol/vol calf serum as the bath. Perfusion rate was slow, varying between 1 and 5 nl/min. Equivalent counts of [^{14}C]urea were added to the perfusion fluid and bath. The concentration of ^{14}C was measured in the collected fluid and compared to the counts in the perfusate and the

TABLE III

Bidirectional Permeability Coefficient for [^{14}C]Urea in the Two Types of Proximal Straight Tubules

Superficial segments; perfusate = solution A					Superficial segments; perfusate = equilibrium solution					Juxtamedullary segments; perfusate = solution A				
Exp. no.	Length		V_i	J_v	P_{urea}	Exp. no.	Length		V_i	J_v	P_{urea}	Exp. no.	Length		V_i	J_v	P_{urea}
	mm		*nl min^{-1}*	*nl min^{-1} mm^{-1}*	*10^{-5} cm s^{-1}*		*mm*		*nl min^{-1}*	*nl min^{-1} mm^{-1}*	*10^{-5} cm s^{-1}*		*mm*		*nl min^{-1}*	*nl min^{-1} mm^{-1}*	*10^{-5} cm s^{-1}*
1	1.44	bl	20.85	0.71	3.50	1	1.94	lb	16.88	0.12	1.21	1	0.73	bl	24.12	0.91	7.46
		lb	19.82	0.22	1.17			bl	12.88	−0.05	3.08			lb	18.97	0.38	1.56
2	1.00	lb	12.14	1.28	1.01	2	1.60	bl	18.61	0.06	4.04	2	1.50	lb	8.44	0.19	2.00
		bl	24.48	0.37	5.57			lb	17.86	0.04	2.39			bl	10.70	0.13	3.55
3	1.60	lb	19.43	0.43	1.83	3	2.36	lb	13.83	0.02	2.84	3	0.82	bl	13.87	0.23	6.15
		bl	19.78	0.52	1.43			bl	14.66	−0.26	3.62			lb	11.18	0.06	2.94
4	1.10	lb	21.57	0.58	1.066	4	1.36	bl	15.32	0.18	4.71	4	1.00	lb	10.19	0.20	3.80
		bl	23.47	0.48	3.89			lb	16.09	0	2.66			bl	11.88	0.34	4.06
5	1.40	bl	16.64	0.42	3.63	5	1.80	lb	15.04	0.09	1.67	5	1.50	bl	7.56	0.15	4.68
		lb	22.72	0.57	0.52			bl	13.84	0.05	4.36			lb	11.85	0.13	3.41
6	1.56	bl	19.70	0.95	3.04	6	2.00	bl	17.27	0.09	4.45	6	1.24	lb	14.17	0.47	1.14
		lb	15.11	0.34	2.06			lb	15.68	−0.04	1.45			bl	18.09	0.46	2.41
						7	2.40	lb	15.13	−0.04	1.46						
								bl	15.82	−0.15	4.25						
	Mean	lb	18.47	0.40	1.28			lb	15.79	0.03	1.95			lb	12.47	0.24	2.48
	SE		±1.65	±0.06	±0.23				±0.50	±0.02	±0.25				±1.51	±0.06	±0.44
	Mean	bl	20.87	0.58	3.51			bl	15.49	−0.01	4.07			bl	14.37	0.37	4.72
	SE		±1.18	±0.09	±0.55				±0.74	±0.06	±0.21				±2.42	±0.12	±0.75

Bath in each case was solution A plus 5% vol/vol calf serum; bl = bath-to-lumen; lb = lumen-to-bath. Both lb and bl permeabilities were measured in the same tubule. The order of measurement was randomized.

bath. At the beginning, bath ^{14}C counts were 5% lower than those of the perfusate but on average the two solutions were the same at the time of the collection. A rise in the ratio of [^{14}C]urea counts in collected fluid to perfusate was then compared to the rise in ^{125}I volume marker ratio. The results of these experiments are shown in Fig. 1 and indicate a highly significant ($P < 0.001$) rise in the ^{14}C ratio as compared to the [^{125}I]-iothalamate ratio. The mean CF/PF urea ^{14}C over CF/PF iothalamate ^{125}I ratio was 1.07±0.01 in SF–PSF ($n = 15$, mean length 1.7 mm) and 1.09±0.01 in JM–PSF ($n = 12$; mean length 1.3 mm). These results indicate net urea secretion in the direction opposite to that of net water movement.

