

# The Clinical and Metabolic Effects of Porcine Calcitonin on Paget's Disease of Bone

FLORENCE SHAI, RICHARD K. BAKER, and STANLEY WALLACH

*From the Department of Medicine and the U. S. Public Health Service Clinical Research Center, State University of New York, Downstate Medical Center, Brooklyn, New York 11203*

**ABSTRACT** The clinical and metabolic effects of porcine calcitonin were assessed in six patients with Paget's disease and two patients with osteoporosis under metabolic balance conditions. The administration of calcitonin for 4-17 wk resulted in an amelioration of the clinical phenomena associated with Paget's disease, including bone pain, increased skeletal vascularity, congestive heart failure, and neurologic deficits secondary to skeletal impingement. The major metabolic effects of calcitonin in Paget's disease included the induction of positive calcium balance of +50 to +240 mg/day, reduction in hyperphosphatasia and hydroxyprolinuria of 15 to 60%, and a deceleration of radiocalcium turnover by 12 to 46%. Natriuresis, phosphaturia, and reduced urinary calcium excretion were observed, whereas sustained hypocalcemia and hypophosphatemia did not occur. The administration of porcine calcitonin was not associated with adverse objective or subjective reactions, toxic effects, or allergic phenomena. There was no evidence of antibody formation or loss of therapeutic potency. Although the response of individual patients with Paget's disease varied widely, the data indicate that calcitonin, presumably through its skeletal anti-resorptive action, is able to reduce skeletal turnover and volume in Paget's disease, and thereby improve the associated clinical and metabolic abnormalities. Long term therapeutic studies in progress suggest that prolonged periods of control of the generalized condition may be feasible.

In osteoporosis, neither clinical improvement nor

consistent metabolic changes indicative of amelioration of the skeletal disease were observed.

## INTRODUCTION

Paget's disease of the skeleton is associated with extreme acceleration of the rates of bone formation and resorption. The resorption process is generally regarded to be a primary consequence of the disease whereas the increased rate of bone formation is considered a secondary event (1). The increased skeletal turnover results in microscopic and gross distortions of bone architecture, and increased vascularity of the skeleton. Important clinical consequences include bone pain and deformity, pathologic fractures, neurologic deficits, and congestive heart failure. Calcitonin, the polypeptide hormone of ultimobranchial and thyroid parafollicular cell origin, is a powerful *in vivo* and *in vitro* inhibitor of bone resorption (2). The present metabolic and clinical study was undertaken to ascertain whether calcitonin is capable of inhibiting the pathologic bone resorption of generalized Paget's disease.

## METHODS

Six patients with generalized Paget's disease, ranging in age from 55 to 75 yr, were admitted to the hospital for study during treatment with porcine calcitonin. Four of the patients had not received previous therapy for bone disease. One patient (C. E. L.) received sodium fluoride in the distant past and phosphate therapy for 2 yr until 2 wk before admission to the study. One patient (A. V.) received sodium fluoride for 5 wk but discontinued the drug 3 wk before admission. For comparative purposes, two women, ages 56 and 80 yr with postmenopausal and senile osteoporosis, respectively, were studied under identical conditions. One patient (A. D.) had received 2 mg of diethylstilbestrol daily and 100 mg of testosterone cypionate weekly for 3 yr without clinical benefit; these drugs were continued during study. The second patient (J. M.) discontinued daily use of sodium fluoride (20 mg) and Premarin, Ayerst Laboratories, N. Y., (1.25 mg) 6 wk before study.

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Dr. Shai is currently an Adjunct in Medicine at the Jewish Hospital and Medical Center of Brooklyn, Brooklyn, New York. Dr. Wallach is Career Scientist, Health Research Council of the City of New York.

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Metabolic balance determinations were performed according to the principles outlined by Reifenshtein, Albright, and Wells (3) and Furman, Howard, Wells, and MacAulay (4). To achieve conditions as ideal as possible, drugs which might interfere with study, such as diuretics and hormones were discontinued except as noted. Other medications deemed necessary for the well-being of the patients were supplied from a single manufacturer's lot and were administered in constant doses throughout study.

All patients except A. V. received constant metabolic balance-diets. A. V. was given a constant calcium and low hydroxyproline diet. The ranges of daily dietary intake were 440–670 mg of calcium, 980–1150 mg of phosphorus, 12–20 mEq of magnesium, 40–85 mEq of potassium, 25–75 mEq of sodium, and 9–12 g of total nitrogen. 5–10 dietary portions, the rare dietary rejections, and all medications were analyzed chemically during each patient's study. The daily intakes were calculated as the averages of the analytic values for the dietary portions and the administered medications minus those for the dietary rejections. The metabolic balance-studies began with a 3 day period of adjustment followed by 7–14 days of equilibration to the constant diet. After equilibration, 2 or 3 wk of control observations were followed by 4–8 wk of calcitonin administration. In one osteoporotic patient (J. M.), a 2 wk postcontrol period followed the 4 wk calcitonin period. In most patients, the gelatin vehicle was administered during the last control week. Urinary excretion of inorganic constituents, total nitrogen, and total hydroxyproline were determined in 7-day pooled specimens. Quantitative fecal excretion of the same constituents were determined in 7-day pooled specimens corresponding to the urine pools.

Lyophilized porcine calcitonin (Armour Pharmaceutical Co., Chicago, Ill. AL 0831 H.P.), a partially purified extract of porcine thyroid glands containing 25–35% calcitonin, was reconstituted in 16% gelatin and given intramuscularly in three equal daily doses at 8-hr intervals. 2 MRC<sup>1</sup> (Medical Research Council) U/kg per day was administered during the first wk, 4 MRC U/kg per day during the 2nd wk, and 8 MRC U/kg per day thereafter. After completion of the inpatient study four patients with Paget's disease were given single daily intramuscular or subcutaneous injections of 200 MRC U. One patient (M. B.) was readmitted after 8 wk and restudied under metabolic balance conditions while receiving the daily 200 MRC U dose. A pagetic patient (A. W.) who did not continue calcitonin was restudied 9 months later during the administration of a daily subcutaneous dose of 200 MRC U. The two osteoporotic patients did not continue calcitonin after completion of the inpatient study.

