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On the Mechanism of Hyposthenuria in Hypercalcemia *

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Loss of renal concentrating ability is a well-known consequence of hypercalcemia (1). On the basis of experimental observations in animals (2–5) and man (6–8), it has been suggested that one or more specific functional abnormalities of the renal tubules underlie the concentrating defect. The abnormality proposed most often is that the collecting ducts become relatively impermeable to water (3–8). It has also been suggested that sodium transport is impaired at some site in the tubule which contributes to creating and maintaining high concentrations of sodium in the interstitial fluids of the medulla and papilla (4). Aside from these specific transport abnormalities, the possibility exists that obstruction of tubules by intraluminal casts (9) and the consequent decrease in number of functioning nephrons may play an important role in the hyposthenuria.

In the present study, hypercalcemic nephropathy was studied in vitamin D-intoxicated rats and hamsters by utilizing clearance and micropuncture methods simultaneously. The data failed to reveal any evidence of either impaired sodium transport by the tubules or impermeability of the collecting ducts to water. The total amount of sodium delivered to the medulla per unit time was markedly diminished since glomerular filtration rate (GFR) was reduced and reabsorption of the glomerular filtrate by the proximal tubules was unimpaired. It is postulated that this reduction in the supply of sodium to the medulla, probably due to loss of a substantial number of functioning nephrons, is a significant contributing factor to the concentrating defect.

Methods

White male rats weighing between 200 and 300 g were rendered hypercalcemic by daily subcutaneous injection of 500,000 U of vitamin D_2 (calciferol) in oil for a period of 5 days. During this time, they were fed a regular pellet diet and drank tap water ad libitum. After the fifth injection, food and water were withheld for the next 24 to 30 hours. At the end of this period, the rat was stimulated to void and was placed in a metabolic cage for collection of an overnight urine specimen. The osmolality of this specimen was taken as U_{max}. Control rats were deprived of food and water for an equal length of time, and U_{max} was determined in the same way. Acute experiments were performed on the day after the overnight urine collection. The animals were anesthetized by intraperitoneal injection of inactin (sodium ethyl-(1-methylpropyl)-thiobarbiturate), 7 mg per 100 g body weight, and the trachea was cannulated and a jugular vein catheterized. The urinary bladder was exposed through a lower abdominal mid-line incision and a polyethylene catheter inserted and tied. A blood specimen was obtained from the abdominal aorta for calcium determination.

Graded mannitol-saline diuresis. In 27 experiments, an intravenous infusion of 5% mannitol, 150 mmoles per L sodium chloride, and 7.5 mU per ml vasopressin was administered at progressively increasing rates of 0.04 ml per minute, 0.10 ml per minute, and 0.19 ml per minute. At each infusion rate, several urine samples were collected under oil in graduated cylinders. At the end of each urine collection period, the bladder was emptied by direct compression. Blood specimens were collected in heparinized capillary tubes from the cut end of the tail. All plasma and urine samples were analyzed for osmolality.

In most of these experiments, tubular fluid was also collected from surface nephrons by micropuncture techniques previously described (10). A left flank incision was made, and the left kidney dissected free of perirenal fat and immobilized in a Lucite dish. Tubular fluid collections were started only after the infusion had been set at the highest rate. In nine normal and eight hypercalcemic rats, tubular fluid was analyzed for osmolality.

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In four normal and five hypercalcemic rats, C^{14}-labeled inulin carboxylic acid [1] was added to the infusion (15 μc prime, 3.5 μc per ml sustaining), and tubular fluid, plasma, and urine radioactivity were determined in a Tri-Carb liquid scintillation counter (11). Urine sodium was measured in the inulin-infused animals by flame photometry, using lithium as an internal standard. After each collection of tubular fluid, a colored latex suspension was injected into the nephron, and at the end of the experiment the kidney was macerated in 50% HCL and the puncture site identified by microdissection.

Graded urea-saline diuresis. In six hypercalcemic rats, a solution consisting of 5% urea, 150 mmoles per L sodium chloride, and 7.5 mU per ml of vasopressin was administered intravenously. The rates of infusion and methods of collecting blood and urine samples were the same as described in the preceding section. Plasma and urine samples were analyzed for osmolality. In three of the six animals, C^{14}-inulin was added to the infusion, and plasma and urine radioactivity were measured as above.

Hamster experiments. In 50- to 75-g golden hamsters, hypercalcemia was induced by subcutaneous injection of 100,000 U of calciferol daily for 4 days. Food and water were withheld during the second 2 days, at the end of which time a voided urine specimen was obtained for measurement of U_{max}. If the animal could not be stimulated to void, urine for U_{max} was collected from the exposed bladder within 5 minutes after anesthesia had been induced by intraperitoneal inactin. Collections of fluid from adjacent collecting ducts, vasa recta, and loops of Henle were made from the exposed renal papilla.[2] Specimens were analyzed for osmolality. In four experiments, 20% mannitol was infused via a jugular vein at 0.25 ml per hour, whereas in three experiments the animals remained hydropenic. Blood was obtained from the abdominal aorta at the end of each experiment for calcium determination. Control values for U_{max} and serum calcium concentration were obtained from six normal hamsters after 48 hours of food and water deprivation.

All osmolality measurements were made by the freezing point method of Ramsay and Brown (12). Serum calcium was measured by the method of Kingsley and Robnett (13).

