JCI The Journal of Clinical Investigation

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J Clin Invest. 1964;43(6):1073-1082. https://doi.org/10.1172/JCI104991.

Research Article

Journal of Clinical Investigation
Vol. 43, No. 6, 1964

Relation between Urinary Hydroxyproline and Parathyroid Function *

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With the exception of small amounts in elastin, all the hydroxyproline (OHPr) in the body is found in collagen. Evidence has been presented that the urinary peptide-bound OHPr reflects the metabolism of collagen (2–5) except when the dietary intake of gelatin or collagen is excessive. Since a major body depot of collagen is the matrix of bone, it might be anticipated that under some conditions the rate of OHPr excretion would furnish an index of the turnover of bone matrix.

Parathyroid hormone is known to increase bone resorption (6–11). Thus, changes in parathyroid function might be expected to be associated with changes in OHPr excretion. Recently, Klein and Curtiss (12) reported that urinary OHPr first decreased and then increased in rats given parathyroid extract (PTE). Bates, McGowen, and Talmage noted that PTE increased plasma peptide-bound OHPr in rats (13). An elevated urinary excretion of OHPr has been reported in patients with hyperparathyroidism (14–16). The present studies were carried out to examine further the relationship of parathyroid activity to OHPr excretion.

Methods

Three different types of experiments were done.

1) Administration of parathyroid extract. Aqueous PTE [1] was administered to one normal 23-year-old woman and to three women (ages, 40 to 47 years) with surgically induced hypoparathyroidism, who had been treated with dihydrotachysterol (M.W.E. and L.M.) or vitamin D. (F.R.M.). Treatment was discontinued 11 to 32 days before starting the study. These four subjects were studied on a complete balance regimen and received constant diets containing at most 120 g of lean, red meat daily, but no fish, Jello, ice cream, or other gelatin-rich foods. PTE was given intramuscularly at a dosage of 600 U a day for 10 to 12 days. Urine was collected in 24-hour pools under toluene and stored in a cold room at 4° C. Feces were collected in 4- or 6-day pools.

2) Four-hour calcium infusion. Calcium glucoheptonate was given to a man (T.W., age, 46 years) and a woman (A.M.S., age, 40 years) with surgically induced hypoparathyroidism, to four normal men (ages, 20 to 42 years), and to five normal women (ages, 18 to 54 years). A.M.S. had been treated with vitamin D and T.W. had not been treated. All these subjects were fed a constant, liquid diet to give a daily intake of 350 mg of calcium (Ca), 980 mg of phosphorus (P), and less than 10 mg of OHPr in the form of protein. The diet was given in five equal feedings daily for 4 days. On day 3 of the diet, Ca, at a dosage of 15 mg per kg, as glucoheptonate in 0.9% sodium chloride solution, was administered intravenously by constant infusion over a 4-hour period starting at 9:00 a.m. Urine was collected in 4-hour samples for the determination of OHPr, P, and creatinine. Blood for determination of Ca, P, creatinine, and OHPr was drawn at appropriate times.

3) Prolonged calcium infusions. Calcium glucoheptonate was given by continued iv infusion to three patients with hypoparathyroidism for 4 consecutive days while they were on a balance regimen similar to that used in the first group of experiments. The dosage of calcium averaged 7.5, 12.5, and 22.0 mg per kg per day in the three subjects, respectively. The 8 days before and after the infusion served as control periods. Blood was analyzed daily for Ca and P, and urine was collected daily for the determination of P, OHPr, and creatinine.

Chemical assays. Samples of diets, urine, and feces were analyzed for P by the method of Fiske and Subba-Row (17), for total nitrogen by the method of Kjeldahl (18), and for Ca by flame photometry; samples of urine were analyzed for creatinine (19). Total OHPr was determined in hydrolyzed samples of urine and homogenized feces by the method of Prockop and Udenfriend (20). At varying levels of OHPr excretion, less than 3% of the total urinary OHPr was found to be the free amino acid; the remainder is presumed to be peptide-bound. Analysis of samples of fecal homogenate by the method

* Submitted for publication November 29, 1963; accepted January 23, 1964.

Presented in part before the Southern Section of the American Federation for Clinical Research, January 1963 (1).

[1] Eli Lilly & Co., Indianapolis, Ind.

