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Research Article

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Micropuncture Study of Hypertonic Mannitol Diuresis in the Proximal and Distal Tubule of the Dog Kidney

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ABSTRACT Fractional reabsorption of water, sodium, and potassium at proximal and distal tubular sites within the nephron was studied by recollection-micropuncture experiments on dogs undergoing hypertonic mannitol diuresis. After an initial control hydropenic phase, 16% mannitol in modified Ringer's solution was administered intravenously, resulting in marked increases in fractional excretion of water (28.7%), sodium (12.6%), and potassium (63.9%). Inulin clearance decreased significantly from 35.1 to 25.2 ml/min. Analysis of paired micropuncture data revealed a significant decrease in tubule fluid to plasma (TF:P) inulin ratios in both the proximal tubule (1.63–1.45) and distal tubule (5.38–1.94). There was also a significant decrease in proximal TF:P sodium ratios (0.99–0.93) and potassium ratios (1.05–0.98). Distal TF:P sodium ratios, in contrast, rose significantly (0.38–0.59), while TF:P potassium ratios tended towards unity whether initially greater or less than one. Fractional reabsorption of sodium and water decreased by 5% and 10% respectively in the proximal tubule, but to a lesser extent than the resulting increases in fractional urinary excretion. The nonreabsorbed fraction, however, had increased sharply at the point of distal puncture for water (32%), sodium (26%), and potassium (26%), indicating a large inhibitory effect within the loop of Henle in addition to the smaller proximal effects.

INTRODUCTION

Mannitol has been used extensively in the study of renal function over the past 20 yr and has been particularly important in establishing the active nature of sodium transport. Despite the wealth of experimental data that exists, there is a surprising lack of detailed information both as to the extent and sites of inhibition of salt and water transport which occurs within the nephron during hypertonic mannitol infusions. The impressive diuretic effects have generally been attributed to its osmotic action, predominantly within the proximal tubule. This view was originally advanced by Wesson and Anslow (1), in part, because of the isosmotic nature of proximal reabsorption which dictates that the retention of a nonreabsorbable solute, such as mannitol, must be accompanied by a corresponding decrease in the tubule fluid sodium concentration. They felt that the luminal sodium concentration would continue to fall until a critical or limiting gradient was reached, at which point no further net transfer of solute or water could occur. Distal sites within the nephron were thought to play little or no significant role in mediating the over-all diuresis.

This view has generally retained acceptance, especially in view of the support afforded by later micropuncture studies. Stationary microperfusion experiments in *Necturus* of Windhager, Whittembury, Oken, Schatzmann, and Solomon (2) confirmed the presence of a limiting gradient for sodium in the presence of sufficient concentrations of mannitol to maintain isosmotic conditions. They showed, furthermore, that net solute transfer could be directly correlated with the intratubular sodium concentration. It has subsequently been shown by a number of different investigators, using a variety of impermeable solutes, that a limiting gradient of approximately 0.7 exists for sodium within the proximal tubule of the rat (3–7).

It has also been demonstrated in the rat, under free-flow conditions, that tubule fluid to plasma (TF:P) sodium concentration ratios during hypertonic mannitol infusions fall below unity and tend to approach the value of the limiting gradient at the end of the proximal tubule (8, 9). These studies, however, gave no convincing evidence for large reductions in fractional fluid

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reabsorption in the proximal tubule, nor did they indicate the extent to which more distal sites might be involved. Moreover, both Windhager and Giebisch (8) and Ullrich, Schmidt-Nielsen, O'Dell, Pehling, Gottschalk, Lassiter, and Mylle (9) observed that early distal TP:F sodium ratios were, in fact lower during mannitol infusions; thus the actual load of sodium presented to the distal tubule might be little or no greater than in the control antidiuretic state.

Dirks, Cirksena, and Berliner (10) showed, in their study in the dog, that fractional fluid reabsorption in the proximal tubule was readily inhibited by mannitol loads when urine flow exceeded 5% of the glomerular filtration rate. More modest loads failed to significantly alter proximal reabsorption. No tubule fluid electrolyte concentrations were measured in this study which precluded an assessment of fractional sodium handling. The present series of experiments were carried out to study in greater detail the effects of hypertonic mannitol infusions on fractional water and electrolyte reabsorption at both proximal and distal sites within the dog nephron. Our results indicate that the major site of inhibition occurs within the loop of Henle in addition to much smaller effects on the proximal tubule.

METHODS

Acute experiments were performed on 20 mongrel dogs weighing between 10 and 20 kg. The animals were anesthetized with intravenous Pentothal (25 mg/kg) and received supplemental doses during the experiment, as required. A cuffed endotracheal tube was inserted and artificial ventilation with room air was routinely performed, using a Harvard respirator, (Harvard Apparatus Co., Inc., Dover, Mass.). Polyethylene catheters were placed into both foreleg veins, a jugular vein, and a femoral vein and artery. The right ureter was catheterized by a suprapubic incision. The left kidney was freed up on its vascular pedicle via a flank incision, and the left renal artery was catheterized by a 27-gauge needle close to the aorta in a retrograde direction. The left ureter was catheterized close to the hilum and the left kidney prepared for micropuncture as previously described (11). Inulin was infused intravenously after a suitable priming injection to maintain plasma levels of approximately 100 mg/100 ml. Distal tubules were identified by means of the injection of 0.1–0.2 ml of 5% lissamine green dye solution into the renal artery and both proximal and distal puncture sites were marked, using nigrosine dye (12).

