Clarification of the Site of Action of Chlorothiazide in the Rat Nephron

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ABSTRACT The saluretic effect of the thiazide diuretics has been attributed to inhibition of sodium reabsorption in the distal nephron of the kidney. Recent micropuncture studies have shown, however, that chlorothiazide administration can also inhibit sodium reabsorption in the proximal convolution. To clarify the site of the saluretic effect of chlorothiazide, these micropuncture studies examined the effect of chlorothiazide on chloride transport in the nephron. The effect of chlorothiazide on chloride transport was studied because chlorothiazide's effectiveness as a saluretic is largely due to its ability to enhance sodium chloride excretion; if only changes in sodium transport are examined, it would be then difficult to determine if sodium as bicarbonate or as chloride is affected, since chlorothiazide can inhibit carbonic anhydrase. One group of rats was studied before and after 15 mg/kg per h chlorothiazide. For comparison, another group of rats was studied before and after 2 mg/kg per h benzolamide, a carbonic anhydrase inhibitor. Fractional chloride delivery from the proximal tubule was similarly increased in both groups; from 59.4 to 71.0% by chlorothiazide administration, $P < 0.001$, and from 54.3 to 68.2% by benzolamide administration, $P < 0.001$. The increased delivery of chloride from the proximal tubule was largely reabsorbed before the early distal tubule as fractional chloride delivery to this site increased only from 5.08 to 7.40% after chlorothiazide administration, $P < 0.001$, and from 4.50 to 6.29% after benzolamide administration, $P < 0.01$. Benzolamide had no effect on chloride reabsorption in the distal convoluted tubule. However, chlorothiazide administration resulted in a marked decrease in distal tubular chloride reabsorption, the fraction of filtered chloride present at the late distal tubule increasing from 1.24 to 6.25%, $P < 0.001$. Fractional chloride excretion in the urine increased from 0.29 to 3.44%, $P < 0.001$, after chlorothiazide, but did not change after benzolamide. The influence of chlorothiazide on proximal chloride transport presumably is related to its ability to inhibit renal carbonic anhydrase. However, it is not the effect of chlorothiazide in the proximal convolution but rather its effect in the distal convoluted tubule which is primarily responsible for its ability to be an effective saliuretic.

INTRODUCTION

The saluretic effect of the thiazide diuretics has been largely attributed to inhibition of sodium transport in distal portions of the nephron (1–3). However, recent micropuncture studies have shown that under certain circumstances sodium reabsorption in the proximal convolution may be diminished by chlorothiazide (4–6). The character and importance of this proximal effect however is unclear. For example, Fernandez and Puschett have recently demonstrated that the increase in fractional sodium excretion following chlorothiazide was comparable in dogs in which proximal sodium reabsorption was suppressed and in dogs in which no proximal effect was discernible (4). In addition, Edwards, Baer, Sutton, and Dirks have demonstrated that the increment in the quantity of sodium present in the distal tubule (site undetermined) following chlorothiazide was greater than could be attributed to the proximal tubular effect of chlorothiazide (5).

To further clarify the location of the saluretic effect of chlorothiazide we have examined its effect on chloride transport in the rat nephron. The effect of chlorothiazide on chloride transport was studied because chlorothiazide's effectiveness as a saluretic is largely due to its ability to enhance (sodium) chloride excretion; if only changes in sodium transport are examined,

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it would be difficult to determine to what extent sodium as bicarbonate or as chloride is affected, since chlorothiazide inhibits carbonic anhydrase (7). Further, to define changes in chloride transport which may result from the ability of chlorothiazide to inhibit carbonic anhydrase, we have also examined the influence of benzolamide, a selective carbonic anhydrase inhibitor, on chloride transport in the rat nephron.

METHODS

Three groups of male Sprague-Dawley rats weighing 325–398 g were studied. Before the micropuncture study the rats were maintained on regular rat chow and were permitted free access to water. The groups were studied in the manner given below.

Group I, hydropenia, five rats. This group of rats were studied to provide control observations for groups II and III. Throughout the experiment the rats were infused at 20 μl/min with a solution of 125 mM NaCl and 25 mM $NaHCO_3$/liter containing 10% inulin. After an equilibration interval of 75–90 min, the initial proximal and distal tubular fluid samples together with arterial blood and urine samples were obtained (period A). After period A, which lasted between 30–45 min, and an interval of 15 min, tubular fluid samples were collected from the previously studied tubules and arterial blood and urine samples again obtained (period B).

