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Renal Tubular Effects of Ethacrynic Acid *

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Ethacrynic acid,[1] 2,3-dichloro-4-(2 methylenebutyryl)-phenoxyacetic acid, is a recently introduced, orally effective natriuretic agent that is structurally unrelated to thiazides (1) and has proven useful in clinical disorders associated with edema (2–5). The present studies were undertaken to determine the natriuretic potency of this agent, its effect on the excretion of electrolytes other than sodium, and whether a specific site of action within the renal tubule is demonstrable. Current knowledge of the anatomical areas within the nephron where sodium transport leads to urinary concentration and dilution has been employed frequently for the purpose of localizing the tubular sites where natriuretic agents exert their effect (6–12). In the present studies, ethacrynic acid was given intravenously to dogs during water diuresis and during the elaboration of concentrated urine. The unique combination of changes in urinary dilution and concentration associated with the natriuretic effect of this agent is consistent with the concept that a major part of the natriuresis is due to interference with the transport of sodium by the ascending limb of Henle's loop. In addition, two drug-sensitive sites of urinary dilution are apparent, one affected by chlorothiazide and the other by ethacrynic acid.

Methods

Studies were carried out in four unanesthetized female mongrel dogs trained to stand quietly with the support of loose slings. Each animal received a constant intravenous infusion of isotonic saline, at 0.30 ml per minute, which contained inulin and *p*-aminohippurate (PAH) in amounts to deliver 10 mg and 3 mg per minute, respectively. This infusion was begun at least 1 hour before experimental collections were started. Urine was collected into graduated cylinders through an indwelling Foley catheter, and suprapubic pressure was employed at the end of each collection period. Blood samples were collected at the mid-point of experimental periods by free flow from an indwelling venous catheter into heparinized tubes. Studies in individual animals were conducted at intervals of no less than 7 days.

In samples of urine and plasma, inulin was determined by the method of Walser, Davidson, and Orloff (13), PAH by a modification of the method of Smith and his colleagues (14), chloride by amperometric titration (15), sodium and potassium by internal standard flame photometry, and osmolality by the freezing point depression, using the Fiske osmometer.

Maximal hydropenia. Animals were deprived of water for 48 hours, and of food for 24 hours, before the experimental procedure. Sixteen to 18 hours before experiments, the animals received 5 U of vasopressin in oil[2] intramuscularly, and in all except one experiment (Table I), the animals also received 10 mg of desoxycorticosterone acetate (DOCA) intramuscularly. The maintenance infusion contained vasopressin[3] and in four experiments (Figure 1), desoxycorticosterone,[4] in amounts to deliver 50 mU per kg per hour and 20 μg per minute, respectively.[5] Maintenance solutions containing vasopressin were acidified to pH 5.0 to 5.5 by the addition of acetic acid. In each animal, $T^c_{H_2O}$ was determined during the infusion of 15% mannitol in 50 mM NaCl. After collection of control periods, infusion of the mannitol solution was begun and increased in a stepwise fashion until a urinary flow rate of approximately 20 ml per minute was achieved. These studies served as controls for subsequent observations on the effect of ethacrynic acid. In two experiments (under similar preparatory conditions), ethacrynic acid was administered intravenously at a dose of 1 mg per kg acutely, and 1 mg per kg per

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[2] Pitressin Tannate, Parke, Davis, Detroit, Mich.

[3] Pitressin, Parke, Davis, Detroit, Mich.

[4] Steraloids, Inc., Queens, N. Y.

[5] Under the described conditions, $T^c_{H_2O}$ was found to remain stable through a greater range of solute excretion than has been observed in similar studies (12) without the use of DOCA.

TABLE I

*The effects of iv ethacrynic acid on the renal concentrating mechanism**

	Time	Urine vol	GFR	U_{osm}	C_{osm}	$T^c_{H_2O}$	P_{osm}
	minutes	*ml/minute*	*ml/minute*	*mOsm/kg*	*ml/minute*	*ml/minute*	*mOsm/kg*
Dog B	−90	0.8% saline, 0.3 ml/minute; inulin, 10 mg/minute; PAH, 3 mg/minute; vasopressin, 50 mU/kg/hour					
	−68−−31	0.20		1,920	1.30	1.1	296
	−31	15% mannitol in 50 mM NaCl, 5 ml/minute					
	0–13	6.58	67	452	9.65	3.1	308
	13–38	8.32	60	404	10.70	2.4	314
		15% mannitol in 50 mM NaCl, 12 ml/minute					
	38–55	11.7	65	399	14.6	2.9	329
	55–68	14.5	57	388	16.8	2.3	335
	68–79	17.3	63	389	19.5	2.2	345
	79–89	17.6	56	393	19.8	2.2	351
		Infusion of mannitol discontinued					
	89–102	15.4	51	401	17.6	2.2	351
	142–152	6.35	47	487	8.91	2.6	347
		Ethacrynic acid, 1 mg/kg at once, and 1 mg/kg/hour					
	152–164	11.0	42	380	12.1	1.1	344
	164–176	11.3	30	344	11.1	−0.2	350
	160 mEq/L Na, 4.0 mEq/L K, 20 mEq/L HCO_3, 144 mEq/L Cl, 30 ml/minute						
	176–187	13.9	36	341	13.8	−0.1	344
	187–195	21.0	49	339	20.8	−0.2	342
		Hypertonic electrolyte solution slowed to 10 ml/minute					
	195–204	22.7	49	338	22.2	−0.5	345

* GFR = glomerular filtration rate; U_{osm} = urine osmolality; C_{osm} = osmolar clearance; $T^c_{H_2O}$ = renal concentrating capacity; P_{osm} = plasma osmolality; PAH = *p*-aminohippurate.

hour, after the collection of control periods. In two experiments, the drug was administered during an intermediate rate of infusion of hypertonic mannitol, and then the infusion of mannitol was discontinued. In one experiment, the drug was administered at the height of a hypertonic mannitol infusion (which was then discontinued), and in one experiment the agent was given when the flow of urine had receded after an infusion of hypertonic mannitol.

