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Research Article

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A Micropuncture Study of Collecting Tubule Function in Rats with Hereditary Diabetes Insipidus

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ABSTRACT The reabsorption of water and solute by the papillary collecting duct was studied during water diuresis and vasopressin-induced antidiuresis in young rats with hereditary hypothalamic diabetes insipidus. The tip of the left renal papilla was exposed and fluid was obtained by micropuncture from loops of Henle and from collecting ducts at the papillary tip, and at an average of 1 mm proximal to the tip. In water diuresis the ratio of tubule fluid to plasma (TF/P) osmolality (osm) of loop fluid was 1.73 ±0.058 (SE); of fluid from the proximal collecting duct, 0.63 ±0.027; and from the tip, 0.55 ±0.024; indicating a substantial osmotic pressure difference across the collecting duct epithelium. The fraction of filtered water reabsorbed (× 100) by the terminal collecting duct was 1.58% ±0.32. In antidiuresis the TF/P osm of loop fluid was 2.65 ±0.109; of fluid from the proximal collecting duct, 2.20 ±0.093; and from the tip, 2.71 ±0.111; indicating a marked decrease in the driving force for water reabsorption. The fraction of filtered water reabsorbed (× 100) by the terminal collecting duct was reduced to 0.58% ±0.08, while the delivery of solute to the same segment was unchanged from that in water diuresis. The glomerular filtration rate (GFR) of the right kidney declined from 327 ±24.4 μl/min in water diuresis to 274 ±24.4 μl/min in antidiuresis ($P < 0.005$); similar results were obtained in a study comparing right and left GFRs in five additional rats. Thus, fractional reabsorption (and very likely the absolute volume) of water reabsorbed by the terminal collecting duct was less in antidiuresis than in water diuresis (mean difference, 1.01% ±0.29, $P < 0.005$).

INTRODUCTION

During water diuresis, fluid in the lumina of collecting tubules is hyposmotic to systemic plasma, but the renal medullary interstitium remains hyperosmotic (1–4). In antidiuresis, the permeability to water of the collecting tubule epithelium is increased by antidiuretic hormone (5, 6); as a result, more water is absorbed and the osmolality of fluid in the collecting tubules approaches equilibrium with that of the medullary interstitium. At the same time, the osmolality of the medullary interstitium also increases (1, 2, 7). These observations seem inconsistent: how can the medullary interstitium become progressively more hypertonic if, simultaneously, more water is reabsorbed from the collecting duct? Among several hypotheses (8–12) is the suggestion (11, 12) that the quantity of water reabsorbed from the medullary collecting duct in antidiuresis is actually less than that reabsorbed in water diuresis. The recent discovery of a strain of rats with diabetes insipidus (13) coupled with the observation that the papilla in young rats is accessible to micropuncture (14), have made it possible to test that hypothesis. The results are reported in this paper.

METHODS

Young rats with hereditary hypothalamic diabetes insipidus (Brattleboro strain) (12, 14–16) weighing 55–85 g were allowed free access to food and water up to the onset of the experiment. Only animals excreting a hypotonic urine before the experiment were used, and if during the experiment, the urine was not hypotonic in water diuresis, the results were discarded. After each rat was anesthetized

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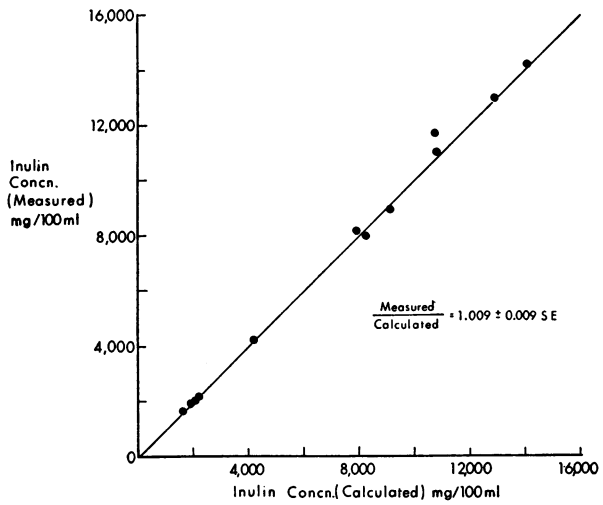

FIGURE 1 Results of analyses of 12 consecutive samples of known (calculated) inulin concentrations by the microinulin technique.

with intraperitoneal Inactin, 80 mg/kg body weight, a tracheostomy was performed, and polyethylene catheters were inserted into both jugular veins and into the left femoral artery. The left kidney was exposed through a midline abdominal incision, the left ureter was excised to uncover the tip of the papilla, and the kidney was placed gently in a glass cup and bathed with mineral oil at 38°C. The papilla was illuminated by a fiber optic light guide (17). The cut end of the left ureter was ligated and a catheter was inserted in the bladder to collect urine from the right kidney. The animal received a prime and maintenance solution of inulin calculated to achieve a plasma concentration of 80 mg/100 ml. In the first period, Tyrode's solution (18), diluted 1:1 with distilled water, was infused at a rate between 0.08 and 0.1 ml/min to insure a brisk water diuresis.

