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Research Article

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Effects of Increased Sodium Delivery on Distal Tubular Sodium Reabsorption with and without Volume Expansion in Man

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ABSTRACT The separate effects of volume expansion and of increased delivery of sodium on sodium reabsorption in the diluting segment of the distal nephron were studied in man. In six normal subjects during a sustained water diuresis, sodium delivery to the distal nephron was increased without volume expansion by the administration of acetazolamide. In these subjects, free water clearance rose linearly as a function of urine flow. In five patients with complete, central diabetes insipidus, distal sodium delivery was increased by the infusion of hypertonic saline during a sustained water diuresis. In four of these five patients, changes in free water clearance were also observed during hypertonic saline diuresis in the presence of distal blockade of sodium reabsorption with chlorothiazide. At high rates of distal delivery the following observations were made: (a) free water clearance was lower and fractional sodium excretion higher during saline diuresis compared to acetazolamide diuresis; (b) although free water clearance was moderately reduced by chlorothiazide at low rates of urine flow, there was no difference in free water clearance between saline loading alone and saline plus chlorothiazide at high rates of urine flow; and (c) during saline loading free water clearance rose without evidence of a limit when increased distal delivery was accompanied by spontaneous increases in glomerular filtration rate, but tended toward a limit when glomerular filtration rate remained constant.

The data indicate that during acute volume expansion with saline, there is a decrease in the fraction of delivered sodium reabsorbed in the distal nephron when compared to the response of the distal nephron to comparable increases in distal sodium delivery in the absence of volume expansion.

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INTRODUCTION

Recent studies of the renal mechanisms of sodium excretion have focused attention on the proximal tubule as the site of an important regulatory function. It has become increasingly apparent, however, that the transport systems in the more distal parts of the nephron are of critical importance in determining the final rate of sodium excretion (1-4). In dogs acutely expanded with saline, Dirks, Cirksena, and Berliner demonstrated a decrease in the fraction of filtered sodium reabsorbed in the proximal tubule (1). However, only a portion of the sodium escaping reabsorption proximally was excreted, indicating increased reabsorption of sodium in more distal sites. Similarly, micropuncture studies in rats have shown that saline loading is associated with a decreased fractional reabsorption of filtered sodium proximally and increased reabsorption in the loop of Henle (3, 4). The magnitude of saline diuresis during saline loading is therefore determined to a large extent by the fraction of the sodium escaping proximal reabsorption which is reabsorbed in the distal nephron. This conclusion has recently received firm support from the study of Howards, Davis, Knox, Wright, and Berliner (5) in the dog which showed that large reductions in proximal tubular sodium reabsorption produced by albumin infusion were associated with little or no natriuresis.

The factors which affect sodium reabsorption in the distal nephron are poorly understood. In hydropenic rats acutely expanded with saline and mannitol, clearance and micropuncture studies have suggested that a relatively constant fraction of sodium delivered to the loop of Henle is reabsorbed with no evidence of a limit to sodium transport up to the highest rates of sodium delivery attainable in the intact animal (4, 6-9). A linear relationship between rate of delivery and sodium reab-

sorption has also recently been shown in single perfused loops of Henle in rats (10). Similarly, in hypertonic man during hypertonic saline diuresis, there is no apparent maximum for sodium transport in the loop of Henle (11). Recently, however, evidence has been presented suggesting that during volume expansion sodium reabsorption in the diluting segment of the nephron in the dog may be inhibited and that under some circumstances sodium reabsorption in this segment tends toward a limit (12).

The present study was undertaken to evaluate the effects of volume expansion on sodium reabsorption in the distal nephron in hydrated man. It is generally accepted that dilute urine is formed in the mammalian kidney by a process of active sodium transport out of water impermeable segments of the loop of Henle and early distal tubule and that during water diuresis free water clearance (C_{H_2O}) has been used as an index of sodium reabsorption at the diluting sites. Attempts to study changes in C_{H_2O} during saline diuresis in normal man have been hampered by the fact that infusions of hypotonic saline solutions do not produce a large diuresis (13, 14). Therefore, for this study we, employed patients with diabetes insipidus in whom hypertonic saline could be used to produce a natriuresis uncomplicated by ADH secretion. Changes in C_{H_2O} in these subjects were studied during saline diuresis alone and during saline diuresis superimposed on the administration of chlorothiazide (CTZ), a diuretic which inhibits a portion of the distal diluting mechanism (15). In addition, the separate effects of increased delivery and volume expansion on the entire distal diluting segment were examined by comparing the effects of saline loading on C_{H_2O} to those produced by the administration of acetazolamide, an inhibitor of proximal tubular sodium and bicarbonate reabsorption.

