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Research Article

ON THE IMPAIRMENT OF RENAL CONCENTRATING ABILITY IN PROLONGED HYPERCALCEMIA AND HYPERCALCIURIA IN MAN *

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Sustained hypercalcemia and hypercalciuria lead to failure of the renal concentrating process. The nature of the changes within the kidney and the manner in which these changes impair concentration of the urine have not been well explained. Recent studies suggest that a decrease in concentrating ability may occur without destruction of renal architecture or loss of nephrons (1). Further, the hyposthenuria accompanying hyperparathyroidism may improve following correction of the abnormal calcium metabolism (2). The present studies were designed to clarify the mechanism whereby an excess of calcium in body fluids interferes with the production of a hypertonic urine.

METHODS

The subjects of this study were six patients, ranging in age from 26 to 52 years, with hypercalciuria, hypercalcemia or both, associated with one of the following conditions: "hyperabsorption hypercalciuria" (we have used this term to describe patients who show hypercalciuria preventable by restriction of calcium intake and ingestion of calcium-binding agents, i.e., sodium versenate); idiopathic hypercalciuria (L.M.), hypercalciuria with sarcoidosis (M.E.); hypoparathyroidism overtreated with vitamin D (M.H., H.E.); and hyperparathyroidism (L.W. and E.H.). L.M., E.H. and L.W. gave a history of passing renal calculi. Diets contained 35 to 70 g of protein a day; calcium intake was varied according to the needs of the study. Except for brief periods of salt restriction, sodium intake was *ad libitum*.

For 18 to 30 hours prior to the period of study, the patients received no liquids and were given a dry diet containing 50 g protein. All observations were made in the fasting state in the morning. A priming dose of 100 mU of Pitressin, Parke-Davis (vasopressin) was given intravenously, followed by a sustaining dose of 200 mU per hour in physiological saline, delivered at a rate of 1 ml per minute. The sustaining infusion was delivered with a Bowman constant infusion pump so as to maintain a maximal urinary osmolality (U_{max}). Urine for measurement of osmolality and total solutes was collected by indwelling bladder catheter, with air washes, at 10-minute intervals for five consecutive periods. Venous blood was obtained through an indwelling Cournand needle. Osmotic diuresis was then produced by infusing, at a rate of 18 ml per minute, a solution of 10 per cent mannitol containing vasopressin in a concentration sufficient to deliver 200 mU per hour. Glomerular filtration rate (GFR) was measured by inulin clearance. Inulin was determined by the method of Walser, Davidson and Orloff (3); osmolality of serum and urine by Bowman osmometer (4); and serum and urine calcium by flame photometer. The quantity of solute-free water (T^cH_2O) absorbed in the concentrating process was computed from the formula:

$$T^cH_2O = \frac{U_{osm}V}{P_{osm}} - V$$

from data obtained during the osmotic diuresis (5). For purposes of comparison, the values of T^cH_2O at osmolar clearance of 15 ml per minute were used, and these are the values appearing on the charts.

RESULTS

1. Effect of increased urinary calcium on the concentrating mechanism. To determine the effects of increased renal calcium excretion over a period of time, two patients with hyperabsorption hypercalciuria were studied in three states.

1) After a low calcium intake had produced and maintained for a long period normal levels of serum and urinary calcium, control values (Figure 1A) were obtained.

2) After 2 weeks of hypercalciuria (urinary calcium, about 300 mg per day) without hypercalcemia (serum calcium ranged from 9.2 to 9.9 mg per 100 ml in L.M. and from 8.8. to 10.4 mg per 100 ml in M.E.) produced with a calcium intake of 1.2 g per day, a second set of values (Figure 1B) was obtained: U_{max} and T^cH_2O were markedly depressed.

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3) After 3 weeks or more of treatment with low calcium intake and disodium versenate, 4 g per day (all urinary calcium determinations below 200 mg per day), a third set of values (Figure 1C) was obtained: U_{max} and T^cH_2O had returned to control values. GFR was not affected, and solute excretion (before mannitol) was not increased by these procedures (Figure 1).

