Micropuncture Study of Water, Electrolytes, and Urea Movements Along the Loops of Henle in Psammomys

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ABSTRACT The mechanism by which the osmotic pressure increases in tubular fluid along the descending limb of the loop of Henle was examined in *Psammomys* undergoing salt diuresis. In two series of experiments, micropuncture samples were collected either from proximal and distal convolutions at the surface of the cortex, or from loops of Henle and collecting ducts at the surface of the extrarenal part of the papilla. Inulin-^{3}H, urea-^{14}C, Na^+, and K^+ concentrations, as well as osmotic pressure, were determined in all micropuncture samples.

Net movements of water along the descending and ascending limbs of the loop could not be deduced by comparing inulin data obtained from convoluted tubules and from loops of Henle, since there appeared to be a large difference in the filtration rate of the superficial glomeruli (9 nl/min) and the deep ones (21.4 nl/min) under the conditions of these experiments.

The results indicate that no large net movement of water occurred along the loop since *a*) only 23% of the filtrate was reabsorbed along the loop of Henle (including pars recta) of superficial nephrons despite the fact that all these loops reached markedly hypertonic regions; *b*) there was no positive correlation between $(F/P)_{In}$ in early distal samples and the simultaneous osmotic pressure of the urine; *c*) when $(F/P)_{In}$ and $(F/P)_{Osm}$ in loop samples were correlated, the increase in inulin concentration accounted only for 15% of the increase in osmotic pressure. Therefore, 85% of the concentrating process taking place along the descending limb must have resulted from net addition of solutes; this was directly supported by Na^+ and K^+ measurements in the loop samples, which showed that, at the tip of the loops, the Na^+ and K^+ flow rates were correlated to the osmotic pressure. Moreover, since the load of Na^+ urea flow rate in superficial early distal tubules was constant and independent of the urinary osmotic pressure, it is suggested that a medullary recycling of both ions occurred between ascending and descending limbs.

Urea-^{14}C concentration in the loop samples indicates a net addition of urea into the descending limb; the mean and K^+ delivered to the distal superficial tubules was 4.18 times its filtration rate, suggesting a recycling of urea from collecting ducts to Henle's loops. The permeability properties of the wall of the thin descending limb are discussed in relation to the obtained results.

INTRODUCTION

The mechanism which increases the concentration of the tubular fluid flowing through the descending limb of the loop of Henle along the medullary gradient of osmotic pressure has not yet been clearly established. Two processes might be a priori suggested: the net osmotic withdrawal of water, or the net addition of solute (1–5).

Available micropuncture studies are inconclusive. According to Gottschalk and colleagues (6), inulin concentration increases along the descending limb indicating a net osmotic reabsorption of water. On the other hand, net addition of urea into the loop of Henle, in the concentrating kidney, is clearly indicated by proximal and distal micropuncture studies in the rat (7), *Meriones* (8), and *Psammomys* (9). Indirect evidence suggests that a medullary segment of the nephrons is highly permeable to sodium ions (10–13) and chloride ions (14), an observation which was interpreted as a prerequisite for the second mechanism (15); nevertheless, in previous discussions, the first hypothesis was generally considered more likely (2, 3). The present micropuncture studies on a desert rodent were undertaken to ob-

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tain more direct information on the concentrating mechanism in the descending thin limbs. *Psammomys obesus* was chosen because the extrarenal tip of the papilla is accessible to micropuncture, the concentrating ability of the kidney is very high even during moderate salt diuresis, and almost all the nephrons have long loops.

Osmotic pressure, sodium, potassium, urea, and inulin concentrations were measured in samples of tubular fluid from superficial proximal and distal convolutions and tips of loops of Henle. The correlations between tubular fluid composition and osmotic pressure indicate that net addition of solute is the main process responsible for the increase in osmotic pressure in fluid flowing through the descending thin limb.

METHODS

The *Psammomys obesus* were captured in South Algeria, at Beni Abbès. They were kept for months in the laboratory at 26°C and fed with ordinary rat pellets, supplemented with fresh halophile plants (salicornia collected on the French Mediterranean Coast); with this diet, they survived for long periods in the laboratory and even reproduced. Young adult *Psammomys* were (preferentially) selected for cortical micropuncture, since the kidney capsule becomes thick and opaque in older animals.

The experiments were performed on *Psammomys* anesthetized with sodium pentobarbital (Nembutal 4 mg/100 g body weight). The surgical procedure was similar to that described elsewhere for rats (16, 17) and *Psammomys* (9). In the experiments in which loops of Henle were punctured, the wall of the extrarenal pelvis of the left kidney was cut and dissected towards the renal vein to expose the longest possible part of the papillary tip. The papilla was bathed with mineral oil and illuminated with a flexible light guide. In these experiments, the ureteral urine of the right kidney was collected and used for clearance measurements. In experiments in which cortical proximal and distal tubules were micropunctured, clearances were measured with urine from the experimental kidney.

A moderate salt diuresis was induced (0.0375 ml/min, 2.5% NaCl solution intravenously for the five first animals of the papilla group; 0.0375 ml/min, 4% NaCl solution intravenously for all the other animals).

A priming dose of 30 μc urea-^{14}C (The Radiochemical Centre, Amersham, England, 257 μc/mg) and 40 μc inulin-^{3}H (New England Nuclear Corp., Boston, Mass., 237 μc/mg) was given intravenously and followed by a sustaining intravenous infusion of 0.75 μc urea and 4.5 μc inulin/min in the hypertonic NaCl solution.

After 1 hr of equilibration period (9), 30-min ureteral urine samples were collected into glass tubes; femoral arterial blood samples (about 50 μl) were collected in the middle of each period.

The experiments lasted 3–4 hr; the micropuncture samples were collected during the clearance periods.

In the experiments on the cortex, oil-filled glass micropipettes with a tip of 5–7 μ external diameter were used; the rate of collection was controlled by microscopic observation of an oil dropplet preinjected into the tubular lumen. After a 3–5 min collection period, the punctured tubule was filled with liquid neoprene and the puncture site marked with India ink. Six to 12 proximal and distal samples of tubular fluid were collected in each experiment.

