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Research Article

THE EFFECTS OF INFUSIONS OF CHLOROTHIAZIDE ON URINARY DILUTION AND CONCENTRATION IN THE DOG

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It has been observed that the saluretic agent chlorothiazide (6-chloro-7-sulfamyl-1,2,4-benzothiadiazine-1,1 dioxide) and its analogs produce alterations in the process of urinary dilution which differ from those expected from a simple increase in electrolyte excretion (1–3). Heinemann, Demartini and Laragh (2) observed in human subjects undergoing water diuresis that the augmented electrolyte excretion resulting from the injection of chlorothiazide was associated with no increase, or a fall, in the clearance of free water. In a single experiment the authors observed no effect of the drug on the elaboration of a concentrated urine, and suggested that chlorothiazide may in part interfere with the reabsorption of electrolyte at sites in the distal convoluted tubule where urinary dilution normally occurs. The absence of an effect on the concentrating mechanism has been confirmed by others (4).

Crawford and Kennedy (3) found that the continuous administration of chlorothiazide (or hydrochlorothiazide) to patients with pituitary or nephrogenic diabetes insipidus, and to rats with pituitary diabetes insipidus, resulted in decreased daily volumes of urine with increased concentrations of solute. Similar effects of this agent and its analogs have been reported by numerous other investigators (5–10).

The following studies were undertaken to investigate further the immediate effects of chlorothiazide on the processes of urinary concentration and dilution in the dog. The changes observed during water diuresis were similar to those observed by others in man. A definite effect of this drug during the elaboration of concentrated urine was also observed, and has been interpreted as supporting the previous suggestion that this agent may interefere with electrolyte reabsorption at sites in the nephron where urinary dilution occurs.

PROCEDURE

Water diuresis. Eight experiments were performed on 2 trained unanesthetized mongrel female dogs. The dogs were given their usual diet and fluid *ad lib.* prior to the experiment. At the onset of the experiment, a water load equivalent to 5 per cent of the body weight was administered through a gastric tube. Inulin in 2.5 per cent dextrose in water was infused at a constant rate of 10 to 14 ml per minute. Urine was collected by free flow through an indwelling catheter with the dog in the upright position. After at least 1 hour had been allowed for equilibration, urine specimens were collected and the rate of flow and osmolality determined. When these were stable for 3 consecutive 10-minute periods, collection of "control" periods was begun. Venous blood samples were collected every 30 to 40 minutes by free flow through an indwelling needle into a heparinized tube.

In 4 experiments, after the collection of 3 "control" periods, chlorothiazide (50 mg per kg body weight) was administered intravenously and a like amount was given by constant infusion each hour. Four 10-minute periods were then collected. The acute change in solute excretion and free water clearance produced by the drug under these conditions was thus determined.

In order to examine the relationship between C_{osm} and C_{H_2O} during water diuresis, 2.5 per cent mannitol in water was infused in 3 experiments to produce progressively increasing rates of solute excretion. The relationship was re-examined during the administration of chlorothiazide. The drug was administered either before the 2.5 per cent mannitol infusion was begun or after the mannitol had been infused and a high rate of solute excretion attained. In the former instance the mannitol infusion was then started in order to increase solute excretion, and in the later instance it was then stopped in order to decrease solute excretion. The effect of chlorothiazide was thus examined under conditions of both increasing and decreasing rates of solute excretion.

The effect of an equivalent dose of acetazolamide was examined in the same manner.

Maximal hydropenia and antidiuretic hormone. In order to examine the effects of chlorothiazide on urinary concentration, 11 experiments were performed on 5 trained unanesthetized mongrel female dogs. The dogs were

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dehydrated for 48 hours and 5 U of vasopressin (Pitressin Tannate in Oil) was administered intramuscularly the evening preceding the experiment.

A continuous infusion of inulin in isotonic saline was maintained at a constant rate throughout the experiment. Aqueous vasopressin was added to the solution, the pH having been adjusted to 5.5 with acetic acid, and infused at the rate of 50 mU per kg of body weight per hour during the course of the experiment. After a period of equilibration, urine and blood specimens were collected as indicated above. The periods varied from 5 to 20 minutes depending upon the rate of urine flow.

In order that the relationship between C_{osm} and $T^c_{H_2O}$ be examined over a wide range of solute excretion, one of the following hypertonic solutions was infused: *1*) 7 per cent mannitol in water containing 50 mmoles NaCl per L; *2*) 5 per cent mannitol in water containing 50 mmoles NaCl per L; *3*) 4 per cent mannitol in water containing 150 mmoles NaCl per L; *4*) 2.5 per cent mannitol in water containing 150 mmoles NaCl per L; *5*) 200 mmoles NaCl per L. The stability of the relationship was determined by discontinuing the infusion after a high rate of solute excretion had been obtained and re-examining the C_{osm}-$T^c_{H_2O}$ relationship as solute excretion was decreasing.