In five of the six drug experiments described above, after the peak drug effect was observed, a hypertonic electrolyte solution (Na, 160; K, 4.0; Cl, 144; and HCO_3, 20 mEq per L) was infused at a rate of 30 to 35 ml per minute, until a desired rate of urine flow was achieved. The purpose of this infusion was to restore the electrolyte losses induced by ethacrynic acid. Through experience we learned that the electrolyte losses produced by the drug were associated with decreases in the clearances of inulin and PAH. Restoration of the electrolyte losses increased these measurements of renal hemodynamics and allowed a comparison of concentrating capacity at various rates of solute excretion and glomerular filtration.

Water diuresis. Animals were not fed on the day of study, but otherwise no special preparation was employed. Each animal was given 50 ml per kg of tap water by stomach tube. Two and one-half per cent dextrose was then infused at a rate approximately 1 ml per minute in excess of the rate of urine flow. When the rate of urine flow had been relatively constant for a minimum of 30 minutes and urine osmolality was below 60 mOsm, ethacrynic acid was administered intravenously in a dose of 1 mg per kg acutely, and 1 mg per kg per hour. In four experiments, the rate of hypotonic dextrose infusion was adjusted to partially match the increased rate of urine flow that followed administration of the drug. In five experiments, the infusion of hypotonic dextrose was discontinued at the time ethacrynic acid was administered, and an infusion of hypotonic electrolyte solution (Na, 110; K, 4.0; HCO_3, 15; Cl, 99 mEq per L) was begun and maintained at a rate approximately 1 ml per minute less than the rate of urine flow. In four of the five latter experiments, chlorothiazide was administered at a dose of 5 mg per kg acutely, and 5 mg per kg per hour, at a time when the flow of urine was relatively constant during the administration of ethacrynic acid and the hypotonic electrolyte solution.

In three experiments during the height of water diuresis, chlorothiazide was administered at the above described dose. When collection periods had been made

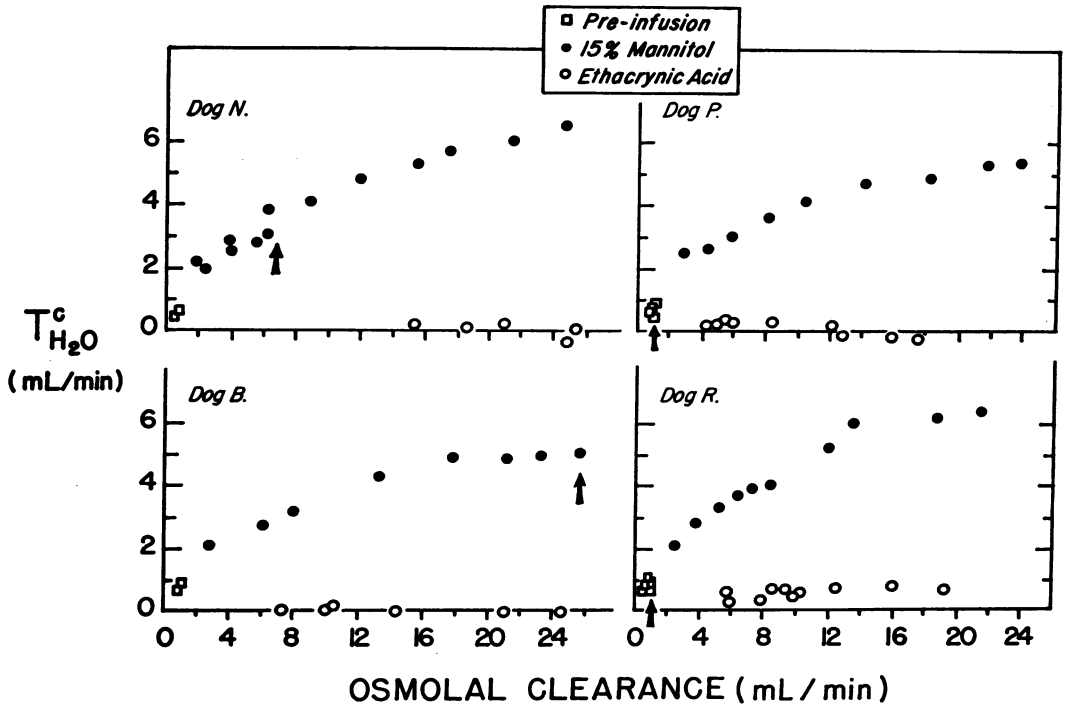

FIG. 1. THE EFFECT OF ETHACRYNIC ACID ON $T^c_{H_2O}$. Vertical arrows indicate the rate of osmolal clearance immediately preceding the administration of ethacrynic acid. In dogs R and P, the studies with hypertonic mannitol infusion were not performed on the same day as the studies with ethacrynic acid. In dog B, ethacrynic acid was administered at the height of the hypertonic mannitol diuresis; infusion of the mannitol was then discontinued and the rate of solute excretion allowed to decrease in the presence of the drug. In dog N, ethacrynic acid was administered at an intermediate rate of mannitol infusion, which was then discontinued. In dogs R, P, and N, hypertonic saline was infused after the effect of ethacrynic acid had been observed; this resulted in an increased GFR and a further rise in osmolal clearance. In each of these experiments, animals were pretreated with desoxycorticosterone acetate (DOCA) and received a constant infusion of DOC.

