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*J Clin Invest.* 1973;52(10):2548-2555. <https://doi.org/10.1172/JCI107446>.

**Research Article**

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# Effect of Extracellular Volume Expansion upon Sodium Reabsorption in the Distal Nephron of Dogs

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**ABSTRACT** Micropuncture studies have disclosed that extracellular fluid (ECF) volume expansion inhibits sodium reabsorption in the proximal tubule. The diuresis that ensues represents only a portion of the increment in sodium and water escaping proximal reabsorption, since a large and variable fraction of the increment is reabsorbed distally. In certain experimental models proximal reabsorption may be depressed by ECF volume expansion, yet only a negligible amount of sodium appears in the final urine. This suggests that saline diuresis is the consequence of depressed distal sodium reabsorption. Previous clearance and micropuncture studies have not conclusively proven this. Eight dogs were studied repeatedly: in some studies glomerular filtration rate and distal delivery were increased markedly without sodium administration; in others comparably high distal sodium loads were achieved by progressive 1/2 isotonic saline infusion.  $C_{H_2O}$  at high distal sodium loads was depressed by expansion of the ECF volume with hypotonic saline. The difference in free water formation between dogs which did and did not receive hypotonic saline was accounted for by the difference in sodium excretion. In one dog hypotonic saline expansion failed to depress free water formation; likewise the level of natriuresis in this dog was severely attenuated. The results of these experiments provide strong evidence that the natriuresis that occurs following ECF volume expansion with saline is a consequence of alteration in function of the distal nephron.

This work was presented in part at the American Society of Nephrology, Washington, D. C. 1969.

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Received for publication 22 February 1973 and in revised form 15 May 1973.

## INTRODUCTION

Extracellular fluid (ECF)<sup>1</sup> volume expansion with sodium containing fluids apparently evokes at least two qualitatively different changes in sodium handling by the renal tubules. In dogs and rats acutely expanded with iso-, hypo-, or hypertonic saline or Ringer's solutions, reabsorption of sodium and water in the proximal tubules is depressed. Most evidence suggests that this is a decrease in the absolute in addition to the fractional rate of net sodium transfer at this nephron site (1, 2). A large fraction of the increment in sodium and water delivered out of the proximal tubules is reabsorbed in Henle's loop, distal tubule and collecting ducts (hereafter referred to as "distal nephron"), and a variable amount is excreted. However, it also has been shown that sodium reabsorption in the proximal tubule may be depressed, without or with a significantly attenuated natriuresis (2-12). It has been hypothesized that the extent of natriuresis following volume expansion is a function of changes in the activity of transport site in the nephron distal to the proximal tubule. Evidence derived from clearance studies, utilizing free water clearance ( $C_{H_2O}$ ) as an index of sodium reabsorption in the diluting segment, has been conflicting; some studies have shown that volume expansion depressed the rate of increase in sodium reabsorption as load increases (13-16), and other more recent studies have failed to show this (17, 18). The use of mannitol or acetazolamide to increase distal delivery in many of these studies makes the data difficult to interpret, since both of these

<sup>1</sup> Abbreviations used in this paper:  $C_{H_2O}$ , free water clearance;  $C_{In}$ , inulin clearance;  $C_{Na}$ , sodium clearance;  $C_{osm}$ , osmolar clearance; ECF, extracellular fluid;  $FE_{Na}$ , fractional sodium excretion; GFR, glomerular filtration rate;  $P_{Na}$ , plasma sodium concentration;  $P_{osm}$ , plasma osmolality;  $U_{K}V$ , urinary potassium excretion;  $U_{Na}V$ , urinary sodium excretion;  $U_{osm}$ , urinary osmolality; Vol, urine flow.

agents affect distal sodium reabsorption (19, 20). In some it is difficult to determine whether or not there was a significant difference in volume expansion between the control and "expanded" groups (14, 17).