Chlorothiazide, in the same dosage as noted above, was administered when solute excretion was either at a high rate (after *one* C_{osm}-$T^c_{H_2O}$ curve had been obtained with the hypertonic solution) or after solute excretion had returned to a low rate (after *two* C_{osm}-$T^c_{H_2O}$ curves had been obtained with the hypertonic solution). In either case, adjusting the rate of infusion of the hypertonic solution permitted observations through a wide range of solute excretion in the presence of chlorothiazide. In all experiments one or more C_{osm}-$T^c_{H_2O}$ curves were obtained with hypertonic mannitol and/or saline, and one curve was obtained during the administration of chlorothiazide.

Chemical methods. Inulin was measured in plasma and urine by the method of Walser, Davidson and Orloff (11). Sodium and potassium were determined by flame photometry. Osmolality was determined cryoscopically by using the freezing point depression apparatus of Bowman, Trantham and Caulfield (12).

RESULTS

Water diuresis. Prior to the administration of chlorothiazide, the relationship between C_{osm} and C_{H_2O} was similar in all experiments. The osmolal clearance prior to the infusion of the hypotonic solution of mannitol ranged from 0.8 to 1.9 ml per minute and the C_{H_2O} ranged from 10.9 to 13.1 ml per minute. When mannitol was infused, the osmolal clearance slowly increased to a maximum of 6.5 ml per minute and the free water clearance increased progressively to a maximum of 15.1 ml per minute (Figure 1, control). The free water

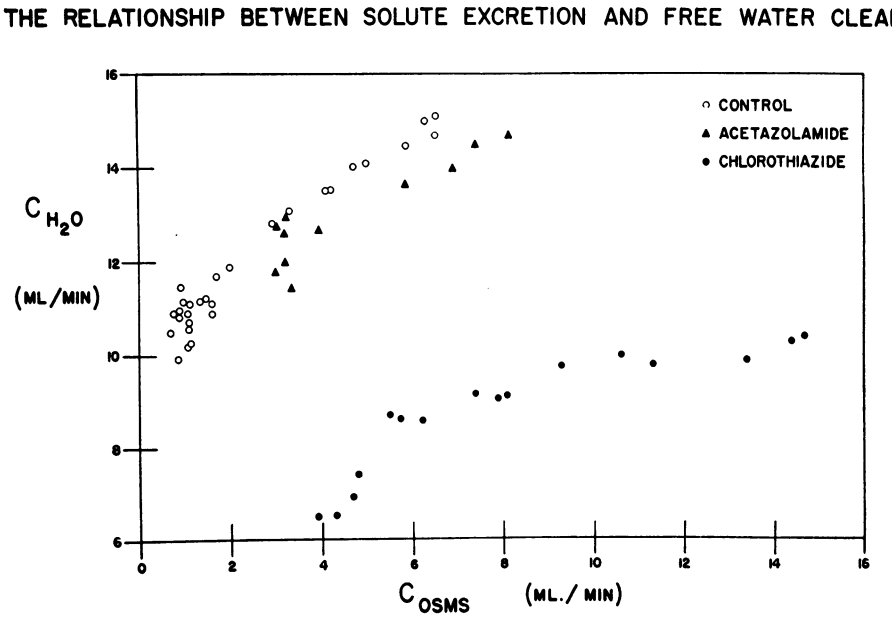

FIG. 1. COMPOSITE OF 5 STUDIES DURING WATER DIURESIS IN A SINGLE DOG. Control points demonstrate increasing clearance of free water with increasing osmolal (mannitol) clearance. A similar relationship is shown during the administration of acetazolamide. Chlorothiazide points represent 3 different experiments; control points immediately preceding each of these experiments are grouped at the left of the upper curve.