during the administration of chlorothiazide, ethacrynic acid was administered and the infusion of hypotonic electrolyte solution adjusted to approximately equal the increased rate of urine flow.

Results

Effects of ethacrynic acid on electrolyte excretion. In five water-loaded and two dehydrated dogs, ethacrynic acid was administered, and the peak diuretic effect was observed before the infusion of sodium-containing solutions. Control rates of sodium excretion ranged from 27 to 31 μEq per minute. An increased rate of urine flow was observed within 2 minutes after the intravenous administration of ethacrynic acid, and the maximal rate of flow was observed usually after 8 to 12 minutes. The rate of sodium excretion increased to a mean of 1,734 μEq per minute (Figure 2), and the peak excretion averaged 17% of the filtered sodium. The excretion of potassium increased from a mean control value of 44 μEq per minute to a mean of 190 μEq per minute during the peak natriuresis. This increased excretion of potassium occurred in the hydrated animals not receiving mineralocorticoid (Tables II and IV), as well as in those experiments employing an infusion of desoxycorticosterone. In all seven experiments, the excretion of chloride exceeded that of sodium and averaged 98.8% (range, 95.5 to 102%) of the sum of sodium and potassium excretion.

Glomerular filtration rate was usually unchanged or slightly increased during the first 10 to 20 minutes after administration of ethacrynic acid but then progressively fell during experiments in which the urinary electrolyte losses were not replaced.

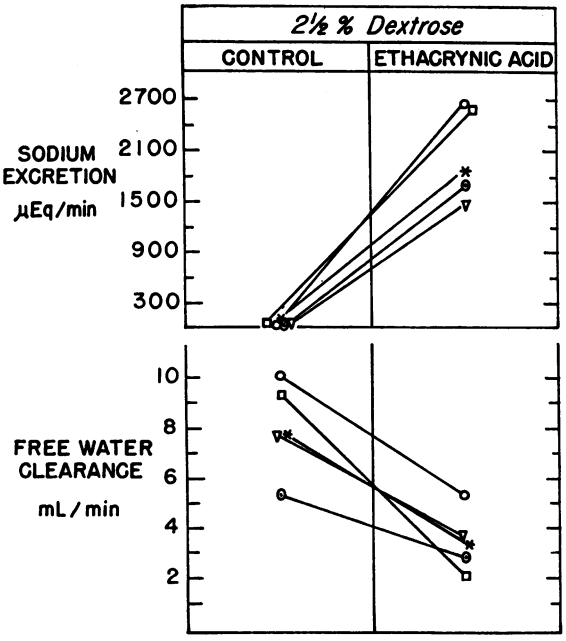

FIG. 2. FREE WATER CLEARANCE DURING THE PEAK NATRIURESIS PRODUCED BY ETHACRYNIC ACID. Control points are the last collection period before administration of the drug. The peak sodium excretion occurred during the second collection period (8 to 12 minutes) after administration of the drug. Five experiments performed in four dogs are shown.

Despite the falling filtration rate, the natriuresis continued throughout the periods of observation (Table II).

The effect of ethacrynic acid on urinary concentrating capacity ($T^c_{H_2O}$). In five experiments, $T^c_{H_2O}$ during the administration of ethacrynic acid was compared to that measured during the infusion of hypertonic mannitol. Three of these experiments were compared to control mannitol infusions performed on different days (dogs N, P, and R, Figure 1). In one experiment (dog B, Figure 1), ethacrynic acid was administered at the height of the hypertonic mannitol diuresis. In the remaining experiment (Table I) ethacrynic acid was administered immediately after the relationship between $T^c_{H_2O}$ and solute excretion had been determined during the infusion of hypertonic mannitol. The continued electrolyte excretion resulting from administration of the drug was always associated with a falling glomerular filtration rate after the first 10 to 20 minutes. In four of the five experiments, infusion of the hypertonic electrolyte solution increased glomerular filtration rate to values approaching those, or in excess of those, present during control studies. It was therefore possible to compare $T^c_{H_2O}$ at