Group II, benzolamide, five rats. This group was studied as group I through period A. After period A, 2 mg of benzolamide/kg body wt was given i.v. and the infusion solution changed to 300 mM $NaHCO_3$/liter to which benzolamide was added in an amount calculated to administer 2 mg/kg per h. This latter solution was chosen to maintain the plasma chloride concentration constant after benzolamide administration (Results). This solution was continued at 20 μl/min throughout the remainder of the study. 15 min after the administration of benzolamide, period B started.

Group III, chlorothiazide, six rats. This group was studied as group I through period A. After period A, chlorothiazide, 15 mg/kg body wt, was given i.v. and was also added to the 125 mM NaCl and 25 mM $NaHCO_3$/liter infusion solution in an amount which administered 15 mg/kg per h. As in group II, the infusion solution used with this drug was selected to maintain a stable plasma chloride concentration (Results). Period B began after an interval of 15 min.

After the administration of Inactin i.p., 80–120 mg/kg body wt, (Promonta, Hamburg, West Germany) the rats were prepared for micropuncture as previously described (8). Polyethylene catheters (PE-50) were placed into the left jugular and femoral veins for the infusion of solutions and into the left femoral artery for the collection of arterial blood samples. Urine for analysis was collected from both kidneys by a catheter (PE-90) in the dome of the urinary bladder.

The recollection micropuncture technique was used in all studies. Tubular fluid samples were obtained from the end of the proximal convolution and from the earliest and latest surface convolutions of the same distal convoluted tubule. End proximal and late distal convolutions were localized after the i.v. administration of 50–75 μl of 5% lissamine green dye (9, 10). To localize the earliest surface distal convolution which was associated with the late distal surface convolution, the selected late distal convolution was punctured with a small (<6 μm OD) micropipette containing 1% FD & C Dye (food, drug and cosmetic dye no. 3, Keystone Aniline & Chemical Co., Chicago, Ill.). With gentle pressure, the dye outlined the late distal surface convolution and any associated early distal convolutions. Usually one or no other convolution was present on the surface.

Subsequent microdissection of the distal tubule was not carried out in the present studies and, therefore, the precise location of the early and late distal puncture sites cannot be made. However, a separate study of 15 distal tubules in which the earliest and latest sites were located as described above and the puncture sites subsequently verified by microdissection, the early distal site ranged from 17 to 37% and the late distal site from 73 to 98% of the length of the distal tubule (11). Because the recollection micropuncture technique was used, comparisons within a group are made in the same tubule, not between different tubules.

In each rat the lissamine green dye was injected no more than three to five times to localize a satisfactory number of tubules. An interval of at least 30 min occurred between the last injection of lissamine green and the collection of any tubular fluid samples. In most studies tubular fluid samples were collected from three end proximal and from the early and late surface convolutions of two distal tubules.

To determine the single nephron filtration rate, the proximal tubular fluid samples were obtained over an accurately timed interval of 60–120 s. The volume of proximal tubular fluid obtained was determined in a calibrated quartz microcapillary with a constant interval diameter. The inulin concentration in the tubular fluid samples was determined in triplicate in the proximal tubular samples and usually at least in duplicate in the distal tubular samples with the microfluorescence technique of Vurek and Pegram (12). The chloride concentration in the tubular fluid samples and the urine was measured by the method of Ramsay, Brown, and Croghan (13). This micromethod was modified for use with a bucking potentiometer with an electrometer serving as a null meter. In this way, readings could reliably be made to ±1 mV. The slope of the line relating millivolts to chloride concentration with the sample micropipette used was approximately 3.3 mV/meq chloride. Duplicate determinations on the same sample were, with rare exception, between 0–2 mV. The chloride concentration in the plasma was measured with a Cotlove chloridometer. The inulin concentration in urine and plasma was determined with the method of Führ, Kaczmarczyk, and Krüttgen (14). The plasma inulin concentration was corrected for plasma water; the latter determined by refractometry. The plasma chloride concentration was corrected for plasma water and the Donnan effect. For the latter, 1.02 was used (15).

The results are expressed as the mean ±1 SEM. Statistical comparisons of the results were performed with the Student's *t* test.

RESULTS

Clearance data: (Table I)

In group I, the GFR[1] and the urinary excretion of chloride were similar and not statistically different in periods A and B. The administration of benzolamide (group II) after period A resulted in a marked increase

[1] *Abbreviations used in this paper:* GFR, glomerular filtration rate measured in both kidneys; TF/P, tubular fluid to plasma; In, inulin.