TABLE I

The effects of chlorothiazide on the excretion of water and electrolyte during water diuresis

Experiment	Time	V	U_{osm}	C_{osm}	$U_{Na}V$	U_KV	C_{H_2O}	C_{In}	per 100 ml GFR V	per 100 ml GFR C_{osm}	per 100 ml GFR C_{H_2O}
	min	*ml/min*	*mOsm/kg*	*ml/min*	*μEq/min*	*μEq/min*	*ml/min*	*ml/min*	*ml/min*	*ml/min*	*ml/min*
A5		900 ml H_2O by gastric tube, 2.5% dextrose in water, 12.0 ml/min i.v.									
	0–10	14.2	30	1.6	11	8	12.6	109	13.0	1.5	11.6
	10–20	14.7	32	1.8	12	9	12.9	109	13.5	1.6	11.9
	20–30	14.6	32	1.8	12	8	12.8	111	13.2	1.6	11.6
		Chlorothiazide, 5 mg/kg and 5 mg/kg/hr i.v.									
	30–40	15.4	85	5.0	418	86	10.5	94	16.5	5.3	11.2
	40–50	14.4	109	6.0	558	98	8.5	89	16.3	6.7	9.6
	50–60	13.6	109	5.7	544	94	7.9	88	15.5	6.5	9.0
	60–70	12.7	110	5.2	485	89	7.4	87	14.7	6.1	8.6
J9		1,000 ml H_2O by gastric tube, 2.5% dextrose in water, 12.0 ml/min i.v.									
	0–10	12.0	22	1.0	7	7	10.9	109	11.0	0.9	10.0
	10–20	12.0	24	1.1	7	7	10.9	110	10.9	1.0	9.9
	20–30	12.2	21	1.0	8	7	11.2	117	10.4	0.9	9.6
		Chlorothiazide, 5 mg/kg and 5 mg/kg/hr i.v.									
	30–40	14.8	92	5.2	513	95	9.6	101	14.7	5.2	9.5
	40–50	14.4	110	6.1	638	119	8.3	107	13.5	5.7	7.8
	50–60	14.4	104	5.7	596	109	8.6	113	12.7	5.1	7.6
	60–70	14.2	101	5.5	567	102	8.7	119	11.9	4.6	7.3

clearance during the infusion of chlorothiazide was consistently lower than that noted with mannitol alone at all rates of solute excretion examined (Figure 1).

In four experiments, chlorothiazide was administered before mannitol was infused. The protocols of two of these experiments are shown in Table I. There was a mean increase in osmolal clearance of 4.2 to 5.4 and a mean decrease in free water clearance of 2.4 to 3.2 ml per minute per 100 ml glomerular filtration rate (GFR). When the free water clearance obtained during the administration of chlorothiazide was compared with the free water clearance obtained with mannitol alone *at the same rate of solute excretion,* there was a net decrease in free water clearance of 5.3 to 5.8 ml per minute per 100 ml GFR. It is apparent from Table II that in all four experiments the increased osmolal clearance closely approximated the decreased free water clearance (mean $C_{osm}/C_{H_2O} = 0.87$).

In the four experiments noted above there was an initial increase in the rate of urine flow following the administration of chlorothiazide. In three of these there was a subsequent decrease in the rate of flow during the 40 minutes of drug ad-

TABLE II

The relationship between changes in the rates of osmolal and free water clearance produced by chlorothiazide during water diuresis

Experiment	Mean C_{osm} Before chloro.	Mean C_{osm} After chloro.	ΔC_{osm} (increase)	Mean C_{H_2O} After chloro.	Mean C_{H_2O} During mannitol*	ΔC_{H_2O} (decrease)	$\frac{\Delta C_{osm}}{\Delta C_{H_2O}}$
	ml/min		*ml/min*	*ml/min*		*ml/min*	
J1	0.9	5.3	4.4	8.2	13.5	5.3	0.83
J2	0.9	5.1	4.2	7.6	13.3	5.7	0.74
A1	1.4	6.5	5.1	9.1	14.8	5.7	0.89
A2	1.4	6.8	5.4	9.2	15.0	5.8	0.93

* C_{H_2O} during water diuresis with superimposed mannitol infusion selected at rates of osmolal clearance (C_{osm}) equal to rates obtained during the respective experiments with chlorothiazide.

ministration. This decreased flow rate was associated with a persistent fall in glomerular filtration rate which averaged 13, 20 and 22 per cent, respectively. The mean rate of flow was *increased* 1.5, 2.3 and 2.8 ml per minute per 100 ml GFR above prechlorothiazide levels when the adjustment for filtration rate was made. The protocol of one of these experiments is shown in Table I, A5. The one experiment in which the rate of urine flow did not fall is shown for comparison (Table I, J9). In this latter experiment the fall in filtration rate following the administration of chlorothiazide was only transient. The increased rate of solute excretion and decreased free water clearance are similar in both experiments in spite of these differences in filtration rate.

In an experiment in which acetazolamide was administered, the mean osmolal clearance increased 2.4 ml per minute, and the filtration rate fell 14 per cent, values similar to those noted above following chlorothiazide administration. As can be seen in Figure 1, the free water clearance increased during the administration of acetazolamide in a manner similar to that with mannitol alone. Adjustment for changes in filtration rate did not alter this identity.