In both patients, sodium deprivation was produced by a mercurial diuretic (Thiomerin) and a sodium intake of 9 mEq per day for 6 days at the time concentrating power was impaired. In each patient (L.M. and M.E., Table I) urinary

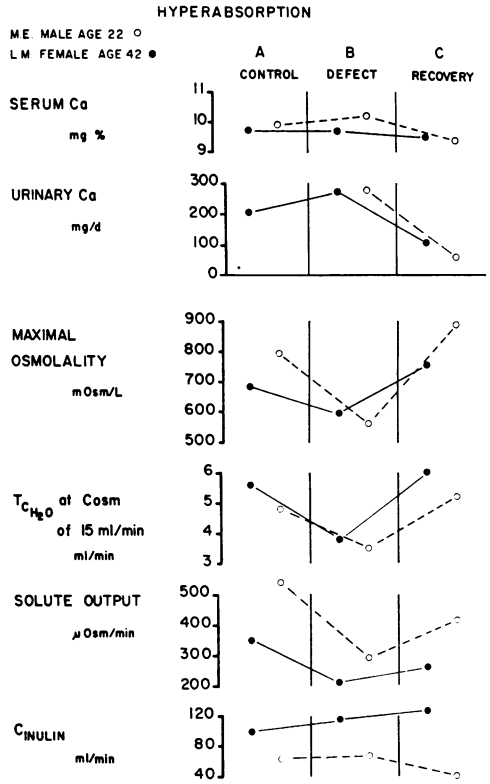

FIG. 1. SERUM AND URINARY CALCIUM, MAXIMAL URINARY OSMOLALITY, T^cH_2O, SOLUTE OUTPUT AND INULIN CLEARANCE IN TWO SUBJECTS WITH HYPERABSORPTION HYPERCALCIURIA: A) DURING THERAPY; B) AFTER TWO WEEKS OF HIGH CALCIUM INTAKE; AND C) AFTER RESUMPTION OF TREATMENT FOR THREE WEEKS. Note depression of concentrating ability with calcium feeding and recovery with treatment. "Normal" T^cH_2O values in B are clearly below normal for these subjects. Figures for solute output are averages of 4 to 7 values obtained before mannitol was given. Figures for C_{Inulin} are means of 4 to 7 periods. Figures for serum and urinary calcium are those obtained the day before or day of the test.

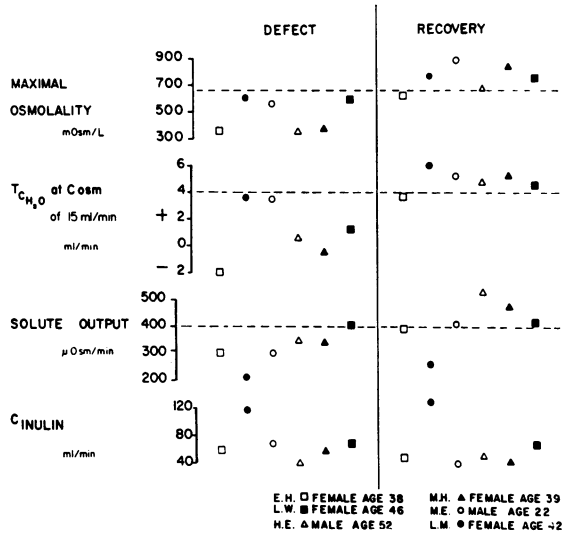

FIG. 2. MAXIMAL URINARY OSMOLALITY, T^cH_2O, SOLUTE OUTPUT AND INULIN CLEARANCE IN SIX SUBJECTS BEFORE AND AFTER RECOVERY. Note that improvement of the concentrating defect is independent of GFR and solute output. Solute output and GFR are averages of 4 to 7 periods.

sodium was less than 4 mEq per day by the fourth day. These findings suggest that an increased renal excretion of calcium without accompanying hypercalcemia may lower maximal urinary osmolality and T^cH_2O without affecting GFR or increasing solute excretion. Further, this impairment of the concentrating process may be reversed by correcting the hypercalciuria.

