STUDIES ON THE RENAL CONCENTRATING MECHANISM. V. EFFECT OF DIURETIC AGENTS *

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Evidence for the site of action of diuretic drugs in man can be derived from observations of their effect on different tubular functions. Previous studies have shown that mercurial diuretic agents have little influence on water diuresis or antidiuresis (1–5) and hydrogen ion secretion (6, 7). These observations have been taken to indicate that the predominant action of these mercurial agents is to decrease sodium reabsorption in the proximal tubule. A proximal site of action has also been suggested by stop flow analysis of tubular function during combined mercurial and mannitol diuresis (8–10). The similarity between osmotic and mercurial diuresis has been emphasized (5, 8, 11, 12) but these states have not been compared directly in man. The present study concerns the effects of diuretic drugs on renal concentrating ability in healthy hydropenic young men. The urine was found to be less concentrated during mercurial diuresis when compared with mannitol diuresis at similar urine flows. This difference was greatest when mercurial action was potentiated by the prior administration of ammonium chloride. The data provide evidence that mercurial diuretic agents can act on those portions of the nephron involved in the renal concentrating process, presumably beyond the proximal tubule. Acetazolamide and chlorothiazide, drugs which probably act both distally and proximally (8, 13–15) were also studied. The former was found to decrease concentrating ability, while the latter had little effect.

METHODS

Experimental procedure. The subjects were 6 paid volunteer medical students, ages 25 to 28 years, who were selected by a "bladder emptying test" for their ability to void small volumes of urine consistently (16). The experiments were begun after 18 hours of water deprivation, at 7 a.m., except in Subject A.G., in whom experiments were begun at 11 a.m. Infusions containing the diuretic agents were administered after 2 to 3 preliminary periods, in which osmotic urine/plasma ratio ranged from 3.0 to 4.6, and urine flow was less than 0.6 ml per minute. Urine samples were collected at intervals of 30 minutes and blood samples every 2 hours.

The following experiments were performed in a random sequence for each subject, at intervals of at least 1 week between studies. In all experiments 200 mU of vasopressin [1] was administered initially. Vasopressin was then added to each infusion to provide 200 mU per hour, except in the repeat experiments with mercaptomerin.

1. Mannitol infusion (all subjects); 5 per cent mannitol in 0.5 per cent NaCl solution was infused at a rate of 10 ml per minute for 5 hours.

2. Urea infusion (3 subjects); 30 per cent urea was infused at 1 to 3 ml per minute for 4 hours.

3. Isotonic sodium chloride infusion (3 subjects); 0.9 per cent NaCl solution was infused at 30 to 40 ml per minute for 1 hour, then at 10 ml per minute for the next 3 hours, giving a total volume of about 4 L.

4. Mercaptomerin (all subjects); a solution of mercaptomerin containing 40 mg Hg in 50 ml of isotonic sodium chloride was injected intravenously, followed by an

TABLE I

*Mannitol loading "reference" experiment**

Subject	No. of periods	a	b	R
M.G.	8	0.0874	0.3886	0.9929
P.M.	8	0.1222	0.2839	0.9928
A.M.	7	0.0910	0.3817	0.9961
J.P.	8	0.0940	0.3031	0.9953
J.S.	7	0.1041	0.2442	0.9931
A.G.	8	0.1168	0.3202	0.9948

* The equation, $\frac{1}{U/P - 1} = aV + b$, for the mannitol loading "reference" experiment for each subject, is presented in terms of the regression constants (a, b) and the correlation coefficient (R).

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[1] A single lot of Pitressin (Parke, Davis) was used which had been shown to cause maximal antidiuresis in hydrated normal man in doses of 25 to 50 mU per hour.