The puncture site was located by microdissection (18). In the experiments on the papilla, oil-filled glass micropipettes with a longer tip and smaller external diameter (4 μ) were used. The rate of collection of loop fluid was

TABLE I

*Plasma Composition and Urinary Excretion of Water and Solutes during Micropuncture Experiments in the Psammomys Undergoing Salt Diuresis**

				Urea	Na^+	K^+	Osm
				mmoles/liter	*mEq/liter*	*mEq/liter*	*mOsm/liter*
Plasma	First series			4.9 ±1.0 (7)	163 ±1 (40)	4.45 ±0.09 (40)	340 ±3 (40)
	Second series			7.5 ±0.7 (10)	170 ±2 (36)	4.56 ±0.13 (36)	351 ±5 (37)
			C_{In}	$(U/P)_{In}$	$(U/P)_{Urea}$	$(U/P)_{Na}$	$(U/P)_{K}$	$(U/P)_{Osm}$
			ml/min					
Urine	First series		0.697 ±0.025 (40)	126 ±10 (40)	25.7 ±0.8 (40)	6.22 ±0.35 (39)	9.88 ±0.88 (39)	7.68 ±0.41 (40)
Urine	Second series	Experimental kidney		108 ±10 (29)	18.5 ±1.5 (29)	4.49 ±0.34 (24)	11.15 ±1.94 (24)	5.46 ±0.33 (29)
		Control kidney	0.591 ±0.046 (37)	119 ±10 (37)	18.9 ±1.8 (36)	5.16 ±0.36 (37)	12.93 ±1.96 (37)	6.78 ±0.40 (37)

* All data are mean values ± SE; in parentheses the number of measurements; U/P = urine-to-plasma concentration ratio; C_{In} = inulin-^{3}H clearance (ml/min per kidney). The first series refers to the animals in which micropunctures were performed on the surface of the cortex. The second series refers to the animals in which micropunctures were performed at the tip of the extrarenal papilla; urine samples of the experimental kidney were collected directly at the tip of the dissected papilla, and urine samples of the control kidney were collected from the ureter.

controlled as in cortical experiments, the direction of the oil movement indicating whether a descending or an ascending limb had been punctured. The site of puncture was determined by measuring the distance to the tip of the papilla. The loop collection had a duration ranging from 1 to 7 min. In these experiments, micropuncture of collecting ducts at the same level of the papilla was also performed, and samples of final urine flowing out of the papilla were periodically collected.

The following determinations were done on all samples: osmotic pressure by the method of Ramsay and Brown (19); simultaneous measurement of the Na^+ and K^+ concentrations by flame photometry (8, 20); urea-^{14}C and inulin-^{3}H were measured by introducing a measured volume (9, 17) of collected fluid into 10 ml scintillating solution [according to Bray (21), modified by Lassiter, Mylle, and Gottschalk (22)] in plastic vials and counting in a liquid scintillation spectrometer.[1] The urea-^{12}C concentration was determined by photocolorimetry[2] on the urine samples and on a larger plasma sample collected at the end of the experiments.

[1] Packard Instrument Co., Downers Grove, Ill.

[2] AutoAnalyzer, Technicon Corporation, Ardsley, N. Y. Diacetyl monoxime method.

RESULTS

Infusion of hypertonic NaCl during micropuncture experiments appears to increase the concentrating ability of the *Psammomys* kidney since mean C_{In}, urine flow rate ($\dot{v}$), and urinary osmolality (U_{osm}) were markedly greater than previously observed in experiments done without such a perfusion (9) ($C_{In} = 0.65$ ml/min kidney compared with 0.25 ml/min kidney, $U_{osm} = 2300$ mOsm/liter compared with 1900 mOsm/liter).

Despite large variations from animal to animal, the osmotic pressure of the urine (range from 800 mOsm/liter up to 4200 mOsm/liter) was generally high. In papilla experiments, there was a small difference in the mean values of the osmotic pressure of the urine from the two kidneys. This difference could have resulted from some impairment of the concentrating ability of the experimental kidney due to the surgical procedure and to the exposure of the papilla. Although significant, the differ-

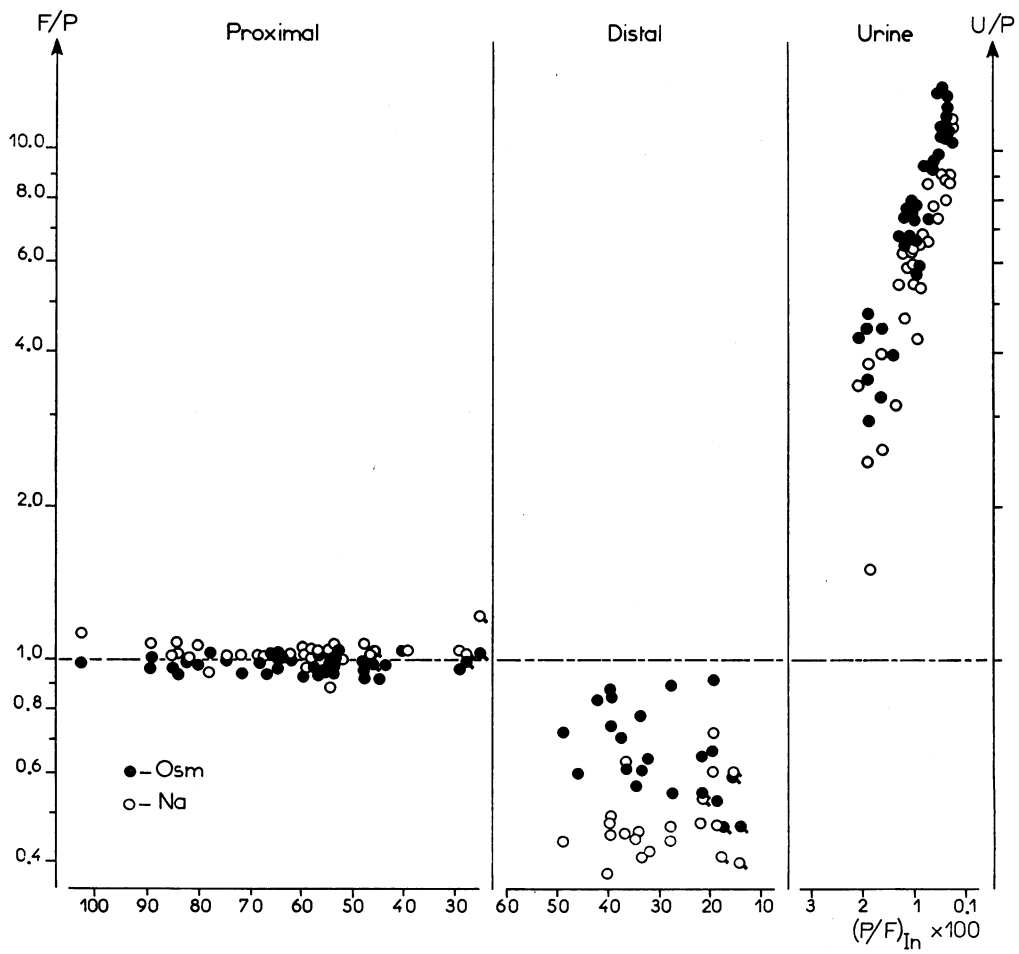

FIGURE 1 Tubular fluid over plasma ratios (F/P) for osmotic pressure and sodium concentration plotted against the per cent of glomerular filtrate remaining at the collection site. Tailed circles refer to the animal which had higher $(F/P)_{In}$ ratios (see text).