II. Effect of large doses of vitamin D on the concentrating mechanism. A patient with hypoparathyroidism (Table II, M.H., 4-17-58), treated with large doses of vitamin D for 16 days, developed hypercalcemia. Urinary calcium averaged 281 mg per day. Maximal urinary concentration was 375 mOsm per L and T^cH_2O was -0.5 ml per minute.[1] Five weeks after vitamin D had been stopped, (Table II, M.H., 5-19-58) serum and urinary calcium were normal and maximal osmolality and T^cH_2O were markedly improved (838 mOsm per L and 5.2 ml per minute, respectively); GFR was lower, and solute excretion was greater at that time.

A second patient (H.E., Table II), with hypoparathyroidism was given large doses of vitamin

[1] A negative T^cH_2O with osmotic diuresis is not infrequently found despite maximal "basal" urine osmolality above that of serum (*vide infra*).

TABLE I

Response to sodium deprivation *

Patient			Days 1†	2†	3	4	5
	Serum	Ca mg%	9.7		9.9		9.6
		Na mEq/L			140		136
L.M.	Urine	Ca mg/day	203	576	341	304	286
		Na mEq/day	130	57.6	4.9	3.4	0
	Serum	Ca mg%	9.9	10.3		10.4	
		Na mEq/L		141		137	
M.E.	Urine	Ca mg/day	395	260	162	182	
		Na mEq/day	351	87	3.2	2.0	
	Serum	Ca mg%	11.6	11.7	10.1	10.3	10.6
		Na mEq/L		139			139
M.H.	Urine	Ca mg/day	319	298	227	214	221
		Na mEq/day	139	30	0	0	0
	Serum	Ca mg%	11.6		11.1		10.9
		Na mEq/L					140
H.E.	Urine	Ca mg/day	409	450	355	349	256
		Na mEq/day	378	177	6	4	2
	Serum	Ca mg%	11.9		11.8		11.9
		Na mEq/L		138			137
L.W.	Urine	Ca mg/day		229	159	116	118
		Na mEq/day		50.4	4.5	2	2.4
	Serum	Ca mg%	15	14		12.4	12
		Na mEq/L	137				135
E.H.	Urine	Ca mg/day	210	41	12	12	14
		Na mEq/day	28	20	14.2	14‡	15.3‡

* 9 mEq sodium intake.
† Thiomerin 2 ml, i.m.
‡ Desoxycorticosterone acetate, 15 mg, i.m.

D after control maximal osmolality and T^cH_2O determinations had been obtained. Both T^cH_2O and U_{max} fell significantly in 2 weeks (Table II, 12-1-58). Both urinary and serum calcium were elevated. Vitamin D was continued, but calcium intake was decreased to 200 mg per day, and disodium versenate, 4 g per day, and neutral phosphate, 8 g per day, were given. Hypercalciuria was corrected, but hypercalcemia persisted. T^cH_2O was 0.5 ml per minute, and U_{max} was 350 mOsm per L (Table II, 1-12-59), suggesting further deterioration. Vitamin D was then stopped. Four months later urinary and serum calcium and renal concentrating ability had all returned to control levels (Table II, 5-18-59). Solute excretion was never abnormally high. The GFR was somewhat lower when concentrating ability was markedly impaired than it was after improvement 4 months later. In general, however, GFR changed very little with recovery (Figure 2). Sodium deprivation, carried out in both patients at the time concentrating power was impaired, showed no gross compromise of sodium conservation (Table I).

The effect of vitamin D on urinary concentration in the absence of hypercalciuria and hypercalcemia was measured by giving large doses (600,000 U per day) to Patient M.H. at a time when concentrating ability was normal (Table II, 3-5-59), and limiting calcium intake (200 mg per day), so that after 3 weeks of vitamin D therapy urinary and serum calcium were still normal. Re-evaluation of the concentrating process (M.H., Table II, 3-24-59) revealed that U_{max} and T^cH_2O had not changed significantly.