After at least 1 hr of infusion to permit equilibration, a loop was identified and punctured. A droplet of oil was injected and fluid collected so as to maintain the droplet in a stationary position or to allow it to move slowly downstream. Usually, after a single loop puncture, the collecting duct was punctured, first at the papillary tip and then as far proximal as exposure permitted. The fluid was collected more slowly than the flow of fluid within the collecting tubule. In most experiments, at least two samples each from the tip of the collecting duct and from the proximal site were obtained.

TABLE I
*Arterial Blood Measurements**

	Water diuresis	Antidiuresis	P
Blood pressure, *mm Hg*	96.7 ±2.6	96.8 ±3.1	>0.5
Hematocrit, *ml/100 ml*	41.9 ±0.86	38.8 ±1.04	<0.001
Plasma osmolality, *mOsm*	290 ±5.7	293 ±6.0	>0.4
Plasma sodium, *mEq/liter*	145 ±1.7	145 ±2.4	>0.5

* Data presented are the mean ±SE.

At the end of the first (water diuresis) period, the infusate was changed to undiluted Tyrode's solution containing inulin and antidiuretic hormone (Pitressin, Parke, Davis & Co., Detroit, Mich.), 2.2 mU/ml. The infusion rate was set to equal exactly half the initial measured rate. Thus, these animals received antidiuretic hormone at a rate ranging from 88 to 110 μU/min. (Preliminary studies showed that under the above experimental conditions, the urinary osmolality increased as the infusion rate of antidiuretic hormone increased until it reached 50 μU/min; above that rate there was no further increase in urinary concentration). After an hour for equilibration, a loop different from the first one was punctured. Next, the collecting duct was punctured, usually at the same sites as those in the first period.

Samples of blood (65 μl) were obtained from the femoral catheter approximately every 30 min during puncture. The blood pressure was measured through the same catheter. At least one urine collection from the right kidney was made during each of the two periods.

In a separate study of five rats, the experimental conditions were identical with those described, including placing the left kidney in the glass cup, except that micropuncture was not performed. The left ureter was catheterized rather than excised, in order to compare the unilateral GFRs of right and left kidneys during water diuresis and antidiuresis.

The manufacture and treatment of the pipettes and the analytic methods have been described previously (19). Standard statistical methods were used (20).

12 consecutive specimens containing known quantities of inulin were measured by the microinulin technique. The results are illustrated in Fig. 1. Among samples ranging in inulin concentration from 1600 to 14,100 mg/100 ml, the mean ratio, measured/calculated inulin concentration, was 1.009 ±0.009.

RESULTS

A summary of arterial blood measurements in 18 rats is presented in Table I. Of the variables listed, only the hematocrit changed significantly. The reduction in packed cell volume reflected the repeated arterial blood sampling throughout the experiment.

Right kidney

The mean weight of the right kidney was 310 mg ±11. A summary of various measurements of renal function in the right kidney in 18 rats is presented in Table II. Comparing values obtained in antidiuresis with those in water diuresis, there was a striking decrease in urine flow and fractional excretion of water, a marked increase in urine osmolality, no change in solute excretion, and a decrease in sodium excretion. The mean glomerular filtration rate decreased 15%.

Left kidney

The protocol of a typical experiment is presented in Table III and its results are illustrated in Fig. 2. The

TABLE II

*Renal Function of the Right Kidney in 18 Rats**

	Water diuresis	Antidiuresis	P
Urinary flow rate, *μl/min*	19.49 ±1.42	1.81 ±0.16	<0.001
U/P osm	0.51 ±0.022	4.12 ±0.238	<0.001
P/U inulin × 100	6.33 ±0.62	0.73 ±0.09	<0.001
GFR, *μl/min*	326.6 ±24.4	274.4 ±24.4	<0.005
U/P osm/inulin × 100	3.17 ±0.48	2.84 ±0.29	>0.2
U/P Na/inulin × 100	0.31 ±0.05	0.07 ±0.02	<0.001

* P, plasma concentration; osm, osmolality.
Data presented are the mean ± SE.
U, urinary concentration.

data obtained in water diuresis (first period) are shown in Fig. 2*a* and in antidiuresis (second period) in Fig. 2*b*. The results of duplicate punctures were averaged. Note the hypertonicity of the fluid in the loop of Henle during water diuresis. As the fluid passed down the collecting tubule it became more dilute despite the fact that there was continued reabsorption of water. The fraction of filtered water reabsorbed (× 100) was 91.7% at the proximal portion of the collecting duct and 93.9% at the tip, indicating the reabsorption of 2.2% of the filtered load of water by the terminal portion of the collecting duct and the excretion of 6.1%. The papilla became more hypertonic in antidiuresis, as indicated by the higher

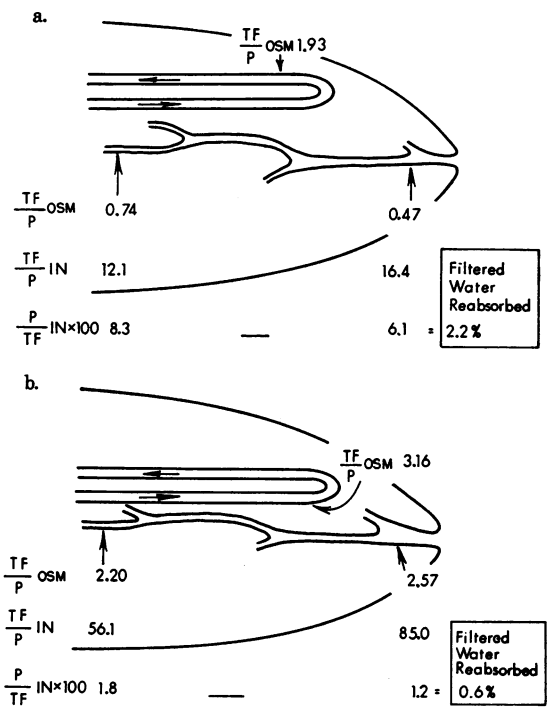

FIGURE 2 Results of experiment outlined in Table I, *a*. Water diuresis, *b*. Antidiuresis. The distances from the tip are drawn roughly to scale. For further description see text. OSM, osmolality; IN, inulin.