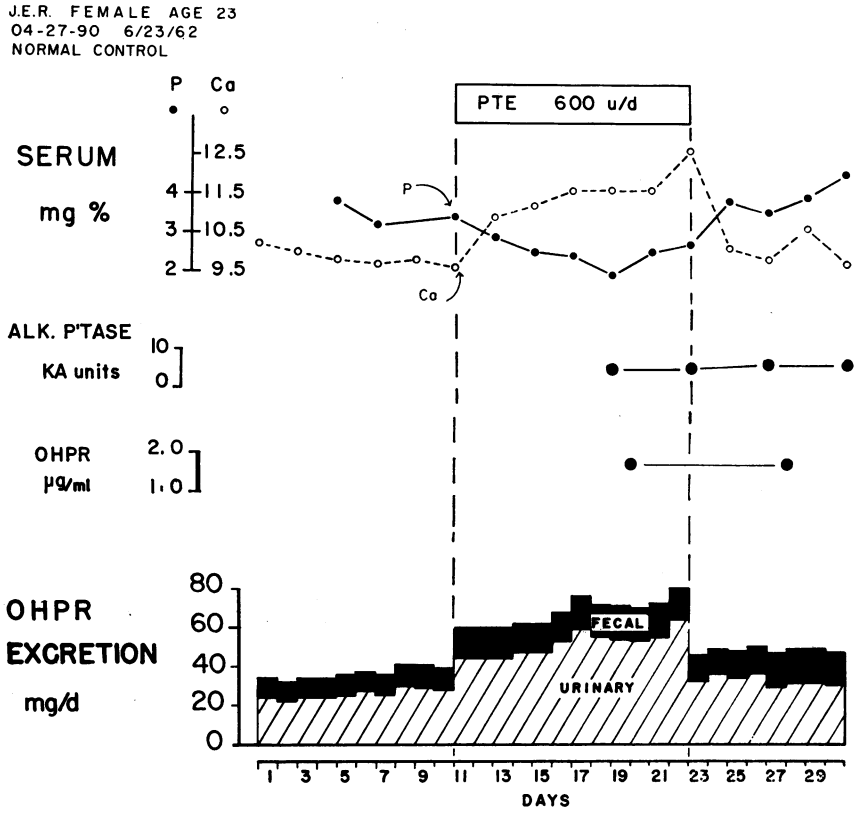

FIG. 1. EFFECT OF PARATHYROID EXTRACT (PTE) ON SERUM CALCIUM, PHOSPHORUS, ALKALINE PHOSPHATASE, AND HYDROXYPROLINE AND URINARY AND FECAL HYDROXYPROLINE (OHPR) IN A NORMAL WOMAN.

of Hamilton and Ortiz (21) produced comparable results. OHPr was measured in dialyzates of serum or plasma (22). The quantity of plasma peptide-bound OHPr was taken as the difference between the total OHPr in an acid-hydrolyzed sample and the free OHPr in an unhydrolyzed sample. The validity of this procedure has been established (23).

Results

Effects of PTE. The effect of PTE in a normal subject is shown in Figure 1 and Table I. Serum Ca rose from 9.6 to 12.6 mg per 100 ml, and serum P fell from 3.4 to 1.9 mg per 100 ml. From a mean control value of 29 mg per day, urinary OHPr rose to 46 mg per day on the first day of treatment with PTE (an increase of 67%) and continued to rise, reaching a peak on day 7. It was 66 mg per day (an increase of 140%) on the last day of treatment. OHPr excretion decreased promptly toward control values on the first day after PTE was discontinued. The con-

TABLE I

Effect of parathyroid extract (PTE) on urinary hydroxyproline

Patient	Mean Control ± SEM*	Mean PTE ± SEM	Mean post control ± SEM
	mg/day	*mg/day*	*mg/day*
	Normal		
J.E.R.	29 ± 1 (6)†	54 ± 2 (12)	34 ± 1 (8)
	Hypoparathyroidism		
M.E.	19 ± 1 (8)	34 ± 2 (12)	26 ± 1 (12)
F.R.M.	16 ± 1 (8)	21 ± 1 (12)	15 ± 1 (12)
L.M.	36 ± 1 (6)	66 ± 2 (10)	32 ± 1 (8)

* SEM = standard error of the mean.
† The figures in parentheses are the number of observations.

