Micropuncture Study of Net Transtubular Movement of Urea and Water in Rats Expanded with Isotonic Saline[1] (34443)

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Previous micropuncture experiments in rats have demonstrated that in antidiuresis a significant amount of the urea reabsorbed from the collecting ducts is returned to tubular fluid flowing through the loops of Henle (1, 2). In contrast, no recirculation of urea from the collecting ducts to Henle's loops was found in rats made diuretic by intravenous infusion of hypertonic saline or mannitol solutions (2, 3). In nondiuretic hamsters and presumably in rats the concentration of urea is much higher in collecting duct than in loop fluid. An intermediate concentration is present in vasa recta plasma (4). We have interpreted these and other data to indicate that the transtubular movement of urea is largely passive and that during diuresis the reduced transtubular urea gradient and decreased contact time result in reabsorption of a lower total amount as well as a lower fraction of filtered urea across the collecting duct epithelium.

A similar mechanism was expected to operate under other diuretic conditions since urea excretion is related to urine flow (5, 6). Recently, however, Roch-Ramel and associates reported [(7) and personal communication] that the amount of urea entering the distal convolution was much greater than that present at the end of the proximal convolution in rats loaded with isotonic saline, indicating addition of urea to loop fluid under this diuretic condition. Since this finding was unexpected, we have studied transtubular urea movement in rats made diuretic by infusion of isotonic saline solution. No evidence of addition of urea to loop fluid was present in our experiments.

Methods. Fifteen male Wistar rats, weighing 275–390 g, were anesthetized with intraperitoneal sodium pentobarbital, 35 mg/kg of body weight. The animals were infused with a volume of 0.85% saline equivalent to 10% of their body weight at the rate of 0.5 ml/min. A prime of 70 μCi of ^{3}H-inulin and 30 μCi of ^{14}C-urea was then administered intravenously, followed by a sustaining infusion of 2.0 μCi/min of ^{3}H-inulin and 0.3 μCi/min of ^{14}C-urea in 0.195 ml/min of 0.85% saline. After an equilibration period of 45 min or longer, tubular fluid samples were collected from the left kidney by micropuncture (8). Tubular transit times were measured following intravenous injection of 0.05–0.10 ml of a 5% solution of lissamine green. Transit time was recorded as the elapsed time from the green flush of the kidney to the arrival of the dye front in the last visible convolution of a proximal tubule or in the first visible convolution of a distal tubule. Some of the puncture sites were also located by microdissection at the end of the experiment. Blood samples were collected from the right carotid artery at the middle of each clearance period. Urine was collected from the catheterized left ureter and its rate of flow was doubled for clearance calculations. In all samples ^{3}H and ^{14}C activities were measured simultaneously in a liquid scintillation spectrometer by a method similar to that which has been

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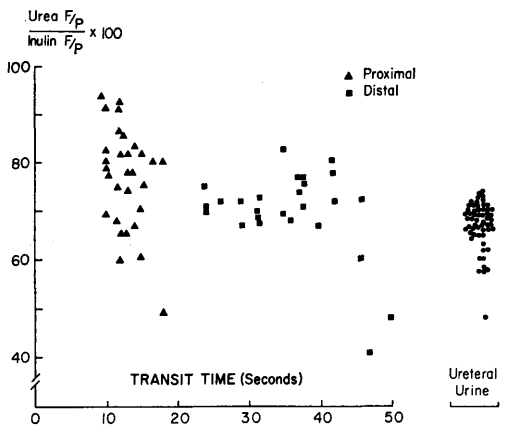

FIG. 1. Relation between tubular transit time and percentage of filtered urea in tubular fluid and urine of 15 rats expanded with isotonic saline solution.

previously described (3). The concentration of ^{14}C-urea in blood was quite constant and did not change by more than 10% in successive samples, except in one instance when glomerular filtration rate (GFR) changed markedly. Osmolality of plasma and tubular fluid was determined microcryoscopically by the method of Ramsay and Brown (9).