TABLE III

Experimental Protocol

Experiment 9/14/70	
8:10	Anesthetized with Inactin (8 mg/100 g body wt). Surgery: rat infused with Tyrode's solution diluted with an equal volume of water at 0.03 ml/min.
10:03	P_0, Hct 44%.
10:05	Prime and maintenance infusion of Tyrode's solution diluted with an equal volume of water at 0.082 ml/min.
1:09	S_1, loop, 0.65 mm from tip of papilla.
1:11	P_1, Hct 47%.
1:12	Begin collection of urine (U_1) from right kidney.
1:16	S_2, CD (tip), 0.29 mm from tip.
1:24	S_3, CD (prox), 1.24 mm from tip.
1:29	S_4, CD (tip), same site as S_2.
1:35	P_2, Hct 45%.
1:39	S_5, CD (prox) same site as S_3.
1:43	End U_1·(V = 15.06 μl/min).
1:47	P_3, Hct 46%. Mean arterial pressure, 90 mm Hg.
1:52	Begin second infusion of undiluted Tyrode's at 0.041 ml/min containing 2.2 mU ADH/ml (=90 μU/min).
3:07	P_4, Hct 47%.
3:08	Begin collection of urine (U_2) from right kidney.
3:40	S_6, loop, 0.78 mm from tip.
3:46	P_5, Hct 44%.
3:52	S_7, CD (tip), 0.20 mm from tip.
4:00	S_8, CD (prox), 1.24 mm from tip.
4:08	S_9, CD (tip), same site as S_7.
4:13	P_6, Hct 43%.
4:20	S_{10}, CD (prox), same site as S_8.
4:25	End U_2·(V = 0.91 μl/min).
4:28	P_7, Hct 44%. Mean arterial pressure, 94 mm Hg. End experiment.

ratio of tubule fluid to plasma osmolality (TF/P osm)[1] of the loop sample. In contrast to water diuresis, the osmolality of fluid in the collecting tubule at the tip of the papilla was higher than that of fluid in the proximal collecting duct in antidiuresis. The filtered water reabsorbed was 98.2% at the proximal portion of the collecting duct and 98.8% at the tip. Thus, 0.6% of the filtered water was reabsorbed in antidiuresis, and 1.2% was excreted.

The micropuncture data obtained from experiments in 18 rats are presented in Table IV and the analyses of fluid samples from the collecting tubules are summarized in Tables V–VII. The results obtained at each collecting duct site (tip and proximal) were averaged and are given as single values. The mean weight of the left kidney was 309 mg ±10.

[1] *Abbreviations used in this paper:* GFR, glomerular filtration rate; TF/P, tubule fluid to plasma ratio; U/P, urine to plasma ratio; osm, osmolality.

TABLE IV

Micropuncture Data from Left Kidney

Rat number	Infusion period	TF/P osm loop	TF/P osm		P/TF inulin × 100			(TF/P Na/inulin) × 100		(TF/P K/inulin) × 100	
			CD prox	CD tip	CD prox	CD tip	Diff.	CD prox	CD tip	CD prox	CD tip
1	WD	2.09	0.57	0.47	6.5	5.2	1.3				
	AD	2.26	1.77	2.14	1.9	1.5	0.4				
2	WD	1.81	0.72	0.61	2.5	2.4	0.1				
	AD	3.42	2.79	2.97	1.0	0.7	0.3				
3	WD	1.55	0.49	0.43	6.5	5.9	0.6				
	AD	2.62	2.08	2.29	1.4	1.1	0.3				
4	WD	1.83	0.51	0.57	3.8	2.6	1.2				
	AD	2.34	1.89	2.25	1.9	1.7	0.2				
5	WD	1.54	0.69	0.54	5.0	4.0	1.0				
	AD	2.75	1.69	2.04	1.8	0.9	0.9				
6	WD	1.93	0.74	0.47	8.3	6.1	2.2				
	AD	3.16	2.20	2.57	1.8	1.2	0.6				
7	WD	1.63	0.56	0.52	4.5	4.0	0.5	0.41	0.32	23.30	25.10
	AD	2.32	2.32	2.86	1.9	1.4	0.5	0.58	0.98	49.20	44.90
8	WD	1.67	0.52	0.54	4.9	3.7	1.2	0.24	0.06	14.00	16.00
	AD	3.04	2.43	2.83	1.6	1.1	0.5	0.74	0.93	37.80	32.00
9	WD	1.59	0.78	0.74	10.2	8.8	1.4	—	—	—	—
	AD	2.68	1.90	2.73	3.7	2.6	1.1	—	—	—	—
10	WD	1.78	0.67	0.52	6.0	4.4	1.6	1.08	0.11	32.77	29.13
	AD	2.13	1.94	3.43	2.2	1.2	1.0	1.62	0.15	24.01	37.89
11	WD	—	0.76	0.65	15.4	10.4	5.0	2.69	0.54	62.30	26.40
	AD	—	2.39	3.37	2.6	1.6	1.0	0.57	0.35	42.30	3.30
12	WD	1.54	0.48	0.48	9.0	4.5	4.5	0.60	0.30	65.20	18.40
	AD	2.78	2.60	2.58	1.2	1.0	0.2	0.41	0.72	46.30	34.80
13	WD	—	0.83	0.71	1.8	1.7	0.1	0.34	0.10	1.50	0.10
	AD	2.08	3.23	3.62	1.1	0.8	0.3	0.77	0.11	13.00	12.40
14	WD	1.66	0.56	0.45	7.0	5.2	1.3	1.90	0.40	24.80	12.50
	AD	—	1.87	3.09	1.9	0.8	1.1	1.10	0.10	31.20	41.90
15	WD	1.86	0.62	0.63	4.2	3.2	1.0	0.80	0.20	3.00	1.80
	AD	2.64	2.30	2.71	1.2	0.9	0.3	0.65	0.43	23.00	23.90
16	WD	2.25	0.75	0.74	3.1	2.7	0.4	0.40	0.36	11.60	7.50
	AD	3.25	2.30	2.95	1.4	0.9	0.5	0.70	0.66	39.20	24.30
17	WD	1.52	1.58	0.40	9.0	6.9	2.1	1.65	0.20	50.25	20.80
	AD	2.93	1.90	2.17	1.3	0.9	0.4	0.70	0.20	23.65	20.95
18	WD	1.36	0.50	0.46	7.6	5.2	2.4	1.20	0.27	38.80	15.15
	AD	2.00	2.00	2.24	1.8	1.0	0.8	0.70	0.13	40.35	38.33