TABLE I

Clearance Results

		V*		GFR		U_{Cl}-V		FE-Cl^-		P-Cl^-	
		A	B	A	B	A	B	A	B	A	B
		μl/min		*ml/min*		*μeq/min*		%		*meq/liter*	
Group I		5.4	6.0	2.54	2.86	0.67	0.55	0.24	0.17	112.1	111.7
(five rats)	SEM	0.2	0.2	0.19	0.07	0.05	0.05	0.02	0.02	2.52	1.70
	P	<0.01		>0.2		>0.1		>0.1		>0.7	
Group II		6.3	49.4	2.79	2.15	1.03	0.69	0.33	0.30	112.4	113.2
(five rats)	SEM	0.2	3.5	0.17	0.11	0.16	0.12	0.04	0.06	2.05	1.77
	P	<0.001		<0.005		<0.05		>0.6		>0.4	
Group III		6.1	49.6	2.83	2.15	0.86	8.09	0.29	3.44	111.1	111.5
(five rats)*	SEM	0.0	3.0	0.31	0.21	0.14	0.48	0.06	0.12	0.36	0.49
	P	<0.001		<0.05		<0.001		<0.001		>0.4	

Abbreviations used: V, urine flow rate; GFR, glomerular filtration rate; U_{Cl}-V, minute excretion of chloride; FE-Cl^-, fractional excretion of chloride; P-Cl^-, plasma chloride.

* Clearance data was not available on one rat.

in the urine flow rate but a decrease in the GFR and absolute chloride excretion. Fractional chloride excretion was not altered by benzolamide. The administration of chlorothiazide (group III) resulted in a decrease in the GFR and a marked increase in the urine flow rate similar to that observed after benzolamide. However, in contrast to benzolamide, chlorothiazide administration markedly increased both absolute and fractional chloride excretion, the latter increasing from 0.29±0.06 to 3.44±0.12%, $P < 0.001$.

Micropuncture data: (Table II)

Group I. The collection, and then the recollection, of tubular fluid samples from the proximal and distal tubules in this group was separated by an interval of 15 min during which the infusion of the isotonic saline-bicarbonate solution was continued at 20 μl/min. As shown in Table II, the changes which occurred in the parameters studied were minor. In the proximal tubule, the TF/P_{In} and TF/P_{Cl^-} ratios both decreased slightly, but significantly, when period B was compared to period A. The decrease in the TF/P_{In} ratio noted under these circumstances is in contrast to our findings when the recollection immediately followed the initial collection of proximal tubular samples as no change is noted in the TF/P_{In} ratios under the latter conditions (8). Similarly, the small decline in the TF/P_{Cl^-} ratio which occurred between periods A and B, 1.28±0.02 to 1.23±0.02, $P < 0.05$, was not observed when the recollection of tubular fluid was performed as soon as possible after the initial collection. In 10 proximal tubules in which this was examined, the TF Cl^- concentration was 139.3±1.23 meq/liter in the initial collection and 140.4±2.33 meq/liter in the recollection, $P > 0.7$. As a result of the changes in the proximal tubular TF/P_{In} and TF/P_{Cl^-} ratios which occurred in group I, fractional chloride delivery from the proximal convolution increased modestly from 51.4±2.79% in period A to 56.0±2.14% in period B, $P < 0.05$.

Although slight decreases in both the early and late distal tubular TF/P_{In} ratios were noted in period B, in comparison to period A, these changes were minimal and not statistically significant. Furthermore, the distal tubular TF/P_{Cl^-} ratios were similar when period A is compared with period B. However, as in the proximal tubule, the small changes in the distal tubular TF/P_{In} and TF/P_{Cl^-} ratios which occurred were sufficient so that when periods A and B were compared by paired analysis small but statistically significant differences in the fraction of filtered chloride present at the early and late distal tubular sites were noted. In the early distal tubule, the fraction of filtered chloride present increased from 3.66±0.56 to 4.66±0.54% of the filtered load, $P < 0.05$, and in the late distal tubule from 1.01±0.16 to 1.67±0.28%, $P < 0.02$.

Group II. As previously noted (16), the administration of 2 mg/kg body wt benzolamide results in a significant decrease in the proximal tubular TF/P_{In} and TF/P_{Cl^-} ratios. Consequently, administration of benzolamide increased the fractional delivery of chloride out of the proximal convolution from 54.3±2.28 to 68.2±1.94% of the filtered load, $P < 0.001$. This increase in chloride delivery out of the proximal convolution was significantly greater, $P < 0.05$, than that observed in group I.