TABLE II

*The effects of iv ethacrynic acid on water diuresis and electrolyte excretion**

	Time	Urine vol	GFR	C_{PAH}	U_{osm}	C_{H_2O}	$U_{Na}V$	U_KV	$U_{Cl}V$
	minutes	*ml/minute*	*ml/minute*	*ml/minute*	*mOsm/kg*	*ml/minute*	*μEq/minute*	*μEq/minute*	*μEq/minute*
Dog N	−180	1,200 ml tap water by stomach tube; 2.5% dextrose intravenously at rate of urine flow							
	−90	0.8% saline, 0.3 ml/minute; inulin, 10 mg/minute; PAH, 3 mg/minute							
	0–15	9.9	98	363	41	8.4	20	20	20
	15–30	11.5	102	374	38	9.9	23	35	23
		Ethacrynic acid, 1 mg/kg at once, and 1 mg/kg/hour; 2.5% dextrose increased to 14 ml/minute							
	30–39	22.2	105	442	208	5.7	2,000	177	1,554
	39–47	26.0	104	409	224	5.2	2,626	208	2,730
	47–57	19.5	96	354	213	4.2	1,775	156	1,910
	57–72	12.4	88	308	240	1.3	1,230	149	1,377
Dog R	−195	1,200 ml tap water by stomach tube; 2.5% dextrose intravenously at rate of urine flow							
	−165	0.8% saline, 0.3 ml/minute; inulin, 10 mg/minute; PAH, 3 mg/minute							
	0–23	8.3	75	326	40	7.1	8	25	17
	23–35	8.9	78	324	39	7.6	18	37	9
		Ethacrynic acid, 1 mg/kg at once, and 1 mg/kg/hour; 2.5% dextrose increased to 13 ml/minute							
	35–47	16.4	78	323	210	3.5	1,460	148	1,428
	47–59	15.0	74	314	211	3.0	1,260	120	1,335
	59–71	14.8	74	303	190	3.3	1,150	118	1,302
	71–89	10.1	72	252	193	2.7	748	101	980

* See Table I for abbreviations. In addition, C_{PAH} = clearance of *p*-aminohippurate; C_{H_2O} = free water clearance; $U_{Na}V$, U_KV, and $U_{Cl}V$ = excretion of sodium, potassium, and chloride, respectively.

various rates of solute excretion and glomerular filtration rate during administration of ethacrynic acid to that during the infusion of hypertonic mannitol. The maximal $T^c_{H_2O}$ during the infusion of mannitol ranged from 2.6 to 6.5 ml per minute and was stable throughout a wide range of solute excretion (Table I, Figure 1). $T^c_{H_2O}$ during the administration of ethacrynic acid was decreased to values ranging from − 0.6 to + 0.6 ml per minute. This decreased $T^c_{H_2O}$ was unaltered by different rates of solute excretion or glomerular filtration rate. The results of these experiments are shown in Figure 1 and Table I.

The effects of ethacrynic acid on urinary dilution (C_{H_2O}). When ethacrynic acid was administered at the height of water diuresis, there was an immediate increase in urine flow and solute excretion and an immediate decrease in free water clearance (C_{H_2O}). When urinary electrolyte losses were not replaced, C_{H_2O} continued to decrease, as sodium depletion continued and glomerular filtration rate fell. Glomerular filtration rate was not decreased during the first or second collection period after administration of the drug (Tables II and IV), and C_{H_2O} at this time was decreased to a mean of 47% of the control values preceding drug administration. The results of these studies are shown in Table II and Figure 2.

When hypotonic saline was infused in four experiments (in order to replace the electrolyte losses induced by the drug), glomerular filtration rate was maintained relatively stable, and C_{H_2O} was maintained at a reduced, but stable, value (Table III). In all experiments, ethacrynic acid clearly reduced C_{H_2O}, but some diluting capacity was maintained if the drug-induced electrolyte losses were replaced by infusion of the hypotonic electrolyte solution.

Combined effects of ethacrynic acid and chlorothiazide on urinary dilution. In seven experiments the sequential effects of ethacrynic acid and chlorothiazide on free water clearance were

TABLE III

The combined effects of iv ethacrynic acid and chlorothiazide on water diuresis during replacement of electrolyte losses

	Time	Urine vol	GFR	C_{PAH}	U_{osm}	C_{osm}	C_{H_2O}	P_{osm}
	minute	*ml/minute*	*ml/minute*	*ml/minute*	*mOsm/kg*	*ml/minute*	*ml/minute*	*mOsm/kg*
Dog N	−400	1,200 ml tap water by stomach tube, 2.5% dextrose intravenously at rate of urine flow						
	−300	0.8% saline, 0.3 ml/minute; inulin, 10 mg/minute; PAH, 3 mg/minute						
	0–10	10.7	96	271	39	1.6	9.1	267
	10–16	11.3	96	307	39	1.6	9.7	
		Ethacrynic acid, 1 mg/kg at once, and 1 mg/kg/hour; 2.5% dextrose discontinued; 110 mEq/L Na, 4.0 mEq/L K, 15 mEq/L HCO_3, 99 mEq/L Cl, begun at rate of urine flow						
	16–25	25.7	106	361	220	21.3	4.4	266
	25–35	29.1	91	341	226	24.8	4.3	265
	35–45	23.9	87	325	218	19.6	4.3	266
		Ethacrynic acid, 1 mg/kg at once						
	45–55	26.8	102	367	236	23.9	3.1	265
	55–65	29.3	112	406	230	25.9	3.6	262
		Chlorothiazide, 5 mg/kg at once, and 5 mg/kg/hour						
	65–75	26.4	77	302	244	24.6	1.8	262
	75–85	26.4	73	340	249	25.0	1.4	263
	85–95	24.3	74	316	244	22.6	1.7	263
Dog R	−160	1,200 ml tap water by stomach tube; 2.5% dextrose intravenously at rate of urine flow						
	−120	0.8% saline, 0.3 ml/minute; inulin, 10 mg/minute; PAH, 3 mg/minute						
	0–10	10.9	87	338	60	2.4	8.5	274
	10–21	10.6	85	316	56	2.2	8.4	275
		Ethacrynic acid, 1 mg/kg at once, and 1 mg/kg/hour; 2.5% dextrose discontinued; 110 mEq/L Na, 4.0 mEq/L K, 15 mEq/L HCO_3, 99 mEq/L Cl, begun at rate of urine flow						
	21–32	17.8	75	300	202	13.2	4.6	273
	32–40	25.2	81	331	219	19.4	5.8	271
	40–47	25.3	83	354	210	21.1	6.2	273
	47–55	25.2	77	338	202	18.7	6.5	273