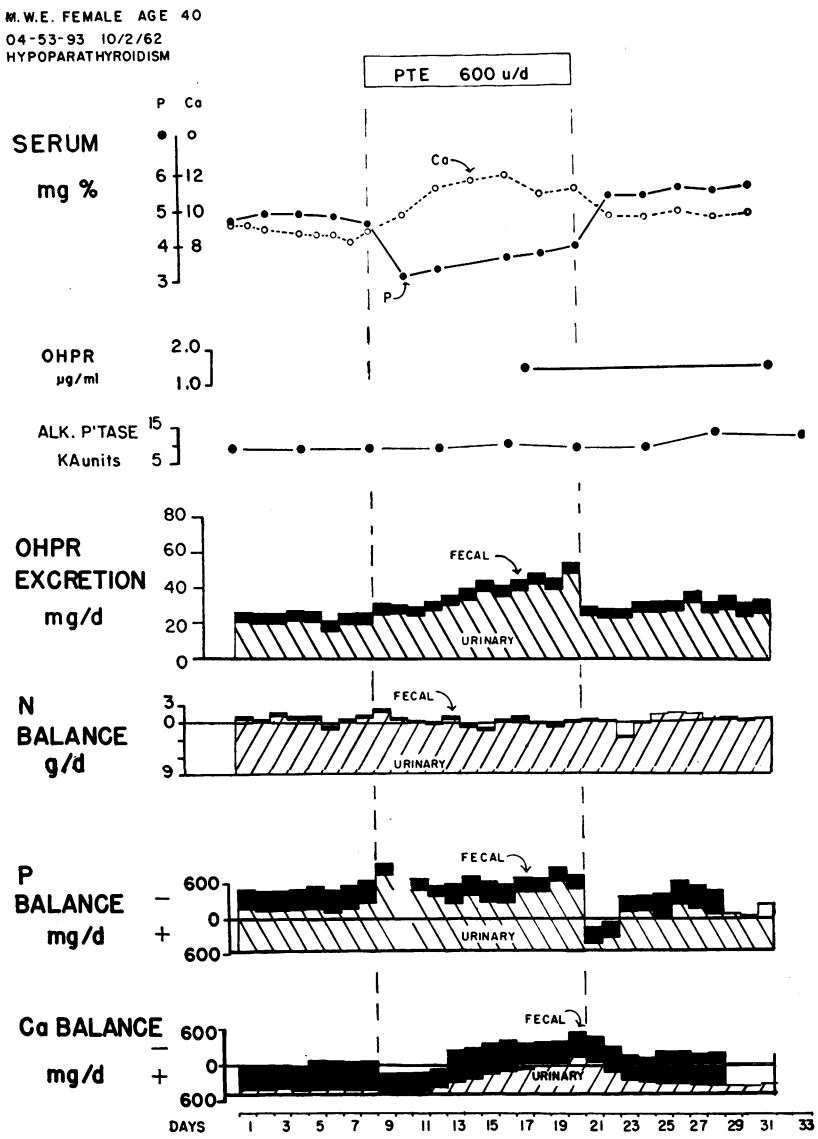

FIG. 2. EFFECT OF PARATHYROID EXTRACT ON SERUM CALCIUM, PHOSPHORUS, ALKALINE PHOSPHATASE, AND HYDROXYPROLINE, FECAL HYDROXYPROLINE AND URINARY HYDROXYPROLINE, AND NITROGEN, PHOSPHORUS, AND CALCIUM BALANCE IN A 40-YEAR-OLD WOMAN WITH HYPOPARATHYROIDISM.

centrations of serum alkaline phosphatase and of plasma peptide-bound OHPr were the same during treatment as 5 days after treatment.

The effect of PTE in a patient with hypoparathyroidism is shown in Figure 2. Serum Ca rose from 8.5 to 11.9 mg per 100 ml, and serum P fell from 4.8 to 3.1 mg per 100 ml. From a mean control value of 19 mg per day, urinary OHPr rose to reach a peak value of 49 mg per day (an increase of 152%) on the last day of treatment and returned to control values within 1 day after treatment was stopped. Serum alkaline phosphatase did not change, and plasma OHPr concentration during treatment was the same as that 11 days after treatment. Nitrogen balance, slightly negative throughout the study, was unaffected by PTE. Urinary P and Ca increased from control values of 700 and 60 mg per day, respectively, to reach values of 1,275 and 618 mg per day, respectively, with negative Ca and P bal-

ance persisting until treatment with PTE was stopped.