TABLE II

*Summary of individual diuresis experiments**

Experiment	Subject	Range V	Range U/P		Mean ΔU/P	Mean $\Delta T^c_{H_2O}$	Mean C_{creat}
		ml/min/1.73 m²					*ml/min/1.73 m²*
Mannitol	M.G.	1.39–6.35	2.07–3.05				140
	P.M.	1.91–7.37	1.85–3.01				145
	A.M.	1.41–7.43	1.95–3.01				105
	J.P.	1.31–4.74	2.35–3.44				128
	J.S.	0.89–5.36	2.27–3.91				150
	A.G.	1.60–7.34	1.86–3.06				131
				Mean			133
Urea	M.G.	1.37–4.17	2.30–3.02		−0.06	−0.19	111
	P.M.	1.10–4.56	2.21–3.01		−0.16	−0.31	166
	A.M.	1.52–4.28	2.31–3.30		0.06	0.10	132
				Mean	−0.05	−0.13	136
Saline	M.G.	1.06–2.58	2.58–3.03		−0.04	−0.06	180
	P.M.	0.78–2.33	2.49–3.42		−0.24	−0.39	145
	J.P.	0.96–2.47	2.90–3.72		0.08	0.10	193
				Mean	−0.07	−0.12	173
Mercaptomerin	M.G.	2.77–6.98	1.54–2.21		−0.44	−1.85	142
	P.M.†	2.40–6.92	1.46–1.99		−0.55	−2.34	130
	A.M.	1.20–4.21	1.68–2.85		−0.53	−1.63	122
	J.P.	1.28–8.97	1.42–2.45		−0.61	−3.16	125
	J.S.	1.72–5.27	1.64–2.40		−0.71	−2.76	124
	A.G.	3.87–9.79	1.30–1.73		−0.41	−2.68	132
				Mean	−0.54	−2.40	129
Meralluride	J.P.	1.06–6.38	1.49–3.00		−0.68	−2.36	122
	J.S.	1.52–6.74	1.64–2.18		−0.71	−2.49	135
	A.G., 1	1.61–3.29	2.49–3.15		0.07	0.15	154
	A.G., 2	1.86–4.18	2.17–2.74		−0.10	−0.32	150
				Mean‡	−0.69	−2.42	128
NH_4Cl + mercaptomerin	M.G.	1.56–8.99	1.28–2.16		−0.78	−4.08	143
	J.P.	4.47–16.1	1.19–1.33		−0.64	−5.45	132
	J.S.	1.41–11.9	1.16–2.22		−0.85	−5.27	103
				Mean	−0.76	−4.94	126
Chlorothiazide	M.G.	2.52–3.34	2.27–2.69		−0.09	−0.27	139
	P.M.	2.47–5.66	1.81–2.44		−0.15	−0.60	153
	A.M.	2.36–3.26	2.33–2.76		−0.02	−0.05	114
	J.P.	1.66–3.87	2.36–2.86		−0.15	−0.36	131
	J.S.	2.02–2.70	2.48–2.81		−0.43	−1.05	117
	A.G.	1.91–3.27	2.04–2.77		−0.13	−0.37	106
				Mean	−0.16	−0.45	127
Acetazolamide	J.P.	1.56–2.42	2.32–2.74		−0.44	−1.01	125
	J.S.	1.56–2.82	2.10–2.32		−1.02	−1.89	142
	A.G.	1.14–3.58	2.14–2.97		−0.23	−0.63	166
	P.M.	1.90–4.08	1.91–2.50		−0.46	−1.19	134
				Mean	−0.54	−1.18	142

* Including range of urine flow (V), osmotic U/P ratio (U/P), mean creatinine clearance (C_{creat}), and deviation of osmotic U/P ratio (ΔU/P) and net water reabsorption ($\Delta T^c_{H_2O}$) from the values predicted from the mannitol loading "reference" experiment.

† Three 60 minute periods only.

‡ Mean of Subjects J.P. and J.S. only.

infusion containing 40 mg Hg in 240 ml of isotonic NaCl solution at a rate of 1.0 ml per minute. This experiment was repeated in Subjects J.S. and J.P. with the addition of 1,000 mU per hour of vasopressin.

5. Meralluride (3 subjects); the procedure and dose were identical with that for mercaptomerin. The experiment was repeated in Subject A.G.

6. Ammonium chloride and mercaptomerin (3 subjects); the mercaptomerin experiment was repeated after the subjects had received 9 to 12 g ammonium chloride orally per day for 3 days.