Osmolar clearance (C_{osm}) was calculated from the equation $V \times U/P_{osm}$, where V equals urine flow per minute per kilogram body weight. Solute-free water reabsorption ($T^c_{H_2O}$) was calculated from the equation $C_{osm} - V$. Plasma inulin concentrations were corrected for a plasma water content of 94% and the corrected values used in the calculations of tubular fluid/plasma inulin ratios (TF/P_{In}) and inulin clearance (GFR). Per cent water reabsorption was calculated from the expression $(1 - P/TF_{In}) \times 100$.

[1] New England Nuclear Corp., Boston, Mass.

[2] By Dr. Donald J. Marsh of the Department of Physiology.

TABLE I

*Effect of calciferol injections upon serum calcium concentration and maximal urinary osmolality**

	Serum calcium	U_{max}	$U_{osm}V$
	mg/100 ml	*mOsm/kg H_2O*	*mOsm/24 hr/kg*
Experimental group (19)†	14.9±1.5	1695±232	42.3±11.7
Control group (14)	9.4±0.4	2956±292	38.0± 6.5

* Values expressed as mean ± standard deviation.
† Number in parentheses is the number of rats.

Results

Effect of calciferol upon serum calcium, maximal urinary concentration, and solute excretion (Table I). The average serum calcium concentration in 19 rats injected for 5 days with 500,000 U of calciferol was 14.9 mg per 100 ml, whereas in 14 normal controls the average was 9.4 mg per 100 ml. Maximal urine osmolality was markedly diminished in the hypercalcemic animals, but solute excretion in the fasting hydropenic state was not significantly different for the two groups. These observations are in agreement with those reported by Epstein, Rivera, and Carone (2).

Graded mannitol-saline diuresis (Figures 1 to 5, Tables II and III). As shown in Figure 1, with progressive osmotic diuresis $T^c_{H_2O}$ rose in the hypercalcemic rats as well as in the normals. At all rates of osmolar clearance, however, $T^c_{H_2O}$ was lower in the experimental animals. In contrast to hypercalcemic dogs (3) and man (6–8), urine osmolality was always higher than plasma osmolality, and free water clearance (C_{H_2O}) was not observed. There was no evidence of a maximal rate of solute-free water reabsorption ($T^cm_{H_2O}$) in either the hypercalcemic or normal rats.

Upon exposure of the kidney for tubular fluid collections, numerous yellowish-white intraluminal deposits were seen scattered over the surface of the kidney in the hypercalcemic animals. Under the microscope, they were seen to lie within surface tubules and also could be seen just below the surface. The number of these deposits varied from one rat to another, but seemed to correlate roughly with the severity of the hypercalcemia. Most of the deposits completely obstructed a segment of the tubule causing collapse of the lumen distally, whereas others only narrowed the lumen

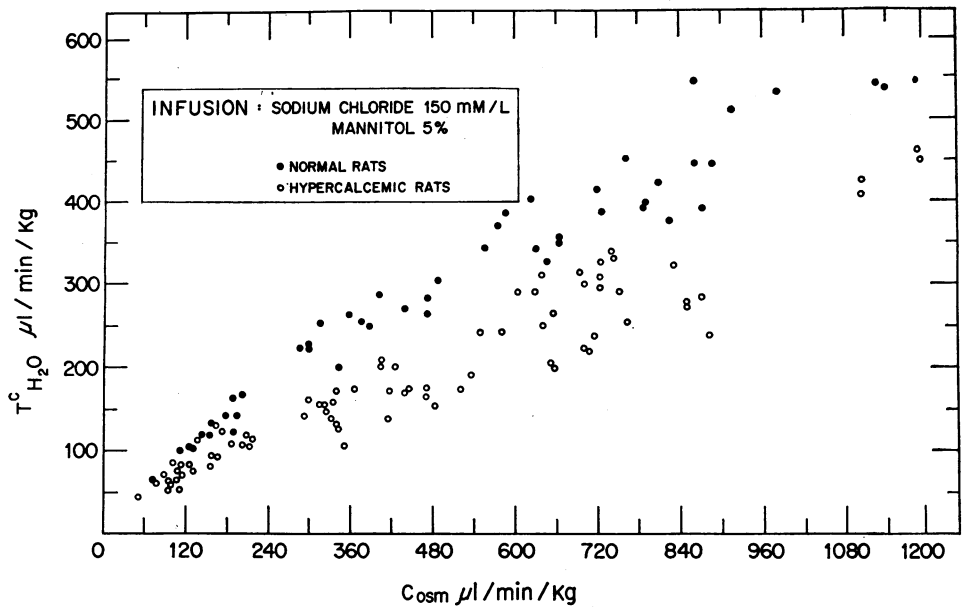

FIG. 1. SOLUTE-FREE WATER REABSORPTION ($T^c_{H_2O}$) DURING MANNITOL-SALINE DIURESIS IN HYPERCALCEMIC AND NORMAL RATS.

without occluding it. No tubular fluid could be obtained distal to the complete obstructions, and the deposits could not be dislodged with a micropipette but were firmly adherent to the tubule wall. On microdissection, they were found to lie within surface distal convoluted tubules.

