CHANGES IN RENAL CONCENTRATING ABILITY PRODUCED BY PARATHYROID EXTRACT *

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Polyuria is a frequent symptom of hyperparathyroidism. Impairment of renal concentrating ability, often out of proportion to other signs of renal insufficiency, has been noted in patients with hyperparathyroidism (1–3) as well as in patients and animals with hypercalcemia due to other causes (4–6). In many patients with hyperparathyroidism, extensive deposits of calcium in the renal parenchyma, secondary fibrosis, superimposed pyelonephritis and vascular disease have combined to destroy considerable amounts of renal substance. The mechanism of the reported decrease in renal concentrating ability in such patients is not clear, since fixation of urinary specific gravity is a well-known accompaniment of the simple ablation of renal tissue (7).

In the present experiments, renal concentrating capacity was found to be depressed in dogs in which hypercalcemia had been induced for only 24 hours by injecting parathyroid extract. This functional defect was associated with morphologic changes in the distal portions of nephrons and the collecting ducts.

MATERIALS AND METHODS

Mature female dogs weighing 8 to 22 Kg. were maintained on Purina Chow® supplemented with horsemeat. All experiments were carried out under light pentobarbital anesthesia. Inulin was determined by the method of Walser, Davidson and Orloff (8), osmolality of serum and urine with a Fiske osmometer, serum calcium according to Kingsley and Robnett (9), and sodium in serum and urine by flame photometry.

Group I. Studies of concentrating ability during graded osmotic diuresis

Four dogs were studied. Prior to the experiment, food and water were withheld for 24 hours. Five units of vasopressin in oil [1] was injected subcutaneously on the evening before the experiment. A control study was carried out on each dog one week before it received parathyroid extract. A total of 90 units of parathyroid extract [2] per Kg. was injected subcutaneously in three divided doses during the 24 hours preceding the second study. During the experiment, 0.5 per cent inulin in physiological saline was infused at a rate of 1 to 1.5 ml. per minute, together with 50 to 100 milliunits of vasopressin [1] per Kg. per hour. Samples of urine and blood were collected at 10 to 15 minute intervals. After a suitable period of equilibration, urine flow was slowly increased from less than 1 ml. per minute to more than 10 ml. per minute by gradually increasing the rate of infusion of 5 per cent mannitol in 0.72 per cent saline. This technique permitted examination of the concentrating function over a wide range of urine flows and rates of solute excretion.

Group II. Studies of concentrating ability during a constant infusion of mannitol

Seven dogs were studied before and after receiving parathyroid extract in doses comparable to those given to the preceding group. Five per cent mannitol in 0.72 per cent saline was infused at a rate of 0.50 to 0.75 ml. per Kg. per minute throughout the experiment. Inulin and vasopressin were infused simultaneously as in Group I. Following an equilibration period of one hour, when urine flow had reached a constant value, collections of urine were made every 10 to 15 minutes. Samples of blood were obtained from the jugular vein at intervals of 20 to 30 minutes. Three dogs were restudied in the same way at intervals up to five weeks after treatment with hormone.

In six additional dogs not given parathyroid extract, the effect of progressive osmotic loading on T^cH_2O was studied by increasing the rate of infusion of 5 per cent

mannitol in saline stepwise to three or four times the initial rate of 0.50 to 0.75 ml. per Kg. per minute.

Dogs were autopsied at intervals up to two months after receiving parathyroid extract. The kidneys were fixed in formalin and studied by microdissection as well as by standard histological techniques.

RESULTS

Subcutaneous injection of 60 to 97 units of parathyroid extract per Kg. over a period of 24 hours resulted in *an increase in serum calcium* to 15 to 20 mg. per 100 ml. During this time the animals usually looked sick and listless and exhibited polyuria. Serum calcium returned to normal after 48 to 72 hours and the dogs had generally regained their usual vigor by one week after injections of parathyroid extract were discontinued.

Glomerular filtration rate (GFR) usually decreased, but remained essentially unchanged in two dogs and rose in one animal, following the administration of parathyroid extract.

The *maximum urinary concentration rate* (U_{osm}/P_{osm}) achieved before mannitol loading in dehydrated dogs injected with vasopressin in oil was considerably diminished following parthyroid extract (Figure 1) but tended to rise to or toward the control level several days to weeks after the serum calcium had fallen to normal.