7. Chlorothiazide (all subjects); 0.5 g of chlorothiazide dissolved in 50 ml isotonic NaCl solution was injected intravenously followed by an infusion of 0.5 g chloro-

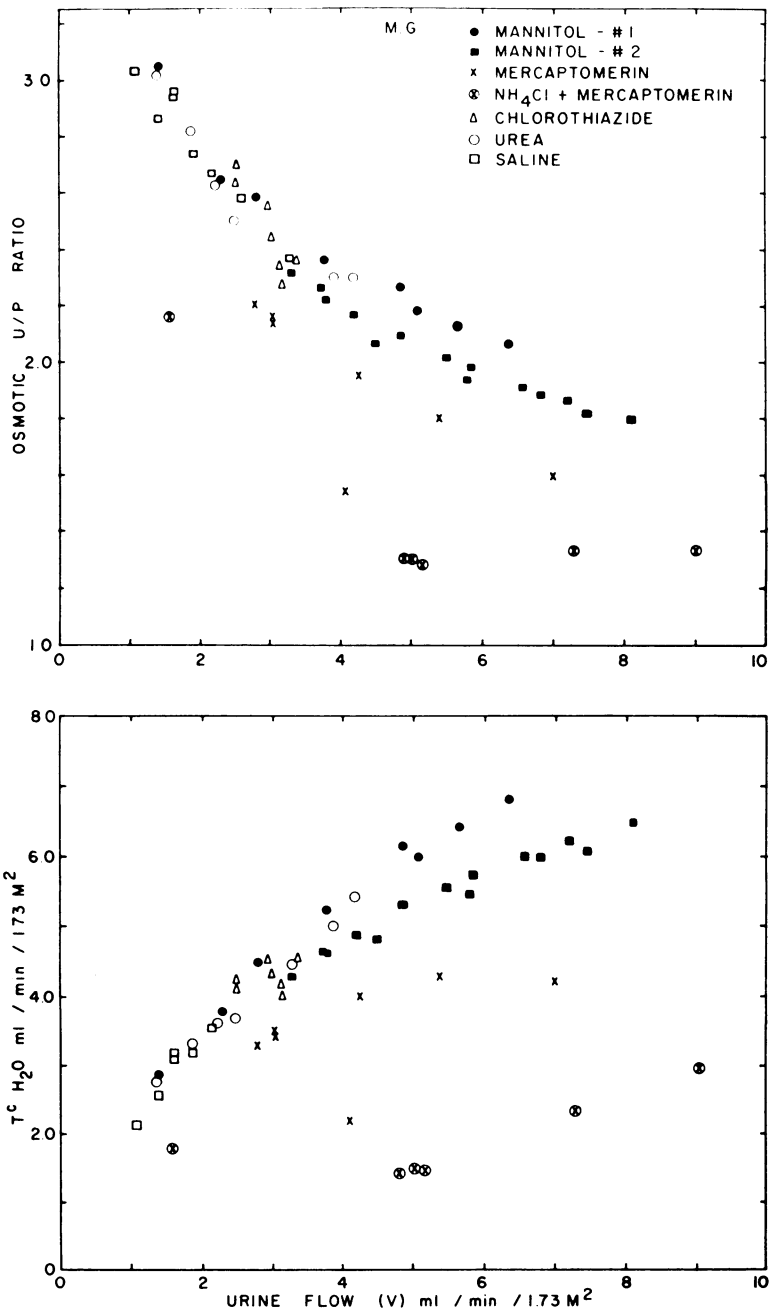

FIG. 1. CONCENTRATING RESPONSE TO VARIOUS DIURETIC AGENTS IN SUBJECT M.G. Mannitol no. 1 indicates the "reference" mannitol loading experiment for this subject. Mannitol no. 2 is a study done one year previously.