These data suggest that the effect of large doses of vitamin D on the concentrating mechanism is probably mediated through the accompanying elevated serum and/or urinary calcium rather than through a direct effect of the vitamin itself on the kidney. As in the case of hyperabsorption hypercalciuria, this impairment of renal function

TABLE II

Effect of large doses of vitamin D on renal function in patients with hypoparathyroidism

Date	Patient	Serum Ca	Urinary Ca*	C_{In}†	P_{osm}	U_{max}	T^cH_2O‡	U_{osm}†	Remarks
		mg%	*mg/day*	*ml/min*	*mOsm/L*	*mOsm/L*		*μOsm/min*	
				Effect of vitamin D; hypercalcemia and hypercalciuria					
4-17-58	M.H.	11.6	281	55	275	375	−0.5§	334	Vitamin D, 600,000 U/day from 3-28-58 to 4-13-58.
5-19-58	M.H.	8.9	(215)	43	263	838	5.2	470	Recovery; compare with 3-5-59 below.
11-17-58	H.E.	9.2	207	94	278	613	5.4	585	Control.
12- 1-58	H.E.	11.6	405	58	283	502	3.2	704	Vitamin D, 500,000 U/day beginning 11-15-58.
				Effect of vitamin D; hypercalcemia without hypercalciuria					
1-12-59	H.E.	12.0	(180)	40	263	350	0.5	352	Vitamin D plus Na versenate, neutral phosphates and Ca restriction from 12-2-58 to 1-12-59.
5-18-59	H.E.	8.2	47	52	275	675	4.8	535	Recovery after stopping vitamin D.
				Effect of vitamin D; no hypercalcemia or hypercalciuria					
3- 5-59	M.H.	8.6	109		266	1021	4.9	180	Control.
3-24-59	M.H.	8.6	131		274	900	4.5	370	Vitamin D, 600,000 U/day from 3-6-59 to 3-24-59; compare with 4-17-58.

* The values are an average of the calcium excretion obtained during the 3 days preceding or following the clearance. Values in parentheses were obtained 4 days preceding the clearance and are representative of urinary calcium during pretesting period. Values for days immediately preceding test were not available.

† Average of 4 to 7 values.

‡ T^cH_2O at C_{osm} of 15 ml/min.

§ A negative value for T^cH_3O is to be understood as C_{H_2O}.

TABLE III

Effect of hyperparathyroidism on renal function

Date	Patient	Serum Ca	Urinary Ca*	C_{In}†	P_{osm}	U_{max}	T^cH_2O‡	U_{osm}	Remarks
		mg%	*mg/day*	*ml/min*	*mOsm/L*	*mOsm/L*	*ml/min*	*μOsm/min*	
7- 2-58	L.W.	11.9	229	86	288	555	3.8	524	
12- 4-58	E.H.	18.0	594	55	280	347	−2.0§	300	
				Effect of neutral phosphate; hypercalcemia without hypercalciuria					
7-23-58	L.W.	11.5	83	68	269	590	1.2	410	Neutral phosphates and Na versenate from 7-7-58 to 7-23-58.
12-29-58	E.H.	12.6	42	35	269	384	−0.7§	310	Neutral phosphates from 12-10-58 to 12-29-58.
				After removal of adenoma					
9-26-58	L.W.	9.5	(67)	67	272	750	4.5	420	2 Mos post-op.
1-30-59	E.H.	9.4	(15)		278	470	0.5	305	2 Weeks post-op.
6-23-59	E.H.	9.5	15	46	278	625	3.7	395	5 Mos post-op.

* The values are an average of the calcium excretion obtained during the 3 days preceding or following the clearance. Values in parentheses were obtained 5 to 7 days preceding or following the clearance and are representative of urinary calcium during pretesting period. Values for days immediately preceding test were not available.

† Average of 4 to 7 values.

‡ T^cH_2O at C_{osm} of 15 ml/min.

§ A negative value for T^cH_2O is to be understood at C_{H_2O}.

is reversible, improving as the serum and urinary calcium decrease to normal values.

III. Effect of hyperparathyroidism on the concentrating mechanism. Two patients with hyperparathyroidism, with elevation of both serum and urinary calcium, showed marked limitation of concentrating ability (Table III). One of them, L.W., showed a T^cH_2O within the normal range (3.8 ml per minute) but a maximal urinary osmolality of only 555 mOsm per L. Oral phosphates, which have been shown to correct the chemical abnormalities of hyperparathyroidism (6), might be expected to improve concentrating ability. When these patients were given neutral phosphates orally, 8 g per day (equivalent to 1.8 g P), urinary calcium returned to normal in both patients and remained normal for 3 weeks, with the exception of 4 days when E.H. showed values above 160 mg per day. Although serum calcium fell, it was still consistently above normal in E.H. (12.5 mg per 100 ml or above) and intermittently elevated in L.W. (9.4 to 11.5 mg per 100 ml). At the end of 3 weeks, E.H. was not significantly improved, while L.W. showed a further decline in concentrating ability (Table II, 12-29-58 and 7-23-58, respectively). Attempts to produce a comparable correction of the hypercalciuria of hyperparathyroidism with disodium versenate alone failed.