TF/P, tubular fluid to plasma ratio; osm, osmolality; P/TF inulin × 100, per cent filtered water unreabsorbed; (TF/P osm/inulin) × 100, per cent filtered solute unreabsorbed; (TF/P Na/inulin) × 100, per cent filtered sodium unreabsorbed; (TF/P K/inulin) × 100, per cent filtered potassium unreabsorbed; CD prox, sample obtained by micropuncture of papillary collecting duct as far proximal as exposure allowed; CD tip, sample obtained by micropuncture of collecting duct at papillary tip; Diff., difference, CD prox value minus CD tip value; WD, water diuresis; AD, antidiuresis.

WATER DIURESIS

The results of the collecting tubule punctures during water diuresis are summarized in Table V.

1. Comparison of urine obtained from the tip of the collecting duct of the left kidney (Table V) with urine collected from the right kidney (Table II). The urine excreted from the left and right kidneys was diluted to a similar extent (U/P osm, 0.55 ±0.024 and 0.51 ±0.022, respectively); the fractional excretion of water (× 100) was approximately the same from the left and right kidney (4.82% ±0.53 and 6.33% ±0.62, respectively, $P > .05$), and the fractional excretion of solute (× 100) was also similar (2.68% ±0.38 and 3.17% ±0.48, respectively, $P > 0.4$).

2. Comparison of collecting tubule fluid obtained at the tip with that obtained at the proximal site. The mean

TABLE V
*Water Diuresis**

	CD prox	CD tip	P	N
TF/P osm	0.63 ±0.027	0.55 ±0.024	<0.001	18
TF/P inulin	20.3 ±2.82	25.3 ±2.89	<0.001	18
P/TF inulin × 100	6.41 ±0.77	4.82 ±0.53	<0.001	18
(TF/P osm/inulin) × 100	4.05 ±0.59	2.68 ±0.38	<0.001	18
(TF/P Na/inulin) × 100	1.03 ±0.23	0.26 ±0.04	<0.005	11
(TF/P K/inulin) × 100	29.8 ±6.73	15.7 ±2.91	>0.1	11

* CD prox, sample obtained by micropuncture of papillary collecting duct as far proximal as exposure allowed; CD tip, sample obtained by micropuncture of collecting duct at papillary tip; TF/P, tubular fluid to plasma ratio; osm, osmolality; P/TF inulin × 100, per cent filtered water unreabsorbed; (TF/P osm/inulin) × 100, per cent filtered solute unreabsorbed; (TF/P Na/inulin) × 100, per cent filtered sodium unreabsorbed; (TF/P K/inulin) × 100, per cent filtered potassium unreabsorbed; data presented are the mean ±SE.

interval between tip and proximal punctures was 0.98 mm. The fraction of filtered water not reabsorbed (× 100) at the proximal collecting duct was 6.41% and at the tip 4.82%. Thus, the terminal collecting duct reabsorbed 1.58% ±0.32 of the filtered load of water ($P < 0.001$). There was a decrease in tubular fluid osmolality as the fluid passed through the terminal portion of the collecting duct; the mean difference in TF/P osm was 0.078 ($P < 0.001$). The fraction of filtered solute reabsorbed (× 100) by the terminal collecting duct was 1.37% ±0.27, ($P < 0.001$). In 11 experiments, sodium and potassium concentrations of the tubule fluid samples were measured. There was significant reabsorption of sodium by the exposed portion of the papillary collecting duct (0.77% ±0.19 of the filtered load, $P < 0.005$).