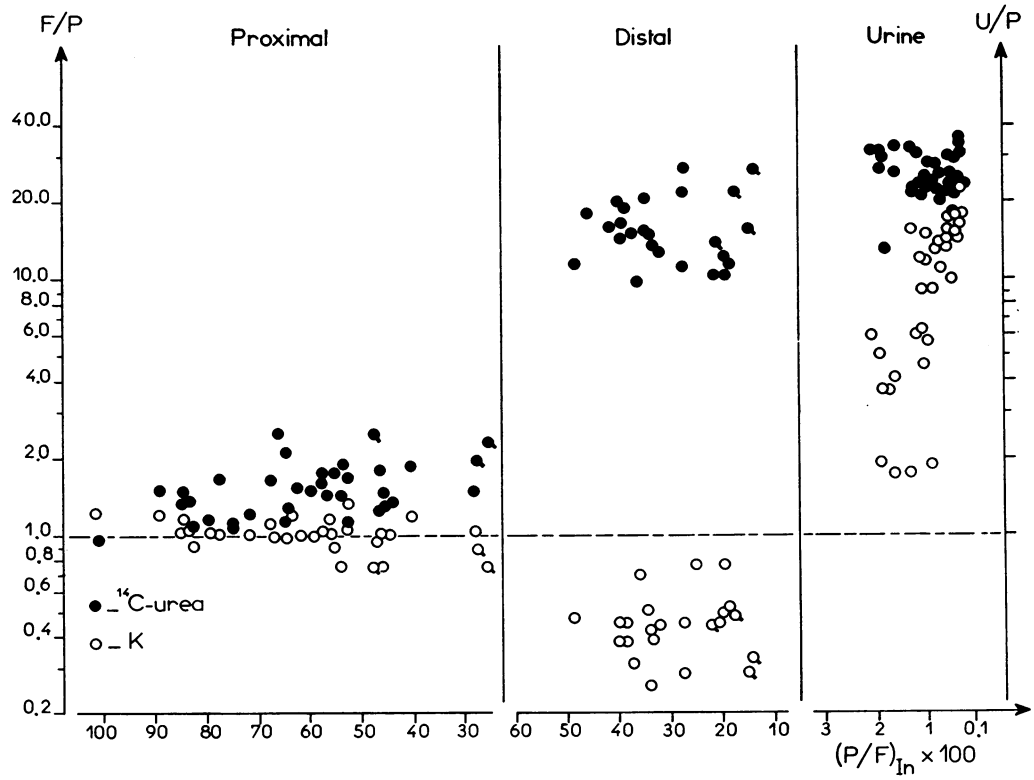

FIGURE 2 Tubular fluid over plasma ratios (F/P) for urea-^{14}C and potassium concentrations plotted against the per cent of glomerular filtrate remaining at the collection site. Tailed circles refer to the animal which had higher $(F/P)_{In}$ ratios (see text).

ence is not large: $((U/P)_{Osm} = 5.46$ on experimental side and 6.78 on the control side).

The general conditions for experiments on papilla and cortex were similar (Table I). The small differences observed in the mean values of the inulin clearance and urinary flow rate between the two experimental series may have resulted from the fact that an homogeneous group of young animals was used for the experiments on the cortex, whereas the animals with exposed papilla received either 2.5% or 4% NaCl infusions and varied more in weight and age.

Cortical micropuncture data. The results of 35 proximal and 24 distal tubular fluid samples collected in seven different *Psammomys* are presented in Figs. 1–3. In these figures, the (F/P) ratios for osmotic pressure (Fig. 1), sodium (Fig. 1), potassium (Fig. 2), and urea (Fig. 2) concentrations have been plotted against the per cent of glomerular filtration rate (GFR) remaining at the puncture site $((P/F)_{In} \times 100)$. This mode of presentation was chosen since only 12 of the 35 proximal micropunctures were accurately located by microdissection. Furthermore, in this series of proximal samples, both representations appear to be roughly equivalent, since for the 12 located micropunctures, there is a good linear correlation ($r = 0.81$, $P < 0.01$) between $(F/P)_{In}$ and the per cent of the tubular length. The $(F/P)_{In}$ ratio appears to reach a mean value of 2 at the end of the accessible part of the proximal tubule. One animal had higher $(F/P)_{In}$ ratios both in proximal and distal samples, for unknown reasons. The data obtained in this animal have been distinguished in the figures. As previously observed (9), the low fractional water reabsorption along the *Psammomys* proximal tubule can be correlated with the short pars convoluta of superficial nephrons [mean 2.1 mm compared to 5.4 mm for the rat (23)]. Assuming water is reabsorbed at the same rate in the pars recta (2.0 mm) as in the punctured segment, extrapolation of the regression line to the end of the proximal tubule suggests an $(F/P)_{In}$ ratio of 3.8 at the beginning of the thin limb. Proximal tubular fluid was iso-osmotic to plasma and had sodium and potassium concentrations nearly equal to those of the plasma, despite a somewhat larger range of variation in the potassium measurements. Urea concentration increased along the proximal tubule and the ratio $(F/P)_{urea}/(F/P)_{In} = 0.7$ at the end of the accessible part indicates that about 30% of the filtered urea was reabsorbed along the pars convoluta.

TABLE II

*Composition of Fluid from Proximal and Distal Tubules Micropunctured at the Surface of Psammomys Kidneys Undergoing Salt Diuresis**

	Proximal	Distal
GFR, *nl/min*	8.4 ±0.62 (34)	9.6 ±1.0 (22)
$(F/P)_{In}$	1.84 ±0.12 (35)	3.71 ±0.30 (24)
$(F/P)_{Urea}$	1.55 ±0.07 (35)	15.5 ±0.93 (24)
$(F/P)_{Na}$	1.03 ±0.01 (32)	0.49 ±0.02 (22)
$(F/P)_{K}$	1.01 ±0.03 (32)	0.44 ±0.03 (22)
$(F/P)_{Osm}$	0.98 ±0.01 (35)	0.67 ±0.03 (22)

* All values are mean values ± SE; in parentheses, the number of determinations. GFR = glomerular filtration rate per nephron; for each sample, GFR was calculated from the rate of fluid collection ($\dot{v}$) and inulin-^{3}H concentration by the equation GFR = $\dot{v} \times (F/P)_{In}$; (F/P) = tubular fluid-to-plasma concentration ratio.

Distal samples were obtained from punctures at between 20% and 50% of the distance separating the macula densa and the first branching with a collecting duct; most of them were, in fact, very early distal samples. The $(F/P)_{In}$ ratio did not correlate with the position of the puncture sites along the distal tubule, and all distal samples had an equally low osmotic pressure and potassium concentration (Figs. 1 and 2).

Table II shows the mean value of the early distal tubular fluid composition. The hypo-osmolality, $(F/P)_{Osm} = 0.67$, almost entirely resulted from the net reabsorption of salt in excess of water along the ascending limb, $(F/P)_{Na} = 0.49$. Potassium concentration was equally low, $(F/P)_{K} = 0.44$, and the difference in osmotic pressure and salt concentration was accounted for by the high distal urea concentration, $(F/P)_{urea} = 15.5$.