Each experiment was carried out in two phases. An initial hydropenic (control) phase usually lasting 60–90 min was performed, during which time several proximal and/or distal tubules were identified and sampled. This was then followed by an intravenous infusion of 16% mannitol in modified Ringer's solution or "saline" (composition: Na 150 mEq/liter, K 3.5 mEq/liter, Cl 133.5 mEq/liter, and HCO_3 20 mEq/liter) at a rate of 12 ml/min until 400–600 cc had been given. This was thereafter continued at 3 ml/min. Additional saline was given to match the urinary losses. When the urine volume had stabilized, recollections of tubule fluid were made from as many of the previously marked sites as possible. Long distal oil blocks were employed to prevent retrograde collection of tubule fluid. Clearance periods of 15 min duration were performed during both phases from the micropuncture kidney. Blood was drawn at the midpoint of each period for the determination of inulin clearance which was used as a measure of glomerular filtration rate.

Urine was analyzed for sodium and potassium on a flame photometer (model 143 Instrumentation Laboratory Inc., Watertown, Mass.). Plasma osmolality was determined on an Advanced Osmometer (Advanced Instruments, Inc., Newton Highlands, Mass.). Urine and plasma inulin concentrations were determined by the anthrone method of Führ, Kaczmarczyk, and Krüttgen (13). The mean inulin recovery from 14 standards containing mannitol (1 g/100 ml), analyzed by the anthrone method, was 99.0% (±4.4% SD). Inulin in tubule fluid samples was analyzed by the fluorometric method of Vurek and Pegram (14), modified to increase the heating time to 10 min. A set of 24 prepared inulin standards, handled as unknowns by this method, gave a mean recovery of 100.4% (±2.3% SD). An additional set of 21 standards containing mannitol (2 g/100 ml) was also determined by fluorometric method and gave a mean recovery of 100.4% (±6.5% SD). A set of 24 plasmas was analyzed simultaneously by both anthrone and fluorometric methods and the fluorometric value expressed as a percentage of the anthrone result. The mean recovery was 98.7% (±4.0% SD) which indicated satisfactory agreement between these two methods. Tubule fluid samples were analyzed for sodium and potassium with a helium-glow photometer[1] by the method of Vurek and Bowman (15). 28 prepared electrolyte standards, handled as unknowns, gave mean recoveries for sodium of 98.8% (±5.3% SD) and for potassium of 97.9% (±9.1% SD). The mean recoveries from 15 standards containing mannitol (2 g/100 ml) were 100.3% (±2.2% SD) for sodium and 100.9% (±13.7% SD) for potassium. The plasma values for inulin, sodium, and potassium which corresponded to the individual tubule fluid samples were interpolated from the values obtained at the midpoint of each clearance period for the determination of TF:P inulin and electrolyte ratios. Arterial blood pressure was monitored from an indwelling femoral arterial catheter by a Statham pressure (Statham Instruments, Inc., Los Angeles, Calif.) connected to a Sanborn recorder (Sanborn Co., Waltham, Mass.). The nonreabsorbed fraction of water, sodium, and potassium was calculated in all tubule fluid samples and in the final urine of all experiments by standard formulas.

RESULTS

Clearance data

In the majority of experiments, paired tubule fluid samples were obtained only from proximal or distal convoluted tubules during the two phases. Since the protocol employed was identical in all cases, and since the over-all effects on urine flow and electrolyte excretion in those experiments in which distal pairs were collected did not differ significantly from those that yielded proximal data, the clearance results from all experiments have been pooled and analyzed together. In each animal, all consecutive clearance periods during

[1] The helium-glow photometer was constructed by Montreal Polycrafters Limited, Montreal, Canada.

TABLE I

*Summary of Plasma Composition, Clearance Data, Urine Flow, and Electrolyte Excretion during Hydropenia and Hypertonic Mannitol Diuresis**

	P_{Na}	P_{Osm}	C_{IN} R	C_{IN} L	V R	V L	$U_{Na}V$ R	$U_{Na}V$ L	$U_{K}V$ R	$U_{K}V$ L	V/C_{IN} R	V/C_{IN} L	FE_{Na} R	FE_{Na} L
	mEq/liter	*mOsm/kg H_2O*	*ml/min*		*ml/min*		*μEq/min*		*μEq/min*		%		%	
Hydropenia	150.7 ±1.2	304 ±2	37.5 ±2.4	35.1 ±2.8	0.52 ±0.07	0.40 ±0.05	76.1 ±9.7	56.5 ±9.8	27.8 ±2.0	28.1 ±2.2	1.58 ±0.27	1.28 ±0.19	1.36 ±0.16	1.06 ±0.15
Mannitol	144.7 ±1.1	364 ±5	26.8 ±2.3	25.2 ±2.0	6.83 ±0.54	6.61 ±0.30	444.6 ±57.9	425.3 ±46.5	60.6 ±5.6	59.0 ±6.8	27.3 ±2.0	28.7 ±2.0	11.5 ±1.2	12.6 ±1.1

Abbreviations: R = right; L = left; P_{Na} = plasma sodium concentration; P_{Osm} = plasma osmolality; C_{IN} = inulin clearance; V = urine flow; $U_{Na}V$ and $U_{K}V$ = urinary excretion of sodium and water; FE_{Na} = fractional excretion of sodium $[(U_{Na}V/P_{Na} \times C_{IN}) \times 100]$.
* Values shown are means ±1 SEM.

which micropuncture was performed were averaged for the two phases for both the right and left kidney. These results are summarized in Table I.