In the distal tubule, benzolamide administration decreased the TF/P_{In} ratio in both the early and late sites. A comparison of the early distal TF/P_{Cl^-} ratio

TABLE II

Micropuncture Results

		SNGFR*		Proximal tubule						Early distal tubule						Late distal tubule					
				TF/P_{In}		TF/P_{Cl^-}		$\frac{TF/P_{Cl^-}}{TF/P_{In}} \times 100\%$		TF/P_{In}		TF/P_{Cl^-}		$\frac{TF/P_{Cl^-}}{TF/P_{In}} \times 100\%$		TF/P_{In}		TF/P_{Cl^-}		$\frac{TF/P_{Cl^-}}{TF/P_{In}} \times 100\%$	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
		nl/min																			
Group I		45.0	46.0	2.55	2.21	1.28	1.23	51.4	56.0	6.5	5.5	0.21	0.25	3.66	4.66	14.3	11.2	0.14	0.16	1.01	1.67
(five rats)	SEM	1.39	1.33	0.12	0.28	0.02	0.02	2.79	2.14	0.70	0.36	0.01	0.02	0.56	0.54	1.81	1.44	0.03	0.02	0.16	0.28
	P	>0.3		<0.01		<0.05		<0.05		>0.1		<0.1		<0.05		<0.1		>0.5		<0.02	
	n	14		14		13		13		9		9		9		9		9		9	
Group II		50.3	47.9	2.37	1.61	1.26	1.09	54.3	68.2	5.3	3.07	0.22	0.19	4.50	6.29	11.3	5.0	0.14	0.07	1.46	1.43
(five rats)	SEM	1.55	2.37	0.10	0.04	0.01	0.01	2.28	1.94	0.35	0.14	0.02	0.03	0.57	0.71	1.50	0.40	0.02	0.01	0.31	0.20
	P	<0.1		<0.001		<0.001		<0.001		<0.001		>0.2		<0.01		<0.005		<0.02		>0.7	
	n	14		14		14		14		10		10		10		9		9		9	
Group III		45.4	41.2	2.19	1.61	1.26	1.13	59.4	71.0	5.0	3.30	0.24	0.24	5.08	7.40	10.7	5.8	0.13	0.36	1.24	6.25
(six rats)	SEM	2.03	1.62	0.07	0.05	0.01	0.01	2.08	2.13	0.26	0.12	0.02	0.01	0.70	0.51	0.48	0.32	0.03	0.03	0.21	0.51
	P	<0.005		<0.001		<0.001		<0.001		<0.001		>0.8		<0.001		<0.001		<0.001		<0.001	
	n	16		16		15		15		11		11		11		11		11		11	

Abbreviations used: SNGFR, single nephron filtration rate; TF/P_{In} and Cl^-, tubular fluid to plasma inulin and chloride ratios; $\frac{TF/P_{Cl^-}}{TF/P_{In}} \times 100\%$, represents quantity of chloride as a fraction of the filtered load; *n* = number of tubules.

before and after benzolamide indicates that this ratio did not change significantly, 0.22±0.02 vs. 0.19±0.03, $P > 0.2$. However, in the late distal convoluted tubule, benzolamide administration resulted in a significant decrease in the TF/P_{Cl^-} ratio, as it fell from 0.14±0.02 to 0.07±0.01, $P < 0.02$.

Although the delivery to chloride from the proximal tubule after benzolamide increased by 14% of the filtered load, chloride delivery to the early distal tubule increased much less, i.e., from 4.50±0.57 to 6.28± 0.71% of the filtered load, $P < 0.02$. Clearly, therefore, almost all of the increment in chloride delivery from the proximal convolution was reabsorbed before the early distal tubule. Furthermore, the small increase in chloride delivery to the distal tubule after benzolamide was all reabsorbed therein as the quantity of chloride present at the late distal site was identical in both periods A and B, 1.46±0.31 vs. 1.43±0.20%, $P > 0.7$.

Group III. The influence of chlorothiazide administration on chloride transport in the proximal convolution was similar to that observed after benzolamide. After the administration of chlorothiazide, the proximal tubular TF/P_{In} and TF/P_{Cl^-} ratios fell significantly. As a result of these changes, the fractional chloride delivery from the proximal convolution increased from 59.4±2.08 to 71.0±2.13%, $P < 0.001$. The increase in chloride delivery out of the proximal convolution which resulted from chlorothiazide administration was significantly greater than that observed in group I, $P < 0.005$, but was not different from group II, $P > 0.98$.