Microperfusion studies of the distal tubule in hydroperic and volume expanded animals have failed to disclose a depression of sodium reabsorption in Henle's loop which could be attributed to volume expansion per se (4, 21-24). However, it is not possible to determine whether ECF volume expansion might not have depressed the rate at which sodium reabsorption increases as load increases, since "distal load" could not usually be measured with accuracy. Furthermore it was not possible to increase equally the distal load in control and expanded animals.

The present studies were designed to reexamine the problem, using clearance techniques and free water excretion as an index of sodium reabsorption in the entire distal nephron. In some experiments glomerular filtration rate and distal delivery were increased markedly without sodium loading; these were compared with others in which distal delivery was increased by administering 1/2 isotonic saline. Care was taken to measure sodium balance and each dog, acting as its own control, was studied several times. The results indicate that ECF volume expansion per se has a depressive effect upon sodium reabsorption in the diluting sites in the distal nephron.

## METHODS

The studies were performed on eight female mongrel dogs, weighing 15-25 kg; the dogs were fed regular commercial diets. Measurement of free water clearance was performed under anesthesia induced by sodium pentothal. The dogs were deprived of food but not water for the 24 h preceding the study. The bladder was catheterized under anesthesia, the dog was placed on an electric heating pad and the temperature was constantly monitored and kept at 37-39°C. A total of 25-30 ml/kg of 2.5% dextrose in water was infused over a period of 45-60 min. After an appropriate loading dose, a sustaining infusion of inulin in 2.5% dextrose in water, containing potassium chloride 36 mmol/liter was infused at 2 ml/min throughout the remainder of the experiment. When a brisk water diuresis (urine osmolality less than 75 mosm) was instituted the sustaining infusion was begun and the first urine collection was initiated. The sustaining infusion consisted of 1/2 isotonic saline in the case of the saline expansion studies or 2.5% dextrose in water in the case of the no saline expansion studies. The rate of the sustaining infusion was set at 5-10 ml/min in excess of urine flow and increased by 3-5 ml/min at the end of each urine collection. Urine collections were 10 min in duration, and collections were made at 30-45 min intervals until urine volume reached a maximum or began to fall.

In the first series of experiments (group 1), five dogs were studied a total of 18 times. In nine studies (group 1A) 1/2 normal saline was the sustaining infusion without any other preparation. The same five dogs were also studied nine times (group 1B), during which delivery to the distal

nephron was increased without administration of saline. Following one or two control urine collections, in nine studies 20 mg of dexamethasone (Decadron Phosphate, Merck, Sharp & Dohme, West Point, Pa.) was administered intravenously and two urine collections were made after 1 h. In five studies (five dogs), following the dexamethasone collections, dopamine was administered at a rate of 5-10 µg/min per kg, and two more urine collections were made 30 min after beginning this infusion. These experiments were then terminated. In two studies (two dogs) following the dexamethasone collections, 100 ml of 25% salt-poor human albumin was administered over 10-15 min, and two urine collections were made after 1/2 h. Following these urine collections dopamine was begun at 10 µg/min per kg and two more urine collections were made after 30 min. The order of the A and B studies was randomized.

In preparation for a second series of paired experiments (group 2), on two occasions each of three dogs was given 40 mg of depo methylprednisolone (Depo-Medrol; The Upjohn Company, Kalamazoo, Mich.) on days 1 and 3, and four cans high protein dog food daily (days 1-4). On the morning of day 5, each dog was given two cans of dog food approximately 1-2 h prior to anesthesia. Similar to group 1 studies, in random order each dog either received 1/2 isotonic saline as a sustaining infusion, (group 2A), or received 2.5% dextrose in water, and in sequence, dexamethasone, albumin, and dopamine (group 2B).

After administration of each agent, at least two, and sometimes three urine collections were made before another agent was administered. Venous blood was collected at the midpoint of each urine collection. These studies usually lasted 3-5 h. The bladder was carefully emptied before and at the end of each urine collection, by manipulation or air washout. At the conclusion of each study small doses of iron-dextran complex (Imferon; Lakeside Laboratories, Inc., Milwaukee, Wisc.) based on body weight, estimated blood loss and hematocrit were administered i.m. The bladder was irrigated with a neomycin solution and the animal was placed in a heated recovery room. The animals were not restudied for 1-2 wk and all studies were accomplished over a period of 4-5 mo.