In 16 animals, a loop of Henle was punctured in water diuresis. The mean TF/P osm of loop fluid was 1.73 ±0.058.

ANTIDIURESIS

The results of the collecting tubule punctures during antidiuresis are summarized in Table VI.

1. Comparison of urine obtained from the tip of the collecting duct of the left kidney (Table VI) with urine collected from the right kidney (Table II). The fractional excretion of water (× 100) in antidiuresis was slightly but significantly greater from the left kidney, 1.18% ±0.07, than from the right kidney, 0.73% ±0.09, ($P < 0.001$), while fractional excretion of solute (× 100) was not significantly different, 3.23% ±0.31 and 2.84% ±0.29, respectively, ($P > 0.4$). The average urinary osmolality from the left kidney (TF/P osm, 2.71 ±0.111) was significantly less than that of urine from the right kidney (4.12 ±0.238), ($P < 0.001$).

TABLE VI
*Antidiuresis**

	CD prox	CD tip	P	N
TF/P osm	2.20 ±0.093	2.71 ±0.111	<0.001	18
TF/P inulin	62.7 ±4.29	93.8 ±6.19	<0.001	18
P/TF inulin × 100	1.76 ±0.15	1.18 ±0.07	<0.001	18
(TF/P osm/inulin) × 100	3.73 ±0.28	3.23 ±0.31	<0.001	18
(TF/P Na/inulin) × 100	0.78 ±0.10	0.43 ±0.10	>0.1	11
(TF/P K/inulin) × 100	33.6 ±3.45	28.6 ±3.9	>0.4	11

Data presented are the mean ±SE.
* Abbreviations defined in Table V.

2. Comparison of collecting tubule fluid obtained at the tip with that obtained at the proximal site. The mean interval between tip and proximal punctures was 1.00 mm. In contrast to water diuresis, there was a rise in osmolality of tubule fluid between the proximal site and tip of the collecting duct in antidiuresis, mean difference, −0.51 ±0.091 ($P < 0.001$). The fraction of filtered water not reabsorbed (× 100) at the proximal collecting duct was 1.76% ±0.15 and at the tip 1.18% ±0.07. The difference, 0.58% ±0.08 ($P < 0.001$), represents the filtered water reabsorbed by the terminal collecting duct. The fraction of filtered solute reabsorbed (× 100) by the terminal collecting duct was 0.49% ±0.07 ($P < 0.01$). In the 11 samples in which the electrolytes were measured, no statistically significant net reabsorption of either sodium or potassium was detected.

In 16 animals, a loop of Henle was punctured in antidiuresis. The mean TF/P osm of loop fluid was 2.65 ±0.109.

TABLE VII
*Comparison, Water Diuresis, and Antidiuresis**

	CD prox‡	CD tip‡	P	N
TF/P osm	−1.57 ±0.087	−2.16 ±0.10	<0.001	18
P/TF inulin × 100	4.64 ±0.69	3.64 ±0.47	<0.005	18
(TF/P osm/inulin) ×100	0.33 ±0.46	−0.55 ±0.25	=0.005	18
(TF/P Na/inulin) ×100	0.25 ±0.24	−0.17 ±0.11	>0.1	11
(TF/P K/inulin) ×100	−3.86 ±5.91	−12.89 ±4.24	>0.1	11

Data presented are the mean ±SE.
* Abbreviations defined in Table V.
‡ Each value is the mean of the differences obtained by subtracting the value obtained in antidiuresis from the respective value obtained in water diuresis for each rat.

DIFFERENCE IN COLLECTING TUBULE FUNCTION BETWEEN WATER AND ANTIDIURESIS

These data are presented in Table VII.

1. Comparison of the changes in urine excretion from the left and right kidney. In antidiuresis, on the right side, the U/P osmolality increased by 3.6 and the fractional excretion of water (× 100) decreased by 5.6%. The respective changes in fluid emerging from the tip of the exposed papilla on the left side were significantly less marked ($P < 0.01$): The TF/P osm increased by 2.2 and the excretion of water decreased by 3.6%.

2. Comparison of changes in reabsorption by the exposed portion of the collecting tubule. The TF/P osm of fluid in the proximal collecting duct increased by 1.57 ±0.087 and that of fluid in the tip of the collecting duct by 2.16 ±0.10 ($P < 0.001$). The fraction of filtered water unreabsorbed (× 100) by the collecting duct decreased by 4.64% ±0.69 at the proximal site and by 3.64% ±0.47 at the tip of the collecting duct ($P < 0.005$). Of special importance is the fact that the fraction of filtered water reabsorbed (× 100) by the exposed portion of the collecting duct in water diuresis exceeded that reabsorbed in antidiuresis by 1.01% ±0.29, ($P < 0.005$).

Net fractional reabsorption (× 100) of solute by the exposed portion of the collecting duct in water diuresis exceeded that in antidiuresis by 0.88% ±0.27 ($P = 0.005$). The reabsorption of sodium and potassium by the exposed portion of the collecting duct was less in antidiuresis than in water diuresis but the differences do not achieve statistical significance.

In 15 rats in which the loop was punctured in both periods, the mean TF/P osm of loop fluid in antidiuresis exceeded that in water diuresis by 0.96 ±0.108 ($P < 0.001$).