Studies of *concentrating ability during graded osmotic diuresis* (Group I) demonstrated impairment of water conservation at all levels of urine flow and solute load. The four experiments of

this group are summarized in Figures 2A through 2D. Despite the continuous infusion of large amounts of exogenous vasopressin, urine became hypotonic to plasma at relatively low levels of solute excretion in three out of four experiments and the excretion of free water increased thereafter with increasing solute diuresis.

Similar changes in $T^c H_2O$ were observed in the experiments of Group II (Figure 3, Table I). Hypotonic urine was excreted during moderate mannitol loading in five of seven dogs. Solute loads of much greater magnitude in proportion to GFR did not suffice to produce hypotonic urine in six normal dogs infused rapidly with 5 per cent mannitol in 0.72 per cent saline (Figure 4).[3]

In experiments of Group I and Group II, the ratio of urinary sodium to total urinary solutes was not consistently altered by parathyroid extract.

Lower doses of parathyroid extract (30 to 35 units per Kg.) produced elevations of serum calcium of only 1 to 2 mg. per 100 ml. in two dogs, with no changes in renal concentrating ability or glomerular filtration rate.

The subcutaneous injection of 4.5 to 7.0 Gm. of calcium as calcium acetate into three animals resulted in increased urinary excretion of calcium. Serum calcium levels were slightly elevated two to four hours after the injection but had returned to normal by the following day. At this time $T^c H_2O$ was found to be unaltered.

Morphological changes

Dogs autopsied within a few days after receiving parathyroid extract had focal tubular lesions of irregular patchy distribution. These were demonstrated by microdissection to lie along the *ascending limbs of Henle's loop*, the *distal convolutions* and the *collecting system*. They consisted of foci of hydropic swelling of the tubular epithelial cells, acute necrosis of the epithelium, frequently with calcification, and often calcification of basement membranes. The necrotic, calci-
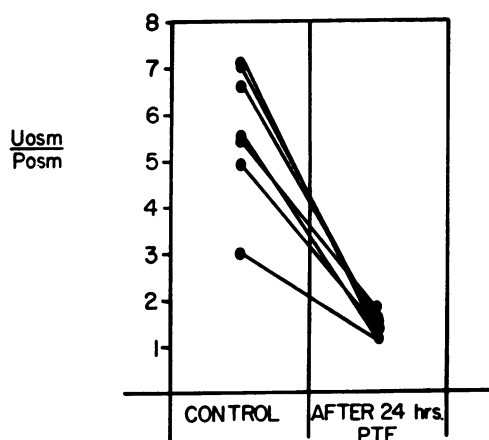
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[3] Others (10, 11) have reported that $T^c H_2O$ may disappear during massive osmotic diuresis in normal dogs. We have observed hypotonic urines in normal dogs loaded with mannitol in saline only with solute loads in excess of those depicted in Figure 4. A possible explanation for the maintenance of hypertonic urine during osmotic diuresis in our normal dogs may lie in their high protein diet (12).
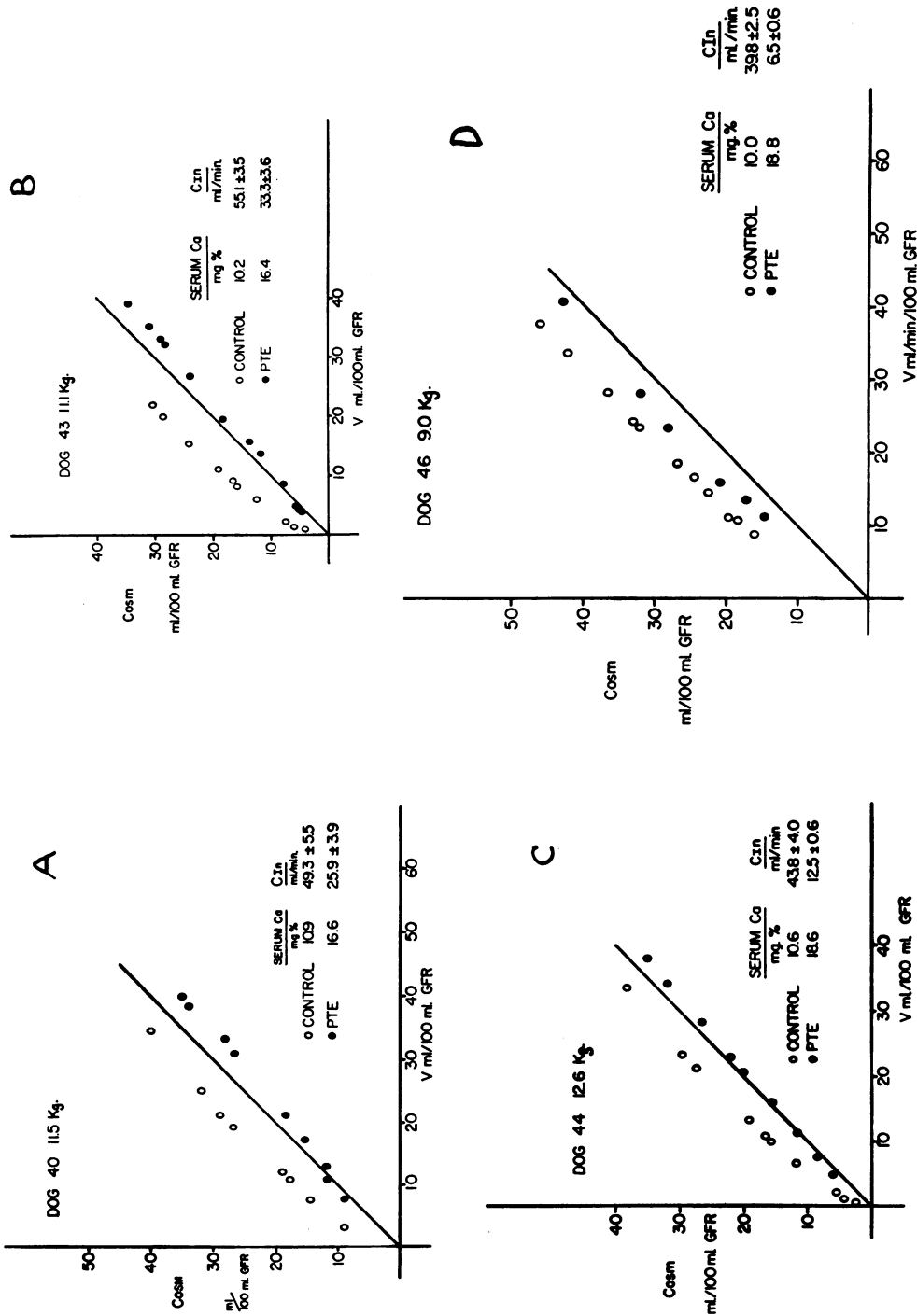
FIG. 2. IMPAIRMENT OF T^{c}H$_2$O AFTER TREATMENT WITH PARATHYROID EXTRACT (PTE)