Fasting blood specimens, taken at the beginning of each collection period, were analyzed for their content of serum calcium, magnesium, inorganic phosphorus, creatinine, alkaline phosphatase, and electrolytes. The following additional tests were performed weekly: blood count, sedimentation rate, platelet count, prothrombin time, urinalysis, urine culture, 24 hr urine protein, fasting blood sugar, blood urea nitrogen, serum protein-bound iodine, bilirubin, uric acid, cholesterol, total protein, albumin and serum protein electrophoresis, glutamic oxalacetic transaminase, glutamic pyruvic transaminase, creatinine phosphokinase, anti-thyroglobulin precipitins, Coombs' test, anti-nuclear antibody, and electrocardiogram. Dr. Alan Josephson periodically determined the level of serum complement (C<sub>3</sub> component) by

<sup>1</sup> Abbreviations used in this paper: MRC, Medical Research Council units; KAU, King-Armstrong units.

radial immunodiffusion (5) and screened sera for precipitin antibodies to the injected calcitonin by the agar diffusion-plate method of Ouchterlony (6). Neurologic evaluation, audiometry, slit lamp examination of the eyes, and skeletal X-ray studies were performed before and at the completion of study. Percutaneous biopsies of bone (iliac crest) were performed by Dr. Bernard Gardner with a Nordin needle during the initial equilibration period and after the metabolic balance studies were completed.

Dietary portions and rejections, medications and fecal pools were prepared for analysis by homogenation in water and dry-ashed in a muffle furnace. The ash was redissolved in dilute HCl. Sodium, potassium, chloride, carbon dioxide, inorganic phosphorus, creatinine, and alkaline phosphatase were determined by modified Technicon AutoAnalyzer (Technicon Instruments Corporation, N. Y.) methods to yield data to  $\pm 1\%$  precision and accuracy. The method for serum alkaline phosphatase-activity utilized phenyl-disodium phosphate as substrate and was expressed as King-Armstrong units (KAU). Calcium and magnesium were determined by an automated adaptation of standard atomic absorption spectrophotometry in a Perkin-Elmer model 303 spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.) to a precision and accuracy of  $\pm 1\%$ . Total nitrogen was determined by an automated Kjeldahl micro method and total urinary hydroxyproline by an automated modification of the method of Bergman and Loxley (7, 8), to a precision and accuracy of  $\pm 2\%$ . Individual analysis of the constant diets agreed within  $\pm 5\%$  of average values.

Radiocalcium turnover studies were performed during the control period and during the 4th to 7th wk of calcitonin therapy. 50  $\mu$ Ci of <sup>45</sup>Ca (Cambridge Nuclear Corp., Cambridge, Mass.) was injected intravenously under basal conditions between 8:00 and 9:00 a.m. During the following 12 hr, multiple heparinized-blood samples were obtained; blood samples were taken daily thereafter for 7 days. The <sup>45</sup>Ca content of the plasma specimens, daily urine specimens, 7-day fecal pools, and of appropriately diluted standards prepared from the injected material were measured at constant geometry in a gamma scintillation spectrometer. The isotopic data were expressed as the per cent of the total dose administered per liter of sample, and specific activities as the per cent dose per milliequivalents of calcium. Specific activity-curves were derived solely from plasma values. The data were subjected to digital computer analysis utilizing the four-compartment open model shown in Fig. 10. Fractional-transport coefficients among the four compartments, compartment sizes, and total-flux rates were determined by the computer analysis.

## RESULTS

*General.* All patients, although severely affected by their disease (Table I), tolerated the study without incident. No adverse objective or subjective reactions developed as a result of calcitonin administration and no allergic reactions were noted. Pain was a prominent feature in five pagetic patients, but in only three (A. W., M. B., and F. J. C.) was the pain clearly due to pagetic bone involvement. One patient (C. E. L.) had marked motor and sensory neurologic changes and causalgia could not be ruled out as the cause for lower extremity pain. The fifth patient (P. K.) had severe venous insufficiency of the lower extremities and bilateral acetabu-

TABLE I  
Clinical Data of Patients with Paget's Disease and Osteoporosis

Patient	Age, sex	Duration of disease	*Serum Ca	*Serum phos.	*Serum Alk. ptase. KAU	*Urinary total hydroxyproline	Clinical findings			
							Bone pain	Bone deformity	Abnormal neurologic	Heart failure
		yr	mg/100 ml		KAU	mg/24 hr				
Paget's disease										
A. W. I	63, M	19	9.3	3.8	119	662	+	+		+
A. W. II	64, M	20	9.8	3.8	131	586	+	+		+
M. B.	62, M	7	9.5	3.3	46	186	+		+	
A. V.	75, F	7	9.6	3.9	85	249		+	+	
C. E. L.	55, M	19	9.0	4.2	55	347	+		+	+
F. J. C.	62, M	7	9.5	3.3	47	192		+	+	
P. K.	60, M	5	10.3	3.4	53	166	+	+		
Osteoporosis										
A. D.	56, F	3	8.9	2.6	6.6	19	+			
J. M.	80, F	2	9.3	3.0	6.6	26	+			

\* Normal ranges: Serum Ca: 8.8–10.6 mg per 100 ml; Serum phos: 3.0–4.5 mg per 100 ml; Serum alk. ptase.: < 16 KAU; Urinary total hydroxyproline: < 46 mg/24 hr.

lar protrusion; his pain appeared related to these problems rather than to pagetic bone involvement. A. W., M. B., and F. J. C. showed significant relief of bone pain within 2 wk of the initiation of calcitonin and this sequence was repeated in A. W. during the second course of treatment. Two patients (A. W. and C. E. L.) had congestive heart failure with edema formation. While receiving constant daily doses of maintenance digoxin, A. W. manifested marked diuresis and return of cardiac compensation during calcitonin, losing 5.8 kg and 5.1 kg during two courses. C. E. L. received maintenance doses of digoxin and hydrochlorothiazide throughout study. He lost 2.2 kg during the 1st 3 days of calcitonin. The

diuresis was not sustained, however, and no further loss of edema occurred. The pagetic tibial deformity present in A. W. was most marked on the left where a 5–6°F increase in surface temperature was recorded by comparison to the right tibia. During calcitonin, a gradual equalization of tibial surface temperature occurred. The bone deformities present in three other patients were minimal and not accompanied by a demonstrable increase in skin temperature.