Maximal hydropenia and antidiuretic hormone. A consistent relationship between C_{osm} and $T^c_{H_2O}$ was noted when hypertonic mannitol and/or saline was infused. If the rate of solute excretion was initially low, a progressive rise in $T^c_{H_2O}$ with increasing C_{osm} was generally observed. With further increases in the rate of solute excretion, a "plateau" was apparent with only minimal changes in $T^c_{H_2O}$ (Figure 2, control). In six experiments, the plateau was followed by a progressively decreasing $T^c_{H_2O}$ as the rate of solute excretion was further increased (Figure 3, control). In two of the latter experiments an initial rise in $T^c_{H_2O}$ was not observed, and the urine became hypotonic to plasma, resulting in the excretion of free water at high rates of solute excretion

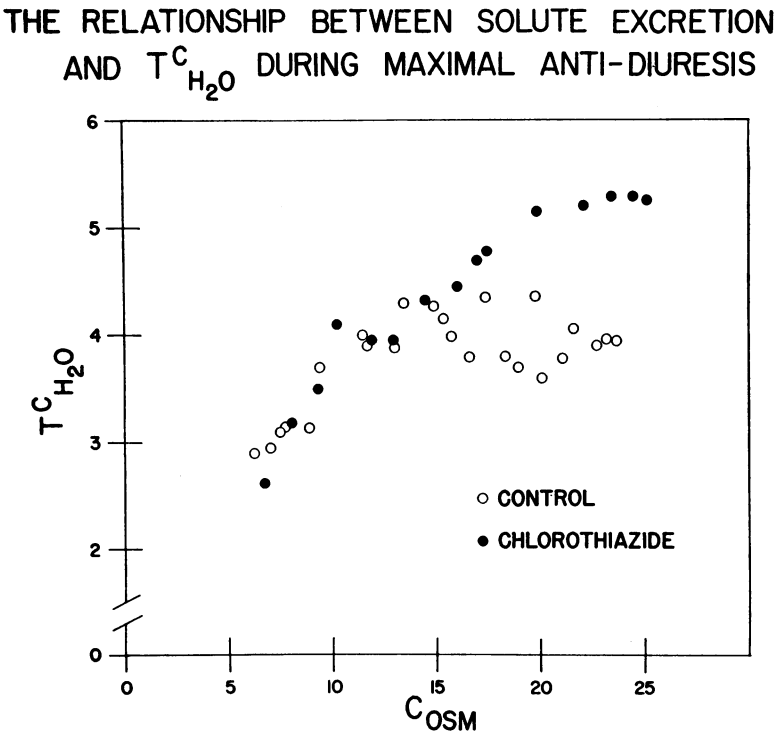

FIG. 2. CONTROL POINTS REPRESENT BOTH INCREASING AND DECREASING RATES OF SOLUTE EXCRETION. Chlorothiazide was administered when solute excretion had returned to a low level (see text). In the presence of the drug the curve did not differ from control at low rates of solute excretion. At higher rates of solute excretion, $T^c_{H_2O}$ during the administration of chlorothiazide is distinctly greater than without the agent.