The changes in urinary OHPr, P, and Ca with PTE are summarized in Figure 3 and Table I. In all four subjects, urinary OHPr rose on the first day of PTE and remained elevated throughout the treatment period. The mean control values ranged from 16 (F.R.M.) to 36 (L.M.) mg per day; with treatment the mean values were higher and ranged from 21 to 66 mg per day. The values on the first day of treatment ranged from 127 to 187% of control values, and the peak values ranged from 142 to 228% of control values. There was a rapid decrease in urinary OHPr towards control levels after PTE was stopped. Urinary P increased on the first day of PTE in all subjects. In the normal subject, J.E.R., urinary Ca rose slowly when PTE was given and fell slowly to control levels when it was stopped. In the patients with hypoparathyroidism,

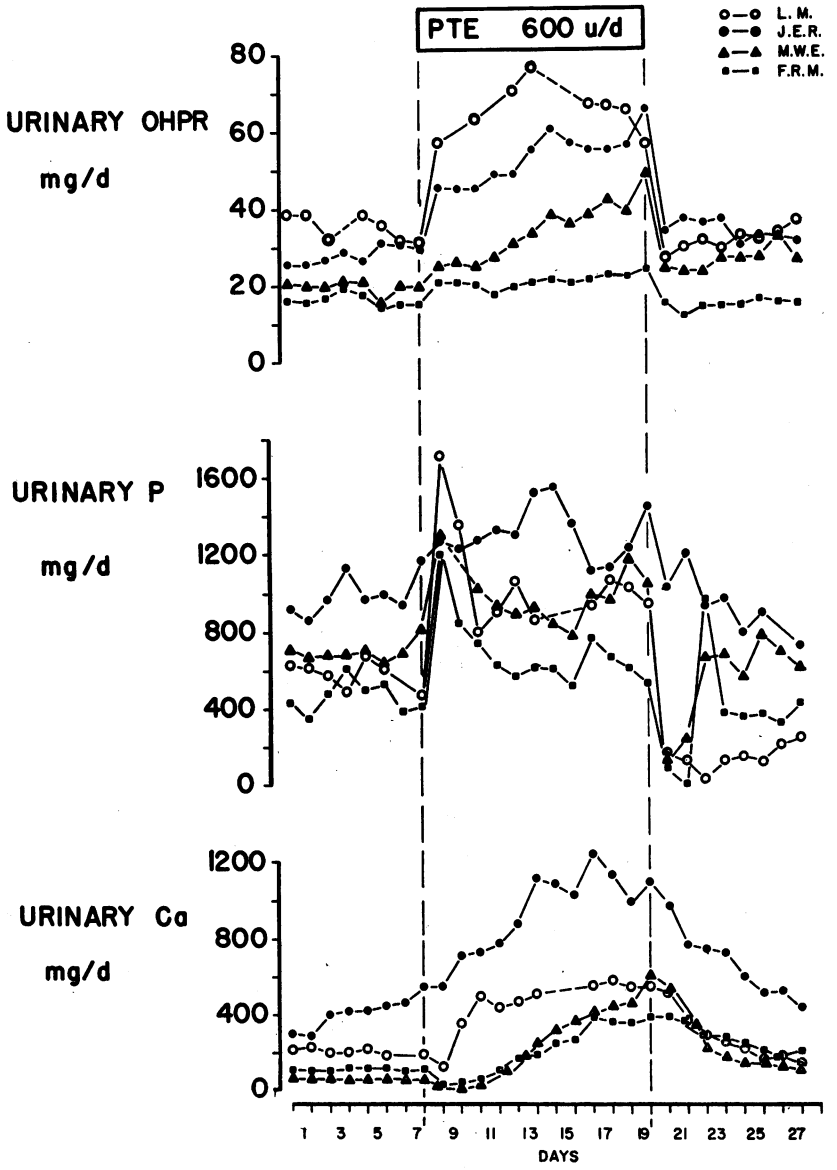

FIG. 3. SUMMARY OF THE EFFECTS OF PARATHYROID EXTRACT ON URINARY HYDROXYPROLINE, PHOSPHORUS, AND CALCIUM IN A NORMAL SUBJECT (J.E.R.) AND IN THREE PATIENTS WITH HYPOPARATHYROIDISM.