METHODS

Five patients with complete, central diabetes insipidus were subjects for the studies with hypertonic saline loading alone and in combination with chlorothiazide. The diagnosis of complete diabetes insipidus was established by demonstrating an inability to raise urine osmolality above 80 mOsm/kg in response to a standardized water deprivation test (16). None of the patients had any evidence of renal disease by the usually accepted criteria and their mean glomerular filtration rate (GFR) was 98 ml/min (range 82–110). R. A. had a pinealoma with both anterior and posterior pituitary insufficiency and was on adequate steroid hormonal replacement therapy at the time of study. The other four subjects had idiopathic central diabetes insipidus. All subjects were studied in the Clinical Research Center of the Hospital of the University of Pennsylvania and were given a diet containing at least 100 mEq of sodium per day and water ad lib.

Saline diuresis studies. Changes in the rate of C_{H_2O} were measured during hypertonic saline diuresis in four of the patients once, and twice in a fifth patient, according to the following protocol. The patients were allowed to drink

fluids, but food was withheld for 12 hr before the study. A maximum water diuresis was induced by the oral administration of 20 ml/kg of tap water acutely and maintained by replacement of urine volume plus 0.8–1.0 ml/min for insensible losses. After a steady-state water diuresis had been established consisting of three consecutive collection periods during which the rate of urine flow did not vary by more than 1.5 ml/min, an infusion of 3% saline was begun intravenously with a constant infusion pump, initially at a rate of 10–12 ml/min. The infusion rate was progressively increased to a maximum of 15 ml/min during the study in order to maintain a rising urine flow rate. Urine was collected through an indwelling Foley catheter, with air washout to insure complete collections. Informed consent was obtained from each subject. Urine cultures obtained 24 hr, 48 hr, and 1 wk after each study all revealed no growth of organisms. Appropriate amounts of inulin and para-aminohippuric acid (PAH) were given as a loading dose and in a sustaining infusion of isotonic saline at a rate of 1 ml/min. Urine was collected at 10- to 20-min intervals during periods of lower urine flows and as often as every 5 min during the later periods of very high urine flows. Blood was collected at approximately 1 hr intervals and immediately preceding any change in the rate of saline infusion. These studies will be referred to in the subsequent discussion as "saline studies."

Chlorothiazide-saline diuresis studies. In five studies in four of the above five patients, saline diuresis was induced during a sustained diuresis produced by chlorothiazide (CTZ). The protocol for these studies was basically the same as outlined above for the saline studies. After the institution of a maximal, sustained water diuresis, a priming dose of CTZ of 500 mg was given intravenously, and a constant infusion of 250 mg/hr was begun. This procedure caused a rise in urine flow and sodium excretion; and when a steady state of urine flow had been established at a new level, hypertonic saline was given as described. These studies will be referred to in the subsequent discussion as "CTZ-saline studies."

Acetazolamide diuresis studies. In six normal volunteers, the effects of acetazolamide on C_{H_2O} were studied. After a maximum sustained water diuresis was obtained, 500 mg of acetazolamide was given intravenously in a single dose, and the acute effects were observed on urine flow, urine osmolality, C_{H_2O} , and inulin clearance.

Blood and urine specimens were analyzed for osmolality, inulin, PAH, sodium, and potassium by methods previously reported (17). The rates of urine flow (V), inulin clearance (C_{in}), PAH clearance (C_{PAH}), sodium and potassium clearance (C_{Na} and C_K), and osmolar clearance (C_{osm}) were obtained by standard calculations. Values for C_{H_2O} were calculated according to the formula: $C_{H_2O} = V - C_{osm}$. Standard statistical techniques were employed (18).