The heavy diagonal line is the isotonic parameter. Points above this line represent urines more concentrated than plasma; points below the line, urines hypotonic to plasma. Note that after administration of parathyroid extract (black dots), urine became progressively more hypotonic with increasing mannitol diuresis, despite a constant intravenous infusion of vasopressin. In D note that after parathyroid extract, concentrating ability was diminished, but urine hypotonic to plasma was not produced even during heavy osmotic diuresis. It is noteworthy that this dog had the most severe impairment in glomerular filtration of all four animals in Group I.

TABLE I

Effects of parathyroid extract on renal function (Group II)

Dog No.	Wt.	Procedure	No. of periods	Serum Ca*	V	Uosm V	$T^c_{H_2O}$†	C_{In}	$\dfrac{U_{Na}}{U_{osm}}$	$\dfrac{T^c_{H_2O}†}{C_{In}} \times 100$	Maximum $\dfrac{U_{osm}}{P_{osm}}$
	Kg.			mg./100 ml.	ml./min.	mOsm./min.	ml./min.	ml./min.			
3	8.4	Control	4	9.7	7.5‡ ±0.5	2,575 ±56	0.5 ±0.17	29 ±2.0	0.28	1.7 ±0.83	
		90 units PTE/Kg.	5	13.1	11.0 ±0.5	3,474 ±236	−1.1 ±.01	27 ±1.2	0.31	−4.2 ±0.39	
		9 days later	5	8.4	10.7 ±1.2	3,878 ±387	1.0 ±0.30	28 ±0.8	0.30	3.6 ±0.40	
4	18.6	Control	5	8.7	11.9 ±1.6	5,118 ±607	4.3 ±0.48	65 ±7.2	0.25	6.6 ±0.15	
		77 units PTE/Kg.	4	14.7	16.9 ±0.94	6,449 ±379	2.7 ±0.14	83 ±3.3	0.25	3.3 ±0.14	
5	20.7	Control	5	10.5	19.0 ±1.0	7,407 ±265	4.5 ±0.21	80 ±3.2	0.32	5.7 ±0.41	
		97 units PTE/Kg.	5	15.0	6.7 ±0.48	2,039 ±188	−0.4 ±0.08	13 ±0.51	0.29	−3.4 ±0.61	
8	13.5	Control	5	10.4	9.0 ±0.68	3,905 ±243	3.6 ±0.25	70 ±8.3	0.25	5.2 ±0.38	4.9
		60 units PTE/Kg.	5	16.0	11.7 ±1.0	3,760 ±357	−0.4 ±0.08	34 ±1.2	0.27	−1.1 ±0.19	1.1
9	17.5	Control	5	8.9	17.7 ±0.96	7,134 ±339	5.0 ±0.19	73 ±3.7	0.28	6.9 ±0.11	5.4
		60 units PTE/Kg.	5	12.3	20.1 ±1.2	6,619 ±150	−0.5 ±0.14	53 ±1.5	0.32	−1.0 ±0.27	1.8
		5 weeks later	6	7.6	15.6 ±1.7	6,427 ±623	4.4 ±0.22	70 ±2.1	0.26	6.3 ±0.38	5.7
19	15.0	Control	4	9.4	11.0 ±0.29	4,736 ±25	3.9 ±0.17	49 ±1.4	0.31	7.9 ±0.43	3.0
		60 units PTE/Kg.	5	14.5	4.2 ±0.20	1,463 ±105	0.2 ±0.10	10 ±0.42	0.28	2.4 ±0.98	1.1
		8 days later	4	7.0	5.8 ±0.30	2,011 ±99	0.1 ±.08	13 ±0.24	0.22	0.6 ±0.59	1.8
		23 days later	4	7.6	10.4 ±0.36	3,633 ±109	0.4 ±0.15	26 ±0.42	0.26	1.4 ±0.58	2.5
6	16.0	Control	7	8.7	10.5 ±2.5	4,374 ±834	3.2 ±0.21	49 ±3.2	0.26	6.6 ±0.58	
		94 units PTE/Kg.	7	13.0	12.4 ±0.82	3,873 ±313	−0.5 ±0.15	22 ±1.1	0.32	−2.3 ±0.68	

* During infusion of mannitol in saline.
† A negative value indicates urine more dilute than plasma.
‡ Mean ± standard deviation of all the periods listed in Column 4.

fied, cellular debris formed obstructing casts with dilatation proximally.

In the kidneys of dogs autopsied two weeks to two months after receiving parathyroid extract there was "fatty change" of the tubular epithelium similar in distribution to the early alterations. Proliferative lesions, seen only in the medullary portion of the collecting system, consisted of intratubular cellular masses, often calcified, which produced obstruction and proximal dilatation, and
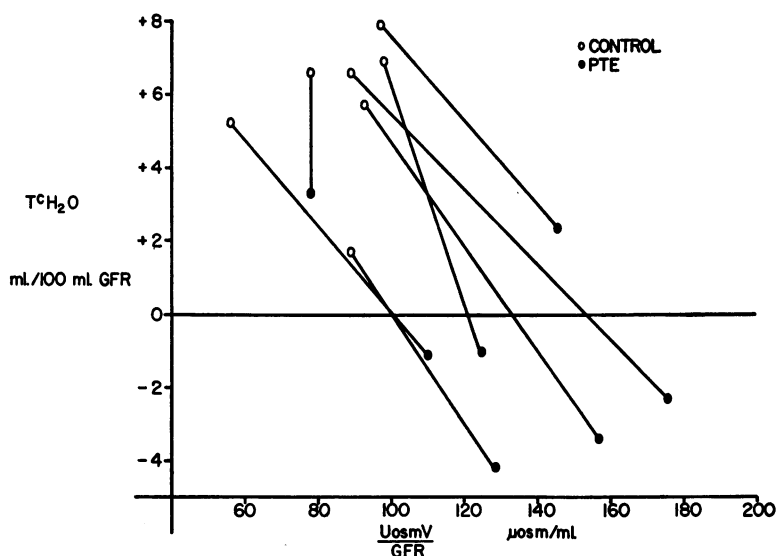
FIG. 3. EFFECT OF PARATHYROID EXTRACT (PTE) ON $T^c H_2 O$