*Neurologic.* All six pagetic patients had audiometric abnormalities with greatest impairment in the higher frequencies. Improvement in the air conduction component of greater than 15 decibels was noted in three

TABLE II  
Pure Tone Audiogram Data—Air Conduction Component

Patient	Time of test	Frequency									
		250	500	750	1000	1500	2000	3000	4000	6000	8000
<i>cycles per second</i>											
A. W., II,	Before calcitonin	65	70	65	70		85			105	
	After 6 wk	40	55	60	65		85			85	
M. B., right ear	Before calcitonin	40	45	45	40	50	55	55	60	85	>110
	After 17 wk	25	25		30		40	45	45	65	>110
	After 29 wk	20	30		30		40	40	50	70	85
M. B., left ear	Before calcitonin	35	35	35	30	40	45	50	45	75	>110
	After 17 weeks	35	35		30		45	50	50	70	85
	After 29 weeks	25	25		25		40	50	55	70	70
C. E. L., right ear	Before calcitonin	55	45		30	50		70	75	85	85
	After 6 weeks	40	30		35	55		85	80	>110	>110

Values represent decibels required for detection. A change of 15 decibels is considered significant.

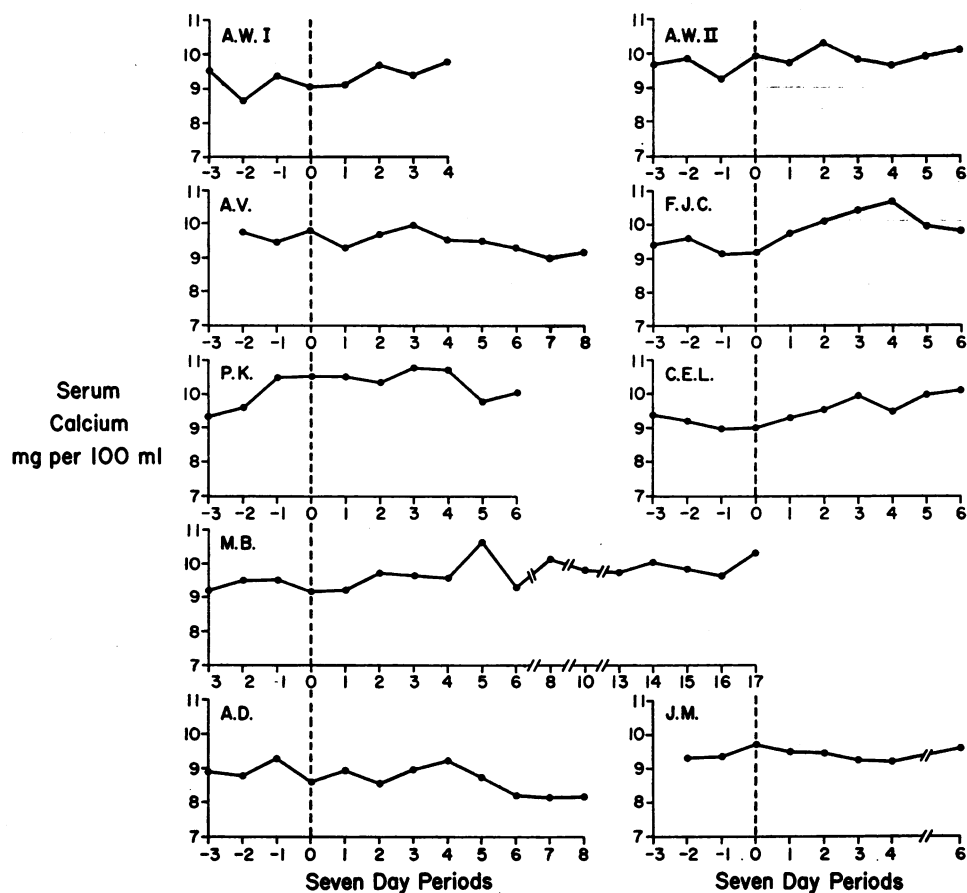


FIGURE 1 Weekly fasting serum-calcium levels in six patients with Paget's disease (A. W., A. V., F. J. C., P. K., C. E. L., and M. B.) and two patients with osteoporosis (A. D. and J. M.) before and during calcitonin. The vertical broken line indicates the initiation of calcitonin. In J. M., calcitonin was discontinued after 4 wk. The serum calcium increased to the upper limit of normal in three pagetic patients. A. D. was the only patient who developed sustained hypocalcemia during calcitonin.

patients (A. W., M. B., and C. E. L.) after 6 to 17 wk of calcitonin (Table II). In C. E. L., improvement in the lower frequencies occurred as the higher frequencies became worse. Three pagetic patients had no significant changes in the audiograms. No patient showed any change in bone conduction.

Four patients had severe impingement upon the thoracic spinal cord or corda equina and resultant motor weakness and sensory changes in the lower extremities. In two of the patients with thoracic cord involvement (M. B. and A. V.), muscle atrophy was absent and marked improvement in muscle power and in the sensory deficits was evident by the 4th wk of calcitonin. After 6 wk of calcitonin, both patients left the hospital without the aid of the cane and walker which they had required at admission. One (F. J. C.) of the two patients with a corda equina lesion, sensory deficits, and severe muscle atrophy, showed a small but significant

improvement in toe and ankle movements and a complete reversal of the sensory defect. The other patient (C. E. L.) did not improve neurologically. Three of the four patients with neurologic involvement also manifested subjective and objective decreases in skin temperature in the lower extremities before calcitonin and these abnormalities disappeared after 1 wk of calcitonin treatment. A detailed study of the response to calcitonin of these four patients and other pagetic patients with neurologic complications will be reported separately.