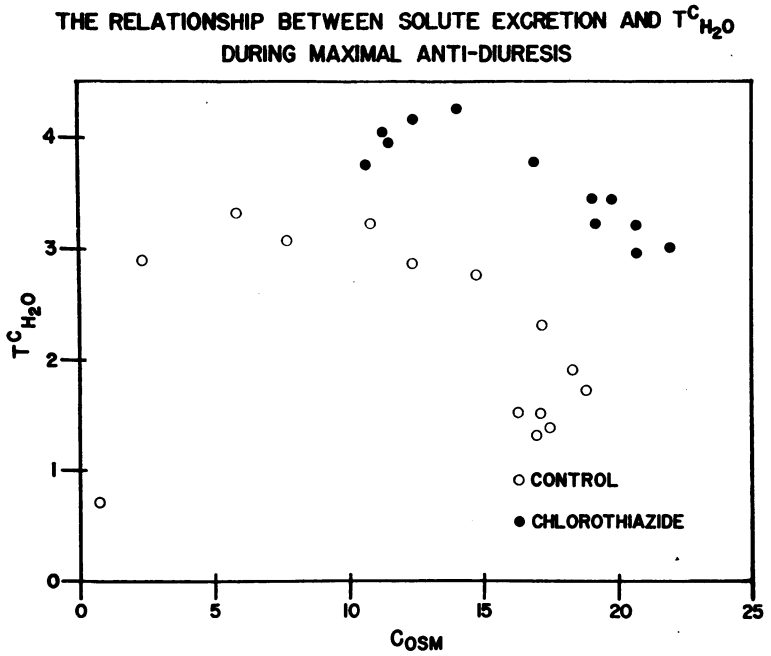

FIG. 3. THE CONTROL POINTS REPRESENT AN EXPERIMENT IN WHICH $T^c_{H_2O}$ PROGRESSIVELY DECREASED AT HIGH RATES OF SOLUTE EXCRETION. Chlorothiazide was administered while high rates of solute excretion were maintained, and $T^c_{H_2O}$ immediately increased. Solute excretion was then allowed to decrease and $T^c_{H_2O}$ remained greater than control points.

(Table III). These relationships were qualitatively the same regardless of which hypertonic solution was infused.

In each experiment, the C_{osm}-$T^c_{H_2O}$ relationship during the infusion of hypertonic solution was reproducible. This is well illustrated in those experiments in which two curves were obtained with the hypertonic solution during a 3 to 5 hour period by first increasing and then decreasing the rate of infusion. The "control" curve shown in Figure 2 is a composite plot of all of the periods from *two* curves obtained in this way. The minimal scatter emphasizes the stability of the C_{osm}-$T^c_{H_2O}$ relationship. This is also apparent in the protocol of Table III. (For example, during progressively *increasing* solute excretion, at a C_{osm} of 9.7 ml per minute the $T^c_{H_2O}$ is 0.6 ml per minute. During progressively *decreasing* solute excretion, at a C_{osm} of 10.4 ml per minute, the $T^c_{H_2O}$ is 0.5 ml per minute.) Differences, when noted, are both quantitatively and qualitatively quite distinct from those obtained during the administration of chlorothiazide.

In spite of this stability during each experiment, there was often marked variability in the C_{osm}-$T^c_{H_2O}$ relationship between experiments on different dogs and between experiments on the same dog on different days. It was therefore necessary to obtain at least one C_{osm}-$T^c_{H_2O}$ curve during the infusion of hypertonic mannitol-saline solution and one curve during the administration of chlorothiazide in each experiment in order to make valid comparisons.

Within 5 to 15 minutes after chlorothiazide was administered, the osmolal clearance increased 2.1 to 4.9 ml per minute (mean 3.8) and the rate of urine flow increased 1.2 to 4.5 ml per minute (mean 3.0). Following the administration of chlorothiazide, in three experiments the filtration rate fell less than 5 per cent. In eight experiments the decrease was 7 to 15 per cent during the first 20 minutes but in seven of these the filtration rate returned to control levels or above in spite of the continued chlorothiazide infusion. The characteristic C_{osm}-$T^c_{H_2O}$ relationship noted during the administration of the drug was neither dependent upon nor qualitatively altered by these variations in filtration rate.

The C_{osm}-$T^c_{H_2O}$ relationship during the administration of chlorothiazide had a characteristic pattern when compared with the relationship obtained during the infusion of the hypertonic solutions alone. This pattern was noted in experiments in which the drug was initially administered at either high rates of solute excretion (Figure 3) or at low rates of solute excretion (Figure 2, Table III). At low rates of solute excretion (corresponding to the phase of rapidly rising $T^c_{H_2O}$ of the control curve), the $T^c_{H_2O}$ during chlorothiazide administration was not apparently different from the hypertonic mannitol-saline solutions alone at the same C_{osm} (Figure 2). At rates of solute excretion corresponding to the late part of the "plateau" of the "control" curve (Figure 2) and in all six experiments in which the rate of solute excretion during the infusion of the hypertonic solutions alone was great enough to obtain a falling $T^c_{H_2O}$ (Figure 2, Table III), the curves differed appreciably and consistently. The $T^c_{H_2O}$ obtained at these rates of solute excretion was greater during the administration of chlorothiazide than that obtained with the hypertonic solutions alone at equivalent rates of solute excretion. This divergence was noted at or slightly beyond the point of maximal $T^c_{H_2O}$ of the "control" curve. Once this divergence was apparent, the $T^c_{H_2O}$ with chlorothiazide remained greater than with the mannitol-saline solutions alone at the same

TABLE III

The effect of chlorothiazide on urinary concentration during maximal hydropenia and solute diuresis