After 24 hours of treatment with parathyroid extract, $T^c H_2 O$ was greatly
diminished in all animals; in five, hypotonic urine was elaborated despite the
administration of vasopressin.

in places extended beyond the tubular basement
membrane into the connective tissue stroma.

Chemical analysis of the kidneys in two in-
stances (Table II) demonstrated an increased
calcium content of the renal medulla. The amount
of calcium in the renal cortex was not greatly
altered by injections of parathyroid extract.

These changes will be described more fully
elsewhere (13). The character and distribution
of the renal lesions resembled those observed in
rats intoxicated with vitamin D (6).

TABLE II

Calcium content of serum, urine and kidneys of dogs treated
with parathyroid extract and of normal dogs *

	Dog No.	Serum Ca	Urine Ca	Ca content, mg./Kg. FFWT†	
				Cortex	Medulla
		mg. %	mg. %		
Parathyroid	8‡	16	6	179	832
	19‖	7	7	173	1,007
Normal	24	6	2	188	141
	25	7	4	116	103

* All animals were sacrificed while undergoing mannitol
diuresis.
† Fat-free wet tissue.
‡ Sacrificed at the conclusion of the experiment sum-
marized in Table I after 24 hours of parathyroid extract.
‖ Twenty-three days after parathyroid extract.

DISCUSSION

Renal function is often impaired in hyperpara-
thyroidism (14) and although renal insufficiency
may prove reversible (3, 14, 15) it frequently
progresses even after the concentration of cal-
cium in the serum is restored to normal by para-
thyroidectomy. Edvall (14) found the clearances
of inulin and para-aminohippurate (PAH) as well
as $T_m PAH$ to be depressed in most but not all
patients with hyperparathyroidism. Hellström
(2) noted that patients with this disease frequently
excreted urine with maximum specific gravity be-
low normal, even when azotemia was absent. Af-
ter operation, the ability to concentrate the urine
was often restored. Cohen, Fitzgerald, Fourman,
Griffiths and deWardener recorded maximum uri-
nary concentrations below the concentration of
solutes in plasma in two patients with hyperpara-
thyroidism who in addition had reduced levels of
glomerular filtration rate and (in one) a decreased
renal plasma flow (3).

The present experiments suggest that the abil-
ity of the kidneys to produce a concentrated urine
was impaired after only 24 hours of hypercalcemia
produced by injections of parathyroid extract.
Interpretation of the data is complicated by the
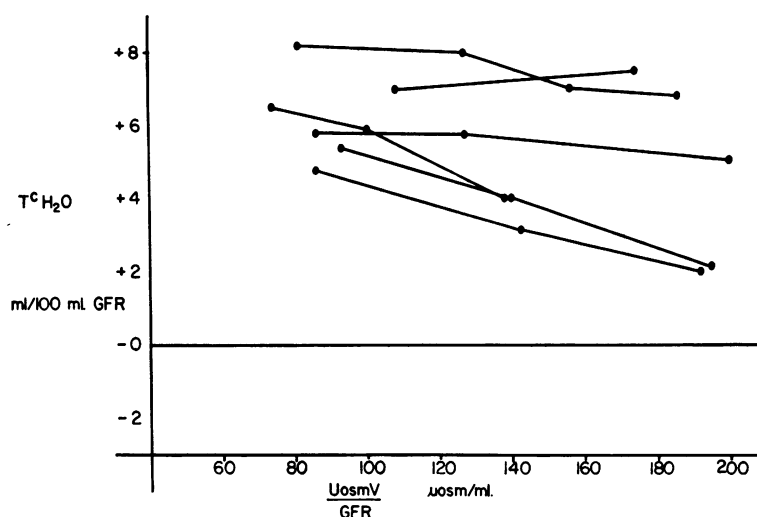fact that in most cases glomerular filtration rate,