Results. The results of individual experiments are presented in Table I and Fig. 1. Urine flow averaged 49.0 ± 28.6[5] (N = 56) μl/min 100 g of body weight. The GFR was increased above nondiuretic values and averaged 1029 ± 199 μl/min 100 g, comparable to that previously observed under similar experimental conditions (10). The mean inulin and urea U/P ratios were 28.3 ± 15.6 and 18.5 ± 9.2 (N = 57), respectively. The mean urea/inulin ratio was 0.67 ± 0.05 indicating that an amount of urea equivalent to 67% of that filtered was excreted in the urine.

In 15 animals, fluid collected from the last visible convolutions of proximal tubules had an average inulin F/P of 1.85 ± 053 (N = 32) and an average urea F/P of 1.39 ± 0.28 (N = 32). The mean urea/inulin ratio was 0.77 ± 0.11, indicating that 55% of the filtered water and 77% of the filtered urea remained within the tubules. Transit time to these collection sites averaged 12.8 ± 2.3 sec. (N = 30).

[5] Mean ± SD.

In nine rats the transit time to early distal convolutions averaged 35.8 ± 7.2 sec (N = 26); osmolality F/P, 0.69 ± 0.15 (N = 16); inulin F/P, 6.67 ± 4.14 (N = 28); and urea F/P, 4.67 ± 2.75 (N = 28). The mean urea/inulin ratio was 0.71 ± 0.09. Thus an amount of urea equal on the average to 71% of that filtered was present in these early distal convolutions. Microdissection of six of these tubules demonstrated the puncture site to average 30% of distal length.

Discussion. Net transtubular urea movement in these rats loaded with isotonic saline was as predicted from previous experiments in rats loaded with hypertonic saline (3). On the average there was 77% as much urea as filtered present at the end of the convoluted portion of the proximal tubule, 71% in the early distal convolution and 67% in the ureteral urine. Thus, there was no evidence of addition of urea to fluid flowing through the loop of Henle. The possibility cannot be excluded, however, of the addition of a small amount of urea to loop fluid. Although we consider it unlikely, if urea and water reabsorption were to proceed at the same rate in the inaccessible proximal pars recta as in the pars convoluta, the amount of urea entering the thin descending limb of Henle's loop would be less than that found in the early distal convolution. A small amount of the urea reabsorbed from the collecting ducts might diffuse into the thin descending limb of the loop and be recirculated, but it seems unlikely that the trapping mechanism would be so efficient as to trap all of the urea reabsorbed from the collecting ducts under these conditions (about 5% of filtered load). We do not know the cause(s) for the disagreement between our results and those of Roch-Ramel and associates, who reported that the amount of urea entering the distal convolutions greatly exceeded the amount filtered (7).

The percentage of filtered urea and water remaining at various points along the nephron is plotted as a function of transit time in Fig. 2. As under other conditions (Table II), the major net loss of urea from the tubule occurred in the proximal tubule,

TABLE I. Tubular Fluid and Urine Analyses in 15 Rats Expanded with Isotonic Saline.