Urine and plasma were analyzed for osmolality (Advanced Osmometer; Advanced Instruments, Inc., Newton Highlands, Mass.) and sodium and potassium (Flame Photometer; Instrumentation Laboratory, Inc., Lexington, Mass.). Inulin was measured using the anthrone method which was approximately 100 times more sensitive to fructose than to glucose. Plasma inulins were maintained in the range of 60-80 mg/100 ml. Plasma and urine glucose was measured in most dogs by a glucose oxidase method (25), and the inulin values were corrected in instances where glucose introduced greater than a 2-3% error in the inulin values.

*Calculations.*  $C_{H_2O} = Vol - C_{osm}$ , where Vol is urine flow in milliliters per minute and  $C_{osm} = (U/P)_{osm} \times Vol$ . Since all studies were paired, and each dog was his own control, neither  $C_{H_2O}$ , urine volume, nor  $C_{osm}$  were factored by the glomerular filtration rate (GFR). In methylprednisolone-treated dogs (group 2) sodium delivery to the distal nephron was considered to be more closely approximately by  $C_{H_2O} + C_{Na}$  rather than by Vol.

## RESULTS

Table I shows a representative experiment on a dog in group 1B that received dexamethasone, albumin, and dopamine. Table II shows a representative experiment

TABLE I  
Representative No Saline Expansion Study

Dog no.	Time	GFR	Vol	U <sub>osm</sub>	C <sub>osm</sub>	C <sub>H<sub>2</sub>O</sub>	C <sub>H<sub>2</sub>O</sub> /Vol	U <sub>Na</sub>	U <sub>Na</sub> V	U <sub>K</sub> V	FE <sub>Na</sub>	Plasma			Na balance
												Osm	Na	K	
		ml/min	ml/min	mosm	ml/min	ml/min		meq/liter	μeq/min	μeq/min		mosm	meq/liter	meq/liter	meq
27 (23.6 kg)	9:07-10:40	Water load: 460 cc 2.5% DW													
	9:45	Inulin prime: start Inulin + 36 mM KCl in 2.5% DW @ 2 ml/min													
	10:40			54											
	10:40-11:48	2.5% DW 6 cc/min													
	10:52-11:02	85.0	4.2	69	1.01	3.19	0.76	16.3	68.46	16.38	0.56	287	143.5	3.5	
	10:58	Decadron 29 mg i.v.													
	11:48-12:58	2.5% DW 10 cc/min													
	12:33-12:43	95.4	9.0	76	2.43	6.57	0.73	27.3	245.7	85.5	1.82	281	141.3	3.7	-43
	12:43-12:53	109.7	12.1	57	2.46	9.64	0.80	19.5	236.0	108.9	1.52				
	12:57- 1:22	100 cc 25% albumin i.v.													
	12:58- 1:00	2.5% DW 15 cc/min													
	1:50- 2:00	116.9	15.4	27	1.43	13.97	0.91	9.3	143.2	110.9	0.86	289	140.5	3.8	-57
	2:00- 2:10	112.3	15.7	30	1.63	14.07	0.90	9.5	149.2	122.5	0.95				
	1:00- 2:13	2.5% DW 14 cc/min													
	2:13- 2:42	14.2 mg dopamine in 900 cc 2.5% DW 16 cc/min (10 μg/kg per min)													
	2:42- 3:10	Dopamine in 2.5% DW 18 cc/min													
2:50- 3:00	119.8	18.6	38	2.40	16.20	0.87	9.0	167.4	85.6	0.97	294	142.0	3.7	-63	
3:00- 3:10	107.8	17.0	48	2.77	14.23	0.84	9.5	161.5	79.9	1.05					

DW, dextrose in water.

on a dog in group 1A that was infused with 1/2 normal saline. The studies in group 2 were similar to those outlined in Table I and II with the exception that the dogs were preloaded with methylprednisolone several days prior to the experiment.