**Blood data.** In the patients with Paget's disease no tendency to fasting hypocalcemia was observed (Fig. 1) and, in three courses of therapy, the fasting serum calcium rose by 0.5 to 1.5 mg per 100 ml during calcitonin. The pattern of hourly serum calcium levels between 8 a.m. and 8 p.m. was studied before therapy and during the 5th wk of calcitonin, and no differences were noted. The fasting serum inorganic phosphorus levels showed

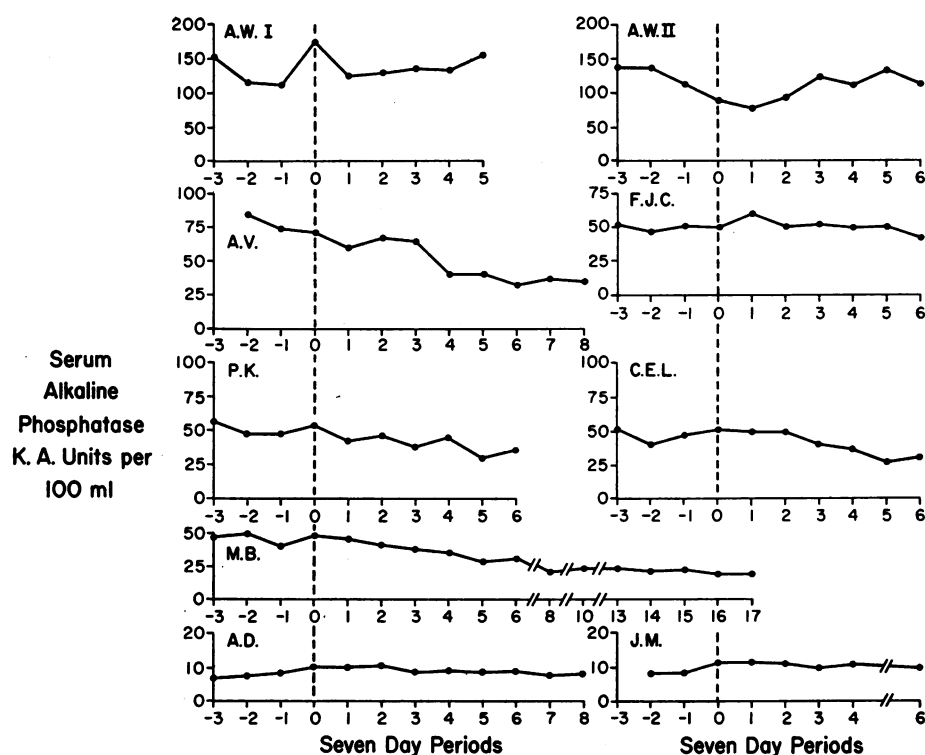


FIGURE 2 Weekly fasting serum alkaline phosphatase levels (King-Armstrong units) in the pagetic and osteoporotic patients. Format is the same as in Fig. 1. The upper limit of normal is 15 KAUs. The serum alkaline phosphatase decreased in four of the six pagetic patients during calcitonin.

no significant trend during calcitonin. The serum alkaline phosphatase activity varied from 40 to 150 KAUs before calcitonin. Decreases of 15–62% occurred in four pagetic patients during calcitonin, whereas no significant changes occurred in two patients (Fig. 2).

**Urinary hydroxyproline.** Urinary total hydroxyproline was increased 4- to 15-fold above the upper limit of normal (45 mg/24 hr) in all six patients with Paget's disease. The average control levels ranged from 168 to 619 mg/24 hr. Calcitonin administration caused a 15–55% reduction in urinary hydroxyproline (Figs. 3–8) but in no case was the normal range approached, even after 17 wk of calcitonin. In A. W., the first course of calcitonin (Fig. 3) caused a marked decrease in urinary hydroxyproline followed by a rebound to initial levels. During the second course at a reduced dose, a less precipitous decline and no tendency to rebound was observed.

**Urinary electrolytes.** Urinary electrolytes were measured in 7-day pools and the data represent mean weekly values. Before calcitonin, urinary calcium was slightly increased in four pagetic patients, ranging from 140 to 210 mg/day on dietary calcium intake of 450–650 mg/day. In two patients, urinary calcium ranged from 60

to 110 mg/day (Figs. 3–8). Urinary phosphorus, sodium, potassium, magnesium, and nitrogen were appropriate to the intake of elements. When calcitonin was administered, mean calcium excretion decreased by 20 to 100 mg/day in six of the seven studies. In M. B., the decrease in urinary calcium was modest during the 1st–6 wk and became marked after 15 wk of treatment (Fig. 4). Mean phosphorus excretion increased by 20 to 140 mg/day in every pagetic patient during the 1st wk of calcitonin (Figs. 3–8). Subsequently, urinary phosphorus excretion returned irregularly toward baseline levels. Marked natriuresis was observed in A. W. on two separate occasions, with increases of 16–33 mEq/day. Transient natriuresis of 4–10 mEq/day occurred in the other pagetic patients, usually during the 1st wk of calcitonin. Transient increase in potassium excretion of 4–7 mEq/day was observed in four studies during the 1st wk of calcitonin. Urinary magnesium and total nitrogen excretion were not influenced by calcitonin.

**Metabolic balance (Figs. 3–7).** Calcium balance was initially negative (–60 to –280 mg/day) in all six patients. In three studies (A. W. I., A. W. II., and M. B.), sustained reversal of the negative calcium balance occurred during calcitonin, and calcium balance be-

came positive (+ 50 to + 240 mg/day). In a fourth study (C. E. L.), calcium balance became transiently positive (+ 114 mg/day) but then reverted to negative. In all four instances of sustained or transient positive balance, net gastrointestinal absorption of calcium increased by 130–310 mg/day. In two patients, calcitonin had no influence on calcium balance despite decrease in urinary calcium of 20–100 mg/day.

Base line phosphorus balance was variable in the pagetic group, varying from – 300 to + 300 mg/day. In the three studies in which a sustained increase in calcium balance occurred, phosphorus balance followed calcium balance, becoming more positive or less negative by approximately 150 mg/day. The improvement in phosphorus balance during calcitonin occurred despite an initial phosphaturia, and was due primarily to