RESULTS

Saline diuresis studies. Results of the saline studies in each of the five subjects are shown in Fig. 1. Both C_{H_2O} and C_{H_2O} factored for GFR are plotted as a function of V in each patient. In two patients (R.A. and L.G.), C_{H_2O} clearly tended toward a limit at high rates of V . A protocol of the study for patient R.A. is shown in Table I. V increased from 17.1 to 38.8 ml/min as a result of the saline infusion. C_{H_2O} rose initially from a control value of 14.5 ml/min but approached an apparent limit of approximately 19.5 ml/min over a range of V

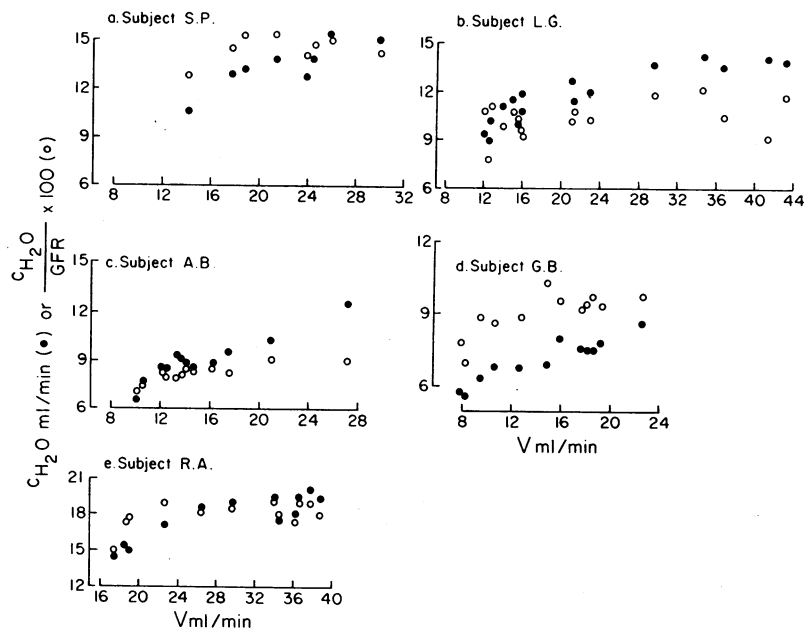


FIGURE 1 Urine flow (V) versus free water clearance (C_{H_2O}), closed circles, and V versus C_{H_2O} factored for inulin clearance (C_{In}), open circles, in each of five subjects during hypertonic saline loading superimposed upon a maximal sustained water diuresis.

from 34 to 38.8 ml/min. C_{In} fell from a mean of 106 ml/min during the control period to a low of 86 ml/min during the first 30 min of saline infusion and then returned to control levels as V increased from 26.5 to 38.8 ml/min.

In the three remaining subjects (A.B., G.B., and S.P.), C_{H_2O} tended to rise without evidence of a limit, even at high rates of V . (A second saline study performed in A.B. gave identical results qualitatively to the

TABLE I
Saline Diuresis Study in R.A.*

Time	V	C_{Osm}	C_{H_2O}	C_{In}	C_{H_2O}/C_{In}	U_{NaV}	C_{Na}/C_{In}	C_{PAH}
min	ml/min	ml/min	ml/100 ml of GFR.	$\mu Eq/min$	%	ml/min		
20 ml/kg of water by mouth followed by replacement of urine volume plus estimated insensible loss								
0-14	18.1	2.9	15.2	106	14.3	256	1.7	683
14-25	17.8	2.8	15.0	104	14.4	237	1.6	650
25-34	19.5	2.9	16.6	108	15.4	236	1.6	658
40	Begin infusion of 3% NaCl							
34-49	17.1	2.6	14.5	96	15.1	197	1.5	657
49-63	18.6	3.2	15.4	88	17.5	285	2.3	688
63-73	19.0	3.8	15.2	86	17.7	383	3.1	606
73-85	22.6	5.4	17.2	91	18.9	597	4.5	742
85-96	26.5	7.9	18.6	102	18.2	963	6.5	780
96-110	29.6	10.7	18.9	101	18.7	1384	9.3	805
110-119	34.0	14.6	19.4	106	18.3	1914	12.1	852
119-125	37.7	17.7	20.0	107	18.7	2374	14.8	855
125-130	36.6	17.2	19.4	104	18.7	2506	15.9	809
130-135	34.5	16.9	17.6	98	18.0	2413	16.1	740
135-140	38.8	19.4	19.4	108	18.0	2820	16.9	818

* Abbreviations: V = urine flow; C_{Osm} , C_{H_2O} , C_{In} , and C_{PAH} = solute, free water, inulin, and PAH clearances, respectively; U_{NaV} = sodium excretion rate.