*Group 1 studies.* One dog (no. 26) behaved in a manner different from the other four and data from three studies on this dog are treated separately. Table III shows that the initial C<sub>in</sub>, the initial urine osmolality and sodium concentration were similar in the remaining four dogs whether they were infused with saline (1A) or infused with 2.5% dextrose in water (1B). The

average increase in C<sub>in</sub> after saline infusion was 43%; after dexamethasone administration alone, the mean increase was 26% and after dexamethasone and dopamine the increase averaged 44%. The increases in C<sub>in</sub> after albumin in two dogs were 6% and 20%. After saline infusion, the urine osmolality rose to 119, there was a marked natriuresis with the urine sodium excretion ranging from 660 to as high as 1,373 μeq/min. Initial fractional sodium excretion averaged 0.61% (0.2-1.5% range); final averaged 7.2% (4.2-11.0%). Sodium balance at the end of the experiment ranged between +72 and +153 meq. As urine volume increased to a maxi-

TABLE II  
Representative Saline Expansion Study

Dog no.	Time	GFR	Vol	U <sub>osm</sub>	C <sub>osm</sub>	C <sub>H<sub>2</sub>O</sub>	C <sub>H<sub>2</sub>O</sub> /Vol	U <sub>Na</sub>	U <sub>Na</sub> V	U <sub>K</sub> V	FE <sub>Na</sub>	Plasma			Na balance
												Osm	Na	K	
		ml/min	ml/min	mosm	ml/min	ml/min		meq/liter	μeq/min	μeq/min		mosm	meq/liter	meq/liter	meq
24 (15.9 kg)	9:00	19.1 cc inulin prime; start inulin + 36 mM KCl in 2.5% DW @ 2 ml/min													
	9:05-10:22	Water load: 500 cc 2.5 DW													
	10:23-10:40	½ N saline 4 cc/min													
	10:30-10:40	76.0	5.5	42	0.80	4.70	0.85	8.0	44.0	17.6	0.41	290	137.3	3.7	
	10:40-11:21	½ N saline 10 cc/min													
	11:10-11:20	67.8	8.0	46	1.28	6.72	0.84	19.3	154.4	56.0	1.60	288	141.8	4.0	
	11:21-12:01	½ N saline 14 cc/min													
	11:50-12:00	78.8	11.2	76	2.96	8.24	0.74	35.5	397.6	99.7	3.62	288	139.3	4.2	
	12:01-12:50	½ N saline 19 cc/min													
	12:32-12:42	90.3	13.2	96	4.40	8.80	0.67	46.0	607.2	124.1	4.78	288	140.5	3.9	+70
	12:50- 1:56	½ N saline 22 cc/min													
	1:34- 1:44	94.5	18.0	134	8.42	9.58	0.53	60.5	1089	144.0	8.17	287	141.0	3.7	+80
	1:56- 3:23	½ N saline 28 cc/min													
	2:40- 2:50	108.5	21.2	118	8.78	12.42	0.59	41.8	886	84.8	5.87	285	138.8	3.6	+160

TABLE III  
Mean Values for Group 1 (4 Dogs) and Group 2 (3 Dogs) Experiments

	Initial GFR	Initial $U_{osm}$	Initial $U_{Na}V$	Highest $U_{Na}V$	$P_{osm}$	$P_{Na}$
	ml/min/kg	mosm	$\mu\text{eq}/\text{min}$	$\mu\text{eq}/\text{min}$	mosm	meq/liter
$\frac{1}{2}$ N saline infusion						
Group 1A (7)*	4.0	45	67	1,065	290	142.5
Group 2A (3)*	5.15	31	50	(700-2,600)	293	143.0
Medrol						
No saline infusion						
Group 1B (8)*	3.9	47	61	108	289	141.5
Group 2B (3)*	4.9	40	115	193	299	143.0
Medrol						

\* Number in parentheses refers to number of experiments.