Ureteral urine			Proximal tubule			Distal convolution			
μl/min 100 g of body wt[a]	U/P		TT[b] (sec)	F/P		TT[b] (sec)	F/P		
	Inulin	Urea		Inulin	Urea		Inulin	Urea	(osm.)
				390 g rat					
38.5	28.6	18.9	15.0	1.88	1.54	—	—	—	—
38.0	25.2	17.6	15.0	2.03	1.40	—	—	—	—
				350 g rat					
30.6	27.9	19.8	18.0	2.06	1.00	—	—	—	—
46.2	18.1	13.2	18.0	1.91	1.54	—	—	—	—
37.3	27.1	18.4	14.0	1.28	1.07	—	—	—	—
				290 g rat					
25.9	33.9	23.6	10.0	1.13	0.94	—	—	—	—
34.5	25.5	18.9	13.0	1.52	1.12	—	—	—	—
				350 g rat					
15.9	46.2	31.2	12.5	2.58	1.70	—	—	—	—
14.8	53.6	35.7	12.0	1.54	1.42	—	—	—	—
16.3	55.1	34.2	12.0	2.21	1.65	—	—	—	—
9.4	76.6	46.1	—	—	—	42.0	3.06	2.46	—
				315 g rat					
29.1	37.3	21.8	11.5	2.44	1.66	—	—	—	—
15.2	53.7	30.8	—	1.64	1.42	—	—	—	—
—	46.3	26.8	13.0	1.44	1.18	—	—	—	—
				350 g rat					
14.7	65.4	37.5	15.5	2.27	1.73	—	—	—	—
18.6	53.7	33.9	10.0	2.01	1.59	—	—	—	—
16.9	55.9	34.7	12.0	1.41	1.22	—	—	—	—
15.9	65.8	39.6	13.0	1.26	0.98	—	—	—	—
				370 g rat					
—	—	—	—	—	—	46.0	5.45	3.99	—
44.2	24.0	17.2	—	—	—	45.0	3.35	2.03	—
41.4	24.4	16.2	12.0	1.42	1.32	—	—	—	—
32.0	25.2	17.4	—	—	—	46.0	3.83	1.57	—
				360 g rat					
50.5	22.0	14.4	9.5	1.25	1.18	—	—	—	—
—	—	—	—	—	—	24.0	5.43	4.05	—
48.3	22.9	14.9	—	—	—	36.0	3.53	2.39	—
44.0	25.9	17.7	—	—	—	26.0	2.70	1.94	—
19.3	37.7	26.6	12.0	2.01	1.65	—	—	—	—
24.3	35.9	24.8	10.0	1.43	1.16	—	—	—	—
24.8	35.4	25.2	—	—	—	—	5.60	4.52	—

[a] Twice the rate of flow from the left kidney.

[b] TT (transit time) was recorded as the elapsed time from the green flush of the kidney to the arrival of the dye front at the puncture site.

TABLE I (continued).

Ureteral urine			Proximal tubule			Distal convolution			
μl/min 100 g of body wt[a]	U/P		TT[b] (sec)	F/P		TT[b] (sec)	F/P		
	Inulin	Urea		Inulin	Urea		Inulin	Urea	(osm.)
				370 g rat					
56.3	30.7	14.9	—	—	—	50.0	17.29	8.25	—
50.6	21.9	14.7	—	—	—	37.0	12.31	9.45	—
47.0	23.9	15.8	15.0	3.65	2.24	—	—	—	—
48.0	22.5	15.1	—	—	—	42.0	8.92	6.93	—
49.0	21.7	14.9	—	—	—	38.0	12.92	9.93	—
41.4	29.5	19.6	12.0	1.48	1.27	—	—	—	—
38.3	30.8	20.0	—	—	—	31.0	17.66	12.12	—
				275 g rat					
40.9	27.8	19.5	—	—	—	29.0	3.76	2.53	0.42
78.7	16.9	11.6	—	—	—	29.0	7.09	5.12	0.77
115.6	12.3	8.5	10.0	2.02	1.40	—	—	—	—
102.2	14.7	10.0	—	—	—	24.0	4.65	3.30	0.77
82.2	16.6	11.6	—	—	—	24.0	7.12	4.96	0.77
				330 g rat					
40.1	29.3	20.0	10.5	1.34	1.17	—	—	—	—
42.2	25.0	16.8	10.0	1.42	1.30	—	—	—	—
				360 g rat					
45.3	22.2	15.9	—	—	—	35.0	7.92	6.60	0.89
60.2	16.1	11.7	—	—	—	38.0	7.45	5.30	0.95
42.3	20.8	14.9	—	—	—	39.0	11.55	7.78	0.71
47.3	21.9	14.9	12.0	2.36	1.42	—	—	—	—
				315 g rat					
153.5	8.4	5.7	14.0	1.88	1.47	—	—	—	—
—	—	—	—	—	—	—	3.71	2.72	0.62
34.1	15.9	10.5	—	—	—	37.0	3.67	2.73	0.92
				345 g rat					
69.6	13.7	9.8	—	1.67	1.22	—	—	—	—
69.0	15.3	10.6	—	—	—	42.0	6.77	4.87	0.62
79.7	13.1	9.4	12.5	2.42	1.57	—	—	—	—
78.3	13.1	8.8	—	—	—	35.0	5.18	3.59	0.54
65.7	16.3	11.3	—	—	—	35.0	4.06	2.95	0.51
				350 g rat					
95.2	10.0	7.0	16.8	1.64	1.32	—	—	—	—
84.4	12.9	8.2	—	—	—	32.0	4.09	2.79	0.59
85.7	11.7	8.0	—	—	—	38.0	5.46	4.16	0.62
85.7	12.2	7.9	14.0	2.55	1.70	—	—	—	—
69.4	14.4	10.5	—	—	—	31.0	2.38	1.67	0.64

[a] Twice the rate of flow from the left kidney.