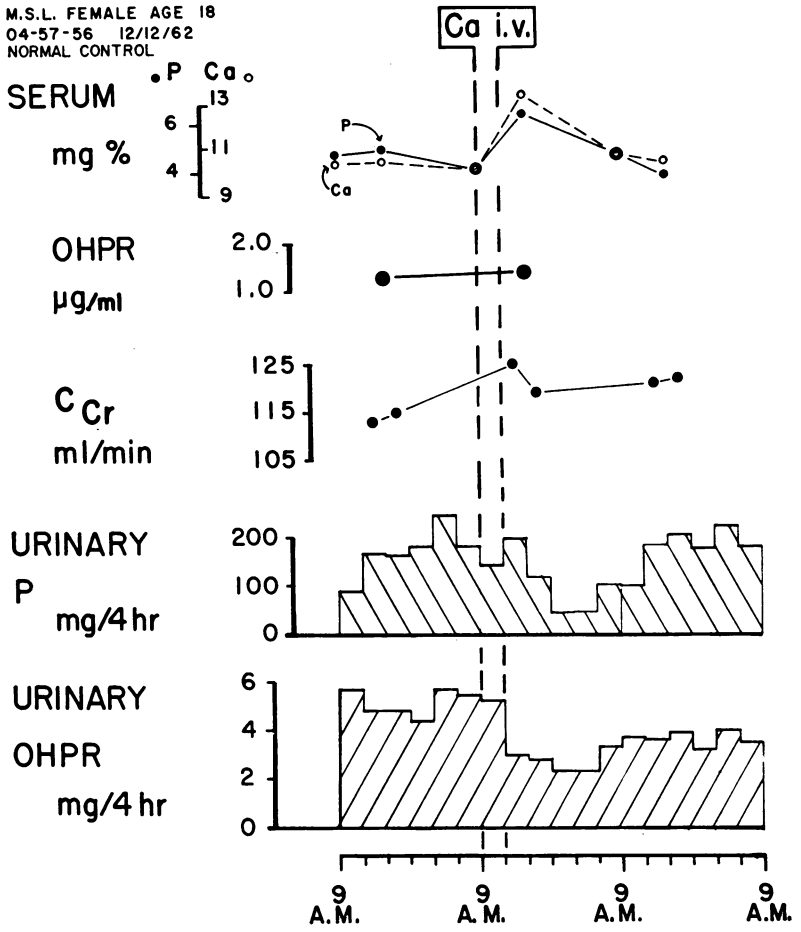

FIG. 4. EFFECT OF INFUSION OF CALCIUM ON SERUM CALCIUM, PHOSPHORUS, AND HYDROXYPROLINE, CREATININE CLEARANCE, AND URINARY PHOSPHORUS AND HYDROXYPROLINE IN A NORMAL WOMAN.

urinary Ca fell when PTE was first given, rose again to control levels within 1 to 4 days, and then rose still higher and remained elevated until PTE was stopped, whereupon it declined slowly towards control levels.

Effects of 4-hour calcium infusions. The rapid decrease in urinary OHPr when treatment with PTE was stopped suggested that significant changes in urinary OHPr might also be observed when endogenous secretion of parathyroid hormone was suppressed by infusion of calcium (24). A typical response of a normal subject to calcium infusion is shown in detail in Figure 4. Urinary OHPr decreased in the 4-hour period immediately following the infusion and remained low for the next 12 hours. A return towards normal levels was evident within 24 hours after the infusion. These changes were not closely correlated in time with the changes in urinary P. They occurred without apparent change in plasma OHPr and in the presence of a rising clearance of creatinine (C_{Cr}).

Pertinent data in nine normal subjects are summarized in Table II. The percentages of changes in the excretion of OHPr and of P from the control day in six of the subjects are plotted for the day of the infusion and the day following infusion in Figure 5. On the day of infusion OHPr excretion decreased by 23 to 43% (mean change, 37%). On the day after infusion urinary OHPr was higher than on the day of infusion in five of the six subjects, but was still less than control values by 6 to 46%. On the day of infusion, phosphorus excretion decreased by 11 to 48%

(mean change, 34%). On the day after infusion, urinary P varied from 72 to 146% of control values. Thus, all six normal subjects showed the classical decrease in urinary P excretion on the day of infusion, but only three had the previously reported rebound in P excretion on the following day (25). Mean control OHPr was 38 mg per day with a range of 24 to 55 mg per day. Mean value on the day of infusion was 25 mg per day with a range of 17 to 32 mg per day.