TABLE III—(*Continued*)

	Time	Urine vol	GFR	C_{PAH}	U_{osm}	C_{osm}	C_{H_2O}	P_{osm}
	minutes	*ml/minute*	*ml/minute*	*ml/minute*	*mOsm/kg*	*ml/minute*	*ml/minute*	*mOsm/kg*
		Chlorothiazide, 5 mg/kg at once, and 5 mg/kg/hour						
	55–63	25.8	64	278	232	21.8	4.0	275
	63–71	26.3	67	300	246	23.8	2.5	272
	71–79	24.6	64	286	240	21.8	2.8	271
	79–87	24.4	67	286	237	21.3	3.1	272
Dog N	−160	1,200 ml tap water by stomach tube; 2.5% dextrose intravenously at rate of urine flow						
	−103	0.8% saline, 0.3 ml/minute; inulin, 10 mg/minute; PAH, 3 mg/minute						
	0–11	13.4	75	338	57	2.6	10.8	289
	11–28	15.0	76	331	55	2.8	12.2	294
		Chlorothiazide, 5 mg/kg at once, and 5 mg/kg/hour						
	28–39	16.1	75	289	117	6.5	9.6	289
	39–45	15.9	73	313	124	6.8	9.1	288
		2.5% dextrose discontinued; 110 mEq/L Na, 4.0 mEq/L K, 15 mEq/L HCO_3, 99 mEq/L Cl, begun at rate of urine flow						
	45–55	15.5	68	298	120	6.5	9.0	287
		Chlorothiazide, 5 mg/kg at once						
	55–65	15.1	60	212	127	6.6	8.5	292
	65–77	13.6	59	238	119	5.6	8.0	290
		Ethacrynic acid, 1 mg/kg at once, and 1 mg/kg/hour						
	77–85	25.6	72	296	245	21.4	3.2	293
	85–93	26.0	67	272	265	23.5	2.5	292
	93–104	17.7	56	205	250	15.2	2.5	291
		Ethacrynic acid, 1 mg/kg at once						
	104–113	26.7	65	254	267	24.5	2.2	291
	113–121	31.3	76	293	266	28.6	2.7	291

determined. In four experiments ethacrynic acid was administered during peak water diuresis, and electrolyte depletion was prevented by the infusion of hypotonic saline. When stable rates of urine flow and reduced C_{H_2O} were present, chlorothiazide was administered. The addition of the latter agent resulted in a further fall in C_{H_2O} and a further increase in solute excretion (Table III, Figure 3). In one experiment (dog N, Table III), a second dose of ethacrynic acid produced only a small further decrease in C_{H_2O}, but an additional larger fall promptly followed the administration of chlorothiazide. In three experiments (Figure 3), chlorothiazide was administered at the height of water diuresis, and after two to four collection periods (during which time urinary losses were replaced in two of the studies by the infusion of hypotonic saline), ethacrynic acid was administered. The administration of chlorothiazide was associated with an initial decrease in C_{H_2O}, as electrolyte excretion increased. The addition of ethacrynic acid produced a further decrease in C_{H_2O} (Table III, Figure 3). In one experiment (dog N, Table III), a second dose of chlorothiazide did not prevent an additional large decrease in C_{H_2O} when ethacrynic acid was administered. Each of these agents alone decreased free water excretion, despite increases in solute excretion. Together they were associated with a greater reduction in C_{H_2O} than was observed when either was given alone. When chlorothiazide was administered in the presence of ethacrynic acid (during relatively stable rates of solute and free water excretion), the further reduction in C_{H_2O} was accompanied by an increase in solute excretion (Table III, Figure 3). When administered first, chlorothiazide produced a smaller increase in the excretion of sodium and total solute than that observed with ethacrynic acid alone (Tables III and IV, Figure 3). A comparison of the