There must have been a large addition of urea to the tubular fluid along the loop of Henle, since the mean urea over inulin clearance ratio equaled 0.7 at the end of the proximal pars convoluta and 4.2 in the early distal tubule. This means that urea entered into the loop at approximately four times the rate of urea filtration at the glomerulus.

The mean value of the $(F/P)_{In}$ ratio in early distal samples was very low (3.7) and almost identical to the value calculated for the end of the pars recta by extrapolation of the proximal micropuncture data. Thus, the net reabsorption of water which takes place along the loops of the superficial nephrons of the *Psammomys* appears to be very low despite the fact that these loops enter the internal medulla and therefore reach areas of high osmotic pressure (24). Moreover, inulin concentration in the distal samples was not correlated to the simultaneous osmotic pressure of the urine (Fig. 3), even in these distal samples collected when the excreted urine had an osmotic pressure as high as 4 Osm/liter.

These obsevations indicate either that the increase in concentration of the tubular fluid along the descending limb almost entirely resulted from the net addition of solutes (urea and NaCl) or that a net water absorption along this segment was balanced by a net addition of water along the ascending limb.

Papillary micropuncture data. The results for 46 samples of fluid from loops of Henle and 13 samples from collecting ducts in 13 different *Psammomys* are presented in Table III. In Fig. 4, the osmotic pressure calculated

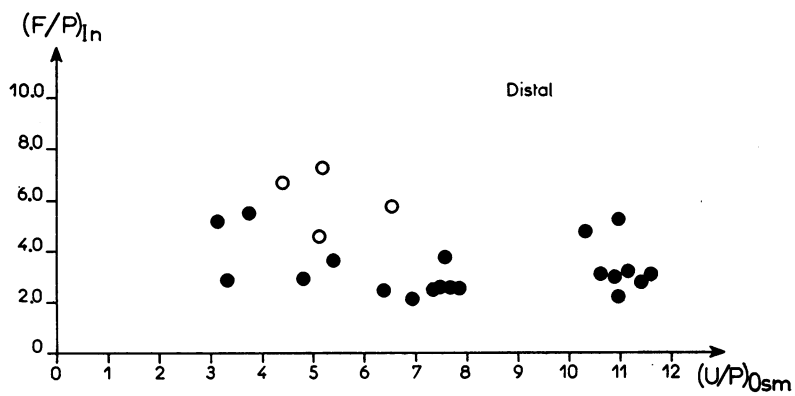

FIGURE 3 Lack of positive correlation between (F/P) ratios for inulin-^{3}H in early distal fluid samples and simultaneous (U/P) ratios for osmotic pressure in the ureteral urine. Open circles refer to the animal which had higher $(F/P)_{In}$ ratios (see text).

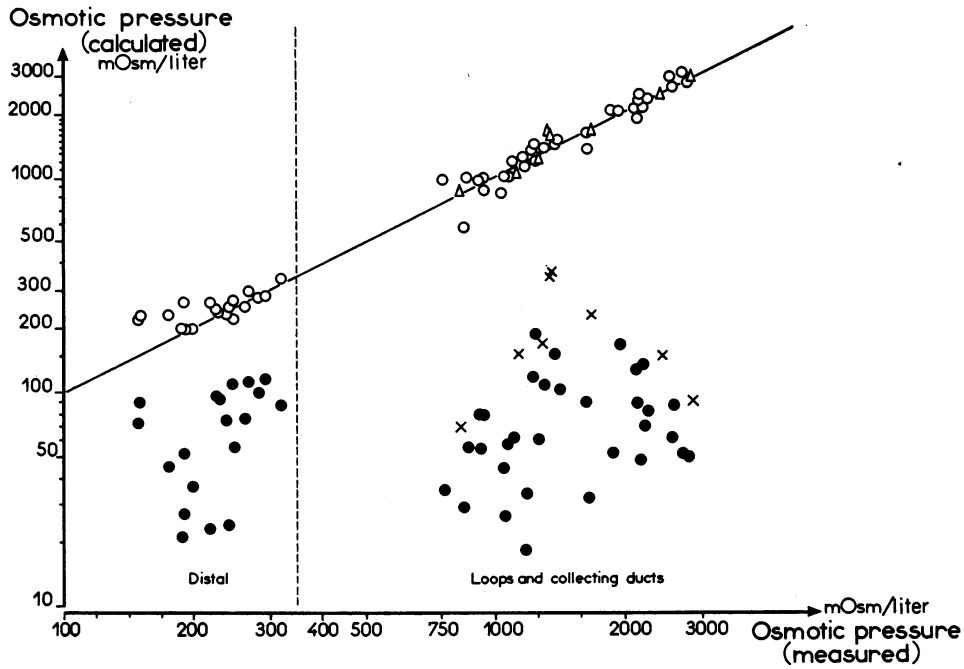

FIGURE 4 Correlation between calculated and measured osmotic pressure for distal and papillary micropuncture samples. The dashed line indicates the mean value of the osmotic pressure measured in the plasma. **Distal** refers to the values measured in the distal micropuncture samples of the cortical series of experiments. *Ordinate:* Open symbols: indicate the calculated osmotic pressure = sum of urea + 2($Na^+ + K^+$) concentrations; circles = tubular fluid, triangles = collecting duct fluid. Other symbols: indicate the osmotic pressure due to urea alone: solid circles = tubular fluid, crosses = collecting duct fluid. Urea concentration was calculated by dividing the urea-^{14}C concentration by the specific activity of the urea in the urine. *Abscissa:* The measured osmotic pressure of the same samples. Double logarithmic scale; most of the data fall near the line of identical values (solid line).

as the sum 2(Na + K) + urea concentrations has been plotted against the measured osmotic pressure. The figure shows that the larger part of the osmotic pressure of the loop fluid can be attributed to sodium ions and corresponding anions.

Nephrons punctured at the tip of the papilla had a much higher calculated mean glomerular filtration rate (21 nl/min) than those punctured at the surface of the cortex (9 nl/min). Although there is a large scatter in the data, this difference is highly significant ($t = 7.6$, $P < 0.001$). The probable origin of this discrepancy, which also accounts for a higher $(F/P)_{In}$ ratio in the loop than in the distal samples, will be analyzed in the discussion. Puncture sites in the ascending and descending limbs of the loops as well as in the collecting ducts were all relatively close to the tip of the papilla (0.5–2.7 mm) as compared to the total length of the medulla, which measures approximately 12 mm in this species. One observes a large range of variation in the osmotic pressure from animal to animal: $(F/P)_{Osm}$ varies from 2.2 to 7.8; but, for a given animal and at a given level, there was no detectable difference in the osmotic pressure of the fluid collected successively from loops and collecting ducts, although the osmotic pressure of the urine collected at the tip of the papilla at the same time was always definitely higher (Table III).