In no case did the clearance results from the right kidney differ significantly from those of the left (micropuncture) kidney. This indicates that the experimental procedures involved in the preparation and performance of micropuncture had a negligible effect on function of the micropuncture kidney. In the subsequent presentation and discussion of the results, the data from the left kidney only have been used.

Striking increases in urine flow and electrolyte excretion occurred in all experiments. Urine flow rose from a mean of 0.40 to 6.61 ml/min, while sodium excretion rose from 56.5 to 425.3 μEq/min. Potassium excretion increased significantly from 28.1 to 59.0 μEq/min. The mean inulin clearance decreased by 27% (35.1–25.2 ml/min) which was highly significant ($P < 0.01$). Plasma osmolality was measured on alternate blood samples on all but the initial two experiments. The mean value during hydropenia was 304 mOsm/kg H_2O and this rose to 364 mOsm/kg H_2O during the mannitol phase. This was accompanied by a small but highly significant decrease in plasma sodium from 150.7 to 144.7 mEq/liter. The fractional excretion rates for sodium and water significantly increased from 1.06 to 12.6% and 1.28 to 28.7%, respectively. Fractional excretion of potassium, not shown in Table I, also rose significantly from 23.8 to 63.9%. The venous hematocrit was measured in 16 experiments and the mean fell significantly from 37.8 to 29.1. The arterial blood pressure remained unchanged throughout the two phases of these experiments.

Micropuncture data

The micropuncture results have been analyzed and presented in terms of the total number of paired tubule fluid samples that were obtained. These results, from both proximal and distal convoluted tubules, are summarized in Tables II and III, and all tubule fluid to plasma inulin and electrolyte ratios are illustrated in Figs. 1–4. We have also analyzed the data using the mean value

TABLE II

*Summary of Tubule Fluid to Plasma (TF:P) Inulin, Sodium, and Potassium Ratios in the Proximal and Distal Tubule during Hydropenia and Hypertonic Mannitol Diuresis**

	Proximal tubule				Distal tubule			
	N	H	M	*P*	N	H	M	*P*
TF:P, inulin	30	1.63 ±0.06	1.45 ±0.04	<0.01	16	5.38 ±0.70	1.94 ±0.15	<0.001
TF:P, sodium	39	0.97 ±0.01	0.93 ±0.01	<0.001	23	0.38 ±0.05	0.59 ±0.05	<0.001
TF:P, potassium	32	1.05 ±0.02	0.98 ±0.02	<0.05	23	1.25 ±0.17	1.05 ±0.08	NS

Abbreviations: N = number of paired tubule fluid samples; H = hydropenia; M = mannitol.
* Values shown are means ±1 SEM.

TABLE III

*Summary of the Nonreabsorbed Fraction (Per Cent) of Filtered Water, Sodium, and Potassium in the Proximal Tubule and Distal Tubule during Hydropenia and Hypertonic Mannitol Diuresis**

	Proximal tubule				Distal tubule			
	N	H	M	P	N	H	M	P
P:TF, Inulin	30	63.7 ±2.4	70.8 ±1.9	<0.001	16	22.6 ±2.4	55.3 ±3.5	<0.001
TF:P: Na/inulin	30	62.3 ±2.4	65.9 ±2.0	NS	16	9.3 ±2.1	35.2 ±4.7	<0.001
TF:P: K/inulin	24	67.3 ±3.1	70.3 ±2.1	NS	16	26.8 ±4.0	52.4 ±6.1	<0.01

Abbreviations: N = number of paired tubule fluid samples; H = hydropenia; M = mannitol.
* Values shown are means ±SEM.

for the two phases from each animal. This method of analysis reduces the number of observations, accordingly. However, the results were initially identical in every case with the analysis using all paired samples and the statistical significance of the differences obtained was essentially unaltered.

Proximal tubule. 39 paired tubule fluid samples were collected from the proximal tubule in 12 experiments and analyzed for sodium concentration. Owing to technical problems, potassium analysis was not performed on seven paired samples from the first two experiments which left only 32 paired TF:P potassium ratios. Because of the larger volume of tubule fluid required for inulin analysis, it was not possible to analyze inulin concentration ratios in nine pairs. A deliberate attempt was made to select late proximal tubule sites for micropuncture by means of lissamine green dye injection in as many instances as possible. The mean TF:P inulin ratio obtained during hydropenia was 1.63 and this fell significantly during mannitol diuresis to 1.45 ($P < 0.01$). These results are illustrated in Fig. 1. The results of TF:P electrolyte ratios are shown in Fig. 2 (sodium) and Fig. 3 (potassium) plotted against their respective inulin concentration ratios as a measure of increasing amounts of fractional fluid reabsorption. The TF:P sodium ratios were clustered around unity during hydropenia with a mean of 0.97. There was a highly significant decrease in the mean to 0.93 during the mannitol phase ($P < 0.01$), which is not so readily apparent when the data are presented in an unpaired fashion, as in Fig. 2. The mean transtubular sodium gradient during mannitol diuresis was 10.4 mEq/liter (±1.3 SE). Potassium concentration ratios showed greater scatter although the results were qualitatively similar to that for sodium. The mean hydropenia value significantly decreased from 1.05 to 0.98 during mannitol loading ($P < .05$).