Time	V	U_{osm}	C_{osm}	$T^c_{H_2O}$	C_{H_2O}	C_{In}	per 100 ml GFR V	per 100 ml GFR C_{osm}	per 100 ml GFR $T^c_{H_2O}$	per 100 ml GFR C_{H_2O}
min	*ml/min*	*mOsm/kg*	*ml/min*	*ml/min*	*ml/min*	*ml/min*	*ml/min*	*ml/min*	*ml/min*	*ml/min*
Inulin, 10 mg/min; vasopressin, 100 mU/kg/hr i.v.										
0–25	1.8	450	2.8	1.0						
Mannitol, 7% in 50 mmoles NaCl, 2.3 ml/min										
30–35	4.4	371	5.7	1.3		92	4.8	6.2	1.4	
35–40	5.5	347	6.6	1.1		95	5.7	6.9	1.2	
40–45	7.3	328	8.3	1.0		85	8.6	9.7	1.1	
Mannitol, 7% in 50 mmoles NaCl, 12.4 ml/min										
45–50	9.1	313	9.7	0.6		86	10.6	11.3	0.7	
55–60	14.0	297	14.0	0.0		83	16.9	16.9	0.0	
Mannitol, 7% in 50 mmoles NaCl, 22.0 ml/min										
65–70	18.2	291	17.6		0.6	79	23.1	22.4		0.7
75–80	23.3	286	22.3		1.0	79	29.5	28.2		1.3
Mannitol, 7% in 50 mmoles NaCl, discontinued										
85–90	23.4	285	22.4		1.4	76	31.2	29.4		1.8
95–100	15.5	296	15.2		0.3	78	19.8	19.5		0.3
100–105	12.2	298	12.0		0.2	77	15.8	15.5		0.3
105–110	9.9	318	10.4	0.5		82	12.1	12.8	0.8	
120–130	6.3	359	7.6	1.3		82	7.6	9.2	1.6	
140–155	3.9	419	5.5	1.6		94	4.1	5.8	1.7	
Chlorothiazide, 5 mg/kg and 5 mg/kg/hr i.v.										
165–170	4.4	431	6.3	1.9		95	4.7	6.7	2.0	
170–175	5.4	427	7.7	2.3		78	6.9	9.8	2.9	
Mannitol, 7% in 50 mmoles NaCl, 12.0 ml/min										
185–190	8.0	382	9.8	1.8		80	9.9	12.2	2.3	
195–200	10.8	368	12.9	2.1		78	14.0	16.6	2.6	
Mannitol, 7% in 50 mmoles NaCl, 22.0 ml/min										
205–210	14.0	353	16.1	2.1		78	18.1	20.8	2.7	
215–220	18.3	345	20.7	2.4		81	22.7	25.7	3.0	
225–230	21.0	337	23.3	2.3		79	26.7	29.6	2.9	
235–240	23.2	338	24.8	1.6		81	29.0	31.0	2.0	

rates of solute excretion. The maximal differences in $T^c_{H_2O}$ between the curves at the same rates of solute excretion were + 1.0 to + 3.5 ml per minute. The *maximal* $T^c_{H_2O}$ obtained during the administartion of chlorothiazide was often greater (1.0 to 1.8 ml per minute) than the *maximal* $T^c_{H_2O}$ obtained with the hypertonic solutions alone, as can be seen in Figures 2 and 3 and Table III.

DISCUSSION

The effects of chlorothiazide on the mechanisms of urinary dilution and concentration observed in the present studies may be interpreted in the light of current views as due to a single action of this agent on the renal tubule. The currently available evidence suggests that urinary dilution begins in the ascending limb of Henle's loop by a process which actively transports solute (sodium) from the lumen into the medullary interstitium without an equivalent movement of water (13, 14). Further dilution of the tubular fluid may occur in the distal convolution by the continued removal of solute, since in the absence of antidiuretic hormone (ADH) the movement of water out of this segment is restricted (13–15). The collecting duct, in the absence of ADH, is also relatively impermeable to water (13, 16), and the diluted fluid which enters this segment is unable to attain osmotic equilibrium with the surrounding medullary interstitium. It is likely that this relative impermeability to water of the distal convolution and collecting duct does not completely restrict the passive outward movement of water along a steep osmotic gradient. Thus, increasing rates of solute excretion during water diuresis may, by decreasing the transtubular osmotic gradient in the presence of increasing rates of flow within the lumen, result in further restriction of the outward flow of water from the distal convolution and collecting duct. As has been suggested, the increasing clearance of free water seen when increasing solute excretion is imposed on water diuresis may be explained in this manner (17, 18). The excretion of free water may also be increased by augmented reabsorption of sodium in the distal portions of the nephron. However, the relative importance of these two factors during solute diuresis cannot readily be assessed.