Changes in OHPr excretion with calcium infusion do not appear to result from the calcium load per se, since a 4-hour infusion of Ca in two patients with hypoparathyroidism raised serum Ca and increased P excretion but did not significantly alter urinary OHPr (Figure 6 and Table II).

Effects of prolonged calcium infusions. A typical response of a patient with hypoparathyroidism to prolonged calcium infusion is shown in Figure 7, and the results for three patients are shown in Table III. The infusion did not decrease urinary OHPr in any of the three patients.

Discussion

The present findings clearly demonstrate a relationship between parathyroid activity and colla-

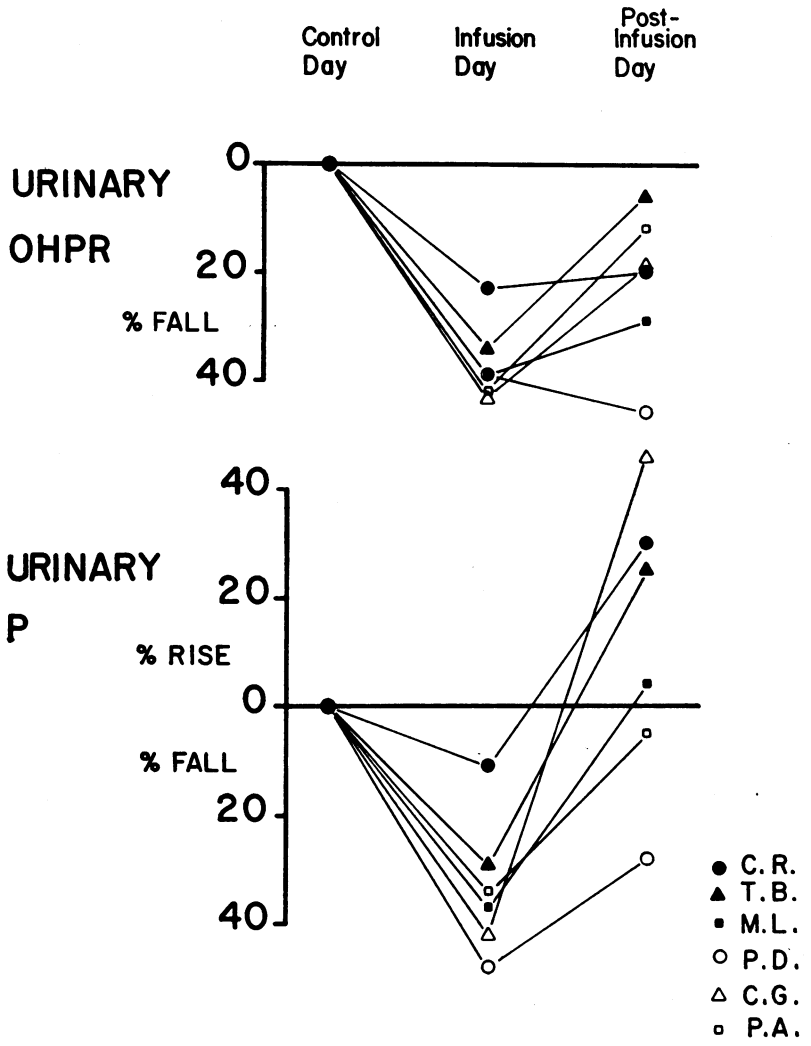

FIG. 5. PERCENTAGES OF CHANGES FROM CONTROL VALUES IN THE 24-HOUR EXCRETION OF HYDROXYPROLINE AND PHOSPHORUS PRODUCED BY THE IV INFUSION OF CALCIUM.

TABLE II

Effect of 4-hour calcium infusion on urinary hydroxyproline

Patient	Control day	Day of calcium infusion	Post control day
	mg/day	*mg/day*	*mg/day*
	Normal		
C.R.	33	26	28
T.B.	39	26	37
M.S.L.	31	19	22
P.D.	50	30	27
C.G.	45	26	37
P.A.	55	32	48
V.D.	38	31	36
M.L.	24	19	21
J.S.	26	17	25
Mean ± SEM	38 ± 3	25 ± 2	31 ± 3
	Hypoparathyroidism		
A.S.	11	10	11
T.W.	15	16	15

gen metabolism, as indicated by urinary OHPr. (Plasma OHPr did not show any apparent changes, but the sensitivity of the method is not great enough to preclude small changes paralleling the urinary ones.) All subjects who received PTE showed a prompt increase in urinary OHPr when treatment was begun and a rapid decrease when it was stopped. The converse was observed when calcium was infused in normal subjects. Since the infusion of Ca did not decrease OHPr (or P) excretion in patients with hypoparathyroidism, we can reasonably conclude that the effect of calcium infusion on OHPr is an indirect one, depending on suppression of parathyroid activity.