imum value, indicating increasing delivery of sodium to the diluting segment, free water clearance tended to plateau at an average value of somewhat less than 10 ml/min (Fig. 1). The difference between  $C_{H_2O}$  and Vol was accounted for almost entirely by  $C_{Na}$  (> 95%). Fractional reabsorption of sodium in the diluting segment, as estimated by  $C_{H_2O}/\text{Vol}$ , fell from an initial value of 0.85 to an average value of 0.59. Urine potassium excretion increased from 28 to 110  $\mu\text{eq}/\text{min}$  during the saline infusion. Fig. 2 shows that in the same dogs (1B studies), over a similar range of urine volumes, free water clearance increased with a steeper and more linear slope, to levels as high as 16 ml/min. As urine volumes increased during these experiments without saline infusion, mean urine osmolality actually fell to 41 mosm; urine sodium concentration did not increase. Urine sodium excretion increased minimally; fractional excretion increased from 0.4 to 0.7%. Fractional reabsorption in the diluting segment ( $C_{H_2O}/\text{Vol}$ ) actually increased on the average from 0.83 to 0.85. In neither of the two dogs did urine sodium excretion increase after receiving albumin; indeed both  $U_{osm}$  and  $U_{Na}$  decreased in the hour after albumin. In 1B studies the sodium balance was negative in every instance from -9

to -63 meq, toward the end of the experiment. Note the striking difference in  $C_{osm}$  depicted in Figs. 1 and 2.

Fig. 3 shows the results of three studies in one dog (no. 26) that had a much less profound natriuresis during the saline infusion, and showed no tendency to reach a maximum value of  $C_{H_2O}$  as volume increased. After saline infusion the highest sodium excretion in this dog was 370 and 260  $\mu\text{eq}/\text{min}$  in two studies. As can be seen from Fig. 3, the increase in free water clearance with increasing urine volume was the same whether or not saline was infused. With respect to initial  $U_{osm}$  and  $U_{Na}$ , and initial  $C_{Na}$  (4.1 ml/min per kg), the studies in this dog were similar to the other studies.

Fig. 4 shows the best fit lines for the relationship between free water excretion and urine volume in group 1 dogs. The equation for the line representing the saline expansion studies is  $y = 1.016 + 0.762x - 0.013x^2$ . This is significantly different from the line representing the studies in which no saline was infused  $y = 0.881x - 0.32$ .

*Group 2 studies.* In these three dogs prior treatment with methylprednisolone resulted in higher initial  $C_{Na}$  (Table III) but initial urine osmolality and urine sodium excretion (0.4%) was similar to group 1 dogs.

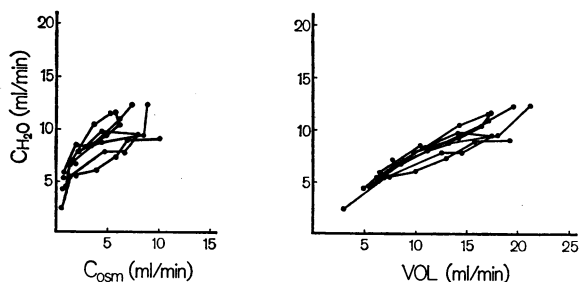


FIGURE 1 Saline expansion studies in four dogs. (1A)  $C_{H_2O}$  excretion estimates  $\text{Na}^+$  reabsorption, Vol estimates  $\text{Na}^+$  delivery to the distal nephron.

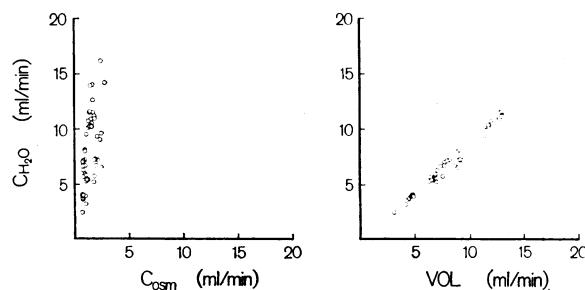


FIGURE 2 Studies in same four dogs, without saline expansion (1B).