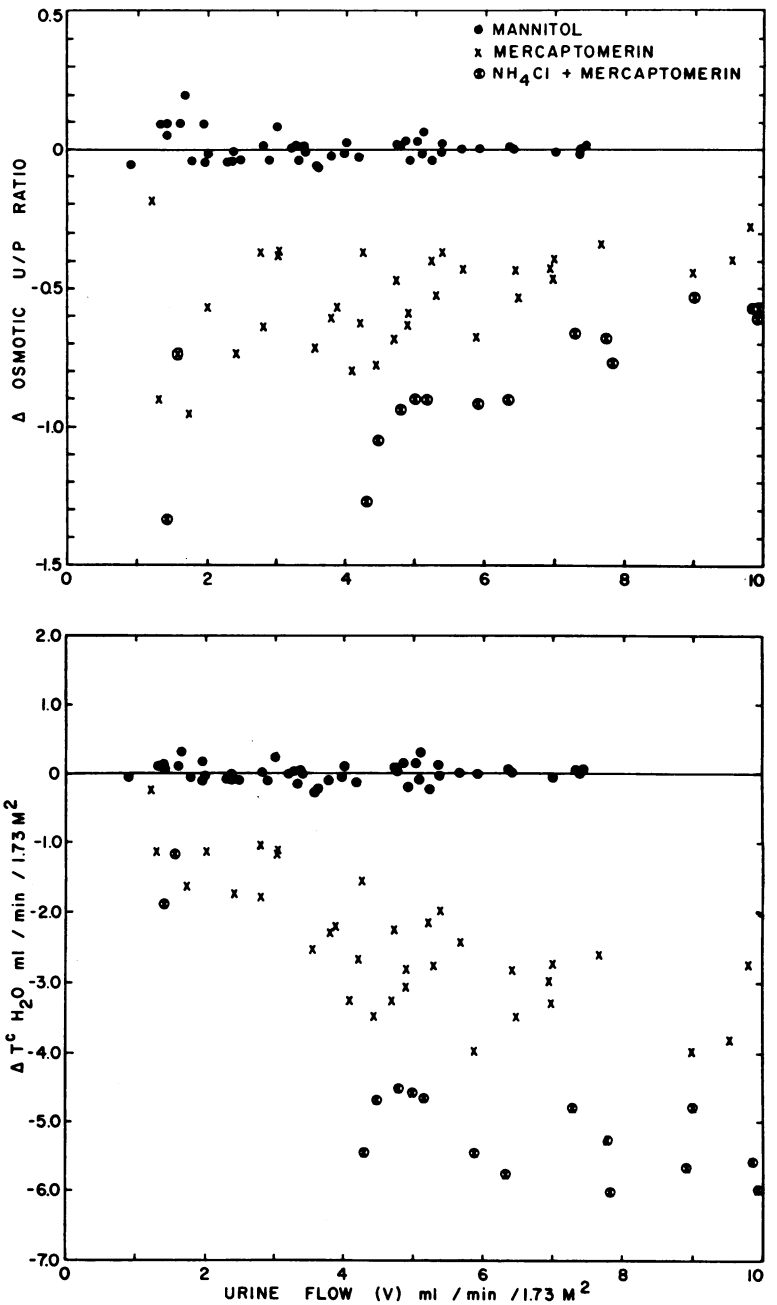

FIG. 2. DEVIATION OF Δ OSMOTIC U/P RATIO AND NET WATER REABSORPTION ($\Delta T^{c}_{H_2O}$) FROM VALUES PREDICTED BY THE MANNITOL "REFERENCE" EQUATION (SEE TEXT). Data include periods for mannitol, mercaptomerin, and ammonium chloride-potentiated mercaptomerin diuresis in six subjects.

thiazide in 240 ml of isotonic NaCl solution at a rate of 1.0 ml per minute.

8. Acetazolamide (4 subjects); the procedure and dose were identical with that for chlorothiazide.

9. Mannitol infusion and mercaptomerin (3 subjects); 10 per cent mannitol was infused at 20 ml per minute until urine flow was 10 to 15 ml per minute for 3 to 4 periods. Mercaptomerin (80 mg Hg) was then given intravenously and the mannitol infusion continued at 10 ml per minute for 3.5 hours.

Chemical methods. Chemical methods were those previously described (16). In addition, chloride was determined by the method of Schales and Schales (17).

Calculations. Osmotic urine/plasma (U/P) ratio,

osmolal clearance (C_{osm}), and net water reabsorption ($T^c_{H_2O}$) were calculated as described previously (16, 18, 19). All data were corrected to 1.73 m^2 of body surface area. The relationship between concentrating response and urine flow (V) during osmotic diuresis, when urine flow is less than 20 ml per minute, can be evaluated in terms of an empirical formula described in the preceding paper of this series (19). In the present study, the mannitol loading experiments on each subject were chosen as the "reference" experiment for comparison of the renal concentrating response with other types of diuresis. The regression constants and the correlation coefficient for the hyperbolic equation, $\frac{1}{U/P-1} = aV + b$, where U/P represents the osmotic U/P ratio, V is urine flow, and a and b are constants, were calculated for each mannitol loading "reference" experiment by the method of least squares [(20) Table I]. Using this equation, it was possible to calculate predicted values for osmotic U/P ratio and $T^c_{H_2O}$, [$T^c_{H_2O} = (U/P - 1) \times V$] for any given rate of urine flow. Differences in concentrating response between the mannitol experiment and other forms of diuresis in each subject could then be quantitated by comparing observed osmotic U/P ratio and $T^c_{H_2O}$ values