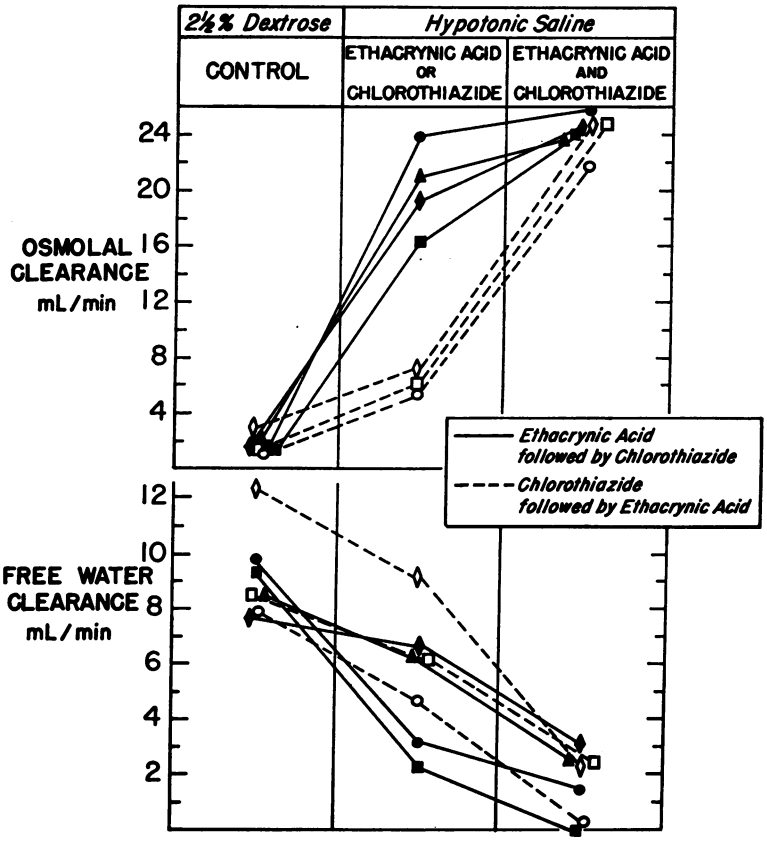

FIG. 3. SEPARATE AND COMBINED EFFECTS OF ETHACRYNIC ACID AND CHLOROTHIAZIDE ON SOLUTE AND FREE WATER CLEARANCE. Control periods are the last collection period before drug administration. Urinary losses were replaced by infusion of a hypotonic solution of electrolyte. The periods of peak solute excretion during each phase of drug administration are shown. Broken lines are experiments in which chlorothiazide was administered first, and solid lines are experiments in which ethacrynic acid was administered first. Seven experiments in four dogs are shown.

effects of each of these agents alone, on water diuresis and electrolyte excretion in the same animals (on different days), is shown in Table IV.

Discussion

Ethacrynic acid represents a new class of natriuretic compounds (1); in early clinical trials it has proven to be an orally effective diuretic agent (2–5). In our present studies, the agent proved to be natriuretic and chloruretic during intravenous administration in the dog, with a potency comparable to that reported for organomercurials (16). Although no systematic attempt was made to determine the maximal effect of the drug, repeated intravenous injections of 1 mg per kg to dogs undergoing diuresis (kept in approximate electrolyte balance by the infusion of a hypotonic solution similar in composition to the excreted urine) did not result in rates of sodium excretion significantly greater than the peak observed after the initial dose. The dose of ethacrynic acid employed in these studies is therefore assumed to have resulted in a near maximal effect of the agent on electrolyte excretion. At the rate of administration of chlorothiazide and ethacrynic acid that we employed, the two agents are clearly additive in increasing solute excretion. The constant administration of ethacrynic acid is associated with continuing electrolyte depletion that ap-

TABLE IV

Comparison of the effects of ethacrynic acid and chlorothiazide on electrolyte excretion and water diuresis

		Urine vol	GFR	C_{H_2O}	$U_{Na}V$	U_KV	$U_{Cl}V$
		ml/minute	*ml/minute*	*ml/minute*	*μEq/minute*	*μEq/minute*	*μEq/minute*
Dog R	Control	8.9	78	7.6	18	37	9
	Ethacrynic acid	16.4	78	3.5	1,460	148	1,428
	Control	8.9	92	7.8	27	44	27
	Chlorothiazide	10.0	78	4.6	530	130	320
Dog P	Control	8.8	90	7.2	18	27	17
	Ethacrynic acid	19.6	90	3.2	1,802	157	2,080
	Control	9.7	84	8.4	39	48	20
	Chlorothiazide	12.3	70	6.1	640	123	430
Dog N	Control	11.5	102	9.9	23	35	23
	Ethacrynic acid	26.0	104	5.2	2,626	208	2,730
	Control	15.0	76	12.2	60	75	45
	Chlorothiazide	15.9	73	9.0	589	191	541

pears to result in compensatory mechanisms (such as the observed decreases in glomerular filtration rate) that preclude a stable diuretic effect. It is therefore difficult to evaluate the relative natriuretic potency of the two agents during sequential administration. In the present studies, the increased excretion of sodium associated with the administration of chlorothiazide alone was similar to that observed in dogs under the same conditions in other studies (12). The natriuretic effect of ethacrynic acid alone was three to four times as great as that observed with chlorothiazide alone.