Bilateral Glomerular Filtration Rates

The GFR of the right kidney in five additional rats averaged 333 μl/min in water diuresis (range: 188–473) and 298 μl/min in antidiuresis (range: 162–412). The mean differences, right minus left, were 49 ±25 SE and 19 ±12 μl/min, respectively.

DISCUSSION

The principal finding in these experiments is that fractional water reabsorption by the terminal collecting duct was greater in water diuresis than in antidiuresis (Fig. 3). The data also show that urinary dilution as well as concentration occurred in the collecting duct.

Water reabsorption by the terminal collecting duct. In the terminal collecting duct, water reabsorption in water diuresis exceeded that in antidiuresis by a volume equal to 1% of the glomerular filtrate. In interpreting the significance of this difference, several factors need to be considered. First, as shown in Fig. 1, the difference is certainly measurable, i.e., the difference in inulin concentration of collecting duct fluid between proximal and tip puncture sites can clearly be distinguished by the microinulin technique. Second, the figure of 1% represents a minimal estimate of the excess water reabsorbed by the medullary collecting duct in water diuresis, since the length of the medulla, papillary tip to cortical-medullary junction is 4–5 mm in these rats.[2] Third, the estimated absolute volume of water reabsorbed by the papillary tip is appreciable. Although the GFR of the left kidney could not be determined during micropuncture, in separate experiments under nearly the same conditions (except that the ureter was not excised), the GFR of the left kidney was only slightly less than that of the right kidney. It seems likely, therefore, that in the micropuncture experiments, the GFR of the left kidney was approximately the same as that of the right kidney. The volume of fluid reabsorbed (estimated by multiplying the fraction of filtered water reabsorbed in the papillary collecting duct of the left kidney by the GFR of the right kidney) was 5.2 μl/min in water diuresis and 1.5 μl/min in antidiuresis. Since the wet weight of the papilla in these rats is approximately 4–5 mg,[3] the calculated volume of water reabsorbed approaches a flow per minute equal to the papillary weight in water diuresis and one-third of the papillary weight in antidiuresis.

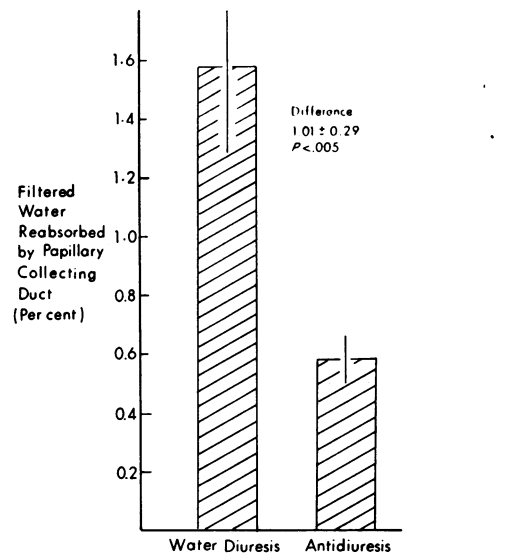

FIGURE 3 Filtered water reabsorbed by papillary collecting duct in water diuresis and antidiuresis. The height of the vertical bar represents ±1 SE.

We have no ready explanation for the decrease in GFR in antidiuresis. If the rats were more volume expanded in water diuresis, the corresponding plasma osmolality and sodium concentration (Table I) and urinary excretion of sodium (Table II) suggest that the difference was small. It is possible that the duration of these experiments was a factor. A similar decline in GFR of adult Brattleboro rats was observed by Schnermann, et al. (21), in antidiuresis, and the decrease was not corrected by a return to water diuresis. (We attempted to reverse the order of our experiments but were unable to achieve a sustained water diuresis after antidiuresis.) It is very unlikely that the decline in GFR by itself could account for the marked changes in urinary osmolality and frac-

[2] Unpublished observations.

[3] Unpublished data.

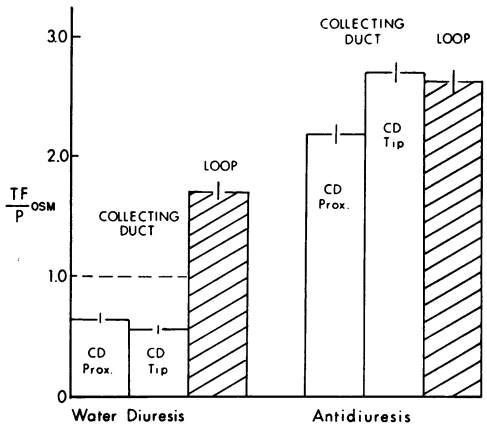

FIGURE 4 Profile in tubule fluid to plasma osmolality of collecting duct and loop fluid in water diuresis and antidiuresis. The height of the verical bar represents ±1 SE.

tional excretion of water. In experiments in dogs in which the presence of vasopressin was excluded, Berliner and Davidson (*22*) found it necessary to reduce GFR by more than 20% before urinary osmolality exceeded plasma osmolality, and the maximum urinary osmolality rarely exceeded 350 mOsm despite a reduction in GFR as great as 70%. According to Valtin (16), when rats with hereditary diabetes insipidus were deprived of water for 12 hr, the animals lost 11% of their body weight, implying that the GFR must have decreased substantially, yet urinary osmolality increased only to 358 mOsm, which was probably little more than the osmolality of the plasma in those rats (16). Nor could a difference in total solute load (*22*) be a factor. The experiments were designed to insure an unchanging solute delivery. At the proximal collecting duct site there was no significant difference between water diuresis and antidiuresis in either (*a*), the fractions of filtered solute or sodium unreabsorbed or (*b*), the product of the fraction of filtered solute unreabsorbed times the GFR (the osmolar clearance) (12.26 ±1.55 μl/min in water diuresis; 9.93 ±0.88 μl/min in antidiuresis, $P > 0.2$).