Following surgical removal of a parathyroid adenoma, concentrating ability improved considerably: U_{max} was 750 and 625 mOsm per L, and T^cH_2O was 4.5 and 3.7 ml per minute in L.W. and E.H., respectively (Table III).

Sodium deprivation, carried out in both patients before operation, showed impairment of sodium conservation in E.H. (Table I), whose urinary sodium remained greater than the intake value of 9 mEq per day even when 15 mg of desoxycorticosterone acetate was given daily. E.H. also showed limited hydrogen ion excretion in response to an acid load (serum carbon dioxide fell from 33 to 10 mEq per L during a 5 day course of ammonium chloride, 130 mEq per day). She was the only one of the patients tested who demonstrated such extensive renal damage.

DISCUSSION

Infusion of solutions containing calcium produces a diuresis of sodium and water in dehydrated subjects (7). Further, a defective urinary concentrating mechanism has been demonstrated in rats given high calcium, high acid-phosphate diets (8) and vitamin D (1, 8), and in dogs given parathyroid extract for 24 hours (9). Thus, the association of impaired concentrating function with four different disease states, having in common an elevation of urinary and (with the possible exception of hyperabsorption hypercalciuria) serum calcium, suggests that the defect is a consequence of the excess of calcium and not of the specific disease. This defect does not result from osmotic diuresis, as solute excretion need not be excessive (Figure 2) nor GFR markedly impaired.

Some of the results suggest that hypercalcemia alone, without hypercalciuria, can maintain and possibly produce the defect (Tables II and III). However, the three patients, E.H., H.E. and L.W., whose concentrating defect persisted or became worse despite absence of hypercalciuria, were receiving neutral phosphate. In the presence of hypercalcemia it is entirely possible that phosphates impair renal function (8). In addition, two of the patients who showed this phenomenon, L.W. and E.H., had hyperparathyroidism. A recent study suggests that parathyroid hormone may itself have a direct nephrotoxic effect (10). It is still possible that parathyroid hormone has no deleterious renal effect independent of an excess of calcium. In contrast, it is very likely that vitamin D has no nephrotoxic effect when serum and urinary calcium are normal (Table II).

Except during the period of treatment with versenate and phosphates discussed above, all patients had hypercalciuria associated with impaired renal function. Inasmuch as hypercalcemia has never been demonstrated in two of them (M.E. and L.M.) or in a third extensively studied subject with hypercalciuria and the concentrating defect (11), it is probable that hypercalciuria alone is sufficient to produce this type of renal impairment.

The renal lesions resulting from increased body fluid calcium are predominantly tubular ones (1, 2). The glomeruli may be minimally affected, if at all; GFR may be relatively normal. The tubules, on the other hand, may show lesions ranging from necrosis of epithelium to spotty deposition of calcium in the basement membranes, or in

"clumps or as a fine dust" (2) in the cells. The collecting ducts are said to be chiefly affected, but the lesions may appear in the convoluted tubules as well (1, 2). Histologically recognizable changes need not be present, however (11); the concentrating defect may be seen despite apparently normal renal architecture.

Figure 3 shows the relationship of urinary flow (V) to solute clearance (C_{osm}) for four patients at a time when concentration was impaired and again after recovery. In three patients, regression lines of C_{osm} on V actually cross the isosmotic parameter (negative T^cH_2O) at osmolar clearances below 12 ml per minute when concentration is defective. Negative T^cH_2O with osmotic diuresis at C_{osm} below 16 ml per minute can be produced by normal kidneys only when vasopressin is present in limited amounts (12). The negative T^cH_2O in our patients despite maximal doses of vasopressin indicates serious impairment of the concentrating mechanism. After recovery, the four patients are comparable to normal subjects who may show positive T^cH_2O at osmolar clearances up to 35 ml per minute (5) or greater (13).