The mean values for the nonreabsorbed fraction (fractional rejection) of water, sodium, and potassium in the proximal tubule during the two phases are shown in Table III along with corresponding data for the distal tubule. The fractional rejection of water in the proximal tubule increased to a small but highly significant degree from 63.7 to 70.8%. Since this value is based on all of the observations from the proximal tubule, a number of which were probably collected from early proximal sites, this value tends to underestimate the degree of fractional inhibition that would be manifested in the late proximal tubule. Assuming that fractional reabsorption at the late proximal site under hydropenic conditions approximates 50% of the filtrate, then extrapolation of these results

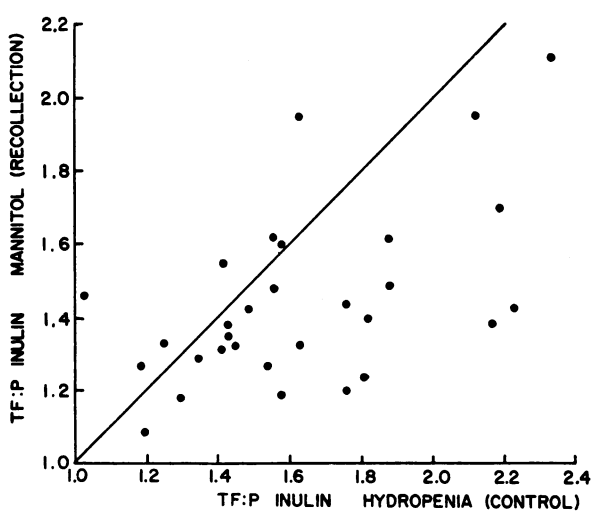

FIGURE 1 Paired proximal tubule fluid to plasma (TF:P) inulin ratios. The results obtained during mannitol diuresis are plotted along the ordinate against the corresponding value obtained during the hydropenic control phase along the abscissa for each tubule sampled.

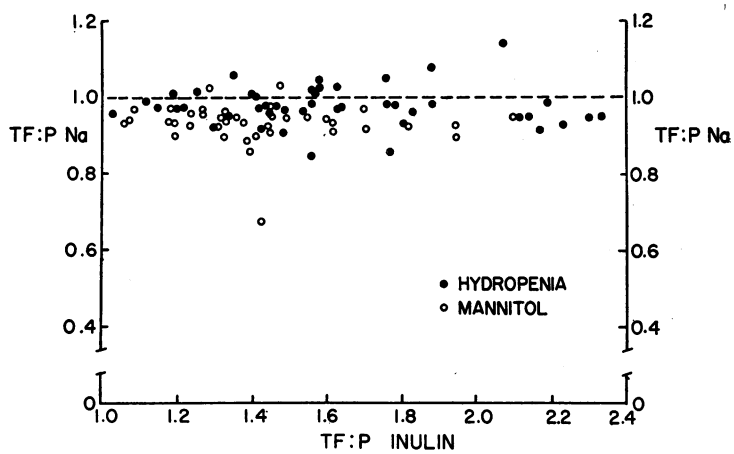

FIGURE 2 Proximal tubule fluid to plasma (TF: P) sodium ratios. The values obtained during mannitol diuresis (shown by the open circles) and hydropenia (closed circles) are plotted against the corresponding tubule fluid to plasma (TF: P) inulin ratio determined in the same sample.

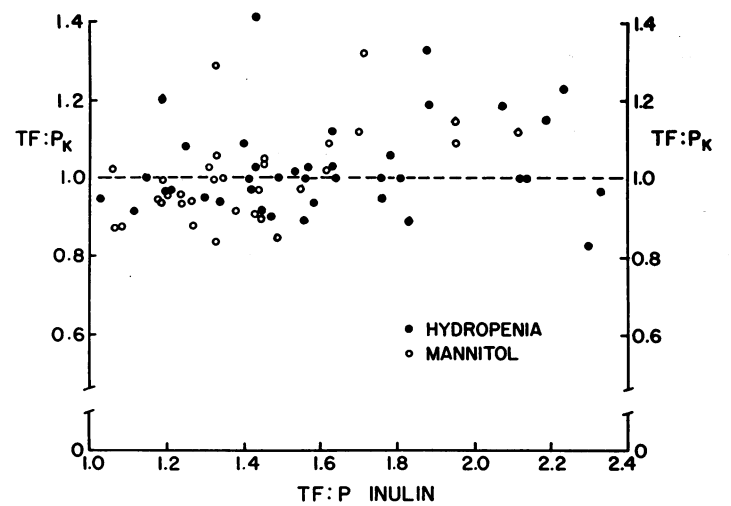

FIGURE 3 Proximal tubule fluid to plasma (TF: P) potassium ratios. The values obtained during mannitol diuresis (open circles) and hydropenia (closed circles) are plotted against the corresponding tubule fluid to plasma (TF: P) inulin ratios determined in the same sample.

increases this estimate of fractional rejection at the late proximal tubule during mannitol diuresis to approximately 10% (i.e. from 50 to 60%). Fractional rejection of sodium and potassium increased, although only by approximately 4%, which was not significant since the reduction in TF: P sodium and potassium ratios partly compensated for the reduced water reabsorption. A similar calculation to that for water would increase this value by only an additional 1–2% in the late proximal tubule. It is apparent that the increases in urinary fractional excretion rates for both water (28%) and sodium (12%) were considerably greater than the degree of fractional inhibition observed in the proximal tubule.