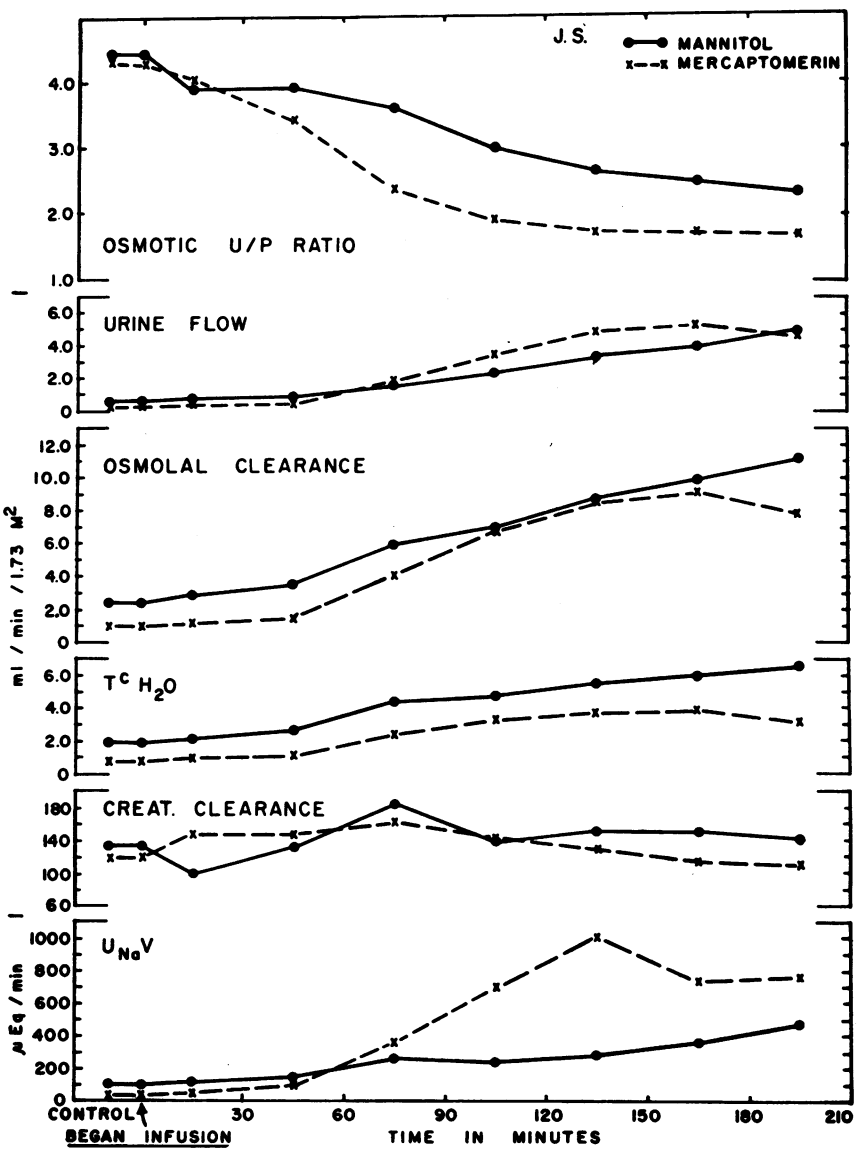

FIG. 3. COMPARISON OF INDIVIDUAL EXPERIMENTS WITH MANNITOL LOADING AND MERCAPTOMERIN DIURESIS IN SUBJECT J.S. Osmotic U/P ratio, urine flow, osmolal clearance, net water reabsorption ($T^c_{H_2O}$), creatinine clearance, and sodium excretion ($U_{Na}V$) are given for individual periods.