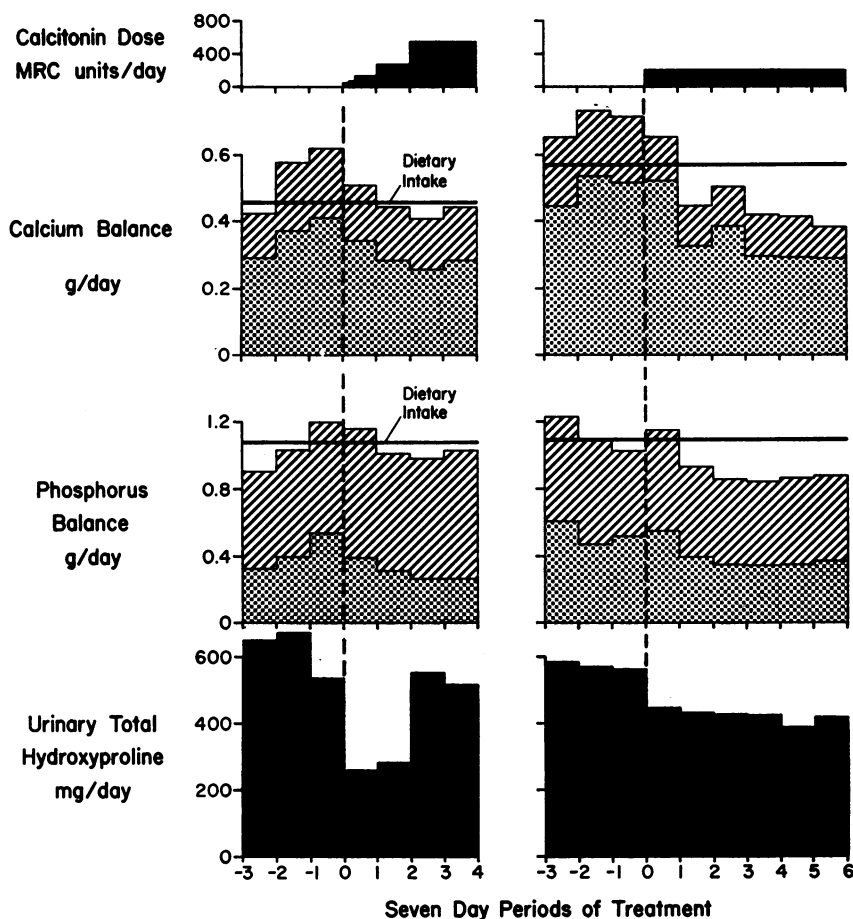


FIGURE 3 Calcium and phosphorus balance and urinary total hydroxyproline excretion in pagetic patient (A. W., 63 M, Paget's disease) before and during two separate courses of calcitonin (I and II). Fecal excretion (stippled area) is plotted upward from the base line and urinary excretion (crosshatched area) is plotted above fecal excretion. A clear space below the dietary intake line indicates positive balance and an extension of the stippled or crosshatched area above the intake line indicates negative balance. The vertical broken line indicates initiation of calcitonin. A. W. I. received chlorthiazide until 2 wk before the first period of balance (– 3 period), accounting for the low urinary calcium and slight positive balance during – 3 base line period. Calcium balance then reverted to negative, a condition the patient was noted to have during previous studies before chlorthiazide. Note the conversion of negative calcium and phosphorus balance to positive and the reduction in urinary hydroxyproline during two separate courses of calcitonin at different dose levels. The first course of calcitonin was administered intramuscularly and the second course subcutaneously.

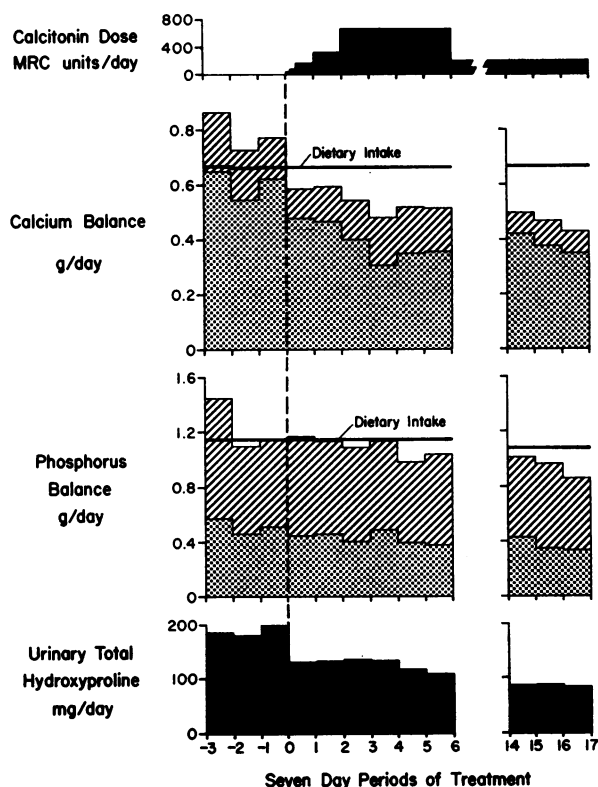


FIGURE 4 Calcium and phosphorus balance and urinary total hydroxyproline excretion in pagetic patient (M. B., 62 M, Paget's disease) before and during prolonged calcitonin administration. Format is the same as Fig. 3. After 6 wk of intramuscular calcitonin in three daily doses, the dose was decreased to a single intramuscular injection of 200 MRC U daily until the 15 wk of treatment when the same dose was continued subcutaneously. Note conversion of negative calcium balance to positive, production of positive phosphorus balance, and a progressive reduction in urinary hydroxyproline during calcitonin.

improved gastrointestinal absorption of phosphorus. In three pagetic patients, phosphorus balance was not influenced by calcitonin.

Except for the natriuresis and consequent negative sodium balance in A. W., sodium balance was not significantly affected by calcitonin administration. Potassium, magnesium, and nitrogen balance were similarly unaltered.

**Radiocalcium turnover (Fig. 9).** Radiocalcium turnover, indicated by the decline in the plasma calcium-specific activity curves, was markedly accelerated in all six pagetic patients. Calcitonin reduced the rate of decline of the plasma calcium specific activity in five patients although an accelerated turnover was still evident after 4–16 wk. The turnover data were subjected to quantitation utilizing a digital computer program and a four-compartment open model (Fig. 10). Compartment

$Q_{1E}$  corresponds to the extracellular calcium pool but in normal individuals it is slightly larger than chemical estimates of the extracellular calcium, suggesting an additional component consisting of a small, rapidly exchanging tissue calcium pool. Pool  $Q_s$  is visualized as a summated intracellular calcium pool. Pools  $Q_s$  and  $Q_i$  are the exchangeable and nonexchangeable bone calcium pools, respectively. The sum of  $Q_{1E}$ ,  $Q_s$ , and  $Q_i$  ( $\Sigma Q$ ) corresponds to the exchangeable body calcium pool of other investigators and rarely exceeds 375 mEq in normal individuals studied for 7 days (9–11). The precise normal ranges in this laboratory for  $Q_{1E}$ ,  $Q_s$ , and  $Q_i$  have not been defined because of the small number of control individuals studied, but liberal upper