There was no difference in the qualitative composition of the fluid samples in the descending and ascending limbs of the loops, whereas in the collecting ducts, the concentration of urea and potassium (in most instances) was higher [(F/P collecting duct/F/P thin limb) for samples taken successively at the same level of the papilla: osmotic pressure = 0.94; sodium = 0.92; potassium = 1.44; urea = 2.08; and inulin = 6.03]. (Table III).

(F/P) ratios for K as well as Na and urea in loop fluid were always much larger than unity.

Since all loop samples were collected at nearly the same level close to the tip of the papilla and since there was no detectable difference between the composition of the ascending and descending thin limb fluid in these experiments, we shall consider that all the loop data apply to tubular fluid flowing at the tip of the longest loops of Henle. The plot of $(F/P)_{In}$ ratio against (F/P)

TABLE III

Composition of Fluid from Loops of Henle and Collecting Ducts Micropunctured at the Surface of the Extrarenal Papilla in Psammomys Undergoing Salt Diuresis

Experiments*		Puncture Site‡	GFR	$(F/P)_{In}$	$(F/P)_{Osm}$	$(F/P)_{Na}$	$(F/P)_{K}$	$(F/P)_{Urea}$
			nl/min					
P2	A1	—	27.4	8.99	6.09	—	—	—
P3	A1	1.0	32.4	10.81	6.67	5.49	—	7.57
	D4	—	26.6	14.01	6.87	6.02	—	11.75
	A5	2.0	23.1	6.73	6.56	5.91	—	11.00
	A7	—	19.4	10.34	5.75	5.36	—	14.48
P4	D1	1.2	6.2	4.88	5.43	5.78	6.65	18.94
	C2	1.2	—	39.76	4.98	5.35	9.93	11.96
	A4	1.6	12.1	4.04	—	—	—	6.66
	C5	1.6	—	26.88	3.46	3.68	12.56	9.34
	A7	0.6	12.6	9.83	2.84	2.85	—	6.61
	D9	—	4.7	6.10	—	—	—	4.45
P5	D1	0.5	10.8	12.03	—	—	—	18.99
	D2	1.5	23.2	7.90	4.69	3.68	2.73	20.96
	A5	1.0	11.2	9.31	—	3.87	4.05	17.77
	C6	1.0	—	61.76	5.80	3.84	4.78	31.77
P6	A1	2.0	15.5	8.97	2.80	2.55	2.67	10.17
	A2	2.0	47.3	5.77	2.80	2.71	3.28	7.02
	A4	1.0	12.6	5.25	3.38	3.23	3.27	7.92
P7	D1	1.4	10.8	8.16	3.63	3.18	1.56	17.01
	A2	1.4	21.1	6.15	3.79	3.16	1.84	9.78
	C3	1.4	—	53.30	3.91	2.93	3.55	33.09
	A5	1.4	5.7	8.20	3.95	3.27	3.20	9.40
	C6	1.4	—	54.33	3.68	2.98	3.40	31.58
	D8	2.4	7.5	4.67	3.00	2.81	4.06	10.81
P8	A1	1.2	34.3	9.63	5.36	5.11	3.81	6.34
	D4	0.8	24.2	8.13	5.86	5.85	6.37	8.13
P9	D1	0.7	16.5	6.62	6.33	6.20	5.49	9.95
	A2	0.7	16.0	5.71	7.09	7.05	7.18	10.92
	C3	0.7	—	52.64	6.71	6.10	10.06	18.05
	A5	1.0	34.7	6.13	4.43	4.18	5.24	11.16
	C6	1.0	—	40.42	4.36	2.80	7.49	—
P10	A1	1.7	18.2	9.01	—	—	—	6.99
	D2	1.7	24.3	10.45	5.82	5.73	12.31	7.75
	C3	1.7	—	57.81	5.71	4.94	13.72	12.74
	A6	2.4	43.3	6.49	4.93	4.84	8.58	8.38
	C7	2.4	—	—	4.42	3.93	7.81	—
P11	A1	—	42.8	12.31	—	—	—	10.34
	A2	1.0	60.9	10.72	7.81	7.03	12.22	9.15
	C3	1.0	—	47.56	7.84	7.11	11.89	16.73
	A5	1.5	24.0	7.23	7.05	7.45	13.08	11.37
	D8	1.7	41.8	9.59	7.45	7.64	12.10	9.40

TABLE III (*Continued*)

Experiments*		Puncture Site‡	GFR	$(F/P)_{In}$	$(F/P)_{Osm}$	$(F/P)_{Na}$	$(F/P)_{K}$	$(F/P)_{Urea}$
			nl/min					
P12	A1	1.5	34.3	7.05	2.68	2.11	3.71	7.93
	D2	1.2	11.3	6.74	2.70	2.65	3.25	4.63
	D3	1.2	—	9.74	2.72	2.24	3.26	6.65
	D9	1.4	9.2	4.29	2.93	3.15	—	3.24
	A13	1.9	5.5	10.62	—	2.68	3.21	5.87
P13	A2	1.1	13.9	4.70	3.12	2.60	1.78	9.48
	D3	1.1	—	3.93	2.68	2.10	1.44	13.15
	D6	1.3	14.1	3.67	2.46	1.48	1.34	4.89
	C7	1.3	—	15.98	2.41	2.16	3.20	11.66
	A9	2.1	37.3	8.14	2.20	2.62	2.67	5.86
P14	A1	0.8	5.5	5.43	3.49	—	—	10.77
	A2	0.7	36.9	5.28	4.24	2.90	4.03	4.53
	C3	—	—	28.39	3.28	2.65	5.05	22.37
	A5	1.2	16.4	6.58	3.08	—	—	5.02
	D6	2.3	16.0	8.00	3.07	2.64	4.10	4.74
	C7	2.3	—	19.08	2.97	2.11	3.53	21.15
	A9	2.7	13.7	5.49	3.38	2.65	4.35	8.50
	D10	2.7	—	6.51	2.70	—	—	8.33

* Experiments: P2–P14 indicate the animals; A = ascending limb; D = descending limb; C = collecting ducts; the joined numbers indicate the sequence of samples collection, the missing numbers corresponding to urinary samples collected at the tip of the papilla (mean values given in Table I).

‡ Puncture site: distance in mm between the puncture site and the tip of the papilla. For other abbreviations, see Table II.