Distal tubule. Since distal recollections have not yet been reported in the dog, we performed recollections of distal tubule fluid samples during a continuous hydropenic phase in a number of experiments. The mean initial TF: P inulin ratio in 34 paired samples was 3.79 ±0.21 (SEM), which did not differ significantly from the recollection value of 3.56 ±0.18. This change represents a 2% decrease in fractional fluid reabsorption.

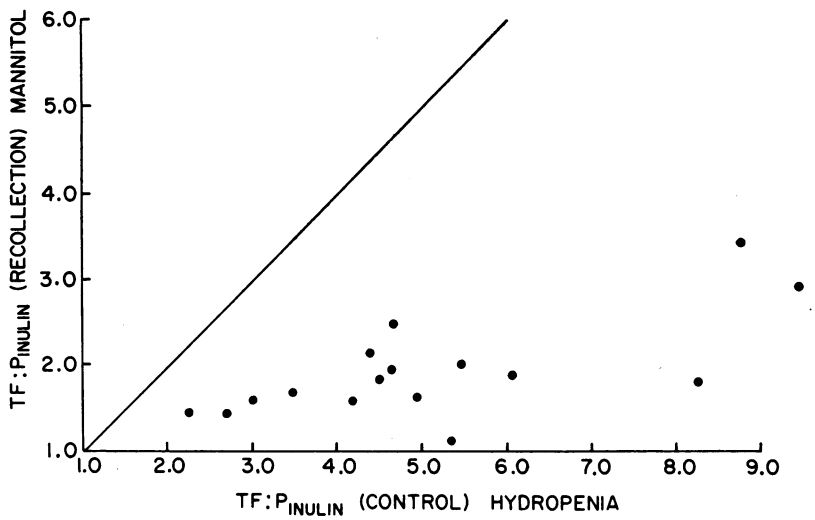

FIGURE 4 Paired distal tubule fluid to plasma (TF:P) inulin ratios. Values obtained during mannitol diuresis are plotted along the ordinate against the corresponding value during hydropenia along the abscissa for each tubule sampled.

Distal electrolyte ratios in 17 paired samples were also essentially unaltered. Initial mean TF:P sodium and potassium ratios were 0.35 ±0.04 and 1.15 ±0.19 compared with the recollection values which were 0.37 ±0.04 and 1.05 ±0.14, respectively. Landwehr, Schnermann, Klose, and Giebisch (16) have previously reported similar distal control data in the rat.

In contrast to the small effects observed in the proximal tubule, mannitol loading resulted in striking changes in distal inulin and electrolyte ratios. These data are summarized in Table II. The results of 16 paired distal TF:P inulin ratios obtained in 11 experiments are illustrated in Fig. 4. A marked drop occurred in every instance. The mean value fell from 5.38 during hydropenia to 1.94 during the diuretic phase ($P < 0.001$). Distal electrolyte ratios were obtained from an additional seven puncture sites, making a total of 23 pairs. The TF:P sodium ratios, depicted in Fig. 5, increased after manni-

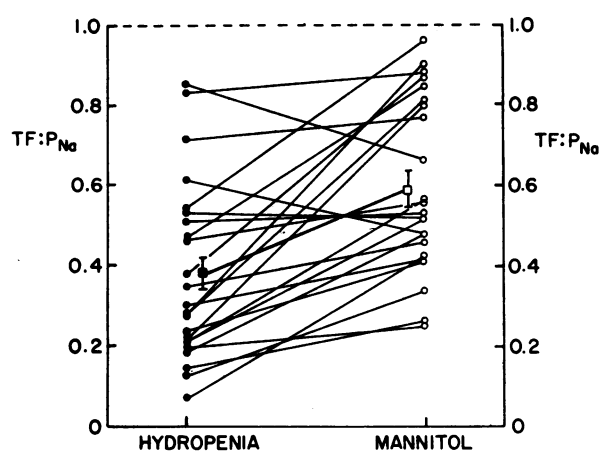

FIGURE 5 Paired distal tubule fluid to plasma (TF:P) sodium ratios. The values obtained during hydropenia (shown on the left in closed circles) are joined to the corresponding value during mannitol diuresis (shown on the right by open circles) for each tubule sampled. The means ±1 SEM are shown by the open and closed squares joined by the dark line

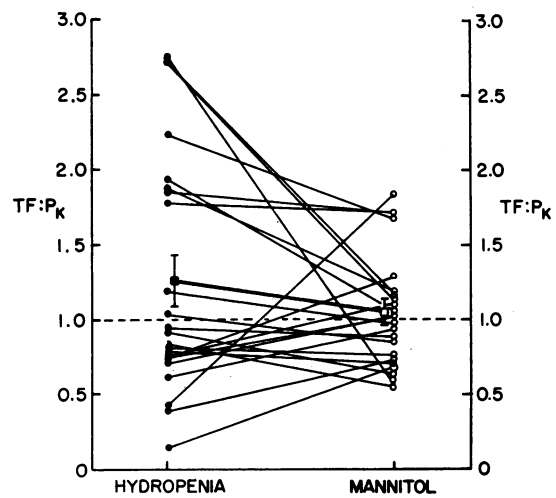

FIGURE 6 Paired distal tubule fluid to plasma (TF:P) potassium ratios. The values obtained during hydropenia (shown on left in closed circles) are joined to the corresponding value during mannitol diuresis (shown on the right by open circles) for each tubule sampled. The means ±1 SEM are shown by the open and closed squares joined by the dark line.