Effect of hypothermia. If net urea secretion occurs by energy dependent processes, then it might be predicted that the unidirectional influx would decrease with a decrease in the ambient temperature. To examine this issue, control periods were first obtained at 37°C when the bath-to-lumen [^{14}C]urea flux was measured together with the transtubular potential difference. This was followed by similar measurements at 28°C. Bath temperature was then returned to 37°C for a recovery period. The results of these experiments are shown in Fig. 2 and indicate a reversible decrease in both the urea influx and the transtubular PD. The efflux (lumen-to-bath) urea permeability also decreased significantly (Table IV); however, in absolute terms, the decrease in efflux of urea was not as great as the decrease in influx of urea.

Effect of sodium cyanide. The effect of 1 mM NaCN (added to the bath) on the bidirectional urea fluxes and transtubular PD was examined in a series of studies in

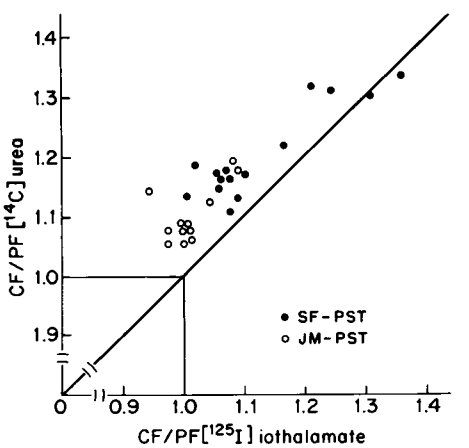

FIGURE 1 The correlation between collected fluid (CF) to perfusion fluid (PF) ratios for [^{14}C]urea and [^{125}I]iothalamate in the SF and JM straight segments of proximal tubules (PST). The bath counts of [^{14}C]urea per nanoliter were identical on the average to the counts in the perfusion fluid. Thus, points above the 45° line of identity indicate net secretion of urea.

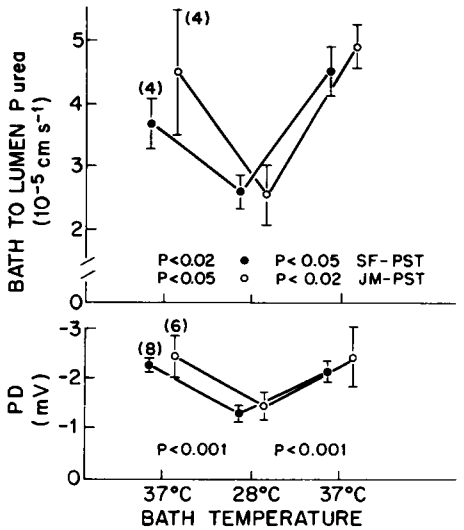

FIGURE 2 The effect of hypothermia on the bath-to-lumen and lumen-to-bath permeability for [^{14}C]urea. Closed and open circles refer to SF and JM segments, respectively. *P* values were calculated by paired *t* test.

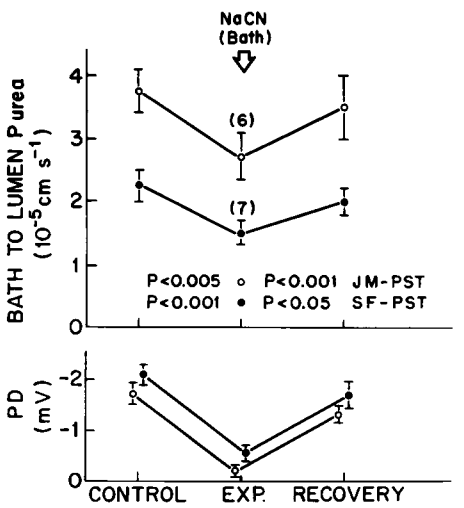

FIGURE 3 The effect of the addition of 10 mM NaCN to the bath on [^{14}C]urea bath-to-lumen influx (top panel) and PD (bottom panel) across the SF and JM segments of proximal straight tubules.

which solution A was used as the perfusion fluid while a similar solution plus 5% vol/vol calf serum was used as bathing fluid. Three control periods were obtained first, followed by three experimental periods, started 10 min after addition of NaCN, and finally three recovery periods, obtained after the NaCN had been exchanged out of the bath.

The results of these studies indicate a highly significant reversible decrease ($P < 0.001$, paired t test) in the bath-to-lumen flux in SF proximal straight tubule (2.3 ± 0.2 to 1.5 ± 0.2, and a return to $2.0\pm0.3 \times 10^{-5}$ cm/s, $n = 6$, (Fig. 3). There was no effect of NaCN on the lumen-to-bath urea fluxes in either of the segments (Table IV). NaCN reversibly decreased the transtubular PD (Fig. 3).