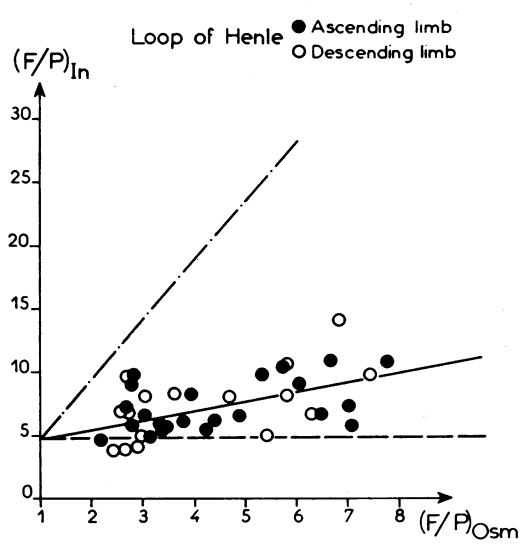

FIGURE 5 Correlation between (F/P) inulin-^{3}H and (F/P) osmotic pressure from the loop micropuncture samples. The solid line indicates the regression line calculated from the data. Traced from the intercept of the regression line with the y axis, the horizontal interrupted line indicates the absence of correlation between the two variables, and the oblique interrupted line indicates the proportional increase of both variables (for explanation see text).

ratio of osmotic pressure measured in the same sample of loop's fluid is shown in Fig. 5. There is a loose, but significant correlation ($r = 0.52$, $P < 0.001$) between these two variables. The equation of the regression line is: $(F/P)_{In} = 0.715\ (F/P)_{Osm} + 4.11$. This equation gives $(F/P)_{In} = 4.8$ if $(F/P)_{Osm} = 1$ at the puncture

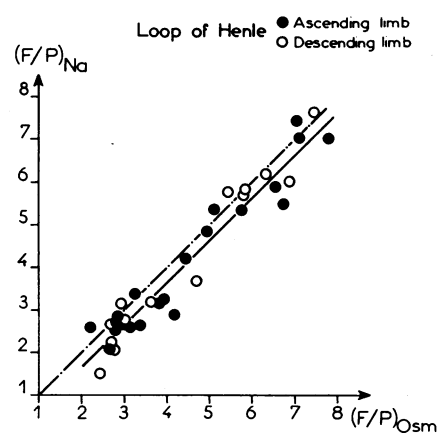

FIGURE 6 Correlation between $(F/P)_{Na}$ and (F/P) osmotic pressure from loop micropuncture samples. Solid line: calculated regression line; the interrupted line bisects the figure.

site. This suggests that iso-osmotic tubular fluid of these very long looped nephrons had a mean $(F/P)_{In}$ of about 4.8 when entering the medullary gradient of osmotic pressure, namely at the beginning of the thin descending limb. Thus, if the concentrating mechanism entirely resulted from osmotic water withdrawal along the descending thin limb, the inulin concentration at the tip of the papilla would have increased with a slope of 4.8 as a function of the osmotic pressure. Despite a large scatter of the data, the results indicate that the inulin concentration increases only to a very limited extent with the osmotic pressure: the slope of the regression line (0.715), when compared to its maximal limit (4.8), suggests that about 15% only of the increase in osmotic pressure resulted from net water reabsorption (limits for $P = 0.05$: 5 and 30%). On the contrary, the $(F/P)_{Na}$ increases more than the $(F/P)_{In}$ with the osmotic pressure (compare Fig. 5 and Fig. 6), indicating that the tubular flow rate of Na ions increases at the tip of the loop when the gradient becomes sharper (Fig. 7); assuming a $(F/P)_{Na}$ of 1 at the beginning of the thin descending limb and a $(F/P)_{In}$ of 4.8 as indicated above, the corresponding sodium over inulin clearance ratio at the entrance of the concentrating system is 0.23; Fig. 7 shows that the values of this clearance ratio is definitely higher at the tip of the papilla, indicating a net addition of sodium ions into the thin segment. Moreover, the steeper the osmotic gradient is, the larger the net sodium addition is. This holds for K^+ ions too, as exemplified by Fig. 8. The data show that the K^+ concentration in the fluid of the loop of Henle increases with the gradient of osmotic pressure, but the scatter is too large to ascertain which kind of function correlates the two variables (in Fig. 8, the line has been drawn free hand).

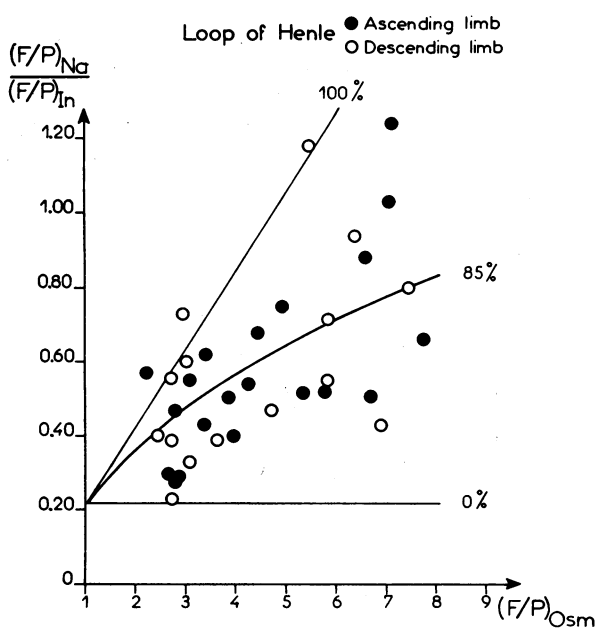

FIGURE 7 Fractional sodium flow $[(F/P)_{Na}/(F/P)_{In}]$ plotted against (F/P) osmotic pressure from the loop micropuncture samples. For explanation of the meaning of the three lines, see footnote 4.

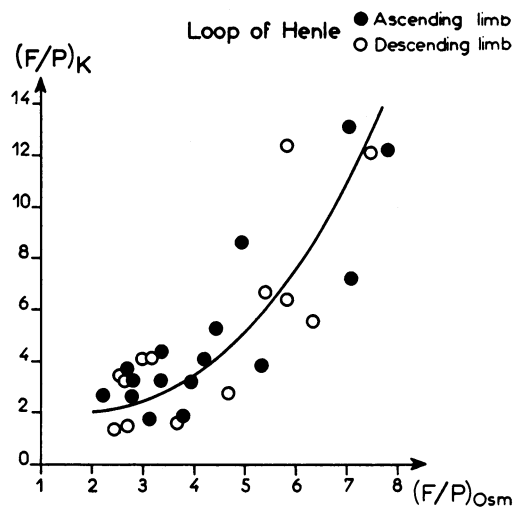

FIGURE 8 Correlation between $(F/P)_K$ and (F/P) osmotic pressure from the loop micropuncture samples. The line of best fit with the data was judged by eye.

Urea concentration measurements also clearly demonstrate the net addition of urea, since the urea over inulin clearance ratio has a mean value of 1.3 at the tip of the loop; nevertheless, there is no clear correlation between the concentration of urea and the osmotic pressure (Fig. 4) of the loop fluid (see Discussion).