Unlike the results seen when mannitol was given during water diuresis, the administration of chlorothiazide uniformly resulted in a decreased clearance of free water. This is in agreement with the findings of others, and suggests that the action of chlorothiazide cannot be limited to diminishing the reabsorption of electrolyte in the proximal tubule. Further, if the increased osmolal clearance associated with this agent is envisioned as the result of interference with the reabsorption of electrolyte at a site where the tubular fluid is normally diluted, then a quantiative relationship should exist between the increased osmolal clearance and the decreased clearance of free water. Such a relationship was observed in the present studies. The decreases in C_{H_2O} closely approximated the increases in C_{osm} (when compared with values obtained during mannitol diuresis), suggesting that the major increase in electrolyte excretion following the administration of chlorothiazide resulted from interference with reabsorption at tubular sites where dilution occurs.

The observed effects of acetazolamide on the clearance of free water are in agreement with previous reports (19, 20), and the qualitatively opposite effects of the two drugs on this measurement make it unlikely that the effect of chlorothiazide on the diluting process is related to the inhibitory effect on carbonic anhydrase common to these two agents.

A similar tubular effect of chlorothiazide was detected in the animal elaborating a concentrated urine. In the absence of the drug, increasing rates of osmolal clearance during the infusion of vasopressin were associated with values for $T^c_{H_2O}$ which increased to a maximum and then either remained constant through the range of osmolal clearance examined, or progressively declined. In some instances dilute urine (C_{H_2O}) was excreted at high rates of osmolal clearance, despite the continued infusion of vasopressin. Such a fall in $T^c_{H_2O}$ at high rates of solute excretion has been occasionally observed by other investigators (18, 21, 22). Since it is this portion of the $T^c_{H_2O}$ curve that is affected by chlorothiazide, it is important to attempt an interpretation of the expected C_{osm}-$T^c_{H_2O}$ relationship in order that the action of the drug may be better defined.

The hypotonic tubular fluid produced in the ascending limb of Henle's loop is allowed to reach osmotic equilibrium with its surroundings in the

distal convolution through a membrane which is made highly permeable to water by the presence of ADH. The fluid will thus become isotonic with plasma in the latter portion of the distal convolution. In the collecting duct the passive movement of water is again enhanced by the presence of ADH, and the tubular fluid will approach osmotic equilibrium with the medullary interstitium which has been made hypertonic to plasma by the countercurrent systems of Henle's loop and the medullary capillaries. Under these conditions the calculated $T^c_{H_2O}$ would represent a true measure of the solute-free water abstracted from the collecting duct. However, as the rate of solute excretion is increased, the increased rate of flow through the distal nephron and the decreased transtubular osmotic gradient may result in a failure of complete passive osmotic equilibration of the diluted fluid which enters the distal convolution (18). Under these conditions the fluid entering the collecting duct would be hypotonic to plasma, and the calculated $T^c_{H_2O}$ would not represent a true measure of the solute-free water removed from the fluid within this segment. If the transport of solute into the medullary interstitium by the ascending limb of Henle's loop [and perhaps the collecting duct (23)] were proceeding at a maximal rate, then the delivery of hypotonic fluid to the collecting duct may impose a limit upon the maximal calculated $T^c_{H_2O}$ and result in its subsequent fall as solute excretion continues to increase. The externally calculated $T^c_{H_2O}$ would then represent the difference between the nonosmotically equilibrated water which enters the collecting duct and the solute free water abstracted by the medullary interstitium. This has been discussed in greater detail elsewhere (18). In support of this concept is the present observation that in some instances urine, hypotonic to plasma, was excreted at high rates of osmolal clearance, indicating that complete equilibration had not occurred even in the collecting duct. Although such marked falls in $T^c_{H_2O}$ were not observed in all studies, the qualitative similarity that exists throughout these C_{osm}-$T^c_{H_2O}$ curves suggests that the same basic mechanisms were operative.