The effect of phloretin. The effect of 0.1 mM phloretin was examined by a similar protocol, as was the effect of NaCN. As shown in Fig. 4, phloretin significantly ($P < 0.001$, paired t) decreased the bath-to-lumen influx of urea (from 2.98 ± 0.14 to 2.09 ± 0.14, and back to $2.22\pm0.20 \times 10^{-5}$), without significant effect on lumen-to-bath fluxes (Table IV). In these studies three SF and three JM straight segments of proximal tubules were used. Simultaneously measured transtubular PD fell minimally but significantly ($P < 0.02$, paired t), and the net water flow fell from 0.71 ± 0.15 to 0.17 ± 0.09 nl mm^{-1} min^{-1} ($P < 0.02$, paired t-test).

The effect of probenecid. The effect of 0.2 mM probenecid was examined only in the bath-to-lumen direction in four SF proximal straight tubules. The probenecid was added to the bath. In these experiments equilibrium solution was used as the perfusate. There was no effect of probenecid either on the influx of urea

TABLE IV

The Effect of Hypothermia, Sodium Cyanide, and Phloretin on the Lumen-to-Bath Permeation of Urea

		n	Control P_{urea}	Experimental P_{urea}	*P*
Hypothermia	SF	6	2.03±0.09	1.25±0.14	0.001
(28°C)	JM	5	2.75±0.63	1.59±0.35	0.05
NaCN, 1 mM	SF	6	1.69±0.24	1.53±0.25	NS
(bath)	JM	5	2.82±0.38	2.62±0.55	NS
Phloretin, 0.1 mM	SF	3	1.96±0.30	1.88±0.29	NS
(bath)	JM	3	3.32±0.47	3.70±0.65	NS

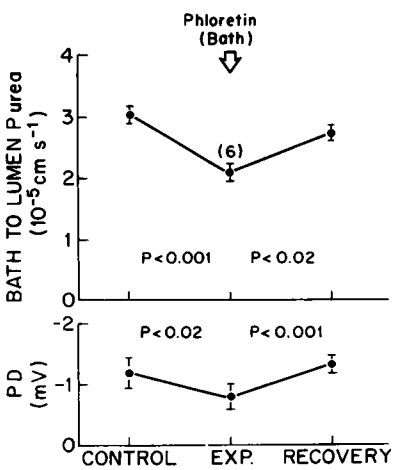

FIGURE 4 The effect of the addition of 1 mM phloretin on [^{14}C]urea bath-to-lumen influx (top panel) and PD (bottom panel) across the two types of proximal straight tubules. Results are grouped and similar.

TABLE V

Reflection Coefficient for Urea across the Two Types of Proximal Straight Tubules

	Length	Bathing medium		V_f	J_v	σ_{urea}
	mm	*mosmol/liter*		*nl/min*	*nl min⁻¹ mm⁻¹*	
SF (n = 5)	0.76	RS	301	20.9±3.6	0.48±0.21	1.01
	±0.12	RS + raffinose	354	24.8±1.9	4.12±0.51	±0.03
		RS + urea	352	25.0±2.3	4.16±0.51	
JM (n = 6)	0.60	RS	299	23.5±1.1	0.62±0.17	0.97
	±0.02	RS + raffinose	353	26.6±2.2	5.22±0.82	±0.02
		RS + urea	353	26.8±2.2	5.13±0.86	

(4.61±0.06 to 4.95±0.23 × 10^{-5} cm/s, $P > 0.05$, paired t) or on the net absorption of water (− 0.05±0.08, control to 0.24±0.11 nl/mm min, experimental, $P > 0.05$, paired t).

Reflection coefficient of urea. The reflection coefficient of urea was measured by the equal concentration technique when the net induced water flow was measured with 50 mosmol/liter raffinose added to the bath. Short segments of tubules and rapid perfusion rates were used in these experiments. The calculated reflection coefficient for urea in SF proximal straight tubules was 1.0±0.03 and in JM proximal straight tubules was 0.97±0.02 (Table V). Neither of these numbers is statistically ($P > 0.05$) different from unity.