DISCUSSION

The transfer of water and solutes along the loop of Henle has not been extensively investigated because the loops are inaccessible to micropuncture in the adult rat unless a partial nephrectomy has been performed (25). Unequivocal information about loop function cannot be extrapolated from cortical micropuncture data since the majority of rat superficial nephrons have short loops (26). A few inulin concentration measurements in micropuncture samples from long loops at the tip of the papilla of nondiuretic golden hamsters and *Psammomys* have been reported by Gottschalk and colleagues (6): the mean value of 11 for the $(F/P)_{In}$ obtained in these experiments was higher than those measured in late proximal and early distal samples collected at the surface of the cortex in the rat. Although the osmotic pressure of the loop fluid was not very high in these experiments, the authors concluded that the observed value of the inulin concentration indicates an osmotic removal of wa-

ter along the descending limb. This conclusion cannot be considered definitive because superficial nephrons of the rat and long-looped nephrons of the golden hamster differ not only in the length of their loops but also in the length of the proximal tubules and the size of the glomeruli (23, 27). In the golden hamster, only 4% of the superficial nephrons have loops penetrating the internal zone of the medulla (26), and the juxtamedullary nephrons, which give the larger part of the long loops, have proximal tubules much larger than the superficial ones (23).

Anatomical considerations

In the *Psammomys,* there is a large heterogeneity in the population of nephrons (23, 27): the glomeruli of the outer cortex are small and give rise to short proximal tubules (pars convoluta 2.1 mm, pars recta 2.0 mm); the juxtamedullary glomeruli are bigger and the corresponding proximal tubules are two times longer (pars convoluta 8.7 mm; no pars recta).

By injecting colloidal carbon into superficial proximal convolutions of living *Psammomys* it can be shown in the macerated kidney (unpublished observations) that their loops definitely penetrate into the internal medulla (long loops) but never reach the extrarenal part of the papilla; therefore, the loops which can be punctured at the tip of the papilla belong to the population of deep nephrons which have large glomeruli and long proximal tubules. This observation could explain some of the results reported here. For example, it is perfectly understandable that a mean filtration rate of 9 nl/min per punctured nephron was found in the cortical experiments and 21 nl/min in the experiments in the papilla, if all the punctured loops issued from large juxtamedullary glomeruli.[3]

The difference in the proximal tubular length of these two kinds of nephrons could also explain the observation that the $(F/P)_{In}$ ratio was higher at the tip of the long loops than in the early distal tubules of the superficial nephrons. Thus, an inulin concentration larger at the tip of the loops than in the early distal tubule, as reported in these experiments on *Psammomys,* can be accounted for without necessarily assuming the existence of net addition of water as a dilution factor along the ascending thin limb of the loops.

[3] Unpublished recent experiments using Hanssen's technique (28) (A. Baines and C. de Rouffignac) confirm this view: $^{14}C\text{-}Fe(CN)_6Na_4$ was injected intravenously into *Psammomys* as a short pulse, and the kidney frozen 8 sec later. After precipitation of the tracer as Prussian blue, a number of proximal tubules were dissected, measured, and their content of radioactivity determined. The mean ratio of the radioactivity of the deep proximal tubules to the superficial ones was found to be 2.5; this value measures the GFR ratio, since $Fe(CN)_6^{=\,=}$ is a glomerular indicator, and since all the filtered tracer was included in the proximal tubule, as controlled by microscopic observation of the bolus of the dye.

Mechanism of concentration

Cortical data. To determine the net reabsorption of water occurring along the loop of the superficial nephrons, it would be necessary to establish the mean value of $(F/P)_{In}$ at the beginning of the thin segment and to ascertain that there is no inulin reabsorption or exchange along the thin limb in this species. Evidence supporting this second point has been obtained in *Psammomys* by the tracer microinjection technique (29): when labeled inulin was injected into proximal convolutions (13), the recovery of the label into the ureteral urine was not significantly different from 100%. $(F/P)_{In}$ ratio at the end of the pars recta of the superficial nephrons, on the other hand, can only be approximated by extrapolating to the end of the whole proximal tubule the regression line obtained from proximal micropuncture data in the experiments reported here. We do not precisely know which kind of function describes the evolution of the inulin concentration along the proximal convoluted tubule, and if the same function would apply along the pars recta as well; moreover, the scatter of the data is large, so that the calculated value 3.8 at the end of the proximal tubule has only to be regarded as an order of magnitude. Comparison of that value to the mean value (3.7) measured in the early distal samples in the same experiments and animals suggests that no net water reabsorption takes place along the loop of Henle of the superficial nephrons in these experimental conditions. Such an interpretation is reinforced by the absence of any positive correlation between the $(F/P)_{In}$ ratio in distal samples and the osmotic pressure of the final urine (Fig. 3). As already mentioned, the superficial nephrons in the *Psammomys* all have long loops which turn in the internal medulla far from the tip of the papilla; even if the tubular fluid, when flowing along these loops, did not reach osmotic pressure values as high as those of the final urine, it nevertheless had to be concentrated to a large extent as a result of the medullary gradient of osmotic pressure (approximately 50% of the actual osmolality of the final urine, according to the measurements performed by Schmidt-Nielsen, O'Dell, and Osaki in salt-loaded *Psammomys* [24]); it is remarkable that distal samples, punctured from long loops in the cortex of kidneys highly concentrating the final urine, $(U/P)_{Osm}$ up to 12, may have $(F/P)_{In}$ ratios as low as 3–4!

Loop data. Micropuncture data from the loops of Henle also indicate that there is minimal water reabsorption along the loops. $(F/P)_{In}$ increases only slightly when $(F/P)_{Osm}$ rises over a wide range at the tip of the long loops; as indicated in the results, the slope of the regression equation suggests that 15% only of the

increase in osmotic presure along the descending limb resulted from water withdrawal. Such an interpretation holds only if in these experiments $(F/P)_{In}$ at the entrance of the system is independent of the actual value of the osmotic gradient. This assumption is supported by the absence of any significant correlation between the inulin clearance and the osmotic pressure of the ureteral urine, and by the already reported constancy of the distal $(F/P)_{In}$ ratio in the superficial distal samples with respect to urinary osmolality.

The Na^+ measurements support the view that the tubular fluid concentrating mechanism results for the larger part from net addition of solutes along the descending limb of the loop. Despite the scatter of the data (Fig. 7), it is obvious that the tubular flow of sodium at the tip of the loop increases with the osmotic pressure.[4]

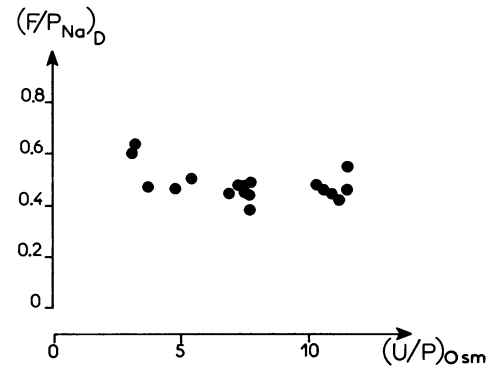

FIGURE 9 Lack of positive correlation between $(F/P)_{Na}$ in early distal fluid samples and the simultaneous (U/P) osmotic pressure in the ureteral urine.