After chlorothiazide administration, there was a marked decrease in the TF/P_{In} ratio in both the early and late distal tubular sites, again similar to the changes after benzolamide. The TF/P_{Cl^-} ratio in the early distal tubule was identical in periods A and B. However, and in striking contrast to benzolamide, chlorothiazide administration resulted in an increase in the late distal TF/P_{Cl^-} ratio from 0.13±0.03 to 0.36±0.03, $P < 0.001$.

Similar to the observations in group II, almost all of the increase in chloride delivery from the proximal convolution after chlorothiazide administration was reabsorbed before the accessible portion of the early distal tubule. At the early distal site chloride delivery increased only from 5.08±0.70 to 7.40±0.51% of the filtered load, $P < 0.001$. However, the quantity of chloride present at the late distal tubule increased markedly from 1.24±0.021 to 6.25±0.51% of the filtered load, $P < 0.001$. This occurred largely as the result of the ability of chlorothiazide to increase the TF/P_{Cl^-} ratio in the distal tubule and was essentially entirely responsible for the increase in chloride excretion noted after chlorothiazide.

DISCUSSION

After the administration of chlorothiazide, fractional chloride excretion increased from 0.29 to 3.44%. On the other hand, benzolamide administration did not increase chloride excretion. Despite these marked differences in their effect on chloride excretion, up to the level of the early distal tubule, the effects of chlorothiazide and benzolamide on chloride transport were very similar. Proximal tubular chloride reabsorption was diminished by both drugs and chloride delivery from the proximal convoluted tubule comparably increased. Neither drug, however, seemed to affect chloride transport in the loop of Henle. In fact, most of the increment in chloride delivery from the proximal convolution which resulted from either chlorothiazide or benzolamide administration was reabsorbed in the loop of Henle as fractional chloride delivery to the distal tubule increased by only about 2%.

It was at the level of the distal convoluted tubule where marked differences in the two agents were apparent. The modest increase in chloride delivery to the distal tubule which resulted from benzolamide administration was reabsorbed at this site so that at the late distal tubule the quantity of chloride present was comparable to that observed during hydropenia. In striking contrast, chlorothiazide administration was associated with inhibition of chloride reabsorption in the distal convoluted tubule. It was as a result of its effect in the distal convoluted tubule that chlorothiazide greatly increased chloride excretion.

The present studies, while demonstrating the effective site of action of chlorothiazide in the rat nephron to be in the distal convoluted tubule, provide no information as to the mechanism(s) by which this agent diminished distal tubular chloride reabsorption. On one hand, as active chloride reabsorption may be present in the distal convoluted tubule (17–19), it is conceivable that chlorothiazide decreased chloride reabsorption at this site by direct inhibition of an active component of chloride reabsorption. Alternatively, the observed effect of chlorothiazide on distal tubular chloride reabsorption may only result from an effect of the drug on other transport systems, e.g., that of sodium. Clearly, additional studies will be required to determine the mechanism by which chlorothiazide decreased chloride reabsorption in the distal convoluted tubule.

The administration of chlorothiazide not only increases the urinary excretion of sodium but of potassium as well. As the distal convoluted tubule is an area of the nephron intimately involved in both potassium and sodium transport, the observed effect of chlorothiazide on chloride transport in the distal tubule may possibly be primarily associated with the increase in urinary potassium excretion. In four additional clearance studies

the excretory rates of chloride, sodium, and potassium were determined before and after 15 mg/kg body wt chlorothiazide. Expressed as microequivalents per minute for purposes of comparison, the excretory rates of chloride, sodium, and potassium were increased by chlorothiazide from 0.87±0.34 to 8.49±1.56; from 0.28± 0.12 to 11.53±1.77; and from 1.28±0.30 to 4.45±0.31 μeq/min, respectively. The increment in the excretion of chloride (7.6 μeq/min) was significantly greater than could be accounted for by the observed increase in potassium excretion, even assuming the unlikely possibility that the increment in the latter was all as potassium chloride. The effect of chlorothiazide in the distal tubule, therefore, is felt to be causually related to the increase in urinary sodium chloride excretion which follows its use and cannot be attributed only or primarily to an enhancement of potassium excretion.