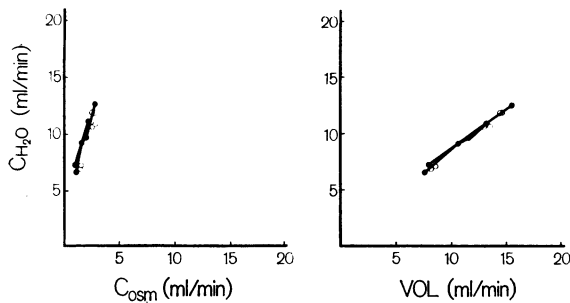


FIGURE 3 Studies in one dog (no. 26). Connected closed circles are during saline expansion; open circles are during no saline expansion.

In saline-loaded dogs (2A)  $U_{Na}V$  reached maximum levels of 653, 1,740, and 2,617  $\mu\text{eq}/\text{min}$ ; this represented fractional excretion of 4, 12, and 16%. In 2B dogs  $U_{Na}V$  rose little (Table III). Fig. 5 shows the clearance data. Since in some of the collections there was glucosuria, particularly at very high rates of 2.5% dextrose infusion, distal nephron sodium delivery was estimated by  $C_{H_2O} + C_{Na}$ . In 2B studies, free water clearance increased in a linear fashion with respect to distal delivery. Moreover at high levels of sodium delivery free water clearance was significantly higher in the non-saline expansion studies (2B) than in the saline expansion studies (2A). For 2A studies,  $y = 1.38 + 0.90x - 0.015x^2$ ; for 2B studies,  $y = 1.89 + 0.77x$ . At the highest distal delivery rates in 2B ( $V > 15$  ml/min),  $C_{H_2O}$  averaged 6.3 ml/min per kg.

## DISCUSSION

Numerous studies of the renal response to acute sodium loads have focused attention primarily on the proximal tubule as the site where both fractional and absolute rates of sodium reabsorption are decreased. However, it is apparent that only a portion of the increment in sodium and water escaping reabsorption proximally appears in the final urine (1). Therefore the inescapable

conclusion is that reabsorption is increased at some site distal to the proximal tubule. There is every reason to believe that this increment in sodium load is reabsorbed all along the distal nephron (ascending limb of the loop of Henle, and distal convoluted tubule and the collecting ducts), since it has been shown that active sodium reabsorption occurs at all of these sites (26). The natriuresis which occurs after acute sodium loads could be explained most simply by postulating incomplete reabsorption of the increment delivered to these sites without suggesting that there are specific mechanisms for adjusting the distal transport rates. However, in 1968 Howards, Davis, Knox, Wright, and Berliner (3) demonstrated that both saline infusions and hyperoncotic albumin or dextran infusions equally depressed proximal fractional reabsorption, whereas sodium excretion increased significantly only during saline infusion. This finding, confirmed by three groups of investigators (2, 4, 5), strongly suggested that the natriuresis that occurs after acute saline loads is a consequence of the alteration of transport function at some site or sites distal to the proximal tubule. Other studies have shown, in a variety of experimental models, that acute saline loads may be accompanied by depression of proximal reabsorption without, or with significantly impaired natriuresis (6-12). In the published reports of such studies there is nearly unanimous agreement that the distal nephron is an important site of regulation of sodium excretion.

Nevertheless in direct studies of the distal nephron it has been extremely difficult to demonstrate that ECF volume expansion per se alters sodium reabsorption at these sites. Clearance studies during water diuresis, using urine flow as an estimate of delivery to, and free water clearance as an estimate of sodium reabsorption in the distal nephron, have been inconclusive (13-18). Perhaps this is due largely to the experimental models employed. Studies comparing mannitol-induced, with saline-induced increases in distal delivery are difficult

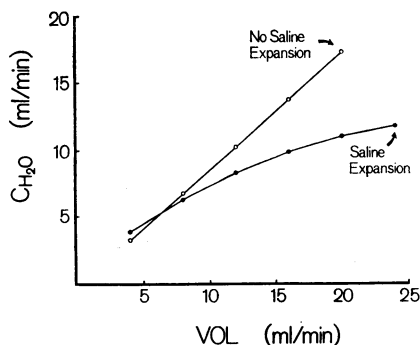


FIGURE 4 Best fit regression lines for group 1 dogs: (1A) given saline; (1B) no saline.