TABLE III

*Effect of mercaptomerin during hypertonic mannitol loading**

Subject	Time	V	U/P	$T^c_{H_2O}$	$U_{Na}V$	$\frac{U_{Na}}{U_{osm}} \times 100$
	min	*ml/min/1.73 m²*		*ml/min/1.73 m²*	*μEq/min/1.73 m²*	
J.P.	−40–0	14.0	1.50	7.0	577	9
	70–110	19.2	1.32	6.2	1,127	15
	150–180	20.6	1.34	6.9	1,144	14
J.S.	−40–0	11.3	1.57	6.4	370	7
	70–110	12.8	1.46	5.8	705	12
	150–170	17.4	1.36	6.2	1,234	17
A.G.	−45–0	10.8	1.48	5.2	515	11
	75–120	21.7	1.18	3.8	1,741	23
	150–195	21.3	1.26	5.6	1,515	19

* Mean urine flow (V), osmotic U/P ratio (U/P), net water reabsorption ($T^c_{H_2O}$), sodium excretion ($U_{Na}V$) and percentage of urine osmolality accounted for by sodium $\left(\frac{U_{Na}}{U_{osm}} \times 100\right)$ are given for periods before, 1 to 2 hours after, and 2.5 to 3 hours after mercaptomerin administration.

with those predicted for the same rate of urine flow by the equation for the mannitol "reference" experiment. The differences between observed and predicted values are termed Δ U/P and Δ $T^c_{H_2O}$. In 3 subjects, M.G., P.M. and A.M., data from previous mannitol loading experiments (19) over the range of 1 to 20 ml per minute urine flow showed good agreement with the mannitol "reference" experiment, so that calculated Δ U/P values for individual collection periods were always between + 0.15 and − 0.30.

RESULTS

The renal concentrating response during different types of diuresis is illustrated for one subject in Figure 1. The mean values for Δ U/P and Δ $T^c_{H_2O}$ for each experiment are given in Table II. The concentrating response to urea and saline diuresis showed no consistent deviation from that observed with mannitol loading, although in individual periods Δ U/P ranged from + 0.38 to − 0.50 and Δ$T^c_{H_2O}$ from + 0.58 to − 0.73 ml per minute.

A consistently lower osmotic U/P ratio and smaller $T^c_{H_2O}$ was observed during mercaptomerin diuresis as compared with osmotic diuresis at similar urine flows (Figure 2, Table II). Mean Δ U/P for all six subjects was − 0.54 (individual periods ranged from − 0.19 to − 0.96) and Δ $T^c_{H_2O}$ was − 2.40 ml per minute (individual periods ranged from − 0.24 to − 4.00 ml per minute). The contrast between mannitol loading and mercaptomerin experiments in one subject, J.S., is shown in Figure 3. Depression of osmotic U/P ratio appeared early in mercurial diuresis and preceded the peak natriuretic response. An even greater change in concentrating response is observed during mercaptomerin diuresis which has been potentiated by prior administration of ammonium chloride (Table II). Although larger peak urine flows were also achieved, greater deviations in the concentrating response were seen even at lower rates of urine flow (Figures 1, 2). When the dose of vasopressin was increased to 1,000 mU per hour in repeat mercaptomerin experiments on J.S. and J.P., the depression of concentrating ability was as great as that observed in the original mercaptomerin experiment (mean Δ U/P values were − 0.65 and − 0.69, respectively).

The concentrating response during meralluride diuresis was the same as that observed for mercaptomerin in Subjects J.P. and J.S. In Subject A.G. there was no impairment of concentrating ability in the first experiment in which meralluride caused only a small increase in urine flow and sodium excretion. In the second meralluride experiment, Subject A.G. showed a greater diuresis but only a slight decrease in concentrating ability.

When mercaptomerin was administered during a hypertonic mannitol infusion there was a small decrease in $T^c_{H_2O}$ despite a further increase in urine flow; 2.5 to 3 hours after mercaptomerin administration $T^c_{H_2O}$ had returned toward control values, although urine flow and sodium excretion remained high (Table III).