Could the difference in water reabsorption be an artifact owing to sampling error, that is, did the composition of the fluid in the collecting duct in deeper layers of the papilla differ from that of the punctured collecting ducts on the surface? It is important to recall that the collecting ducts fuse at least five times before reaching the external portion of the papilla and thereafter fuse two or three times more (23). It seems reasonable to assume that an adequate mixing of fluids from deep and superficial nephrons had already occurred, an assumption inherent in the previous studies by Ullrich and his associates (24, 25).

The fact that the urinary osmolality of the fluid emerging from the tip of the exposed papilla in antidiuresis was less than that from the untouched right kidney implies that the urinary concentrating function of the left kidney was somewhat impaired. The cause of this impairment is not clear but it has previously been observed by others (*26*) and may be attributable to loss of urea from the exposed papilla (*27*). Nevertheless, the papilla clearly retained the capacity to concentrate, as indicated by the fact that in antidiuresis, the osmolality of fluid from the tip of the collecting duct was significantly higher than that from the proximal collecting duct and by the substantial increment in osmolality of the urine excreted from the left side in antidiuresis compared with that in water diuresis.

The results of these experiments confirm the idea expressed by Kiil and Aukland (11), by Berliner (12) and later by Morgan (*28*) that water reabsorption in the medullary collecting ducts is greater in water diuresis than it is in hydropenia. It was reasoned (12) that, even in the absence of antidiuretic hormone, the collecting duct is still appreciably permeable to water, a supposition demonstrated to be true (5, 6, *22*), while in hydropenia, water reabsorption is limited by the small volume entering the papilla and consequent rapid rise of osmolality as water is reabsorbed in that segment. The fraction of filtered water reaching the proximal portion of the exposed collecting duct was reduced in the present experiments from 6.4% in water diuresis to 1.8% in antidiuresis. An estimate of the driving force for water reabsorption may be obtained by calculating the difference in osmolality between the fluid emerging from the tip of the collecting duct and the fluid in the loop of Henle, which reflects the osmolality of the papillary interstitium (*29*) (Fig. 4). In 15 animals in which these values were obtained in both water diuresis and antidiuresis, the osmolality of fluid from the loop of Henle exceeded that of fluid emerging from the tip of the collecting duct during water diuresis by a TF/P osm of 1.2, i.e., by roughly 350 mOsm, while there was no significant difference in osmolality between fluid in the loop of Henle and that in the tip of the collecting duct in antidiuresis. In summary, even though the water permeability of the collecting tubule epithelium is greater in antidiuresis, the driving force for the reabsorption of the collecting duct is reduced to the extent that, at least in the terminal portion, the absolute amount of water reabsorbed is less.

These findings are also in accord with the suggestion (30) that the degree to which the volume of water remaining is diminished between its emergence from the loop of Henle and its reentry into the medullary portion of the collecting duct is of prime importance in the effectiveness of urinary concentrating mechanism. Because neither the end of the ascending limb nor the beginning of the medullary collecting duct is accessible on the

kidney surface, this hypothesis may be difficult to test directly, but a comparison of the volume of fluid reabsorbed by the accessible length of the distal tubule in water diuresis with that in antidiuresis might provide an index of quantitative differences involved.

The terminal collecting duct as a diluting site. An unexpected observation in these studies was the reduction in the TF/P osm of fluid as it passed through the terminal collecting duct in water diuresis from 0.63 to 0.55 (Fig. 3). Wirz demonstrated a higher osmolality in fluid from the distal tubule than that of ureteral urine during water diuresis in rats (31), but Clapp and Robinson (32) reported the opposite findings in dogs. However, the latter experiments, as the authors noted, were not performed during conditions of maximum water diuresis. It is difficult to assess the importance of the present finding in view of the small difference in osmolality between the two sites, but it implies that the collecting duct, at least in the rat, participates in the process of urinary dilution.