Knowledge of the sites within the nephron where the reabsorption of filtered solute leads to urinary dilution and concentration (17, 18) has provided a useful tool for localizing the anatomical areas where natriuretic agents may impair the net reabsorption of sodium. Urinary dilution apparently begins in the ascending limb of Henle's loop, and, in the absence of antidiuretic hormone, probably continues through more distal areas of the nephron (17, 18), although direct evidence for this more distal site of dilution is lacking. Therefore, an agent that impairs electrolyte reabsorption in the proximal portions of the nephron would result in a greater delivery of isotonic fluid to these distal diluting sites and thereby increase the generation of free water during water diuresis. Acetazoleamide has such an effect during water diuresis (6, 7, 12), and, on the basis of such evidence alone, it has been suggested that the natriuretic effect of carbonic anhydrase inhibitors resides largely in the proximal nephron. This interpretation has been supported by the results of micropunctures which demonstrate that the major fraction of filtered bicarbonate is reabsorbed in the proximal convolution (19, 20). Chlorothiazide, on the other hand, results in an increased excretion of solute and a decreased excretion of free water during maximal water diuresis in both man and the dog (11, 12). On the basis of this evidence, it has been suggested that this latter agent interferes with sodium reabsorption in distal portions of the nephron where sodium transport results in the production of dilute tubular fluid (11, 12). Thiazides do not decrease the renal concentrating capacity ($T^c_{H_2O}$), as would be expected if a major decrease occurred in the transport of solute by the ascending limb of Henle's loop. Therefore, it has been suggested that thiazides interfere with net sodium reabsorption at diluting sites distal to Henle's loop (11, 12). This conclusion has received some support from stop-flow analyses that are consistent with a distal site of action of chlorothiazide (21, 22). The effect of organomercurials on the renal concentrating and diluting mechanisms appears more complex than that of carbonic anhydrase inhibitors or thiazides (8–10). However, the natriuresis resulting from the administration of organomercurials may not be associated with major decreases in either $T^c_{H_2O}$ or C_{H_2O} (8–10).

In our studies, the administration of ethacrynic acid was accompanied by the virtual disappearance of the ability to excrete a concentrated urine.

This observation alone is consistent with the view that this agent may markedly decrease the transport of electrolyte by the ascending limb of Henle's loop. However, other factors such as membrane permeability, glomerular filtration rate (23), and the rate of solute excretion (12, 23) may influence the renal concentrating mechanism, as measured by $T^c_{H_2O}$. In the present studies, $T^c_{H_2O}$ was measured, during the administration of ethacrynic acid, through a wide range of solute excretion and at various rates of glomerular filtration (which overlapped with the same measurements during the control studies with hypertonic mannitol infusion). $T^c_{H_2O}$ was completely abolished or markedly decreased, regardless of the rate of solute excretion, and concentrating ability was not restored when glomerular filtration rate was increased by the infusion of hypertonic saline.

If ethacrynic acid does decrease the transport of solute by Henle's loop, then its action should also be manifested by decreased diluting ability, since this tubular transport site results in both urinary concentration and dilution (17, 18). Such an effect on C_{H_2O} was observed in our studies. During the peak natriuresis, free water excretion was reduced from control values by an average of 53%. In earlier studies, we had observed that after approximately 20 minutes of drug administration, C_{H_2O} began to progressively decrease, in association with a decrease in solute excretion. Since this delayed fall also was associated with a decreasing glomerular filtration rate, we considered the possibility that the progressive fall in C_{H_2O} might be the result of the electrolyte depletion induced by the drug. This conclusion appeared to be correct, since the replacement of drug-induced electrolyte losses (by the infusion of a hypotonic electrolyte solution) resulted in relatively stable values for both C_{H_2O} and glomerular filtration rate.

Although ethacrynic acid uniformly reduced the excretion of free water, the effect of the agent on C_{H_2O} during the "steady state" (replacement of electrolyte losses) appeared less marked than the effect on $T^c_{H_2O}$. These observations are consistent with the view that ethacrynic acid may markedly decrease electrolyte transport by the loop of Henle and that a more distal site of urinary dilution may exist which is not impaired by the drug. It has been suggested that chlorothiazide (which decreases C_{H_2O} but not $T^c_{H_2O}$) may interfere with electrolyte reabsorption at diluting sites distal to Henle's loop (11, 12). It therefore appeared likely that this latter drug may produce further impairment of urinary dilution in the presence of ethacrynic acid. When chlorothiazide was given at a time when a reduced, but stable, clearance of free water was present during the administration of ethacrynic acid and hypotonic electrolyte infusion, a further fall in the clearance of free water occurred. However, some minimal ability to dilute the urine persisted in the presence of both drugs in six of the seven experiments. When chlorothiazide was administered first during water diuresis, the same sequence of changes occurred. Free water clearance was reduced immediately and then decreased further when ethacrynic acid was added. The administration of additional drug did not prevent the effect on C_{H_2O} of the second drug; clearly, therefore, two drug-sensitive mechanisms of urinary dilution exist.

It is unlikely that the effect of ethacrynic acid on free water clearance was due to a release of antidiuretic hormone or to an antidiuretic hormone-like effect of the drug, since both the concentrating and diluting mechanisms were affected. The possibility cannot be excluded that a release of antidiuretic hormone accounted in part for the progressive decrease in C_{H_2O} observed when electrolyte losses were not replaced. However, the initial decrease in free water clearance and the increase in solute excretion occurred simultaneously after administering the drug, and a continued fall in free water clearance was prevented by replacement of the electrolyte losses. Therefore, an endogenous release of antidiuretic hormone appears unlikely to have influenced the interpretation of the results of these studies.

On the basis of the present observations, we suggest that a major part of the natriuretic and chloruretic effect of ethacrynic acid is the result of a decreased (and perhaps abolished) transport of electrolyte by the ascending limb of Henle's loop. This interpretation agrees with that of Cannon, Ames, and Laragh, who suggested that this agent has some effect on "distal" reabsorption, since the natriuresis is associated with a de-

creased C_{H_2O} (3). In addition, our data are consistent with the concept that urinary dilution continues at tubular sites distal to Henle's loop and that electrolyte transport at these latter sites is not markedly diminished by ethacrynic acid. On the other hand, chlorothiazide appears to impair electrolyte transport at these more distal diluting sites, but this latter agent may not affect urinary dilution in Henle's loop, since it does not decrease $T^c_{H_2O}$ (12). Although the administration of chlorothiazide often was associated with decreases in glomerular filtration as C_{H_2O} decreased, this was not true in all experiments (dog N, Table III). This is in agreement with the previous report that the thiazide-induced reduction in C_{H_2O} occurs independent of changes in glomerular filtration rate (12).