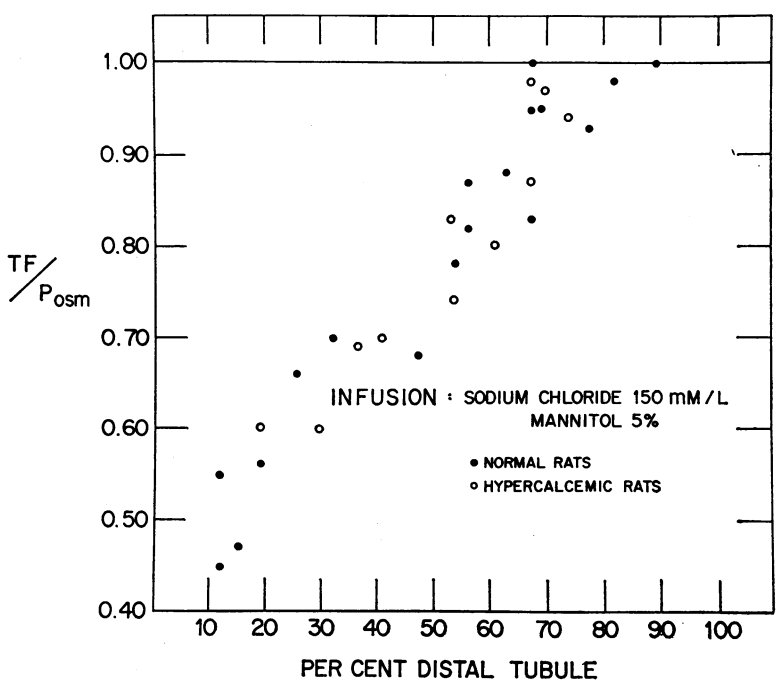

FIG. 2. DISTAL TUBULAR FLUID/PLASMA OSMOLALITY RATIOS (TF/P_{osm}) DURING MANNITOL-SALINE DIURESIS IN HYPERCALCEMIC AND NORMAL RATS.

TABLE II

*Clearance data during mannitol-saline diuresis in hypercalcemic rats**

Rat no.	Clearance period	Urine flow	U/P_{Osm}	C_{osm}	$T^c_{H_2O}$	$U_{Na}V$	GFR	$\frac{C_{osm}}{100\text{ ml GFR}}$	$\frac{T^c_{H_2O}}{100\text{ ml GFR}}$	$\frac{U_{Na}V}{100\text{ ml GFR}}$	% H_2O reabsorbed	Location	U_{max}	Serum Ca
		µl/min/kg		*µl/min/kg*	*µl/min kg*	*µEq/min/kg*	*ml/min kg*	*ml/min/kg*	*ml/min/kg*	*µEq/min/kg*		%	*mOsm/kg H_2O*	*mg/100 ml*
1	1	48.2	2.32	112	63.8	1.2	2.22	5.04	2.86	54.1	92	D81	1,605	13.8
	2	68.9	2.27	156	87.1	1.4	1.84	8.50	4.76	76.1	91	D82		
	3	162	1.96	318	156	6.1	1.65	19.2	9.42	370				
	4	250	1.69	423	173	11.3	1.89	22.4	9.13	598	56	P62		
	5	266	1.65	439	173	13.3	2.01	21.9	8.61	662	35	P55		
	6	275	1.62	446	171	13.1	2.18	20.5	7.83	601	89	D44		
	7	298	1.59	475	177	14.2	2.13	22.3	8.27	667	32	P40		
	8	347	1.55	538	191	17.4	2.36	22.8	8.09	737				
	9	486	1.46	710	224	24.3	2.37	29.9	9.43	1,025				
2	1	153	1.94	296	143	4.6	3.94	7.51	3.63	117			1,755	14.9
	2	184	1.86	341	157	4.6	2.68	12.7	5.86	172	43	P49		
	3	201	1.70	342	141	5.0	2.31	14.8	6.10	216	61	P52		
	4	282	1.50	423	141	5.6	1.62	26.1	8.70	346	25	P39		
	5	310	1.54	477	167	6.2	1.96	24.3	8.52	316	30	P35		
3	1	113	1.92	217	104	1.7	2.22	9.78	4.69	76.6	53	P39	1,280	14.0
	2	406	1.74	706	300	16.2	3.59	19.7	8.37	451	43	P47		
	3	415	1.80	747	332	14.5	3.54	21.1	9.37	410	43	P45		
	4	419	1.74	728	309	16.7	3.69	19.7	8.39	453				
4	1	207	1.96	407	199	3.1	2.68	15.2	7.43	116	50	P57	1,505	14.3
	2	332	1.94	644	312	8.3	3.42	18.8	9.12	243	84	D54		
	3	402	1.85	744	342	12.1	3.89	19.1	8.79	311				
5	1	176	1.83	322	146	4.4	2.04	15.8	7.17	216	91	D60	1,090	15.2

Mean = 2.56 ± 0.76 SD

*Abbreviations: U/P_{Osm} = urine/plasma osmolality; C_{osm} = osmolar clearance; $T^c_{H_2O}$ = solute-free water reabsorption; $U_{Na}V$ = rate of urinary sodium excretion; GFR = glomerular filtration rate; D = distal and P = proximal tubule.