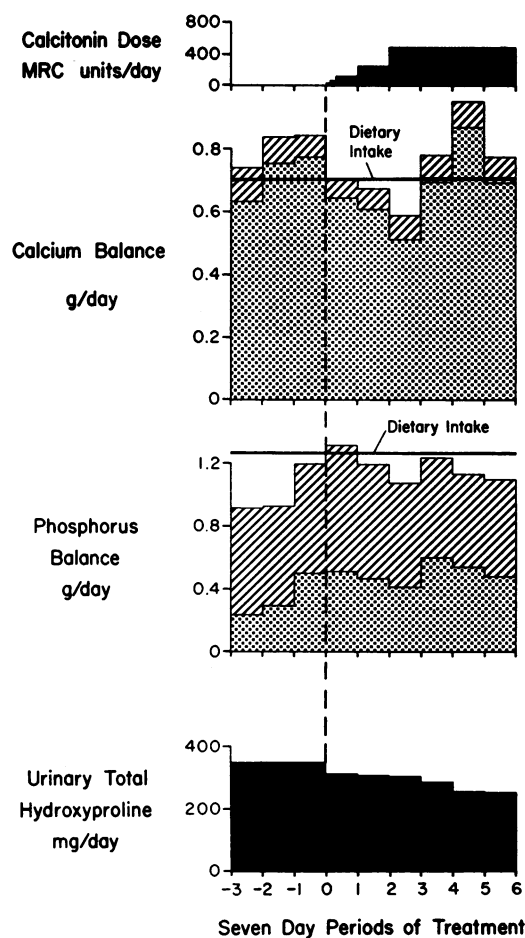


FIGURE 5 Calcium and phosphorus balance and urinary total hydroxyproline excretion in pagetic patient (C. E. L., 55 M, Paget's disease) before and during calcitonin administration. Format is the same as Fig. 3. Negative calcium balance became positive during the first 3 wk of calcitonin, but then reverted to negative. Phosphorus balance was not influenced by calcitonin. Urinary hydroxyproline declined throughout the period of calcitonin administration.

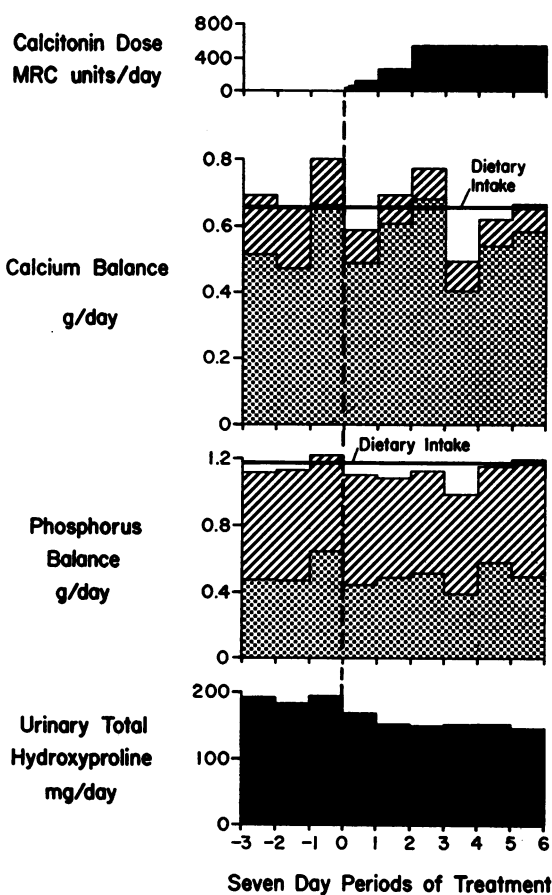


FIGURE 6 Calcium and phosphorus balance and urinary total hydroxyproline excretion in pagetic patient (F. J. C., 62 M, Paget's disease) before and during calcitonin administration. Format is the same as Fig. 3. Because of irregular fecal calcium excretion, calcium balance fluctuated independently of calcitonin. Phosphorus balance was uninfluenced by calcitonin whereas urinary hydroxyproline decreased.

limits can be set at 80 mEq, 100 mEq, and 130 mEq, respectively. As shown in Table III, the sum of the exchangeable pools ( $\Sigma Q$ ) was enlarged by 300–1800 mEq in the six pagetic patients and although all the pools participated, the greatest increase was found in  $Q_2$ . During calcitonin administration,  $\Sigma Q$  decreased by 12–46% (mean decrease 30%), primarily due to a diminution in  $Q_2$  although  $Q_{12}$  and  $Q_3$  also decreased.

The calcium flux parameter  $F_{34}$  corresponds to the bone accretion rate ( $V_{0+}$  or A) of other investigators (9–11) and rarely exceeds 1.5 mEq/hr.  $F_{34}$  was increased by 3.6 to 19.5 mEq/hr in the pagetic patients and decreased by 7 to 36% (mean decrease 21%) during calcitonin. Liberal upper limits for  $F_{12}$  (transport into "cells") and  $F_{13}$  (transport into the exchangeable bone pool) are 50 and 10 mEq/hr, respectively.  $F_{12}$  and

$F_{13}$  were also increased in untreated Paget's disease but the effects of calcitonin on these parameters were variable and inconclusive.

**Other Tests.** Iliac crest bone biopsy-samples processed by standard histologic techniques failed to show consistent changes during 4–16 wk of treatment. There was also no discernible influence of calcitonin on the roentgenographic appearance of the involved bones. Other tests cited under methods were unchanged from control values throughout study.