Chlorothiazide and acetazolamide yielded considerably smaller increases in urine flow than did

TABLE IV

*Effect of diuretic agents on urinary composition**

Diuretic agent	No. of experiments	V	$U_{osm}V$	$U_{Na}V$	U_KV	$U_{Cl}V$	$U_{urea}V$	$\frac{U_{Na}}{U_{osm}} \times 100$	$\frac{U_K}{U_{osm}} \times 100$
		ml/min	*μOsm/min*		*μEq/min*		*μmoles/min*		
Mannitol	6	5.98	3,612	470	80	435	333	13.0	2.2
Isotonic NaCl	3	2.36	1,838	582	116	614	393	31.7	6.3
Urea	3	4.09	2,898	420	103	380	1,925	14.5	3.6
Mercaptomerin	6	6.59	2,903	1,155	68	1,170	317	39.8	2.3
NH_4Cl + mercaptomerin	3	11.0	3,812	1,360	112	1,581	266	35.6	2.8
Meralluride†	2	6.18	2,882	1,192	34	1,035	289	41.4	1.2
Chlorothiazide	6	3.35	2,228	777	187	576	342	34.5	8.4
Acetazolamide	4	2.82	1,776	568	230	244	320	31.6	13.1

* Urine flow (V), total solute ($U_{osm}V$), sodium ($U_{Na}V$), potassium (U_KV), chloride ($U_{Cl}V$), and urea ($U_{urea}V$) excretion, and percentage of total solutes accounted for by Na $\left(\frac{U_{Na}}{U_{osm}} \times 100\right)$ and K $\left(\frac{U_K}{U_{osm}} \times 100\right)$ are given for the maximal hourly urine flow.

† Subjects J.P. and J.S. only.

mercaptomerin. During chlorothiazide diuresis concentrating ability was only slightly less than that observed with mannitol loading, except in Subject J.S. who showed a definite decrease. Acetazolamide caused a distinct decrease in concentrating ability, and in Subject J.S. this decrease was relatively greater than that observed with mercaptomerin.

Endogenous creatinine clearance values were little affected by any of the agents studied, except for an increase with saline loading. Transient depressions of creatinine clearance were not encountered with any of the agents used in these experiments.

The patterns of urinary electrolyte composition observed during the peak response to the various agents are shown in Table IV. Mercurial diuretic agents primarily increased sodium and chloride excretion, while chlorothiazide and acetazolamide increased potassium as well as sodium excretion and caused less increase in chloride excretion. The resultant increase in undetermined anion was presumably due to increased bicarbonate excretion. This difference was greatest with acetazolamide diuresis. There were no consistent changes in plasma composition with any of the agents used except for a decrease in plasma bicarbonate concentration to 17 mmoles per L after three days of ammonium chloride administration.

DISCUSSION

The urine is less concentrated during mercurial diuresis than it is during osmotic diuresis at comparable urine flows in the same subjects. This apparent impairment of the ability of the kidney to elaborate a concentrated urine could be due to inhibition of antidiuretic hormone, to a direct effect on water transport, or to inhibition of a moiety of sodium reabsorption which is essential to the concentrating process. Inhibition of antidiuretic hormone could decrease concentrating ability by altering water reabsorption (21) or by altering sodium reabsorption in the loop of Henle (22) if antidiuretic hormone increases sodium reabsorption at this site as it does in the short-circuited toad bladder (23). A recent study has suggested that antidiuretic hormone might act on the renal tubule by forming disulfide linkages with proteins in the cell membrane (24). Since mercurial diuretic compounds are known to bind available sulfhydryl groups (25) these agents might compete for available binding sites. Competitive inhibition of antidiuretic hormone action could not be demonstrated in the present study, however, since a fivefold increase in the dose of exogenous vasopressin did not alter the mercurial effect on concentrating ability. Mercurial diuretic agents might act on concentrating ability by directly inhibiting water transport. Since net water reabsorption at the concentrating site is probably passive in response to an osmotic gradient (21, 22, 26), such an inhibition should then be due to decreased membrane permeability. However, Garby and Linderholm (27) have shown that mersalyl has no effect on the permeability of frog skin to heavy water, but it does cause a de-

crease in active sodium transport. Could impairment of concentrating ability be ascribed to the direct action of organomercurial agents on sodium transport in the renal tubules? It has been postulated on the basis of stop flow analysis that mercurial diuretic agents act by inhibiting active (9) or passive (10) sodium reabsorption in the proximal tubule. Such an effect would not explain impairment of concentrating ability relative to that observed during osmotic diuresis since the latter also decreases proximal sodium reabsorption (28).