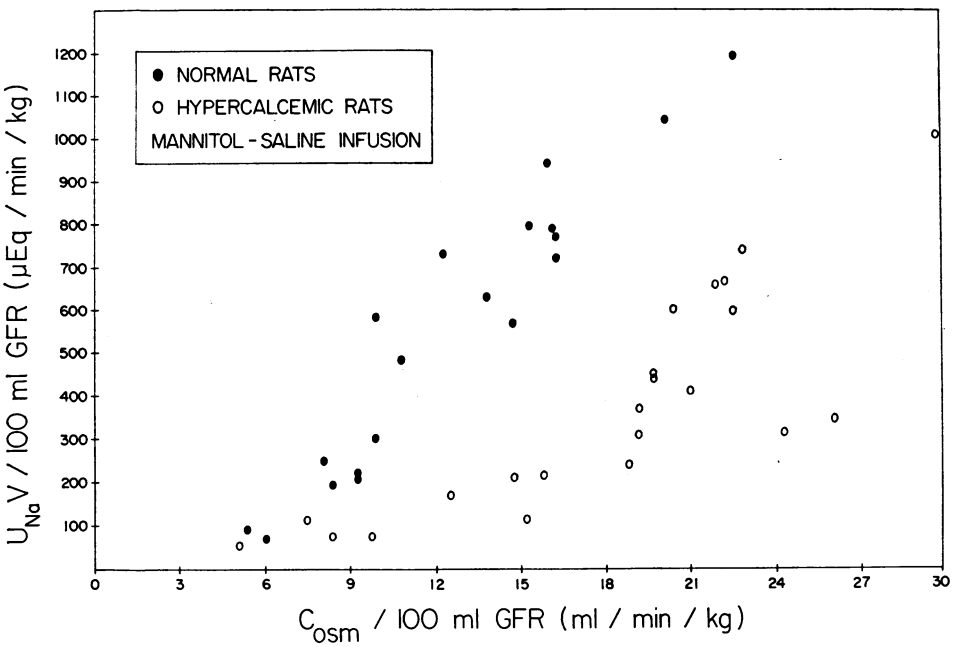

FIG. 3. URINARY SODIUM EXCRETION DURING MANNITOL-SALINE DIURESIS IN HYPERCALCEMIC AND NORMAL RATS. The data have been factored by glomerular filtration rate (GFR) in order to obtain an estimate of reabsorptive capacity per intact nephron at theoretically comparable osmotic loads. C_{osm} = osmolar clearance.

Nineteen samples of proximal tubular fluid were collected from the hypercalcemic rats and analyzed for osmolality. The mean TF/P_{Osm} ratio was 1.00 ± 0.01. Distal tubular samples were collected primarily from normal appearing nephrons in both groups of animals. The findings are shown in Figure 2. Fluid from the early portion of the distal convoluted tubule was markedly hypotonic to plasma, the values at 20% of the length falling as low as 190 mOsm per kg H_2O in the hypercalcemic rats and 180 mOsm per kg H_2O in the normal rats. In both groups, the fluid returned to isotonicity in the second half of the distal convoluted tubule. Three samples were collected from the first half of partially obstructed distal tubules in the hypercalcemic rats. The flow rates were noted to be slow, and the TF/P_{Osm} ratios were 0.82, 0.85, and 0.90, respectively, higher than found in nonobstructed distal tubules. It seems possible that the slow flow, by allowing more time for osmotic equilibration in this water-permeable segment of the nephron, could account for this finding (14).

In Tables II and III are shown the data from the experiments in which inulin clearance, tubular fluid inulin concentrations, and sodium excretion were measured in addition to C_{osm} and $T^c_{H_2O}$. Inulin clearance (GFR) was significantly reduced in the hypercalcemic rats. Sodium excretion ($U_{Na}V$), when compared at equal absolute rates of C_{osm}, was approximately the same in the two groups of animals. Because of pathologic evidence (3, 9) and our own observations that a substantial number of tubules are obstructed and nonfunctioning in hypercalcemia, it seems likely that at equal absolute rates of C_{osm}, C_{osm} per intact nephron was greater in the hypercalcemic rats than in the normals. In order to examine sodium excretion at more comparable osmotic loads per functioning nephron, therefore, the values for C_{osm} and $U_{Na}V$ have been factored by GFR in Figure 3. The derived data in this Figure suggest that the functioning tubules of the hypercalcemic rats were able to reabsorb sodium at least as well as those of the normal controls. In Figure 4, solute-free water reabsorption has been factored by GFR for similar reasons, i.e., to estimate the capacity of the intact nephrons to reabsorb water at comparable osmotic loads (C_{osm}/100 ml GFR). (For a similar analysis of solute-free water reabsorption

TABLE III

Clearance data during mannitol-saline diuresis in normal rats

Rat no.	Clearance period	Urine flow	U/P_{Osm}	C_{osm}	$T^c_{H_2O}$	$U_{Na}V$	GFR	$\frac{C_{osm}}{100\text{ ml GFR}}$	$\frac{T^c_{H_2O}}{100\text{ ml GFR}}$	$\frac{U_{Na}V}{100\text{ ml GFR}}$	% H_2O reabsorbed	Location	U_{max}	Serum Ca
		µl/min/kg		*µl/min/kg*	*µl/min kg*	*µEq/min/kg*	*ml/min kg*	*ml/min/kg*	*ml/min/kg*	*µEq/min/kg*		%	*mOsm/kg H_2O*	*mg/100 ml*
1	1	65	4.41	288	223	3.2	4.73	6.09	4.71	67.7	53	P54		
	2	78	3.87	300	222	4.9	5.51	5.44	4.03	88.9			2,850	9.0
	3	170	2.59	441	271	10.5	4.73	9.32	5.73	222	27	P44		
	4	340	2.14	728	388	33.5	5.28	13.8	7.35	634	33	P49		
	5	438	2.02	884	447	52.3	5.56	15.9	8.04	941	26	P38		
2	1	319	2.03	647	329	37.9	6.48	9.98	5.08	585			2,940	8.5
	2	397	1.99	789	393	35.7	7.34	10.7	5.35	486	28	P43		
	3	451	1.83	825	374	49.6	6.76	12.2	5.53	734	10	P30		
	4	703	1.82	1,280	577	66.8	8.37	15.3	6.89	789	0	P23		
3	1	216	2.58	558	342	17.3	6.90	8.09	4.96	251	32	P52		
	2	308	2.34	720	412	16.9	8.61	8.36	4.79	196	46	P65	2,560	9.5
	3	312	2.44	762	450	17.2	8.12	9.38	5.54	212	88	D56		
	4	451	2.18	982	531	38.3	6.68	14.7	7.95	573	81	D67		
4	1	486	1.80	875	389	26.7	8.85	9.89	4.39	302				
	2	587	1.92	1,126	539	49.9	6.92	16.3	7.79	721	23	P40	3,010	8.8
	3	602	1.89	1,138	536	54.2	7.04	16.2	7.61	770	35	P44		
	4	648	1.84	1,193	545	58.3	7.39	16.1	7.37	789	21	P38		
	5	810	1.83	1,483	673	77.0	7.36	20.0	9.14	1,046	33	P35		
	6	1,019	1.79	1,824	805	96.8	8.10	22.5	9.94	1,195	13	P32		