About 35 to 40% of total body collagen is found in bone matrix (26), which in turn constitutes about 30% of the dry weight of bone (27). Osteoclasts may play a role in the process whereby

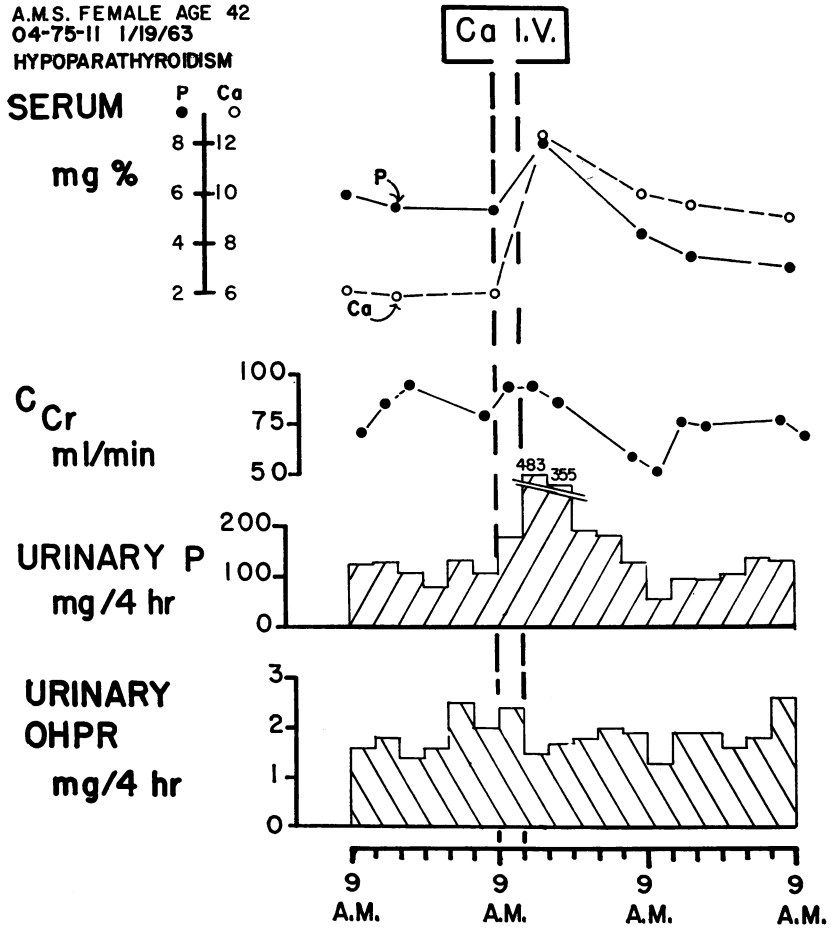

FIG. 6. EFFECT OF INFUSION OF CALCIUM ON SERUM CALCIUM AND PHOSPHORUS, CREATININE CLEARANCE, AND URINARY PHOSPHORUS AND HYDROXYPROLINE IN A PATIENT WITH HYPOPARATHYROIDISM.

TABLE III

Effect of 4-day calcium infusion on urinary hydroxyproline in hypoparathyroidism

Patient	Mean control ±SEM	Mean calcium infusion ±SEM	Mean post control ±SEM
	mg/day	*mg/day*	*mg/day*
A.S.	21 ± 1 (4)*	20 ± 3 (3)	18 ± 1 (4)
F.R.M.	16 ± 1 (8)	20 ± 1 (4)	16 ± 1 (4)
L.M.	32 ± 1 (8)	33 ± 2 (4)	30 ± 1 (8)

* The figures in the parentheses are the number of observations.

parathyroids induce breakdown of bone (28, 29); the increase in OHPr excretion presumably results from this breakdown of bone and of insoluble bone collagen. It is not clear, however, whether the collagen of organic matrix is altered directly, or after the salts have been removed. Bollet, Handy, and Parson (30) found that treatment of guinea pigs with PTE decreased the calcium content of bone without altering the collagen content; accordingly, they favor the latter alternative. In our own experiments, OHPr increased before urinary calcium (Figure 3), but this may merely reflect decreased renal clearance of calcium produced by PTE (31). Similarly, the prompt rise in urinary P probably resulted from a direct effect on renal tubules.