Recent evidence indicates that the renal concentrating process operates by means of a countercurrent multiplier system which is initiated by active sodium transport in the loop of Henle (21, 22, 26, 29, 30). This produces a hypertonic renal medullary interstitial fluid, which results in passive reabsorption of water from the collecting ducts. If this hypothesis is correct, it then appears likely that mercurial diuretic agents impair concentrating ability by inhibiting sodium transport in the loop of Henle. The localization of mercurial diuretic action to this site is further suggested by histochemical data which show that the number of available sulfhydryl binding sites in the ascending portion of the loop of Henle is decreased after the administration of mersalyl (25).

The greater impairment of concentrating ability observed when mercurial diuretics were given during ammonium chloride acidosis is not unexpected if acidosis potentiates mercurial inhibition of sodium transport at the concentrating site. Ammonium chloride acidosis itself did not impair renal concentrating ability in preliminary periods in these subjects or in three additional subjects studied before and after the administration of ammonium chloride for three days. Moreover, intravenous acid loading in hydropenic dogs does not appear to affect renal concentrating ability (31, 32).

Previous studies assigning mercurial action to the proximal tubule have cited the apparent lack of effect of these drugs on net water reabsorption during antidiuresis (3, 5) and on free water clearance during maximal water diuresis (1, 2). In a more recent study, free water clearance was found to increase during combined mercurial and water diuresis (33) but this could still be consistent with a proximal site of action. Although direct comparison of osmotic and mercurial diuresis was not made, in one of the available studies in man (5) concentrating ability may have been depressed during mercurial diuresis, since values of $T^c_{H_2O}$ were generally less than 4 ml per minute when urine flow was 15 ml per minute. In hydropenic dogs, Brodsky and Graubarth (11) found no difference in the relationship between osmotic load and urine flow when they compared meralluride and mannitol diuresis. The effects of meralluride and mercaptomerin on the concentrating mechanism were different in one subject of the present study, but identical in two others.

In the present study there was little alteration in the rate of net water reabsorption when mercurial diuresis was superimposed on a mannitol diuresis at high urine flows. Interpretation of these data is difficult since $T^c_{H_2O}$ can vary with different types of osmotic diuresis at high urine flows (19). If, as Malvin and Wilde (34) have recently suggested, the countercurrent gradient is "washed out" during massive osmotic diuresis then, under these circumstances, the action of inhibitory drugs on the concentrating mechanism could be less apparent.

Impairment of concentrating ability during acetazolamide diuresis suggests that carbonic anhydrase inhibition may affect sodium transport in the loop of Henle. The increased bicarbonate ion in the tubule does not appear to be a factor since sodium bicarbonate infusions in dogs (30) and in one of our subjects (unpublished observations) did not impair concentrating ability. Chlorothiazide, which in addition to inhibiting carbonic anhydrase to a lesser extent appears to act chiefly on sodium chloride reabsorption (8, 13, 35), caused little impairment of concentrating ability. Others have found no impairment of net water reabsorption during mannitol diuresis in subjects given chlorothiazide (33, 36). These results with chlorothiazide would indicate that the difference between mannitol and mercurial diuresis is not due to the fact that sodium chloride is the predominant urinary solute during drug-induced diuresis. Actually, sodium chloride diuresis produces a somewhat more concentrated urine than does mannitol diuresis in hydropenic rats (22).

Impairment of concentrating ability during mercurial diuresis has been quite transient in the subjects of the present study. Further studies are

required to determine whether this effect is similarly transient in patients requiring diuretic therapy. The fact that the urine is less concentrated during mercurial diuresis may actually represent an advantage to the patient with edema, who must excrete an excess of isotonic extracellular fluid, in the presence of maximal antidiuretic hormone action.

SUMMARY

The effect of osmotic (mannitol, urea, saline), mercurial, ammonium chloride-potentiated mercurial, chlorothiazide, and acetazolamide diuresis on renal concentrating ability was determined in six hydropenic young men. There was no consistent difference between the response of the concentrating mechanism to mannitol, urea, and saline diuresis at low rates of urine flow. A marked decrease in concentrating ability was seen during mercaptomerin diuresis as compared with mannitol diuresis at the same urine flows. Meralluride diuresis was associated with a similar decrease in two of three subjects studied. When mercaptomerin action was potentiated by prior administration of ammonium chloride, there was greater apparent impairment of concentrating ability than with mercaptomerin alone. This impairment indicates that mercurial diuretic agents act at the renal concentrating site as well as on the proximal tubule. Acetazolamide was also found to cause a decrease in renal concentrating ability while chlorothiazide had little effect.

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