Mean = 6.88 ± 1.25 SD

TABLE IV

Clearance data during urea-saline diuresis in hypercalcemic rats

Rat no.	Clearance period	Urine flow	U/Posm	Cosm	$T^c_{H_2O}$	GFR	Umax	Serum Ca
		µl/min/kg		*µl/min/kg*	*µl/min/kg*	*ml/min/kg*	*mOsm/kg H_2O*	*mg/100 ml*
1	1	62.8	2.92	183	121	9.96	1,200	17.6
	2	106	2.79	295	189	7.79		
	3	114	2.70	308	194	5.37		
2	1	76.1	3.00	228	152	7.68	1,445	14.6
	2	102	3.01	308	206	6.03		
	3	117	3.07	359	242	6.04		
	4	238	2.90	691	452	5.88		
	5	328	2.48	812	485	6.62		
	6	354	2.62	927	573	7.93		
3	1	112	2.91	326	214	10.5	1,700	13.8
	2	152	2.91	441	289	8.77		
	3	189	2.65	502	313	7.06		
	4	245	2.32	568	323	4.04		
	5	354	2.09	739	385	4.24		
	6	434	1.89	821	387	4.60		
	7	505	1.82	919	414	4.04		
					Mean =	6.66 ± 2.0 SD		

in hypercalcemic dogs, see reference 3). This plot suggests that the remaining nephrons of the hypercalcemic rats were capable of reabsorbing as much solute-free water as those of the normal rats.

In Figure 5, the values for fractional water reabsorption by the tubules, calculated from TF/P_{In} ratios, are shown for the two groups of animals. It is evident that approximately the same per cent of the glomerular filtrate was reabsorbed by the accessible portions of the tubules. No evidence was found for diminished filtrate reabsorption in any of the proximal tubules of the hypercalcemic rats.

Graded urea-saline diuresis (Table IV, Figure 6). During urea-saline infusion, $T^c_{H_2O}$ was considerably higher in hypercalcemic rats than in similarly prepared animals given mannitol. The results of the six experiments are shown in Figure 6, with the data from the mannitol experiments included for comparison. The values for U_{max} before the urea-saline infusion were as low in these six rats as in the other hypercalcemic rats. The data for the three experiments in which GFR was also measured are shown in detail in Table IV. As can be seen, GFR was significantly higher than in the mannitol experiments.

Collecting duct, vasa recta, and loop of Henle fluid osmolality in hamsters (Table V, Figure 7). No significant differences were found between the osmolality of collecting duct fluid and that of adjacent vasa recta and loop of Henle fluid in hypercalcemic hamsters with a marked decrease in U_{max}. This was the case in both hydropenic and diuretic animals receiving 20% mannitol.

Discussion

The renal concentrating defect in hypercalcemia has been attributed by most investigators (3–8) to diffuse changes in tubular function that pre-

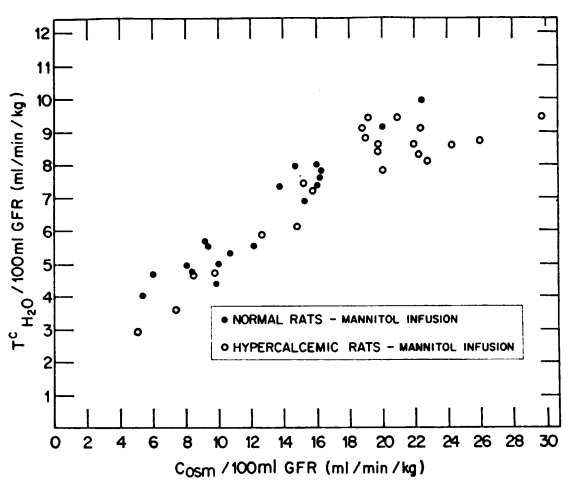

FIG. 4. SOLUTE-FREE WATER REABSORPTION COMPARED AFTER CORRECTION FOR DIFFERENCES IN GFR. These derived data suggest that at comparable osmotic loads per nephron, the intact tubules of the hypercalcemic rats had a normal capacity to reabsorb solute-free water.