TABLE II
Chlorothiazide-Saline Diuresis Study in L.G.*

Time	V	C _{osm}	C _{H₂O}	C _{In}	C _{H₂O} /C _{In}	U _{Na} V	C _{Na} /C _{In}	C _{PAH}
min	ml/min	ml/min	ml/100 ml of GFR	μEq/min	%	ml/min		
0-10	17.4	8.6	8.8	98	9.0	985	7.0	385
10-20	19.5	8.8	10.7	114	9.4	1100	7.2	436
20-30	17.8	8.4	9.4	101	9.3	991	6.9	416
35	Begin 3% NaCl infusion at 12 ml/min							
30-40	17.7	8.2	9.5	102	9.3	993	6.9	415
40-50	20.0	9.2	10.8	112	9.6	1140	7.1	455
50-60	18.6	8.5	10.1	114	8.9	1065	6.5	431
70-80	21.0	9.7	11.3	132	8.6	1296	7.2	405
90-100	22.0	10.3	11.7	126	9.4	1376	7.5	512
100-110	21.0	9.9	11.1	111	9.9	1365	8.2	489
110-120	23.6	11.6	12.0	124	9.8	1590	8.7	513
120-130	28.3	14.7	13.6	122	11.5	2103	11.5	493
130-140	25.0	13.8	11.2	131	8.5	1975	9.9	452
140-150	30.5	17.1	13.4	128	10.5	2537	13.0	564
150-160	34.8	20.3	14.4	123	11.7	3061	16.2	552
160-170	35.7	21.3	14.4	131	10.9	3155	15.6	556
170-180	39.1	24.3	14.8	134	11.1	3678	17.7	605
180-190	44.1	29.0	15.1	135	11.1	4515	21.4	596
190-200	47.6	31.9	15.7	133	11.8	5095	24.5	610
200-210	48.8	33.2	15.6	157	9.9	5175	21.1	657
210-220	50.2	33.1	17.1	153	10.8	5268	22.1	683

* Abbreviations same as Table I.

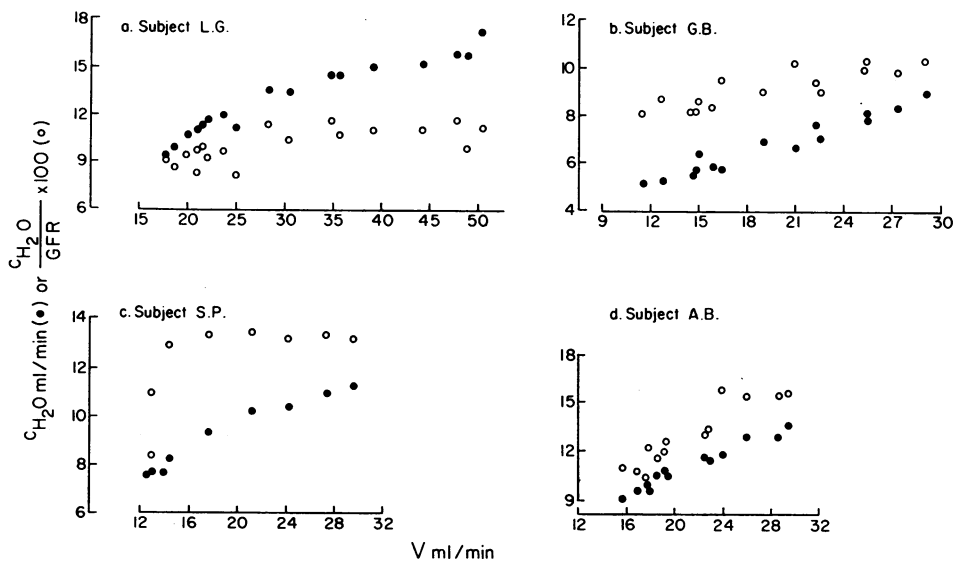


FIGURE 2 Urine flow (V) versus free water clearance (C_{H_2O}) and V versus C_{H_2O}/C_{In} in each of four subjects receiving hypertonic saline during the continuous administration of chlorothiazide. Symbols are the same as Fig. 1.