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REFERENCES

1. Ullrich, K. J., F. R. Drenckhahn, and K. H. Jarausch. 1955. Untersuchungen zum Problem der Harnkonzentrierung und verdunnung: über des osmotische Verhalten von Nierenzellen und die begleitende Elektrolytanhaufung im Nierengewebe bei verschiedenen Diuresezustanden. *Pflüegers Arch.* **261**: 62.
2. Gottschalk, C. W., and M. Mylle. 1959. Micropuncture study of the mammalian urinary concentrating mechanism: evidence for the countercurrent hypothesis. *Amer. J. Physiol.* **196**: 927.
3. Gottschalk, C. W. 1961. Micropuncture studies of tubular function in the mammalian kidney. *Physiologist.* **4**: 35.
4. Bray, G. A. 1960. Freezing point depression of rat kidney slices during water diuresis and antidiuresis. *Amer. J. Physiol.* **199**: 915.
5. Grantham, J. A., and M. Burg. 1966. Effect vasopressin and cyclic AMP on permeability of isolated collecting tubules. *Amer. J. Physiol.* **211**: 255.
6. Morgan, T., and R. W. Berliner. 1968. Permeability of the loop of Henle, vasa recta and collecting duct to water, urea, and sodium. *Amer. J. Physiol.* **215**: 108.
7. Wirz, H., B. Hargitay, and W. Kuhn. 1951. Lokalisation des Konzentrierungsprozesses in der Niere durch direkte Kryoskopie. *Helv. Physiol. Pharmacol. Acta.* **9**: 190.
8. Thurau, K., P. Deetjen, and K. Kramer. 1960. Hamo dynamick des Nierenmarkes II. Mitteilung. *Pflüegers Arch.* **270**: 270.
9. Levitin, H., A. Goodman, G. Pigeon, and F. H. Epstein. 1962. Composition of the renal medulla during water diuresis. *J. Clin. Invest.* **41**: 1145.
10. Gottschalk, C. W. 1964. Osmotic concentration and dilution of the urine. *Amer. J. Med.* **36**: 670.
11. Kiil, F., and K. Aukland. 1960. Renal tubule localization of water and sodium reabsorption in antidiuresis and water diuresis. *Scand. J. Clin. Lab. Invest. Suppl.* **12**: 277.
12. Berliner, R. W. 1961. Mechanisms involved in the formation of dilute and concentrated urine. *Proc. Int. Congr. Nephrol. 1st.* **1**: 5.
13. Valtin, H., and H. A. Schroeder. 1964. Familial diabetes insipidus in rats (Brattleboro strain). *Amer. J. Physiol.* **206**: 425.
14. Lassiter, W. E., M. Mylle, and C. W. Gottschalk. 1966. Micropuncture study of urea transport in rat renal medulla. *Amer. J. Physiol.* **210**: 965.
15. Valtin, H. 1967. Hereditary hypothalamic diabetes insipidus in rats (Brattleboro strain). A useful experimental model. *Amer. J. Med.* **42**: 814.
16. Valtin, H. 1966. Sequestration of urea, and nonurea solutes in renal tissues of rats with hereditary hypothalamic diabetes insipidus: effects of vasopressin and dehydration on the countercurrent mechanism. *J. Clin. Invest.* **45**: 337.
17. Jamison, R. L. 1970. Micropuncture study of single superficial and juxtamedullary nephrons in the rat. *Amer. J. Physiol.* **218**: 46.
18. Weisser, F., F. B. Lacy, H. Weber, and R. L. Jamison. 1970. Renal function in the chinchilla. *Amer. J. Physiol.* **219**: 1706.
19. Jamison, R. L. 1968. Micropuncture study of segments of thin loop of Henle in the rat. *Amer. J. Physiol.* **215**: 236.
20. Snedecor, G. W. 1956. Applied to experiments in agriculture and biology. *In* Statistical Methods. Iowa State University Press, Ames, Iowa.
21. Schnermann, J., H. Valtin, K. Thurau, W. Nagel, M. Horster, H. Fischbach, M. Wahl, and G. Liebau. 1969. Micropuncture studies on the influence of antidiuretic hormone on tubule fluid reabsorption in rats with hereditary hypophalamic diabetes insipidus. *Pflüegers Arch.* **306**: 103.
22. Berliner, R. W., and D. G. Davidson. 1957. Production of hypertonic urine in the absence of pituitary antidiuretic hormone. *J. Clin. Invest.* **36**: 1416.
23. Oliver, J. 1968. Nephrons and Kidneys. *In* A Quantitative Study of Developmental and Evolutionary Mammalian Renal Architectonics. Harper & Row, Publishers, New York.
24. Jarausch, K. H., and K. J. Ullrich. 1957. Zur Technik der Enthnahme von Harnproben aus einzelnen Sammelröhren der Saügetiereniere mittels Polyäthylincapillaren. *Pflüegers Arch.* **264**: 88.
25. Hilger, H. H., J. D. Klumper, and K. J. Ullrich. 1958. Wasserrückresorption und Ionentransport durch die Dammelrohrzellen der Säugetierniere. *Pflüegers Arch.* **267**: 218.
26. Gottschalk, C. W., W. E. Lassiter, M. Mylle, K. J. Ullrich, B. Schmidt-Nielsen, R. O'Dell, and G. Pehling. 1963. Micropuncture study of composition of loop of Henle fluid in desert rodents. *Amer. J. Physiol.* **204**: 532.
27. Schmidt-Nielsen, B., and H. D. Pagel. 1970. Urea and the Kidney. Mechanisms of urea retention in the renal medulla. *Excerpta Med. Found. Int. Congr. Ser. 195.* 393.

28. Morgan, T. 1969. Movement of solute and water in the countercurrent systems of the medulla 1970. *Nephrol. Proc. Int. Cong. 4th 1970.* 1: 156.
29. Jamison, R. L., C. M. Bennett, and R. W. Berliner. 1967. Countercurrent multiplication by the thin loops of Henle. *Amer. J. Physiol.* **212**: 357.
30. Berliner, R. W., and C. M. Bennett. 1967. Concentration of urine in the mammalian kidney. *Amer. J. Med.* **42**: 777.
31. Wirz, H. 1956. Der osmotiche Druck in den corticalen Tubuli der Rattenniere. *Helv. Physiol. Pharmacol. Acta.* **14**: 353.
32. Clapp, J. R., and R. Robinson. 1966. Osmolality of distal tubular fluid in the dog. *J. Clin. Invest.* **45**: 1847.