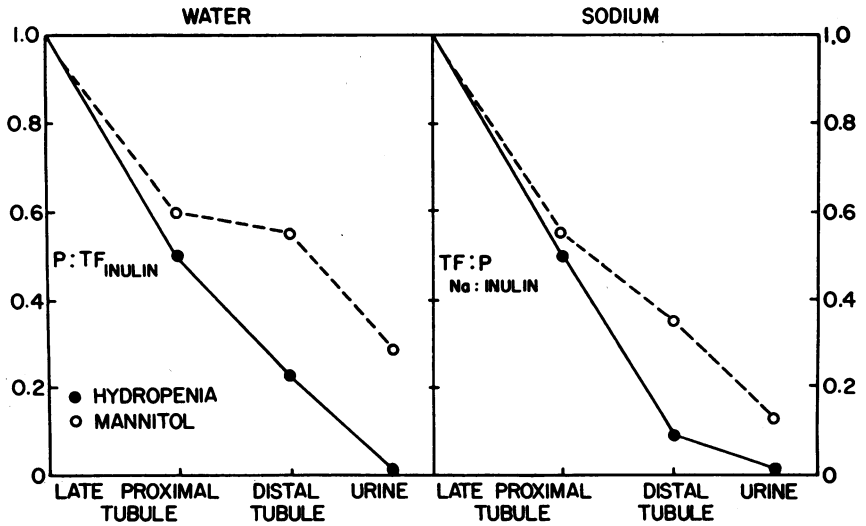

FIGURE 7 Fractional rejection of water and sodium at successive nephron sites during hydropenia (solid line) and mannitol diuresis (dashed line). The values shown for the proximal tubule are estimated values for the late proximal tubule (see text). Values shown for distal tubule are the means of all distal tubule fluid samples; those for the urine were determined from the mean clearance data from the micropuncture kidney in all experiments.

tol in all but two instances. The mean value in the mannitol phase of 0.59 was significantly higher than the mean hydropenic value of 0.38 ($P < 0.001$). The distal sodium ratios during the mannitol phase were also significantly higher than the corresponding final urine to plasma sodium ratios (mean = 0.40 ±0.03, $P < 0.01$). Distal TF:P potassium ratios showed a considerable range during hydropenia (0.15–2.75). There was a noticeable tendency for these ratios to approach unity during the mannitol phase, as illustrated in Fig. 6, and the range was correspondingly reduced (0.56–1.82).

The mean values for the nonreabsorbed fraction of filtered water, sodium, and potassium in the distal tubule are summarized in Table III along with the corresponding values from the proximal tubule. It will be noted that the fractional rejection of water had increased by 32% at the distal tubule during the mannitol phase and that for sodium and potassium by almost the same extent (26%). These large increases provide a striking contrast to the small increases that were observed in the late proximal tubule. It should also be noted that fractional urinary excretion of water in these experiments was augmented by approximately the same extent (27%) as in the distal tubule. Fractional reabsorption of water in the collecting ducts, therefore, appears to be similar during the two phases. Fractional excretion of sodium in the urine, however, increased by only 12% which indicates that substantial further reabsorption of sodium occurs within the collecting duct. These changes in the fractional rejection of sodium and water at different nephron sites are illustrated graphically in Fig. 7. Rejection of potassium at the distal tubule averaged 52% during the mannitol phase, compared with a urinary rejection of 64%. Since the mean distal value probably includes both early and late distal sites, it is not possible to estimate whether additional secretion of potassium has taken place in the collecting duct or not.

We have used the foregoing results to estimate the fraction of filtered sodium and water reabsorbed at various nephron segments, as well as the absolute rate of reabsorption and the reabsorbed fraction of the load presented to each segment. These results are summarized in Table IV. The figures for the proximal tubule were based on estimates of fractional reabsorption in the late proximal tubule. The figures for the distal tubule and urine were based on the means of all observations. The mean inulin clearance and plasma sodium concentration of all experiments were used to estimate the filtered loads of water and sodium. They demonstrate the far greater inhibition of water and electrolyte reabsorption in the proximal tubule, considered in absolute terms rather than in fractional amounts of the filtered load. Note that while sodium reabsorption in the loop of Henle is inhibited to a considerable degree, water reabsorption is almost completely abolished. However, since sodium reabsorption during hydropenia is proportionately far greater than water reabsorption, the increase in sodium delivery to the distal tubule which results during mannitol diuresis is proportionately larger than the increase in water delivery (see Table III). This thus accounts

TABLE IV

*Estimates of Fractional and Absolute Reabsorption of Water and Sodium at Successive Nephron Segments during Hydropenia and Hypertonic Mannitol Diuresis**

Nephron segment	Fraction of filtered load reabsorbed				Fraction of segment load reabsorbed				Calculated absolute reabsorption			
	Water		Sodium		Water		Sodium		Water		Sodium	
	H	M	H	M	H	M	H	M	H	M	H	M
	%		%		%		%		*ml/min*		*uEq/min*	
Proximal tubule	50	40	50	45	50	40	50	45	17.6	10.1	2645	1641
Henle's limb and early distal tubule	27	5	41	20	54	8	82	36	9.6	1.2	2153	722
Late distal tubule and collecting duct	21	27	8	22	96	47	89	63	7.5	6.8	440	820
Totals	98	72	99	87	—	—	—	—	34.7	18.1	5238	3183

* Abbreviations: H = hydropenia; M = mannitol.

for the rise in TF : P sodium ratios in the distal tubule. From the amounts of water and sodium reabsorbed in the loop, it is apparent that the concentration of sodium in the reabsorbate is greatly increased during mannitol (602 mEq/liter) compared with the hydropenic value (224 mEq/liter).