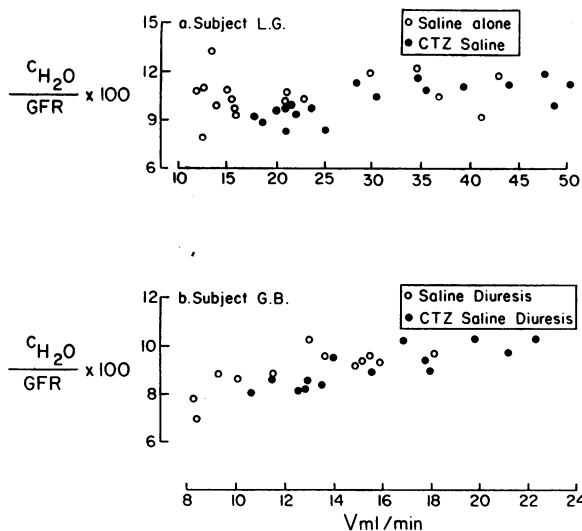


FIGURE 3 A comparison of a saline study, open circles, and a chlorothiazide-saline study, closed circles, in subjects L.G. and G.B.

study shown in Fig. 1.) Since in these latter four studies, at high rates of V a spontaneous rise in GFR occurred which was proportional to the rise in C_{H_2O} , C_{H_2O} factored for GFR reached an apparent limit in each case. Therefore, in all saline studies, C_{H_2O}/GFR remained constant at high rates of V .

CTZ-saline studies. A protocol of a typical CTZ-saline study is shown in Table II for patient L.G. The infusion of hypertonic saline superimposed upon a continuous CTZ diuresis resulted in a rise in V from 18–20 to 50 ml/min and in C_{H_2O} from 9–10 to 17.1 ml/min

with no evidence of a limit of C_{H_2O} . The rise in V from 35 to 50 ml/min was accompanied by a spontaneous rise in C_{In} in a step-wise fashion from 125 to 155 ml/min. As a result, C_{H_2O}/C_{In} approached an apparent limit of 11–12 ml/100 ml of GFR over a range of V from 28 to 50 ml/min. A similar result was obtained in four studies performed in three subjects (two studies were performed in S.P.) illustrated in Fig. 2 where values for both C_{H_2O} and C_{H_2O}/C_{In} are plotted against V for each subject individually. C_{H_2O} rose without evidence of a limit; however, GFR rose proportionally to C_{H_2O} at high rates of V so that C_{H_2O}/C_{In} reached an apparent limit in every subject. Thus in all of the studies of both saline and CTZ-saline, the results were qualitatively similar when C_{H_2O} was factored by GFR at rates of V exceeding approximately 16 ml/min.

During the CTZ-saline studies, sodium transport was inhibited in a portion of the diluting segment of the distal nephron (15). Fig. 3 shows C_{H_2O}/C_{In} plotted against V in patients L.G. and G.B. during saline and CTZ-saline diuresis. It can be seen that, although C_{H_2O}/C_{In} is lower initially in the CTZ-saline study in each subject, at very high rates of V the saline and CTZ-saline studies are indistinguishable. This was also true for the other two subjects in which paired saline and CTZ-saline studies were performed. Thus during saline infusion alone at high rates of V the portion of the distal nephron which is functionally responsive to CTZ appears to make little or no contribution to C_{H_2O} .

Acetazolamide diuresis studies. In six normal subjects undergoing a sustained water diuresis, acetazolamide administration was followed by a linear rise in C_{H_2O} as a function of V as illustrated in Fig. 4. In these

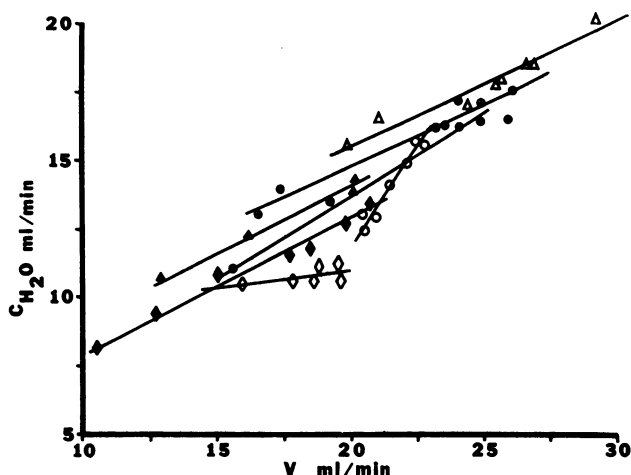


FIGURE 4 Urine flow (V) versus free water clearance (C_{H_2O}) after a single dose of 500 mg of acetazolamide in six normal subjects undergoing a maximal, sustained water diuresis. Each symbol signifies one study in one subject. One subject was studied twice on two separate occasions.