Fig. 4. Effect of Progressive Mannitol Loading on Normal Dogs

Massive solute diuresis was associated with some decline in T^cH_2O but did not, in these experiments, result in the appearance of urine hypotonic to plasma.

was also reduced by parathyroid extract. *Acute* reductions in glomerular filtration rate, induced by compressing the renal artery of dogs undergoing mannitol diuresis, result in a rise, rather than a fall, in maximum urinary osmolality (16). Nonspecific destruction of renal tissue, whether produced by injecting an aminonucleoside into the renal artery, by experimental pyelonephritis or by temporary renal ischemia, does not appreciably reduce the ratio of T^cH_2O to glomerular filtration rate, even though the latter may be considerably depressed (17). This is also true, in general, of patients with chronic nephritis whose glomerular filtration rate is below normal (18). By contrast, when concentrating capacity (T^cH_2O) was referred to GFR in the present experiments, a considerable difference between normal dogs and those treated with parathyroid extract was apparent. Finally, in two dogs of the present series (3 and 4, Table II) T^cH_2O diminished after parathyroid extract without a concomitant decrease in glomerular filtration rate.

Although a progressive increase in the quantity of solute excreted by each nephron may, under some circumstances, result in the excretion of a dilute urine (19) it seems unlikely that such an increase in solute load was responsible for the present results. Massive solute diuresis in normal dogs did not lower T^cH_2O to the degree observed

in animals treated with parathyroid extract. Hypercalcemic dogs of Group I infused with vasopressin produced a hypotonic urine while excreting the same amount of solute per unit of GFR as they had done when they were normal and able to concentrate the urine. Mere destruction of nephron units or an overall decrease in the rate of glomerular filtration could not, therefore, have been primarily responsible for the diminution in concentrating capacity which was observed.

The morphological alterations in these kidneys, localized chiefly to the ascending portion of Henle's loop, the distal convoluted tubule and the collecting ducts, are interesting in the light of current theories concerning the mechanism by which the kidneys reabsorb water and excrete a concentrated urine (20, 21). Impairment of renal concentrating ability might theoretically result from: *1*) a decrease in the reabsorption of sodium without water in the ascending limb of the loop of Henle, *2*) disruption of the counter-current mechanism by which sodium (and urea) are concentrated in the medulla, and *3*) slowing of the back-diffusion of water from the collecting ducts to the hypertonic interstitial fluid of the renal medulla (a process normally facilitated by vasopressin). The data of these experiments are probably compatible with any of the above explanations, although excretion of hypotonic urine by hypercal-

cemic dogs infused with vasopressin implies the continued active reabsorption of sodium without water in distal portions of the nephron.

The changes in concentrating ability observed in these experiments were associated with an elevated serum calcium, yet it is noteworthy that hypercalcemia of the same degree produced acutely by infusions of calcium salts does not result in nearly as large changes in T^cH_2O as those observed here (22). Perhaps the serum level of calcium is less important in this regard than the calcium content of cells lining the medullary tubules, or the structural and metabolic changes induced in the renal medulla by prolonged hypercalcemia and/or hypercalciuria. In addition to producing hypercalcemia, parathyroid hormone probably directly induces certain changes in the metabolism of renal tissue (23) which are not entirely reproduced by increasing the concentration of calcium in the serum, the renal cells, or the urine. It is of interest that morphological and functional changes in the kidneys similar to those of the present experiments can be produced by the administration of large doses of vitamin D to rats (6). The frequent association of polyuria with hypercalcemia and nephrocalcinosis of diverse origins lends support to the thesis that changes in calcium metabolism are at least partially responsible for the hyposthenuria of hyperparathyroidism.

SUMMARY

1. The effects of parathyroid extract upon renal function and structure were studied in dogs.

2. After 24 hours of hypercalcemia induced by parathyroid extract, glomerular filtration rate was usually but not always decreased.

3. Renal concentrating capacity, as measured by the maximum solute concentration of urine during dehydration and by T^cH_2O during mannitol diuresis, was severely impaired. Dogs treated with parathyroid extract excreted a hypotonic urine during moderate mannitol diuresis despite the infusion of large amounts of exogenous vasopressin.

4. These changes were associated with morphological alterations demonstrated by microdissection to lie chiefly in the distal portion of the nephron and the collecting ducts.

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