Ethacrynic acid produced large increases in urine flow, both during water diuresis and during hydropenia. The increased rate of osmolal clearance resulting from the drug was several times as great as the absolute values for the maximal $T^c_{H_2O}$, or drug-depressed C_{H_2O}, observed in the animals. These differences suggest that the agent may decrease the reabsorption of electrolyte at sites within the nephron other than those sites where solute reabsorption leads to concentration and dilution. Since continued distal reabsorption of electrolyte is apparent from the continued excretion of free water during water diuresis, this additional effect of the drug on electrolyte reabsorption probably occurs in proximal rather than distal portions of the nephron. Measurements of $T^c_{H_2O}$ are, however, unlikely to be entirely representative of solute transport by the ascending limb of Henle's loop, since other factors influence this measurement. Likewise, the depression of C_{H_2O} may bear little relationship to the extent to which dilution in Henle's loop is impaired, since the increased delivery of solute to more distal diluting areas may increase solute reabsorption and dilution at these latter sites. It is entirely possible, but perhaps unlikely, that the large fraction of filtered sodium excreted in the presence of this drug could all be the result of an action in Henle's loop alone, in which case the relatively low $T^c_{H_2O}$ (compared to the drug-induced solute excretion) would reflect the inefficiency of the loop transport system in ultimately conserving water. It has been suggested that a significant fraction of the filtered sodium may be reabsorbed "isotonically" in the distal nephron, even during water diuresis (9). An action of ethacrynic acid at such a site, in combination with an action in Henle's loop, could not be distinguished from a combined effect of the agent on the reabsorption of electrolyte by the proximal convolution and Henle's loop.

The relationship between the increased excretion of chloride induced by ethacrynic acid and the total of sodium and potassium excretion is consistent with the premise that this agent acts at tubular sites where reabsorption involves both sodium and chloride. The data provide no information as to how net electrolyte reabsorption is impaired by the drug. The differences that exist between the effects of ethacrynic acid and organomercurials (8–10) on $T^c_{H_2O}$ and C_{H_2O} make it likely that these two agents also have different mechanisms of action within the nephron. This suggestion is supported by reports that these drugs have additive diuretic effects (3, 5). The increased excretion of potassium associated with the natriuresis induced by ethacrynic acid may be due to increased cation exchanges in the distal nephron (24), and, in itself, does not imply a special effect of the drug on the excretion of potassium.

Summary

Ethacrynic acid was administered intravenously to dogs during hydropenia with vasopressin infusion and during maximal water diuresis. The diuretic effect of the drug began within 2 minutes, and peak natriuresis occurred within 20 minutes. Potassium excretion increased, and chloride excretion approached the sum of cation excretion. The agent had a natriuretic potency three to four times that of chlorothiazide, with an average maximal effect resulting in the excretion of 17% of the filtered sodium.

The renal concentrating ability was virtually abolished by ethacrynic acid. During water diuresis, the drug uniformly reduced, but did not eliminate, free water clearance. The addition of chlorothiazide always resulted in a further decrease in free water clearance. The two agents were additive in decreasing free water clearance

and increasing solute excretion, regardless of the sequence of administration.

We suggest that an important part of the natriuresis produced by ethacrynic acid results from an interference with reabsorption of electrolyte by the ascending limb of Henle's loop. In addition, the drug may exert a significant effect on proximal electrolyte reabsorption. These data are consistent with the premise that two drug-sensitive sites of urinary dilution exist. *1*) The loop of Henle, where electrolyte transport is common to both urinary dilution and concentration, is affected by ethacrynic acid and probably not by chlorothiazide. *2*) A more distal site of urinary dilution, not directly involved in the concentrating mechanism, may be affected by chlorothiazide and probably not by ethacrynic acid.

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Addendum

Since this manuscript was completed, Goldberg, McCurdy, Foltz, and Bluemle (25) have reported on the effects of orally administered ethacrynic acid to hydrated and maximally hydropenic humans. These authors observed decreases in C_{H_2O} and nearly complete abolition of $T^c_{H_2O}$ and suggested that a major effect of ethacrynic acid is to decrease or abolish solute transport by the ascending limb of Henle's loop. Our findings in the dog are in entire agreement with this conclusion, but, as discussed, the effects of this agent on the transport of electrolyte also may occur in other portions of the nephron. In addition, the present conclusion that ethacrynic acid and chlorothiazide exert independent effects on urinary dilution should be based only on the combined effect of the two agents during water diuresis, and not on observations made during the administration of either agent alone.

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ERRATUM

In the paper entitled, "Vasoactive Mediators as the 'Trigger Mechanism' of Endotoxin Shock," by Eugene D. Jacobson, Benjamin Mehlman, and John P. Kalas, published in the May issue, the following correction should be noted on page 1000, line 14 of the second column: The sentence beginning *Platelet-free plasma concentrations* . . . should read *Platelet-rich plasma concentrations* . . . .