According to present concepts, maximal concentration of the urine requires antidiuretic hormone (ADH), a distal tubular membrane whose permeability to water is dependent on ADH, and medullary solute adequate in amount and concentration (14).

As tubular flow is increased with mannitol diuresis, a given amount of ADH is rendered less effective both by limitation of distal tubular reabsorption as larger volumes containing unreabsorbable solute are delivered, and by limitation of medullary function as more water is presented for removal. Reduced function of the concentrating mechanism in disease, despite maximal exogenous ADH, might result from a decrease in tubular permeability (either in the distal convolutions or in the collecting ducts) or from a decrease in medullary efficiency (either for solute delivery or for water removal). In this schema, medullary solute content depends ultimately upon tubular reabsorption of sodium.

In rats made nephrocalcinotic (15), maximal urinary concentration was depressed relatively more than medullary concentration of sodium and total solutes, suggesting impairment of tubular

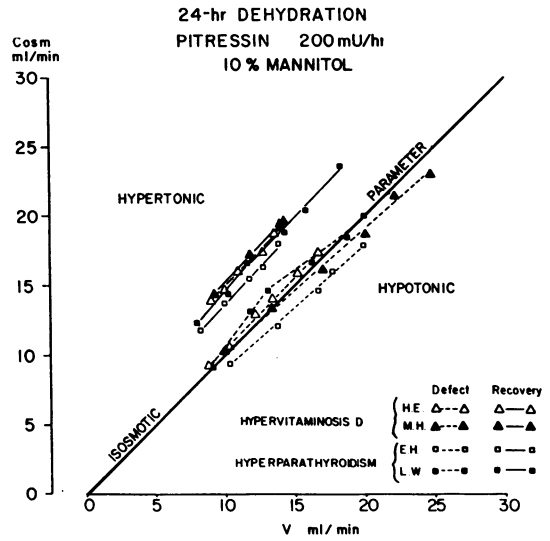

FIG. 3. REGRESSION OF OSMOLAR CLEARANCE (C_{osm}) ON URINE VOLUME (V) IN FOUR SUBJECTS WITH CONCENTRATING DEFECT AND AFTER RECOVERY, DURING OSMOTIC DIURESIS. The data for hyperabsorption hypercalciuria are contained in Figure 1, B and C.

permeability to water. Further, analysis showed a significantly decreased medullary sodium and total solute content which would limit efficiency of the medullary concentrating mechanism. Also, *in vitro* studies indicate that calcium can inhibit sodium (16) and water (17) transport in frog skin by reducing its permeability, and can suppress the action of vasopressin in slices of Necturus kidney (18).

In contrast, our studies demonstrated a failure of renal sodium conservation in only one of the six patients. It is possible, however, that changes associated with sodium restriction could have obscured an altered tubular transport of sodium.

From presently available data, one cannot define precisely the site or mode of action of calcium in limiting urinary concentrating ability.

SUMMARY

Maximal urinary osmolality (U_{max}) and T^cH_2O were measured in subjects with hypercalciuria, hypercalcemia, or both. Patients with "hyperabsorption hypercalciuria," hypervitaminosis D and hyperparathyroidism were studied before and after treatment designed to lower urinary calcium.

All subjects showed impairment in both U_{max} and T^cH_2O, and improvement in them with ther-

apy. The data suggest that excess of calcium, rather than the agent or disease process producing it, is ultimately responsible for the defect.

The concentrating defect was not attributable to an increased solute excretion, which showed negligible changes with improvement.

All subjects but one showed normal sodium conservation with urinary sodium less than 9 mEq per day at this intake, suggesting that the concentrating defect does not depend upon gross failure of sodium transport.

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CORRECTION

On page 590 of the paper entitled "Isozymes of lactic dehydrogenase in human tissues" by Elliot S. Vesell and Alexander G. Bearn (J. clin. Invest. 1961, **40**, 586), lines 12 and 13 of the Summary should read: "Leukocytes and serum showed highest activity in peak 4."