DISCUSSION

It is now generally accepted that transport of urea occurs by passive transport processes both in the proximal convoluted tubule and the descending limb of Henle. However, micropuncture studies have shown that more urea exists in fluid obtained from the bend of the loop of Henle than can be accounted for by filtration (3–7). This suggests that urea enters the nephron somewhere between the end of the proximal convoluted tubule and the end of the descending limb of Henle. Only two general types of epithelia exist between these two points: the pars recta (straight segment of the proximal tubule) and the descending limb of Henle. Direct in vitro studies have suggested that the descending limb of Henle is relatively impermeable to urea (8); therefore, it is likely that significant recirculation or re-entry of urea occurs across the pars recta. No previous studies have examined the role of the pars recta with respect to the transport of urea. In view of these evaluations, the present studies were designed to examine the mechanism of urea transport by the pars recta.

One way to determine whether active urea secretion exists in the pars recta is to measure simultaneous bidirectional urea fluxes. This approach is not feasible since only one isotope of urea is practical to count by currently available technology, [^{14}C]urea. Another approach is to measure the unidirectional fluxes sequentially in the same tubule; i.e., first calculate the lumen-to-bath urea flux from the disappearance rate of [^{14}C]-urea added to the perfusate, then change the perfusate, then calculate the bath-to-lumen urea flux from the appearance rate of [^{14}C]urea added to the bath. Initially, a large series of such studies were conducted in which the unidirectional fluxes were measured sequentially, with the same tubule as its own control. The results of these measurements indicate that in both the SF and JM straight segments, the influx of urea is approximately twice as great as the efflux of urea. The inequality of the bath-to-lumen as compared to lumen-to-bath flux suggests net secretion of urea.

If active secretion of urea exists, then urea concentration of the luminal fluid should rise above the concentration in the bathing and perfusion fluid as the fluid courses through the pars recta. Ideally, these experiments should be designed so that the tubules are perfused slowly, with perfusion fluids and bathing fluids with the same concentration of urea, and then the concentrations of urea in the perfusate, bath, and collected fluid are measured by the same ultra-micro chemical methods. We have been unable to develop a reliable and a reproducible method for the measurement of urea in nanoliter-size samples. Therefore, we used a technique in which equivalent counts of [^{14}C]urea were added to the perfusion fluid and the bath. Since the concentration of urea (1 mM) was the same in the perfusate and the bath, a rise in the counts of ^{14}C per nanoliter of collected fluid would represent secretion or concentration of counts due to net fluid reabsorption. Therefore, a disproportionate rise in [^{14}C]urea counts with respect to ^{125}I volume marker counts would represent urea secretion. In fact, this was found, as demonstrated by the results in Fig. 1. The rise in counts is small but statistically significant ($P < 0.001$).

Thus, active secretion of urea is strongly suggested by the inequality of unidirectional urea fluxes and by the net secretion of urea against a concentration gradient. If active secretion of urea exists, then its influx

component should be decreased by hypothermia and by some transport inhibitors.

The interpretation of temperature effects noted in the present studies is not straightforward. It is known that temperature affects both passive and active transport processes. Temperature can affect transport as a consequence of at least two factors: an effect expressed through free diffusion and an effect expressed through activation energy of membrane permeation. The information concerning temperature dependence of urea permeation across biological membranes is limited. Galey et al. (17) have noted that the temperature coefficient of diffusion, Q_{10}, of urea across the red blood cell membrane is 1.67. Since urea is passively transported across the red blood cell membrane, a Q_{10} of approximately 1.67 (or a rise in permeability coefficient by a factor of 1.67 when the temperature is elevated by 10°C) establishes precedence for a Q_{10} associated with nonactive transport processes for urea. Wright (personal communication) has determined a Q_{10} of passive urea permeation across the toad bladder of 1.3. Again, the interpretation of these findings is complicated since urea is, in part, transported across the toad bladder by a carrier-mediated process. In our experiment, the temperature was lowered from 37°C to 28°C, close to 10°C. The lumen-to-bath flux decreased by 1.6. A value of 1.6 is consistent with a nonactive process. On the other hand, the active secretory component of urea transport (bath-to-lumen flux minus the lumen-to-bath flux) has a Q_{10} of about 2.0. Thus it is interesting that the effects of temperature on the unidirectional fluxes are asymmetrical. The greater inhibitory effect of temperature lowering on the influx component of urea transport supports the concept that some type of carrier-mediated mechanism is involved in the influx of urea from the bath to the lumen of the pars recta.

A purely passive unidirectional flux (a flux describable by Fick equations) should not be inhibited by transport inhibitors, whereas the active component of unidirectional flux may be influenced by an appropriate inhibitor. The effects of three different transport inhibitors were tested. These included cyanide, phloretin, and probenecid. Bidirectional effects on urea transport of cyanide and phloretin were tested, while the effect of probenecid was tested only on the bath-to-lumen flux. Neither cyanide nor phloretin had any effect on the lumen-to-bath flux of urea, whereas both significantly decreased the bath-to-lumen flux of urea. It is of interest that the bath-to-lumen flux was decreased to approximately the same level as the lumen-to-bath flux, suggesting that only the active component was influenced by these two inhibitors. Probenecid had no effect on the bath-to-lumen flux, suggesting that urea transport does not occur by the same pump responsible for transport of organic acids across the proximal straight segments (18).