The absolute amount of water, as well as the fraction of the filtrate reabsorbed beyond the distal tubule within the collecting system, was essentially unchanged during the diuretic phase. However, in terms of the load presented to this segment, which was greatly increased, there was approximately a 50% reduction of reabsorption. Sodium reabsorption, here too, was inhibited to a lesser degree. The absolute rate of sodium reabsorption almost doubled.

DISCUSSION

The present studies place in perspective the changes in fractional salt and water reabsorption that occur at various nephron segments during hypertonic mannitol infusions. Our results indicate that the diuresis is mediated in large part by inhibition of sodium and water transport within the loop of Henle and to a lesser extent by changes within the proximal tubule.

The small extent of proximal inhibition that was observed in this study is of some interest. The decrease in fractional reabsorption, though significant, was less than that seen when extracellular fluid volume was expanded by saline infusions (12). It is also apparent that the fractional inhibition within the proximal tubule after mannitol loading was less than the increased fractional excretion of sodium and water in the urine, in contrast to the effects of saline loading where far less of the proximal rejection fraction appears in the final urine (12). This difference speaks strongly for the role of nephron segments beyond the proximal tubule in determining over-all diuretic effects. It is evident that expressing the results of mannitol in terms of fractional changes masks the large decrease in absolute sodium reabsorption that did occur, since there was a 30% decrease in the filtered sodium load. Koch, Dume, Krause, and Ochwadt (17) have demonstrated in the rat that the reduction in filtration rate during a mannitol diuresis results from an increase in the intratubular pressure which leads to a fall in effective filtration pressure. The drop in filtration rate thus produced may thereby limit the decrease in fractional reabsorption that might otherwise have occurred.

It is reasonable to ascribe the inhibition of salt and water transport within the proximal tubule to the osmotic effects of mannitol within the tubular lumen, as originally suggested by Wesson and Anslow (1). The small extent of the transtubular sodium gradient that developed, due to the retention of relatively nonreabsorbable solute, may initially seem surprising in view of the previously cited studies in the rat (8, 9). However, this results from the smaller degree of fractional reabsorption in the dog proximal tubule than in the rat. If one assumes that mannitol contributed 70 mOsm/kg H_2O to the total plasma osmolality and a mid-proximal TF : P inulin ratio of 1.45, then the maximum transtubular mannitol gradient would be 31 mOsm/kg H_2O. The small but finite permeability of the tubular epithelium to mannitol would reduce this gradient to 20–25 mOsm/kg H_2O. The maximum transtubular sodium gradient that could be created while maintaining isosmotic conditions across the tubule would be 10–12 mEq/liter which is in agreement with the observed gradient (10.4 mEq/liter). The possibility that retrograde collection of tubule fluid occurred during the diuretic phase due to high intratubular pressure should be considered. This would contaminate tubule fluid samples with fluid down-

stream to the collecting pipette and thus result in a smaller reduction of fractional reabsorption at the point of collection. The internal consistency of the inulin and and sodium ratios referred to above, together with the fact that TF : P inulin ratios fell in almost all instances, makes this extremely improbable.

Analysis of the distal inulin data indicated that a profound depression of water reabsorption occurs between the late proximal and distal tubule. We have inferred that most of this decrease takes place within the loop of Henle. It is generally thought that only a small fraction of the filtrate is reabsorbed from the pars recta of the proximal tubule. Moreover, Burg and Orloff have shown in rabbit kidney tubules that fluid transport in the pars recta is considerably less than in the pars convoluta (18). Furthermore, Bennett, Clapp, and Berliner (19) have shown that the distal convoluted tubule of the dog reabsorbs only a small proportion of the filtered water (~8%) and sodium (~3%) under hydropenic conditions. It is probable that even less is reabsorbed under the conditions of hypertonic mannitol diuresis. Therefore, using the mean value of all distal samples will overestimate loop changes to the slight extent that fractional reabsorption takes place in the pars recta and early distal tubule. It is not possible as yet in the dog to estimate the extent of fractional reabsorption within each limb of the loop of Henle. In view of the lesser permeability to water in the ascending limb, it seems likely that most of the water reabsorption in the loop takes place in the descending limb, and therefore, the inhibitory action of mannitol is largely manifested at this point in the nephron. This would readily result from both the osmotic effect of mannitol within the tubular lumen and to dissipation of the medullary osmotic gradient which occurs during mannitol diuresis (20–22).