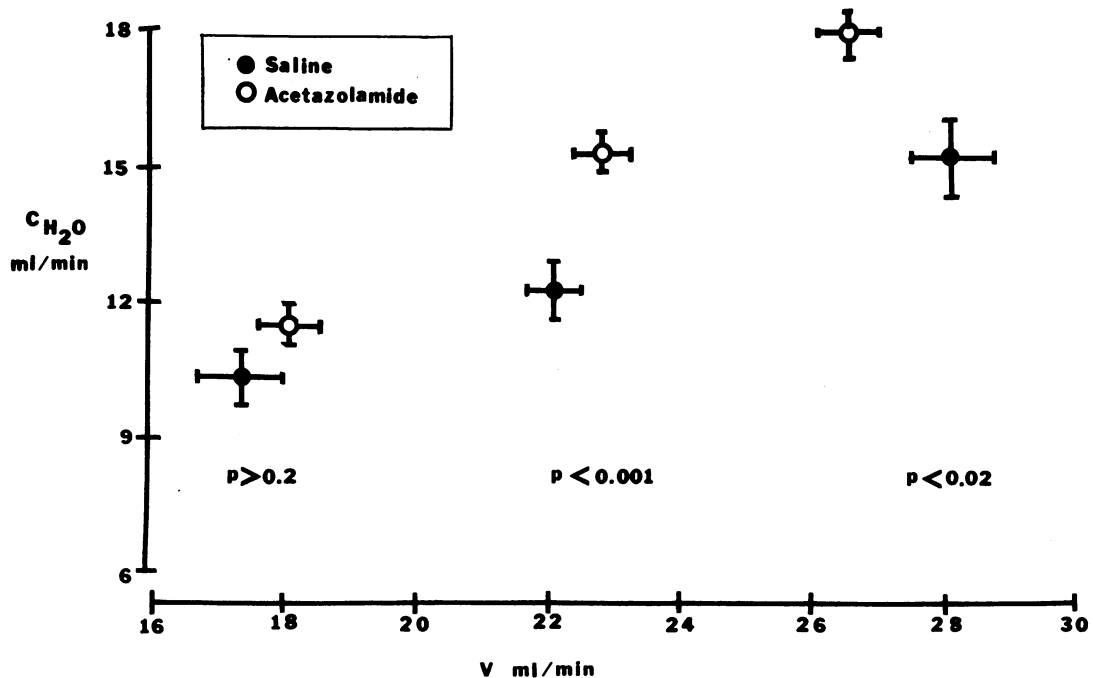


FIGURE 5 A comparison of saline, closed circles, and acetazolamide studies, open circles, over a range of urine flow (V) from 15 to 30 ml/min. Each point is the mean \pm SE of all points in each study at three levels of V (15–19.9; 20–24.9; and 25–30).

normal subjects the mean C_{In} during the control water diuresis was 106 ml/min (range 97–117). During peak acetazolamide diuresis, C_{In} fell an average of 12% to a mean of 93 (range 76–106) as V rose from a range of 15–20 to 19–29 ml/min. In these studies the rise in C_{H_2O} was not associated with a rise in GFR and the relationship between V versus C_{H_2O} was not qualitatively different from that of V versus C_{H_2O}/C_{In} .

Fig. 5 shows a comparison between C_{H_2O} during saline studies and acetazolamide studies over the range of V from 15 to 30 ml/min. The figure was constructed by taking the mean value for all points in the two studies at three levels of V : 15–19.9, 20–24.9, and 25–30 ml/min. At the two higher values of V , mean C_{H_2O} was significantly lower in the saline compared to the acetazolamide studies ($P < 0.001$ and $P < 0.02$, respectively). The validity of this observation was further tested by a statistical analysis of covariance (18) comparing the slope of the relationship between C_{H_2O} and V in the saline and acetazolamide studies over comparable ranges of V . The slope for 43 points in six subjects during acetazolamide diuresis was 0.73, which was significantly higher than the slope of 0.50 for 36 points obtained during the six saline studies ($P < 0.025$).

Fig. 6 shows a comparison of fractional sodium excretion between saline and acetazolamide diuresis at the same levels of V shown in Fig. 5. Mean fractional sodium excretion during control water diuresis was 1.7 ± 0.1

(SE) in the acetazolamide studies and 1.5 ± 0.1 in the saline studies. During saline loading, fractional sodium excretion progressively increased as V increased, reaching a mean of 10.3 ± 0.8 at a mean V of 28.1 ± 0.6 . In contrast, after acetazolamide administration, fractional sodium excretion rose initially to 5.1 ± 0.6 as V increased from a mean control level of 17.0 ± 0.4 to a mean of 18.1 ± 0.4 . However, as V continued to rise, fractional sodium excretion rose only slightly to a mean of 5.8 ± 0.2 at a mean V of 26.5 ± 0.4 . As a result, at the high levels of V when C_{H_2O} was lower during saline diuresis, fractional sodium excretion was significantly higher.