[b] TT (transit time) was recorded as the elapsed time from the green flush of the kidney to the arrival of the dye front at the puncture site.

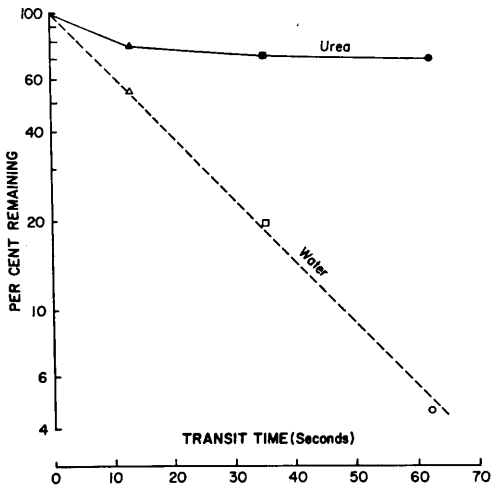

FIG. 2. Relationship between mean transit time and amount of urea and water present expressed as percentage of filtered load in late proximal and early distal fluid and in ureteral urine: (triangular-shaped symbols), determinations on proximal fluid; (square-shaped symbols), distal fluid; and (circular symbols), ureteral urine.

providing further evidence for the relatively high permeability to urea of the proximal epithelium (1, 11, 12). The mean F/P urea of 1.4 at the end of the proximal pars convoluta in these experiments is very similar to that observed in nondiuretic rats (1) and in rats and Psammomys made diuretic by infusion of hypertonic saline solution (3, 13). The relative constancy of this ratio with varying degrees of water reabsorption is consistent with the view that the reabsorption of urea is a function of transtubular urea concentration gradients produced by water reabsorption.

The present observations on water reabsorption are in agreement with previous studies in isotonic saline diuresis and demonstrate decreased fractional and absolute rates of reabsorption in the proximal tubule as a consequence of expansion of body fluids with isotonic saline (10, 14). When plotted as a function of the transit time (Fig. 2), the mean F/P and U/P inulin ratios fall close to a straight line, suggesting an exponential reduction in tubular fluid volume along the entire length of the nephron under the present experimental conditions. Although the observation is interesting, it is difficult to arrive at any conclusions regarding the mechanism of salt and water reabsorption from this relationship because of the large scatter in the early distal and ureteral urine data and the uncertain location of the U/P ratios on the transit time axis. The estimated total tubular transit time of 62 sec was derived from the transit times in these experiments plus the average time for fluid to flow through the remainder of the nephron previously measured by the intratubular microinjection technic in rats with similar rates of urine flow (11). This estimate can be made since loop transit time was the same when measured following intravenous injection of lissamine green (24 sec) or by the intratubular microinjection technic (23 sec).

Summary. Net transtubular movement of urea and water was studied in rats made diuretic by infusion of a volume of isotonic saline solution equivalent to 10% of their body weight. The major site of urea reabsorption was the proximal tubule and there was

TABLE II. Estimates of the Amount of Urea and Water Reaching the Late Proximal Tubule, Early Distal Convolution and Ureter Expressed as Percentage of Filtered Load in Diuretic and Nondiuretic Rats.

Physiological condition	Substance	(%) Late proximal	Early distal	Ureter
Isotonic saline diuresis	Urea	77	71	67
	Water	54	20	4
Hypertonic saline diuresis (3)	Urea	50	60	60
	Water	36	25	4
Nondiuretic (1)	Urea	50	110	10
	Water	36	15	0.1

little urea loss from more distal parts of the nephron. There was no evidence of addition of urea to tubular fluid flowing through the loop of Henle. Throughout the nephron the percentage of filtered water remaining varied exponentially with transit time.

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