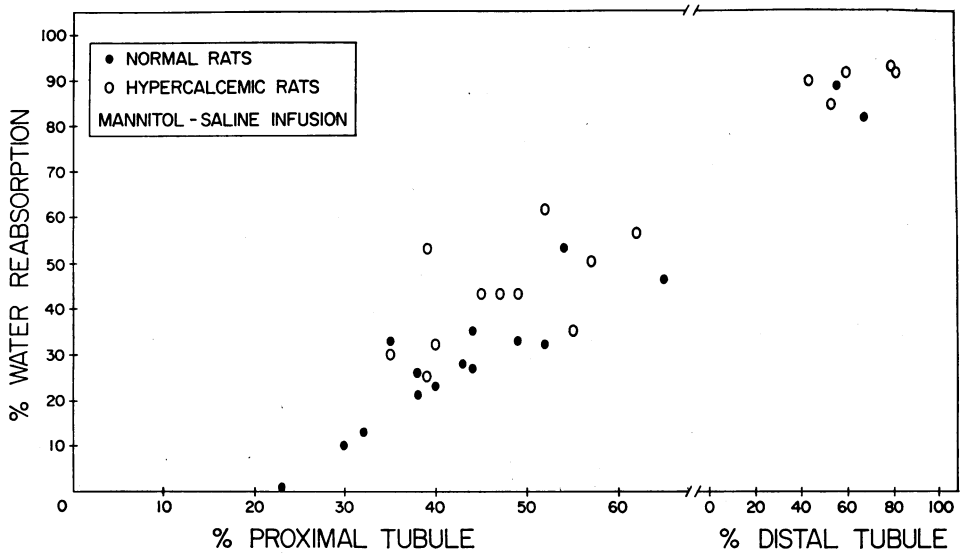

FIG. 5. SEGMENTAL REABSORPTION OF FILTERED WATER DURING MANNITOL-SALINE DIURESIS. Per cent reabsorption was calculated from TF/P inulin ratios.

sumably involve all nephrons to a greater or lesser degree. It has been postulated that the permeability of the collecting ducts to water is diminished in the presence of high concentrations of calcium, the main evidence being that hypercalcemic dogs (3) and man (6–8) excrete a urine hypotonic to plasma during mannitol diuresis despite the presence of excess vasopressin. In hypercalcemic rats, sodium continues to be excreted in the urine after several days of salt deprivation, and it has been proposed that a defect in transport exists at some site in the nephron involved in establishing the high concentration of sodium in the medullary interstitium (4). The

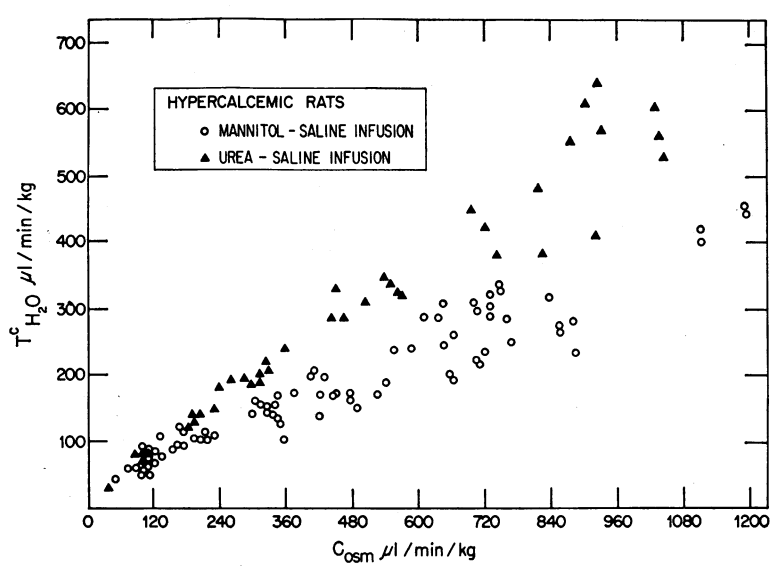

FIG. 6. SOLUTE-FREE WATER REABSORPTION DURING UREA-SALINE DIURESIS IN HYPERCALCEMIC RATS. The data from Figure 1 for mannitol-saline diuresis in hypercalcemic rats are repeated here to allow direct comparison between the effects of mannitol and urea.

TABLE V

Collecting duct, vasa recta, and loop of Henle fluid osmolality

Hypercalcemic hamsters					Normal hamsters		
Animal no.	Serum Ca	U_{max}	Collecting duct	Loop of Henle	Vasa recta	Animal no.	Serum Ca	U_{max}
	mg/100 ml	*mOsm/kg H_2O*	*mOsm/kg H_2O*	*mOsm/kg H_2O*	*mOsm/kg H_2O*		*mg/100 ml*	*mOsm/kg H_2O*
1	12.8	1,740†	820		800	8	9.2	3,240
2	13.8	1,470†	1,280	1,323	1,300	9	9.2	3,140
3	13.8		1,660	1,555		10		2,930
	Infusion: mannitol, 20%; sodium chloride, 150 mmoles/L at 0.25 ml/hr					11	8.1	3,200
4	14.0*	825	560	555		12	9.4	3,140
5	13.6*	1,395†	585	560	540	13	9.9	3,070
			565	475	490			
6	12.1*	940	470	455	503			
			480	462				
			420	405	383			
7	13.9*	1,225†	680	685	620			
			575	590	558			

* Blood collected after mannitol infusion.
† Urine collected from bladder after anesthesia.

present experiments were carried out utilizing micropuncture techniques in order to obtain additional information about hypercalcemic nephropathy and to evaluate directly the functional capabilities of various segments of the nephron thought to be important in the concentrating mechanism.