DISCUSSION

The interpretation of clearance studies such as these requires several assumptions based on current concepts of the tubular mechanisms of sodium and water transport. Dilute tubular fluid is formed by the process of active sodium reabsorption from the water-impermeable portions of the ascending loop of Henle and early distal tubule (19). Calculated C_{H_2O} is therefore an index of sodium reabsorption at these diluting sites. However, during water diuresis, back diffusion of water may occur from the collecting ducts, since this structure maintains some permeability to water even in the absence of antidiuretic hormone (20, 21). This raises the possibility that during solute diuresis in hydrated subjects increments in V and C_{H_2O} could be due to decreased back

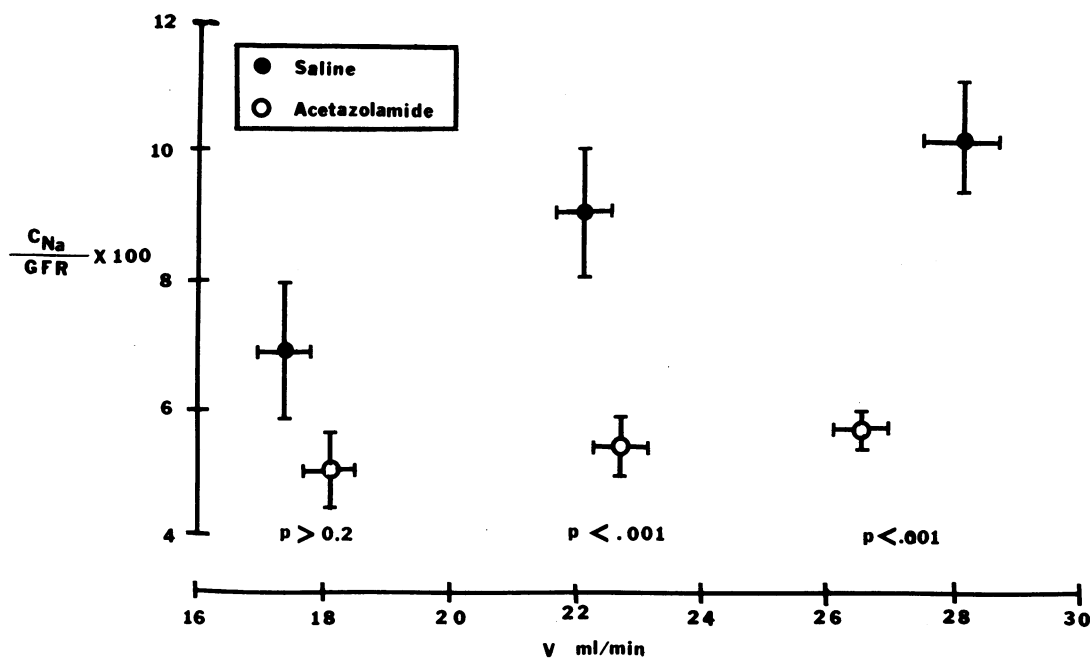


FIGURE 6 A comparison of fractional sodium excretion (C_{Na}/C_{In}) during saline studies, closed circles, and acetazolamide studies, open circles, at the same levels of urine flow (V) shown in Fig. 5. Data are means \pm SE.

diffusion of water from the collecting ducts. Although this latter phenomenon might occur if the medullary solute concentration were diminished by the diuresis, Eknoyan, Suki, Rector, and Seldin (22) demonstrated that the osmotic gradient between papillary water and urine increased as V and $U_{Na}V$ increased during hypotonic saline diuresis in hydrated, deoxycorticosterone-loaded dogs.