Medullary recycling of sodium

Fig. 7 shows that the steeper the medullary gradient of osmotic pressure, the more sodium (and other solutes) has been added along the descending limb (and, as a consequence, delivered to the ascending limb). On the other hand, at the early distal level, both Na (Fig. 9) and inulin (Fig. 5) F/P ratios appear to be unrelated to the osmotic pressure of the final urine; this means that the load of sodium delivered to the distal tubule remains more or less constant irrespective to the medullary gradient of osmotic pressure [mean value of $(F/P)_{Na}/(F/P)_{In}$ for the distal micropuncture samples $= 0.13$)]. Thus, the sodium added along the descending limb must be reabsorbed along the ascending limb, suggesting a large recycling of Na^+ (and Cl^-) ions between ascending and descending urinary limbs of the counter current multiplier system. The possibility of such a recycling has been considered on theoretical grounds with other hypothesis in several occasions in the past (1–3, 15, 30) but it was generally rejected (2, 3); the implication of such a mechanism on the net reabsorption of sodium taking place in the ascending segment has already been stressed (5, 15); as a consequence of the sodium recycling, both the tubular flow rate along the entire loop, and the rate of free water delivery to the distal segment are maintained high and relatively constant (Fig. 10). The large and variable (according to the osmotic gradient) amount of sodium reabsorbed out of the ascending limb suggests that the active transport was not Tm limited, but had the Na concentration gradient as a limiting factor; this has been already suggested by several authors (22, 31–33).

[4] *a*) If one assumes from Fig. 5, that the inulin concentration increases linearly with the osmotic pressure and *b*) if one accepts, that $(F/P)_{Na}$ is equal to $(F/P)_{Osm}$ (Fig. 6), the curve relating $(F/P)_{Na}/(F/P)_{In}$ to $(F/P)_{Osm}$ can be calculated: from *a*) $(F/P)_{In} = A(F/P)_{Osm} + B$, from *b*) $(F/P)_{Na} = (F/P)_{Osm}$; combining *a* and *b*

$$\frac{(F/P)_{Na}}{(F/P)_{In}} = \frac{(F/P)_{Osm}}{A(F/P)_{Osm} + B}$$

which is of the hyperbolic form $y = x/(Ax + B)$. If, as suggested by the inulin data (Fig. 5), 15% of the concentrating mechanism results from water withdrawal ($A = 0.715$ and $B = 4.1$, equation of the regression line given in the results), then the flow rate of sodium at the tip of the loop would increase with the osmotic pressure according to the curve indicated 85% in Fig. 7, which seems compatible with the data. If there was no net reabsorption of water along the osmotic gradient ($A = 0$), the flow rate of sodium would increase linearly with the osmotic pressure (line indicated 100% on Fig. 7); if the concentrating mechanism entirely resulted from water reabsorption along the osmotic gradient ($B = 0$), the flow rate of sodium would obviously remain unchanged (line 0% in Fig. 7).

Potassium

High potassium concentrations in the loops of concentrating rats kidneys have recently been reported by Jamison, Bennett, and Berliner (34). The present data confirm this observation and, moreover, indicate that the K^+ concentration in the loop fluid is correlated with the osmotic pressure. Since K^+ concentration increases more than inulin concentration with the osmotic pressure, net addition of K^+ ions along the descending limb is suggested; on the other hand, the micropuncture data indicate a large net reabsorption of K^+ along the ascending limb; thus, a potassium recycling between ascending limbs, and possibly collecting ducts (34), and descending limbs seems to operate in the concentrating *Psammomys* kidney, but the corresponding osmotic effect, of course, remains very limited as compared to the sodium recycling.

Urea

Schmidt-Nielsen, O'Dell, and Osaki (24) have shown that the salt-loaded *Psammomys* excretes a urine of high

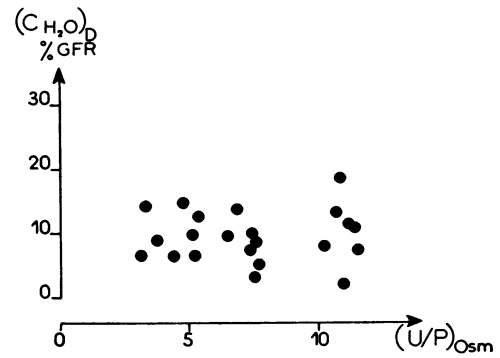

FIGURE 10 Lack of positive correlation between the free water "clearance" $[(CH_2O)_D]$ in early distal tubules and the simultaneous (U/P) osmotic pressure in the ureteral urine. The flow rate of "free water" in distal tubule $(CH_2O)_D$, expressed as a per cent of the GFR for that nephron, was calculated from the equation, $(CH_2O)_D = [1 - (F/P)_{Osm}] \times 100/(F/P)_{In}$.

osmotic pressure, but of relatively low urea concentration; moreover, in these experimental conditions, the medullary gradient of urea concentration reaches a peak in the middle part of the papilla and declines towards the tip. This agrees with our observations that the urea concentration within the loop samples is neither higher than in the distal samples nor correlated with the osmotic pressure.

Nevertheless, a large net addition of urea to the tubular fluid along the loop is obvious from the data. As already discussed (7), the high rate of urea flow delivered to the distal convoluted tubule indicates that the walls of the ascending limb must be relatively impermeable to urea. The larger part of urea entering in the distal tubules is reabsorbed along the distal tubules and collecting ducts, since the final urinary urea clearance is only 20% of the inulin clearance; in the case of this compound, the medullary recycling probably takes place between the collecting ducts and the loops of Henle (7–9). Such a medullary recycling of urea was first described in the rat by Lassiter et al. (7), but it appears to be much more prounounced in the concentrating kidney of desert rodents species such as *Meriones* (8) and *Psammomys* (9). The observed differences may result both from longer loops and from higher medullary gradient of urea concentration in the experiments performed in the two last species.

Permeability of the descending thin limb

It appears from these experiments and others that the walls of the thin descending limb is permeable to Na^+ (10–13), Cl^- (14), K^+ ions, and urea molecules, whereas it is impermeable to larger molecules like inulin, for example. Such a high permeability of the tubular wall for extracellular ions is a prerequisite for the observed net addition of solutes as the major process of the concentrating mechanism along the descending limb of the loop of Henle. It must be stressed that the tubular wall of this segment is highly permeable to water molecules as well. But, if the reflexion coefficient of the membrane for small solute molecules is small, they only exert a very limited osmotic pressure on the membrane, so that the driving force for osmotic outflow of water is small; we do not know what concentration gradient exists for sodium (and Cl^-) ions and water molecules across the membrane; but, it may be calculated that, when expressed in moles per min, the net outflux of water along the descending limb is about 80 times higher than the net influx of salt (assuming from the data that the concentrating process results for 15% from water net outflux, and for 85% from solute net influx). More direct information remains obviously necessary to describe quantitatively the permeability properties of the walls of the descending thin limb to small molecules.

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