Chlorothiazide, by interfering with the tubular reabsorption of solute without water at diluting sites, would result in less solute-free water within the distal convolution, and allow passive osmotic equilibration to occur more readily. Such an action might be expected to increase the maximal $T^c_{H_2O}$ and delay the subsequent fall which normally occurs. At lower rates of osmolal clearance, when $T^c_{H_2O}$ is progressively rising or is stable, passive osmotic equilibration in the distal convolution should be complete, and diminishing the dilution of the tubular fluid under these conditions should not affect the calculated $T^c_{H_2O}$ (provided the rate of solute transport into the medullary interstitium were not simultaneously affected). In the present studies chlorothiazide fulfilled these requirements. At rates of osmolal clearance associated with increasing values for $T^c_{H_2O}$ the administration of this agent did not alter these values. However, the presence of chlorothiazide resulted in maximal values for $T^c_{H_2O}$ greater than the maximal values obtained without the drug. Also, the administration of this agent uniformly resulted in increased values for $T^c_{H_2O}$ at rates of osmolal clearance which were associated with decreasing values in the absence of chlorothiazide. The failure of this drug to lower $T^c_{H_2O}$ at any level of solute excretion is consistent with the previous suggestion that its action is not manifest to any measurable degree in the ascending limb of Henle's loop, and that its primary site of action may be in the distal convolution (2). (However, since factors other than the rate of transport of sodium into the medullary interstitium may be important determinants of the measured $T^c_{H_2O}$, the exclusion of Henle's loop as a site of action of this drug may not be justified.) These conclusions are in contrast to those of Kessler, Hierholzer, Gurd and Pitts (24) who, by utilizing the "stop flow" method in dogs, concluded that the primary action of chlorothiazide on sodium reabsorption was within the proximal tubule. However, since all "stop flow" urine will ultimately be influenced by its passage through the more distal nephron, a quantitative discrimination of functions which occur both proximally and distally may be difficult.

These studies do not exclude the possibility that chlorothiazide, in addition to interfering with the reabsorption of electrolyte, enhances the passive movement of water across the epithelial membrane of the distal convolution and collecting duct in a fashion similar to that of ADH. If such were the case, the increases in $T^c_{H_2O}$ reported here

would necessitate that this agent increase the permeability to water of the distal portions of the nephron to an extent greater than that present during maximal ADH activity. In this study, as well as in those of others, chlorothiazide given during water diuresis never resulted in urinary concentrations as great as those that may be seen when vasopressin is given under similar circumstances (18, 25). The implication, thus, is that these two agents could increase the permeability of the tubular epithelium to water by separate and additive mechanisms. Further, an agent that increases the passive movement of water from the tubular urine should result in decreased urinary volumes with increased concentration of solute. Chlorothiazide uniformly produced an initial increase in urinary volume as the clearance of free water decreased. Since the increased osmolal clearance produced by the drug is quantitatively related to the decreased clearance of free water, it would seem convenient and justified to assign to this agent the single tubular action of interfering with solute reabsorption at sites of urinary dilution.

The administration of chlorothiazide was associated with variable changes in the rate of glomerular filtration, and it would appear unlikely that such changes as did occur were responsible for the observed effects on C_{H_2O} and $T^c_{H_2O}$. The constancy of the changes in C_{H_2O} produced by chlorothiazide, and the finding of similar changes in human subjects with nephrogenic diabetes insipidus (26), argue against the fact that the release of endogenous ADH produces any measurable effect on these results.

The mechanism whereby this drug results in an increase in urine osmolality acutely does not completely account for its chronic action in diabetes insipidus (see above). Additional factors are necessary to account for the associated fall in urine volume observed in these patients.

SUMMARY

Chlorothiazide was administered by continuous intravenous infusion to dogs undergoing maximal water diuresis. This agent resulted in an immediate fall in the clearance of free water (C_{H_2O}). This fall bore a consistent quantitative relationship to the increased osmolal clearance contributed by the drug.

Values for $T^c_{H_2O}$ were determined through a wide range of solute excretion (mannitol and/or sodium chloride) in normal dogs receiving infusions of vasopressin. A characteristic curve was observed through which values for $T^c_{H_2O}$ increased to a maximum and then diminished, with further increases in solute excretion. It is suggested that diminishing values for $T^c_{H_2O}$ may result from failure of complete passive osmotic equilibration in the distal convolution at very high rates of solute excretion. Immediate comparison of values for $T^c_{H_2O}$ at various rates of osmolal clearance with and without chlorothiazide were obtained. The values for $T^c_{H_2O}$ at low rates of osmolal clearance during the administration of chlorothiazide did not differ from values obtained without the drug. At higher rates of osmolal clearance, $T^c_{H_2O}$ during the infusion of chlorothiazide often exceeded the maximal control value, and this agent uniformly resulted in increased values for $T^c_{H_2O}$ at rates of osmolal clearance which, in the absence of the drug, were associated with a decreasing $T^c_{H_2O}$.

Changes in the rate of glomerular filtration during the infusion of chlorothiazide were not uniform and were not always associated qualitatively with the observed changes in C_{H_2O} and $T^c_{H_2O}$.

It is suggested that these data are consistent with the premise that chlorothiazide increases electrolyte excretion by interfering with its reabsorption in portions of the nephron where the tubular fluid is normally diluted. The failure of this agent to lower $T^c_{H_2O}$ suggests that this action takes place predominantly in the distal convolution rather than in the ascending limb of Henle's loop.

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