**Osteoporotic patients (Figs. 1, 2, 9, 11, 12).** Patient A. D. developed persistent hypocalcemia during calcitonin, achieving levels of 8.0 mg per 100 ml. The fasting serum calcium was unchanged in J. M. The serum phosphorus, alkaline phosphatase activity, and urinary total hydroxyproline were unaltered by calcitonin. The rate of decline of the plasma calcium-specific activity after

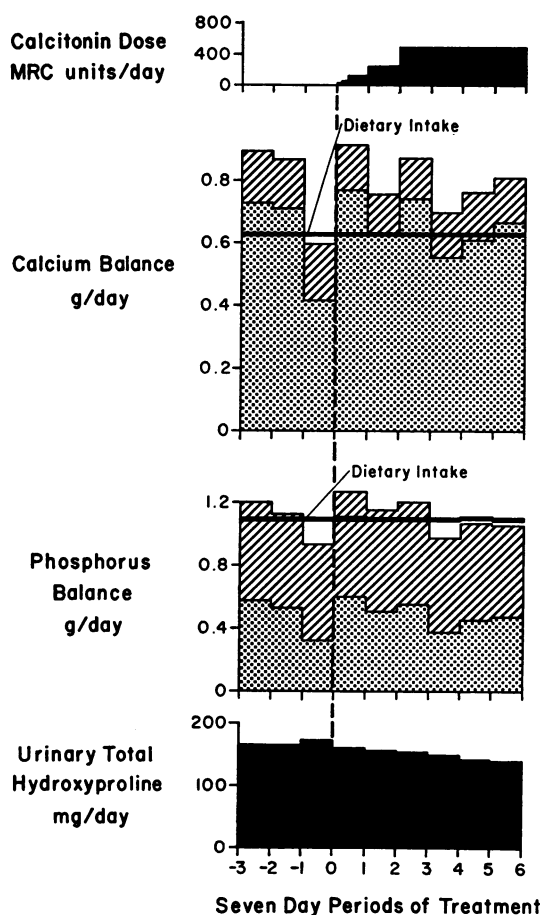


FIGURE 7 Calcium and phosphorus balance and urinary total hydroxyproline excretion in pagetic patient P. K. before and during calcitonin administration. Format is the same as Fig. 3. Calcium and phosphorus balance were unchanged by calcitonin but a slight decrease in urinary hydroxyproline occurred.



radiocalcium injection was slow in both patients compared to the standard normal range defined by Heaney et al. (12). In A. D., calcitonin had minimal effects on the plasma calcium specific activity and on the digital computer-derived parameters of radiocalcium turnover (Table III).

In A. D., calcium balance (Fig. 11) was normal but became negative by 100 mg/day during calcitonin. Nitrogen balance also became negative by 3 g/day. In J. M., a slight tendency to positive calcium balance occurred during calcitonin, and calcium balance became distinctly positive, by 200 mg/day, after calcitonin was stopped abruptly (Fig. 12). Phosphorus balance became slightly less positive during calcitonin in both patients. Sodium, potassium, and magnesium balances were not significantly altered. Iliac crest bone biopsies, skeletal X-rays, and subjective assessments of back and cervical pain were unchanged.

## DISCUSSION

The studies performed provide clinical and metabolic evidence which supports the ability of calcitonin to reverse the skeletal manifestations of Paget's disease. Skeletal pain, bone vascularity, congestive heart failure, and neurologic impairment were variably but dramatically improved. Of the clinical effects, only the pain relief was subjective since the improvement in other clinical parameters was documented by objective changes in physical findings. Prolonged relief of pain was observed in these patients and in a series of 20 additional patients treated for several months on an outpatient basis, suggesting that pain relief is a real rather than a psychic result of calcitonin treatment.

Predominant resorption of bone, indicated by negative calcium balance, is frequently present when pagetic patients come to clinical attention. Reversal of negative calcium balance and a reduction in hydroxyprolinuria can be related directly to the anti-resorptive function of calcitonin. Although the turnover of  $^{45}\text{Ca}$  and the level of serum alkaline phosphatase are generally thought to reflect the rate of bone formation, these parameters were also favorably affected by calcitonin. The improvement in these two parameters is probably due to concomitant decrease in bone formation consequent to primary inhibition of bone resorption.

Bell, Avery, and Johnston (13) have reported the induction of positive or less negative calcium balance of 85–150 mg/day during daily intramuscular administration of 4–8 MRC U/kg calcitonin to patients with Paget's disease. In their report as well as in the present study, both urinary and fecal calcium decreased. On the other hand, Haddad, Birge, and Avioli (14) observed small increases in urinary calcium during the

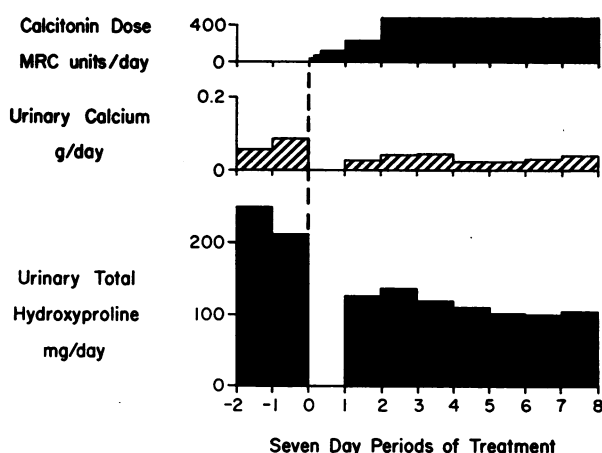


FIGURE 8 Urinary calcium and total hydroxyproline excretion in pagetic patient (A. V., 75 F, Paget's disease). The patient was unable to cooperate in metabolic balance studies because of advanced age and neurologic infirmity. The calcium and hydroxyproline content of the diet was fixed but the diet was otherwise uncontrolled. Urinary creatinine was measured daily. Each week, the two to four 24-hr urines with adequate creatinine levels were selected for analysis. The data represent averages of these two to four excretory values except during the 1st wk of calcitonin when urinary losses occurred every day. Note the decrease in urinary calcium and hydroxyproline during calcitonin administration.

daily administration of 400–1500 MRC U for a longer period, but no balance procedures were employed to assess gastrointestinal effects. In these previous studies, urinary hydroxyproline decreased during the 1st wk of calcitonin. The present data demonstrate that urinary hydroxyproline continues to decrease gradually as treatment proceeds. In studies of the immediate effects of intravenous calcitonin, Bijvoet, van der Sluys Veer, and Jansen (15, 16) showed decreases in hydroxyproline within 2 hr and, in the majority of the patients in the present report, reductions were manifest on the 1st day of calcitonin. The accumulated data also indicate that the level of serum alkaline phosphatase is a less sensitive index of response of Paget's disease to calcitonin than is urinary hydroxyproline since reductions are not evident in the early weeks of treatment. With prolonged treatment, however, the serum alkaline phosphatase almost invariably decreases. (Unpublished observations.)