The initial event in the concentrating process is thought to be transport of sodium (and anions) in excess of water out of the thick ascending limb of Henle's loop (14). Hypotonicity of early distal tubular fluid presumably depends entirely on the integrity of loop sodium transport. Therefore, the finding of normal hypotonicity of early distal tubular fluid in hypercalcemic rats suggests that there is no generalized impairment in sodium reabsorption involving all thick ascending limbs of Henle's loop. Although the osmolality values cannot be equated with sodium concentrations, particularly because of the presence of other important solutes such as urea and mannitol in the tubular fluid, it seems reasonable that if sodium transport were substantially impaired, the early distal fluid would have had higher osmolalities than normal. The data do not exclude the possibility, however, that a defect may have been present in the ascending limbs of deep nephrons that originate in the juxtamedullary zone and are inaccessible to micropuncture. If there was such a defect, it was not apparent from urinary sodium excretion. Thus, at equal rates of osmolar clearance, sodium excretion was about the same in the two groups of animals. Because of the probability that the number of functioning nephrons was considerably reduced in the hypercalcemic rats (*vide infra*), sodium excretion was factored by GFR in Figure 3 in order to evaluate the capacity of residual intact nephrons to reabsorb sodium at comparable osmotic loads per nephron

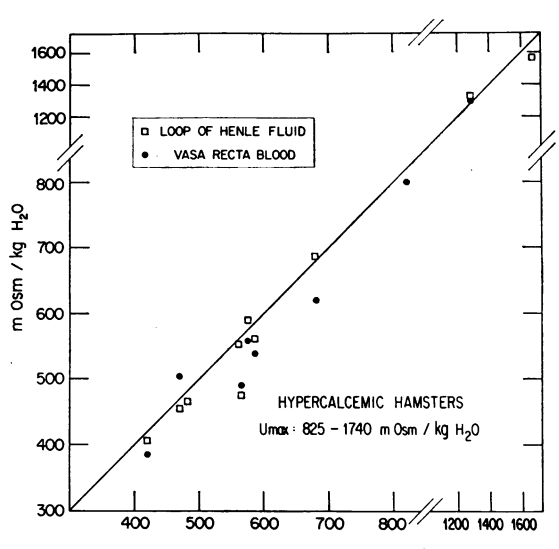

FIG. 7. COMPARISON OF COLLECTING DUCT FLUID OSMOLALITY WITH THE OSMOLALITY OF VASA RECTA OR LOOP OF HENLE FLUID IN HYPERCALCEMIC HAMSTERS. Points above the diagonal line indicate that collecting duct fluid was hypotonic to vasa recta or loop of Henle fluid, whereas points below the line indicate the reverse. Most of the samples were collected during mannitol diuresis.

(C_{osm}/100 ml GFR). The evidence that GFR in a diseased kidney provides an index of the population of residual functioning nephrons has been presented by Bricker, Kime, Morrin, and Orlowski (15). This analysis suggests that over-all sodium reabsorption was not impaired in intact nephrons of the hypercalcemic rats. Gill and Bartter found that hypercalcemic patients can conserve sodium normally on a salt-free diet and also concluded that over-all sodium reabsorption is unimpaired in this condition (6). In contrast to the findings in chronic hypercalcemia, acute elevation of serum calcium concentration in man increases urinary sodium excretion (16), and Walser has found a close parallelism between renal clearances of sodium and calcium in normal dogs under a variety of acute experimental conditions (17). No explanation is apparent to account for the absence of an overt effect of calcium on sodium clearance in chronic hypercalcemia, although it might be postulated that sodium transport was inhibited in some localized segment of the nephron and that the rejected sodium was then reabsorbed at a more distal site.

The micropuncture data obtained from the papilla of hypercalcemic hamsters do not support the view that the collecting ducts develop a selective impermeability to water since no consistent osmotic gradient was found between collecting duct, vasa recta, or loop of Henle fluid, even during mannitol diuresis. This does not entirely exclude the possibility, however, that a more general decrease in permeability occurred in this segment of the nephron. Experiments on isolated amphibian membranes have shown that high concentrations of calcium in the bathing media can inhibit the action of vasopressin on net water (18), sodium (19), and urea (20) transfer. If sodium and urea reabsorption were impaired as well as water in the collecting ducts, a transtubular osmotic gradient might not develop. However, the failure to observe any reabsorptive defect for sodium argues against this possibility.

On the basis of the foregoing, it is evident that no specific abnormalities in sodium or water transfer were observed in two critical segments of the nephron important in the concentrating mechanism, i.e., the thick ascending limb of Henle's loop and the collecting ducts. The data do indicate, however, that the total amount of sodium delivered to the medulla per unit time was considerably less than normal. This was primarily due to the marked fall in GFR in the hypercalcemic rats. The underlying mechanism for this decrease in GFR is not entirely clear. One possibility is that filtration rate was reduced uniformly in all nephrons, due perhaps to a direct effect of calcium on the glomerulus or to a contraction of extracellular fluid volume. A second possibility is that a large number of nephrons became nonfunctioning and that filtration was carried out by the remaining relatively intact population. Several lines of evidence favor this latter view. First, intravenous calcium infusions tend to increase GFR in man (16) and produce only slight decreases in dogs (21). Secondly, very striking reductions in GFR occur in dogs after only 24 hours of hypercalcemia (3), a period of time probably too short to allow for severe contraction of extracellular fluid volume. Finally, pathologic evidence obtained by microdissection of nephrocalcinotic kidneys (3, 9) has demonstrated extensive blockage of tubules by casts, and our own observations in the present study strongly suggest that such blocked tubules do not contribute to the final urine. In the physiological-pathological study by Carone, Epstein, Beck, and Levitin in hypercalcemic dogs (9), a close correlation was found between the degree of reduction in GFR and the extensiveness of intratubular obstruction. Although it cannot be concluded that blockage of tubules was the only cause of the reduction in GFR in the rats in this study, it seems probable that obstruction played a prominent role. Any effect of contraction of extracellular fluid volume, which would have acted to reduce filtration rate throughout all nephrons, was probably minimized by the rapid infusion of sodium chloride and mannitol during the course of the experiment.