The distal electrolyte data indicate that fractional sodium reabsorption within the loop was also markedly retarded owing to the large reduction in fluid reabsorption, coupled with a small decrease in the distal sodium gradient. Distal sodium ratios, under normal hydropenic conditions, are thought to be close to the value of the limiting gradient (19). The addition of nonreabsorbable solute should, therefore, of itself produce no change in this electrolyte ratio or lower the ratio if initially higher than the limiting gradient. The latter situation appears to occur in the rat where early distal sodium ratios are lower after mannitol loading than under hydropenic conditions (8, 9). This difference implies that the distal tubule of the dog is less permeable to water than that of the rat. This conclusion is also supported by the finding that tubule fluid remains hypotonic to plasma throughout the distal tubule of the dog (23). The fact that sodium ratios were significantly higher after mannitol loading in the dog indicates an additional effect on sodium transport apart from its osmotic action within the tubular lumen. The mechanism responsible for this is not clear. It is possible that this reflects a limitation of sodium transport in the ascending limb at high rates of flow through the loop of Henle (24, 25) or is the result of an impairment of active sodium transport in the ascending limb due to altered interstitial factors which affect peritubular capillary absorption, evidence for which has been presented in the proximal tubule (26).

While these findings of a large depression of fractional sodium and water reabsorption within the loop of Henle apply specifically to the superficial cortical nephrons, the possibility that cortical nephrons are not representative of the entire nephron mass must be considered. It has recently become evident that two major functionally distinct groups of nephrons, cortical and juxtamedullary, exist in the rat (27–29). It is not yet clear to what extent such functional differences are present in the dog. Liebau, Levine, and Thurau (30) have suggested that superficial nephron filtration rates in the dog correlate to a high degree with the over-all kidney filtration rate, and furthermore, are consistent with a homogeneous nephron population. The nephron mass in the dog, however, cannot be estimated with the same degree of certainty as in the rat. Preliminary data from a recent study in the dog (31), moreover, indicate that superficial nephron filtration rates increased to a greater extent after saline infusions than did the over-all kidney filtration rate, suggesting that differences probably exist between cortical and juxtamedullary nephrons in the dog as well as in the rat. Since cortical nephrons appear to account for the majority of the nephron population and such nephrons receive a greater proportion of the filtrate during states of extracellular volume expansion, then estimates of nephron function based on cortical nephrons during such conditions are unlikely to be in large error. Obviously, more precise information will be needed before this question can be fully settled.

Finally, the relevance of the present micropuncture study to clearance studies of mannitol infusions must be considered. A number of studies in the dog (22, 32–34) have shown that large increases in both free water clearance (C_{H_2O}) and reabsorption ($T^c_{H_2O}$) occur during mannitol administration, and such data have been used to infer that increased sodium reabsorption occurs within the loop of Henle in response to a large increase in delivery of filtrate from the proximal tubule. These studies, therefore, appear to be at variance with our data which indicate that the predominant site of inhibition is located within the loop of Henle. It is certainly possible that differences in juxtamedullary and cortical nephrons exist which could account for discrepancies when analysis of cortical nephrons is applied to over-all

kidney clearance measurements. There are a number of reasons, however, which support the view that this conflict is probably more one of interpretation than a reflection of a real difference in experimental results. It must be noted that the experimental conditions of free water clearance studies are totally different from those of the present study and therefore extrapolation of our data to such studies is unwarranted. Even so, these data are not inconsistent with large increases in free water excretion, since the major inhibitory effects are exerted before the diluting segment. A large increase in load to the ascending limb, as a result of both proximal tubule and descending limb inhibition will readily lead to large increases in free water excretion despite a small limitation of the transtubular sodium gradient.

The conditions of the present study more closely resemble those used for $T^c_{H_2O}$ studies. Free water reabsorption, as conventionally measured ($C_{osm} - V$), is a valid estimate of the amount of water reabsorbed in the collecting system, and thus considered to be an indirect assessment of sodium transport in the loop, only in so far as two conditions are met: (*a*) tubule fluid reaching the collecting duct is isosmotic and (*b*) osmolar reabsorption in the collecting duct is negligible. Neither of these conditions, however, prevails in the dog. Clapp and Robinson (*23*) have shown that tubule fluid remains uniformly hypotonic to plasma throughout the length of the distal convoluted tubule, and this was subsequently confirmed by Bennett, Brenner, and Berliner in the rhesus monkey (35). Moreover, the studies of Bennett et al. (19), as well as the present study, have indicated that a considerable fraction of the filtered sodium load is reabsorbed in the collecting duct. Both these factors lead to a gross underestimation of collecting duct reabsorption of water by the $T^c_{H_2O}$ formula. During osmotic diuresis, it is likely that the errors thus introduced become less as tubule fluid osmolality rises and the fraction of the filtered solute entering the collecting duct that is reabsorbed there decreases. Measured $T^c_{H_2O}$ may thus rise appreciably even though absolute volume reabsorbed may not have changed or even decreased. Certainly, on the basis of present micropuncture evidence in the dog, it is hazardous to draw any definite conclusions about the function of the loop of Henle from measured changes in $T^c_{H_2O}$. Our data which suggest that large inhibitory effects occur within the loop, moreover, seem more consistent with the finding that strong osmotic diuresis leads to almost total dissipation of the osmotic gradient in the papilla (*20–22*). Clearly, further studies specifically designed to correlate intratubular events with over-all measurements of C_{H_2O} or $T^c_{H_2O}$ under the appropriate experimental conditions are needed.

A comparison of the findings of the present study with the results of extracellular volume expansion in the dog offers perhaps the strongest support for our conclusions. Isotonic saline loading consistently leads to a greater fractional inhibition within the proximal tubule, despite much smaller net diuretic effects than did mannitol loads which resulted in far greater fractional excretion rates of sodium and water. It follows, therefore, that proximal tubule inhibition of itself is insufficient cause for the massive diuretic effects of hypertonic mannitol infusions.

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