Recent micropuncture studies have suggested that chlorothiazide can: (*a*) induce at least a modest natriuretic effect without a detectable suppression of proximal tubular sodium reabsorption, (4), or (*b*) that the effect of the drug in the more distal portions of the nephron is greater than could be accounted for by the observed decrease in proximal reabsorption (5). These direct micropuncture studies, taken together with the more indirect results of clearance studies (1–3), suggest that the major saluretic effect of the thiazides resides in the distal nephron. In the present studies, although the ability of chlorothiazide to increase (sodium) chloride excretion can be largely accounted for by its effect in the distal convoluted tubule, these studies should not necessarily be construed as indicating that its influence in more proximal segments of the nephron are totally unrelated to its saluretic effect. The quantity of (sodium) chloride in the distal tubule which may be influenced by chlorothiazide administration is certainly, in part, dependent upon (sodium) chloride delivery to this site. An factor(s) which would tend to increase, or decrease, distal (sodium) chloride delivery would presumably indirectly influence the character of the chlorothiazide response. Nevertheless, the results of our studies indicate that a response to chlorothiazide is not dependent upon a concomitant increase in delivery to the distal tubule. After chlorothiazide the increment in fractional chloride delivery to the distal tubule of 2.3% was only approximately one-half as large as the increase in the fraction of filtered chloride present at the end of the distal tubule (5%).

Based upon the ability of chlorothiazide to modestly inhibit free water formation during a hypotonic diuresis in the hydrated dog but not inhibit free water reabsorption during a hypertonic infusion in the hydropenic dog, Earley, Kahn, and Orloff suggested that the drug inhibited sodium reabsorption in the distal convoluted tubule (1). In view of micropuncture studies which suggested that the distal convoluted tubule in the rat (20) and subsequently in the dog (21), did not contribute to urinary dilution, findings similar to those of Earley et al. were taken to suggest that sodium reabsorption was inhibited in a nephron segment distal to the medullary ascending limb, a portion of the nephron designated as the cortical diluting segment (2). This nephron segment was presumed to reside between the end of the medullary portion of the ascending limb of the loop of Henle and the macula densa, and to be proximal to that part of the distal tubule accessible to micropuncture study. The findings of the present study may appear to be in conflict with this suggestion in that the primary effect of chlorothiazide was to result in inhibition of chloride reabsorption in the portion of the distal convoluted tubule accessible to micropuncture study but have no influence on chloride reabsorption in the nephron segment immediately proximal to this portion of the distal tubule. Based on the results of the present study, at least two explanations can be suggested to account for the ability of chlorothiazide to inhibit the kidney's ability to maximally dilute the urine, as measured by free water formation, without invoking an effect in either the medullary or cortical segment of the ascending limb. First, the accessible portion of the distal tubule may contribute to urinary dilution, but the inevitable scatter of micropuncture data and the inherent difficulty in inducing a maximal water diuresis in animals subjected to the surgery for micropuncture study may have prevented a modest diluting defect in the portion of the distal tubule accessible to micropuncture from being detected. If this is the case and urinary dilution does occur in the distal tubule, inhibition of distal tubular (sodium) chloride reabsorption by chlorothiazide would result in an impairment in urinary dilution. Second, the decreased free water formation seen with chlorothiazide may, in part, be related to the ability of chlorothiazide to inhibit carbonic anhydrase. The proximal tubular effect of chlorothiazide was remarkably similar to that observed after benzolamide. Although these data do not document that the fall in the proximal TF/P_{Cl^-} and TF/P_{In} ratios and, undoubtedly, the reciprocal rise in the proximal tubular TF/$P_{HCO_3^-}$ ratio (16), noted after chlorothiazide were due to inhibition of carbonic anhydrase, they are consistent with the known capacity of this agent to inhibit carbonic anhydrase (7). Consistent with these results in the proximal tubule, in one study in which bicarbonate in the urine was measured after chlorothiazide, the concentration was 98 meq/liter. Previous clearance studies have demonstrated that when large amounts of tubular fluid rich in sodium bicarbonate are presented to the diluting segment of the nephron, e.g., by acetazolamide

administration or sodium bicarbonate infusion, free water formation is modestly diminished (22). As the effect of chlorothiazide in the proximal tubule would be similar to that of a carbonic anhydrase inhibitor, such as acetazolamide, large quantities of sodium bicarbonate would be delivered to the diluting segment of the nephron and free water formation would be diminished.

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