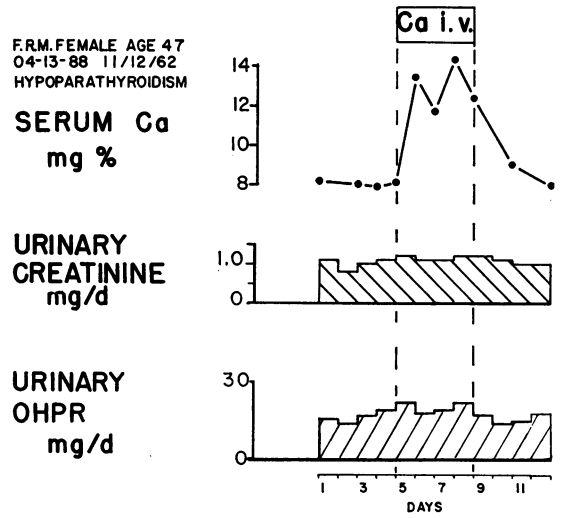

FIG. 7. EFFECTS OF A 4-DAY INFUSION OF CALCIUM ON SERUM CALCIUM AND URINARY CREATININE AND HYDROXYPROLINE IN A PATIENT WITH HYPOPARATHYROIDISM.

There is good evidence that under some circumstances elevated urinary OHPr is related to an increased pool of so-called soluble collagen. This form of collagen is considered to be the precursor of the more inert, insoluble, fibrous collagen. The increased excretion of urinary OHPr observed in experimental lathyrism, in growing children, in patients with acromegaly, and in subjects treated with growth hormone is presumably derived from soluble collagen (2, 4, 5). This may also account for the reported increases of urinary OHPr in other conditions such as rickets (32) and Paget's disease (16), in which bone collagen formation is excessive. To what extent destruction of bone matrix contributes to urinary OHPr in these conditions is difficult to judge. Growth hormone regularly increases calcium excretion and thus may induce destruction as well as increased formation of bone collagen (33, 34). Contrariwise, a direct effect of PTE on over-all collagen synthesis cannot be excluded. Whereas Johnston, Deiss, and Miner (35) found that PTE decreased the incorporation of C^{14}-proline into collagen *in vitro,* there is no doubt that bone formation proceeds rapidly in the osteitis fibrosa cystica of hyperparathyroidism (36). Preliminary reports (14–16) suggest that elevations of urinary OHPr occur in patients with hyperparathyroidism in whom alkaline phosphatase is elevated. In the present work, alkaline phosphatase did not rise with PTE. It was normal (8 King-Armstrong U) in a patient whom we have studied recently who had a parathyroid adenoma and normal bones by X-ray but whose urinary OHPr was significantly elevated (49 mg per 24 hours). Accordingly, if parathyroid hormone increases urinary OHPr by stimulating collagen synthesis, it can do so at doses that do not induce appreciable bone disease as estimated from the alkaline phosphatase. Klein (37) has recently reported that calcium infusion did not lower urinary OHPr in three patients with parathyroid adenomas. In our patient with a parthyroid adenoma, calcium infusion did not lower urinary OHPr.

It is clear that changes in parathyroid activity produce prompt parallel changes in urinary OHPr, presumably through effects on bone. Thus, urinary OHPr may provide a means of studying the

effects of the parathyroids on the bones, independent of their effects on the renal tubules. Indeed, OHPr metabolism may prove to be useful for the study of bone metabolism in general.

Summary

In normal subjects and in patients with hypoparathyroidism, parathyroid extract produced prompt increases in the urinary excretion of hydroxyproline (OHPr); prompt decreases were seen when treatment was stopped. The changes in urinary hydroxyproline preceded those of urinary calcium but not those of urinary phosphorus.

Infusion of calcium produced rapid decreases in urinary hydroxyproline in normal subjects but not in patients with hypoparathyroidism. The decreases appeared within 6 hours and on occasion preceded the decreases in urinary phosphorus. Urinary OHPr, but not urinary phosphorus, was generally below control levels on the day following infusion of calcium.

The metabolism of body collagen, probably that of bone in particular, changes rapidly as parathyroid activity is altered; further evaluation of urinary hydroxyproline as an index of bone metabolism is suggested.

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