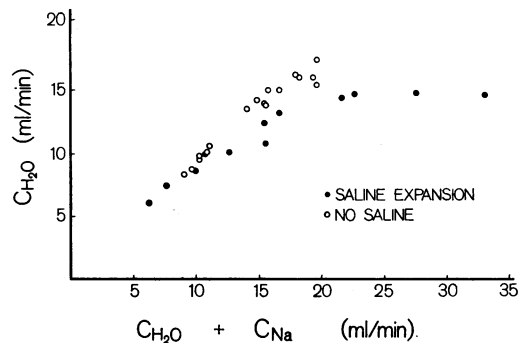


FIGURE 5 Six studies in three dogs preloaded with methylprednisolone.

to interpret, since mannitol itself is known to depress sodium reabsorption in the distal nephron (19). In one widely quoted study, at high rates of urine flow, the maximum  $C_{H_2O}$  in dogs receiving mannitol or saline, by inspection of the graphs, appeared nearly the same (13). Other studies of free water clearance have purported to compare dogs minimally, with those maximally ECF volume expanded, without showing any significant difference between groups, either in ECF volume or sodium balance (14, 16–18). For example, studies in which prior administration of desoxycorticosterone acetate was used to produce a group “maximally expanded,” compared with a “minimally expanded” control group are difficult to interpret (14, 17). This is because during the actual experiment, dogs which did and did not receive DOCA were so massively volume loaded with hypotonic saline, that it seems unlikely that there was a significant difference in ECF volume or sodium balance between the two groups. One study showed a rise in urine sodium concentration after saline infusion, not after albumin infusion, without making any comparison of distal sodium reabsorption at similar distal sodium loads (15).

The present work carefully documents the fact that near and at the end of each study, sodium balance was markedly positive when the dogs were saline expanded (group 1A) and markedly negative when they were not saline expanded (group 1B). Moreover each dog was studied several times in random order, so that saline expansion versus non-saline expansion was compared in each of four dogs. Initial  $C_{In}$  was similar in A and B studies; therefore it is presumed that the relationship between filtered sodium loads and the number of functioning distal nephrons, i.e., the mass of tubules participating in the diluting process, was constant over the few weeks that elapsed during the repeated studies in the same dogs. That is, the potential reabsorptive capacity of the distal nephrons was similar each time the same dog was studied, whether or not it received saline. It should be noted that the problem of comparing animals or subjects of different body weights, kidney weights, and thus sodium reabsorptive capacity of the distal nephrons, cannot be eliminated by factoring by  $C_{In}$ , in studies in which  $C_{In}$  is initially abnormal or is altered by the experimental protocol. This is because  $C_{In}$  or GFR no longer necessarily bears a relationship to the renal mass or to the “capacity” of the distal nephrons. Therefore it is imperative, as we have done in these studies, to use each animal as its own control, so that the studies are matched not only with respect to distal sodium load but with respect to total distal sodium reabsorptive capacity.

It has been shown at low levels of sodium delivery, that distal sodium reabsorption is proportional to load

(27). This presumably is because at low loads, net sodium reabsorption in the loop of Henle and beyond is limited only by the sodium concentration gradient that develops. Our own studies show that at low levels of delivery, the increases in  $C_{H_2O}$  with increases in urine flow are nearly the same whether or not the animal has received saline. The curves that fit the regression of  $C_{H_2O}$  on urine flow from 1A and 1B studies separate significantly only at high levels of urine flow. This phenomenon could be due to the fact that at low flows (early in each study), the difference in Na balance between A and B studies was small; whereas near the end of the study there was greater volume expansion, and thus greater difference in sodium balance between A and B studies. Alternatively, it could be that the depression of distal transport function induced by ECF volume expansion only can be measured at levels near maximum transport. The data from the present study do not answer this question.