Whatever the explanation, the result of the fall in filtration rate is that the over-all filtered load of sodium was substantially reduced. Conceivably, the total delivery of sodium to the medulla might be maintained in the face of such diminished filtered loads if reabsorption by the proximal tubules were significantly impaired. However, no evidence for this was found, as shown by the data

for proximal fluid reabsorption in Figure 5. It seems highly probable, therefore, that the amount of sodium available for transport into the medullary interstitium was much less than in the normal rats, and this could have been an important factor in limiting $T^c_{H_2O}$. This view gains some support from the experiments in which urea was infused rather than mannitol (Table IV, Figure 6). The combination of urea and saline apparently produced an acute rise in GFR, similar to that seen in normal rats (22) and dogs (23, 24). Presumably, only relatively intact nephrons were able to respond to this infusion in a normal fashion. Because of the higher GFR, the total amount of sodium delivered to the medulla was also higher. This could account for the improved ability to generate $T^c_{H_2O}$ in this group of animals, but it is also possible that urea helped to augment $T^c_{H_2O}$ by diffusing into the medullary interstitium from the collecting ducts and raising interstitial osmolality (24–26).

Several considerations suggest that some additional factor, other than a reduction in over-all delivery of sodium to the medulla, may have contributed to the hyposthenuria. Thus, if the capacity to reabsorb sodium and water was undisturbed in individual functioning nephrons, as the present data suggest, one might expect that under hydropenic conditions a high maximal urine concentration (U_{max}) could be achieved even though the total number of nephrons were reduced. One explanation for this failure is that medullary blood flow remained relatively high in proportion to the diminished rate of sodium delivery. A second possibility is that with complete obstruction of a substantial number of nephrons, the remaining nephrons were undergoing a relative solute diuresis that prevented maximal concentration of the urine. This explanation could also account for the inability of hypercalcemic rats to conserve sodium on a salt-free diet (4) and the continued excretion of normal amounts of sodium during mannitol diuresis despite the reduction in filtered sodium. The change in fractional reabsorption of sodium per intact nephron necessary to maintain normal or even excessive rates of excretion would be of the order of only a few per cent, even though the total filtered sodium load were decreased markedly. In this respect, the situation in hypercalcemia might be analogous to that in patients with chronic renal insufficiency in whom sodium excretion is normal or even excessive in spite of marked reduction in GFR and filtered sodium load. This has been attributed by Bricker, Morrin, and Kime to an increased osmotic load per intact nephron (27).

In contrast to the present observations in rats and hamsters, hypercalcemic man and dog may excrete a urine hypotonic to plasma during a moderate mannitol diuresis, despite the presence of adequate amounts of vasopressin (3, 6–8). The reason for this species difference is not certain, but two explanations seem plausible. If it is assumed that tubular fluid in man and dog is isosmotic with plasma at the beginning of the collecting duct, as it is in normal and hypercalcemic rats, hypotonic urine would have to be generated in the collecting ducts by abstraction of a hypertonic reabsorbate. Although this mechanism could account for the findings in these species, it would represent a reversal of what is thought to be the normal function of the collecting ducts, i.e., to reabsorb water in excess of solute. A second explanation is that hypotonic urine excreted by hypercalcemic man and dog comes from the distal convoluted tubule. It has been suggested by Goldsmith and co-workers (28), by Stein and co-workers (29), and by Thurau and Deetjen (30) that in the dog (and perhaps in man at high levels of osmotic diuresis), distal tubular fluid normally does not regain isotonicity before entering the collecting ducts. This would be obscured, however, by reabsorption of water from the collecting ducts. Any abnormality that leads to a fall in medullary hypertonicity, such as a reduction in the supply of sodium to the medulla, could result in excretion of a dilute urine during osmotic diuresis, since the ability to abstract water from a large volume of hypotonic fluid would be greatly diminished. This explanation might also account for the poor correlation between $T^c_{H_2O}$ and GFR in hypercalcemic man (6) and dog (3), since the addition of a variable amount of hypotonic distal tubular fluid to the collecting ducts would markedly affect the calculated $T^c_{H_2O}$. Although this latter view seems the more likely, presently available evidence does not permit a definite choice between these two alternatives.

Summary

1) The renal concentrating defect associated with hypercalcemia was studied in vitamin D-intoxicated rats and hamsters by clearance and micropuncture methods simultaneously. During brisk mannitol diuresis, the osmolality of early distal tubular fluid was as hypotonic to plasma in the hypercalcemic rats as in the normal controls. No evidence was found of excessive loss of sodium in the urine, whether sodium excretion was compared at the same absolute rates of osmolar clearance or osmolar clearance per intact nephron (C_{osm}/100 ml GFR). These observations thus failed to demonstrate any localized or generalized impairment of sodium transport by the tubules.

2) The osmolality of collecting duct fluid from the papilla of hypercalcemic hamsters with a severe concentrating defect was approximately the same as that of fluid from adjacent loops of Henle and vasa recta. This finding indicates that the concentrating defect in hypercalcemia need not be accompanied by a selective impermeability of the collecting ducts to water.

3) The total amount of sodium delivered to the medulla per unit time was much less in hypercalcemic rats than in controls because of a marked reduction in GFR and unimpaired reabsorption of the glomerular filtrate by the proximal tubules. We assume this to be an important factor in the concentrating defect. The observations are compatible with the view that the total number of functioning nephrons is reduced due to blockage of tubules by casts, whereas the ability to transfer sodium and water remains essentially intact in the remaining nephrons.

4) It is postulated that a higher osmotic load per intact nephron could account for the fall in U_{max} under hydropenic conditions and the excretion of normal amounts of sodium during mannitol diuresis in spite of the large reduction in filtered sodium.

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