If the patients with diabetes insipidus had a small residual capacity to secrete ADH in response to the hypertonicity resulting from the saline infusion, the lower C_{H_2O} observed during volume expansion studies might have been due to increased back diffusion of water from the collecting ducts. This possibility seems unlikely since none of the patients could raise urine osmolality above 80 mOsm/kg during a standardized water deprivation test which produced a rise in plasma osmolality and sodium concentration of 8–10%, the same order of magnitude as that produced by the saline infusion. It seems likely that some non-ADH-dependent back diffusion of water occurred during these studies, the extent of which cannot be determined with certainty. However, there is no reason to suppose that more back diffusion of water would have occurred during the saline studies compared to the acetazolamide studies in the absence of ADH. Furthermore, it seems unlikely that the relationship between C_{H_2O} and GFR demonstrated in the saline and CTZ-saline studies could be explained simply by

changes in back diffusion of water in the collecting ducts. Therefore, it appears reasonable to assume that V is an index of delivery of proximal tubule fluid to the diluting segment of the nephron and that the changes in C_{H_2O} demonstrated in these studies largely reflect changes in net sodium reabsorption in this segment.

Based on these assumptions our studies indicate that in man the response of the diluting segments of the distal nephron to increased delivery of sodium depends upon the presence or absence of volume expansion. The data show that over a wide range of distal sodium delivery, C_{H_2O} is quantitatively lower in the presence of volume expansion than in the absence of volume expansion (Fig. 5). In addition, fractional sodium excretion was higher during saline diuresis compared to acetazolamide diuresis at the same levels of V where C_{H_2O} was lower (Fig. 6). These observations taken together provide further evidence that the lower C_{H_2O} observed in the saline studies is not due to differences in back diffusion of water but is due to decreased sodium reabsorption in the diluting segment of the nephron. Thus, these data indicate that volume expansion exerts an inhibitory effect on sodium reabsorption in the distal nephron in man.

The above conclusion is strengthened by the comparison between saline and CTZ-saline studies showing that at high rates of distal delivery distal blockade with CTZ does not diminish C_{H_2O} quantitatively (Fig. 3).

Thus the inhibitory effect of saline loading in the distal nephron occurs in part at a site functionally responsive to CTZ which is presumed to be a locus where urinary dilution occurs unrelated to the concentrating mechanism; hence it has been referred to as the "cortical" diluting site (15, 17). In addition the remainder of the distal diluting sites respond to volume expansion by developing a tendency for C_{H_2O}/GFR to remain relatively stable, in contrast to the acetazolamide studies. This difference between the saline and acetazolamide studies indicates that in the presence of acute volume expansion the distal tubule reabsorbs a smaller fraction of sodium delivered from the proximal nephron. Hence, during saline loading the resultant natriuresis is greater at any given level of distal delivery than in the absence of volume expansion (Fig. 6). The apparent relationship between distal sodium reabsorption and GFR during volume expansion is of interest, since a similar relationship has been demonstrated between GFR and proximal sodium reabsorption (23) and between GFR and tubular reabsorption of other substances such as glucose (24) and bicarbonate (25). The way in which tubular solute reabsorption and GFR are linked is not known, but whatever the mechanism, it appears to influence reabsorption in both the proximal and distal tubules.

Recently, it has been demonstrated that acetazolamide may inhibit C_{H_2O} to some degree in the dog (26, 27). Even if such an effect were present in our studies, we were still able to show clearly a lower C_{H_2O} with saline loading in man at comparable rates of V than with acetazolamide. Thus, if acetazolamide had no distal inhibitory effect, the differences in C_{H_2O} in our experiments would have been even greater.

The mechanism by which volume expansion might depress sodium reabsorption in the distal nephron is not clear from our studies. Work recently reported by Howards et al. would suggest that blood volume expansion per se is not the critical factor decreasing sodium reabsorption in the diluting sites (5). In their study, blood volume expansion with hyperoncotic albumin caused a large decrease in proximal sodium reabsorption but resulted in increased sodium reabsorption distally and very little natriuresis. On the other hand, expansion of the extracellular fluid volume with saline caused a similar degree of inhibition of proximal sodium reabsorption, but resulted in less sodium reabsorption distally, and hence more sodium excretion. It is possible that factors such as renal perfusion pressure, plasma protein levels, and the rate of renal blood flow, which have been shown to affect sodium reabsorption proximally (28, 29), may have similar effects on the distal nephron. Alternatively, it is possible that some humoral factor or factors released in response to volume expansion such as have been recently described (30, 31), which could inhibit sodium

reabsorption distally, might be playing a role in our studies. In any case, it is clear that a decrease in sodium reabsorption proximally occurs before the major distal effects become manifest during the course of acute saline loading and that the magnitude of natriuresis is to a large extent determined by the distal nephron.

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