Though no previous suggestions of urea secretion by the mammalian pars recta have been advanced, the suggestions of active urea transport by the kidney is not without precedent. Clapp (19) and Lassiter et al. (20) have noted that urea reabsorption occurs from the collecting duct against a concentrating gradient in a protein-depleted animal. These findings would suggest that under certain circumstances the collecting duct is capable of active urea reabsorption. No mammalian renal studies have suggested active urea secretion; however, active urea secretion has been demonstrated by frog kidney (21), and carrier-mediated urea transport inhibited by phloretin has been shown in the toad bladder (22, 23). However, phloretin has no effect on transpithelial water permeation across the toad bladder (22). In addition, urea permeability is inhibited by phloretin across the gall bladder (23).

The finding of urea secretion by the pars recta does seem to answer an apparent controversy between published in vitro microperfusion and in vivo micropuncture data. We have previously demonstrated by in vitro microperfusion that the descending limb of Henle is relatively impermeable to urea (8). We reasoned that if urea recirculation takes place, the descending limb of Henle does not participate in this process to any major extent. On the other hand, micropuncture studies of antidiuretic rats have revealed urea concentration at the bend of the loop of Henle in the 300–350 mM range (3–7). We initially felt that these high values of urea are not consistent with a rise in urea concentration purely by water abstraction, but that influx of greater amounts of urea had to be postulated than would be predicted by our previously published urea permeability coefficient of 1.5×10^{-5} cm/s (8). However, Pennell, et al. (7) used a differential mass balance equation and have shown that it is possible by inward diffusion to raise the urea concentration to higher values than we predicted by our measured permeability coefficients, though the levels that they predicted, 106 mM urea in a 5.0-mm descending limb, fall short of their own experimentally determined concentration of urea. In their calculations, it was assumed that urea entering the descending limb of Henle is 0 mosmol/liter. Our present studies suggest that urea concentration is much higher than zero by two different mechanisms: (*a*) passive inward diffusion, and (*b*) active inward secretion. Since the pars recta courses through the outer strip of the outer medulla, and since the urea concentration in the medullary interstitium is higher than in the cortical interstitium, it then follows that there is a favorable inward concentration gradient for urea diffusion. The second mechanism of urea influx would be by active

urea secretion. It is impossible to make firm quantitative estimations as to how much either of these mechanisms contribute. The reason is that the urea concentration of the outer medullary interstitium is variable and highly dependent on the state of hydration of the animal. Also, since the kidney possesses loops of differing lengths, it is not possible to estimate which fraction of the urea concentration at the bend of the loop is the consequence of urea influx across the pars recta. Nevertheless, these studies do show that one source of the high urea concentration is across the pars recta, and together with the recent mathematical approach of Pennell et al. (7) the present findings provide an explanation for the relatively high concentrations of urea at the bend of the loop.

We have previously proposed a model of countercurrent multiplication system, in which high concentrations of papillary urea abstract water out of the thin descending limb of Henle (1). It was postulated that the water abstraction resulted in osmotic equilibration of the thin descending limb fluid and, therefore, generated a NaCl concentration gradient for passive diffusion of NaCl down its concentration gradient. To the extent that urea exists in the descending limb, the magnitude of the NaCl gradient between the loop fluid and adjacent interstitium would be reduced (1). Thus, the maximal amount of NaCl available for passive diffusion out of the thin ascending limb would be reduced (1). However, as discussed in our previous paper (1), the postulated amount of urea entry, consistent with the previous in vivo measurements (3–7), does not negate the operation of the passive equilibration model. Admittedly, the entry of urea into the pars recta would decrease the ability to generate as concentrated a urine as could be generated without the entry of any urea into the descending loop structures. However, the passive model does require that high papillary urea concentrations are maintained to form maximally concentrated urea. Thus, urea must be trapped or recirculated within the medulla. Our previous studies on the thin ascending limb of Henle (14) show that this segment is moderately permeable to urea and therefore would provide a significant pathway for urea recirculation. The present studies would suggest that the pars recta provides another possible mechanism by which urea within the medulla can be conserved without allowing it to "wash out" into the systemic circulation.

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