Micropuncture and microperfusion studies of the loop of Henle and the distal tubule during ECF volume expansion have failed to show depression of sodium reabsorption; indeed micropuncture studies have shown markedly increased sodium reabsorption after saline expansion when compared to hydropenic animals (4, 21–24). However, nothing in the results of the present study is contrary to such findings;  $C_{H_2O}$  increased markedly as distal load increased in both group 1A and group 1B. Unfortunately none of the micropuncture studies have measured sodium reabsorption in the loop and distal tubule during continued hydropenia compared to that after expansion of the ECF volume, at comparably high sodium loads. A microperfusion study by Morgan and Berliner in rats showed that sodium reabsorption in the loop continued to increase in a linear fashion as perfusion was increased from 5 to 40 nl/min (28), i.e., no  $T_m$  was reached. Yet when measuring the effect of saline infusion, they perfused the loop of Henle only at 10–20 nl/min, leaving them vulnerable to the criticism that they were grossly undersaturating the loop transport sites. This may explain why they failed to detect a suppression in the reabsorption after systemic saline infusion.

Our own study shows that in group 1, “fractional reabsorption” in the distal nephron, as estimated by  $C_{H_2O}/Vol$ , fell during saline expansion (1A) but at comparable loads, did not fall in the studies without saline expansion. In these studies it is reasonable to assume that all of the distal nephrons, deep and superficial, from the tips of the loop of Henle to the ends of the collecting ducts, are participating in the generation of free water (29–32). This brings into sharp focus the potential problem with rat micropuncture studies; of necessity only the function of the short loops of Henle and distal tubules

of superficial nephrons can be measured. Therefore the results of rat micropuncture studies previously published, probably are not comparable to the present results; the differences in results and conclusions cannot be explained further. It is of interest that in some micropuncture studies the authors have concluded that ECF volume expansion must have depressed reabsorption beyond the distal sites punctured (23, 24).

Although it is clear that four dogs in group 1 showed a depression of  $C_{H_2O}$  formation when infused with saline, one dog did not behave in this manner. Dog no. 26 had a nearly linear increase in free water formation as load increased and this was the same whether or not the animal received saline. Also it is quite apparent that this dog had a markedly attenuated natriuresis after saline loading; the fractional sodium only reached a little over 2% in contrast to the average of other group 1 dogs which was 7.2%. In absolute amounts the sodium excretion in dog no. 26 averaged 300  $\mu\text{eq}/\text{min}$ , compared with the average in the other four dogs of 1,031  $\mu\text{eq}/\text{min}$ . This phenomenon, reproduced twice, is not easily explained. However, it does show that the magnitude of natriuresis is determined by the degree to which free water formation in the distal nephron is depressed.

The studies in dogs preloaded with methylprednisolone (group 2) show that massive amounts of glucocorticoids given chronically do not alter the depression of  $C_{H_2O}$  formation at high levels of distal delivery which occurs after saline expansion. This is consistent with recent reports that methylprednisolone had no effect on sodium or water reabsorption in the kidney (33, 34). However because only three studies were performed it is not possible to be certain that the glucocorticoids chronically administered did not cause some increase in the capacity to reabsorb sodium in the diluting sites, thus perhaps somewhat diminishing the effect of saline.

In summary these studies provide strong evidence that the so-called "diluting segment" (which probably encompasses the entire distal nephron from the tip of loop of Henle through the collecting ducts), plays a prominent regulatory role in the control of sodium excretion. Furthermore, at high sodium and water loads to this distal nephron, expansion of the extracellular fluid volume with saline significantly inhibits the increase in sodium reabsorption that normally occurs as load increases. It follows therefore that the natriuresis occurring following saline expansion, is a consequence of alterations in the functional properties of the distal nephron, presumably in the ability to transport sodium.

#### ACKNOWLEDGMENTS

I am grateful for the excellent technical assistance of Ms. Lynn Eddy, Mr. Thomas Hines, Ms. Audrey Hines, and Mr. Junious Ingram.

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