The ability of calcitonin to improve net gastrointestinal absorption of calcium was noted in this series of pagetic patients and by Bell, Avery, and Johnston (13) despite previous animal studies indicating that the gastrointestinal tract is not essential for the hypocalcemic effect of calcitonin (17). The gastrointestinal effect of calcitonin may not be direct since several stud-

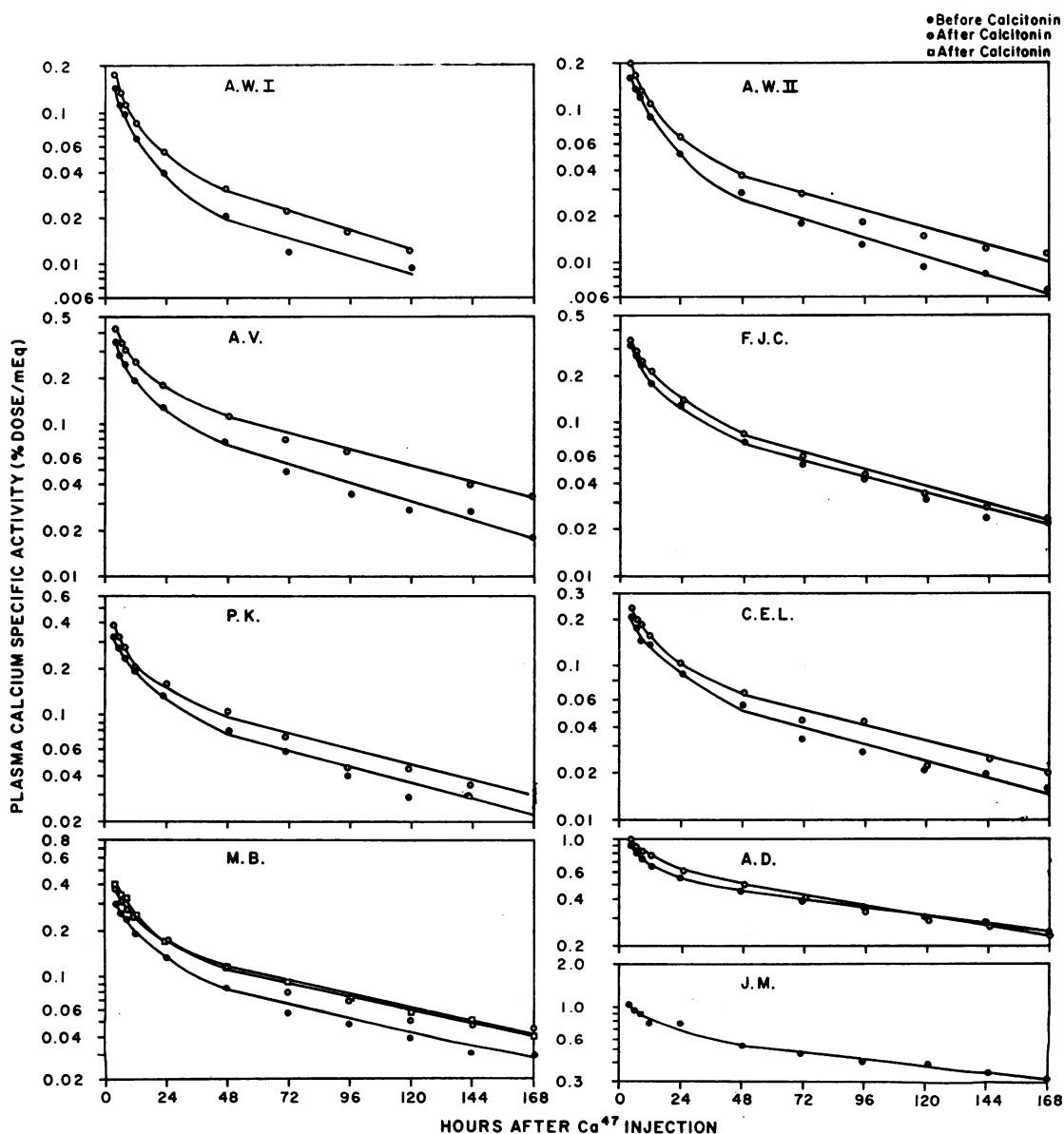


FIGURE 9 Pattern of plasma calcium specific-activity after radiocalcium injection in six pagetic patients (A. W., A. V., F. J. C., P. K., C. E. L., and M. B.) and two osteoporotic patients (A. D. and J. M.). Closed circles represent data before calcitonin and open circles after 4-16 wk of calcitonin. M. B. had studies during the 5th and 16th wk of calcitonin. In A. W. I., the plasma radioactivity was too low to be measured accurately after 5 days. J. M. was not restudied after calcitonin. Note the individual ordinate scales. Radiocalcium turnover was rapid in the pagetic patients and slow in the osteoporotic patients. A decrease in radiocalcium turnover during calcitonin in the pagetic group was evident 1-2 hr after radiocalcium injection.

ies have been presented in which influences believed to be directed at skeletal homeostasis have resulted in parallel but secondary effects on calcium absorption (18). The mechanism by which calcium absorption reacts to skeletal influence is unknown. Nicolayson, Eeg-

Larsen, and Malm have theorized on the existence of an "endogenous factor" capable of acting to couple the two organs (19). The enhanced absorption of calcium might have been due to increased parathyroid hormone secretion resulting from the hypocalcemic action of the

administered calcitonin but there is no positive evidence for this contention.

The phosphaturic effect of calcitonin observed in these studies has been described previously (13, 15, 16). The natriuresis observed in a florid form in one patient and to a minor extent in the remainder has also been noted by others (13, 15, 16) and probably represents a renal rather than a skeletal effect. Natriuresis usually occurred on the 1st day of calcitonin, before the skeletal effects of the hormone could result in reduced vascularity of pagetic bone and improved cardiovascular hemodynamics.

Not all the clinical and metabolic parameters measured were affected in every pagetic patient nor were the changes always quantitatively similar. The variable effect of calcitonin was probably related to the degree of physiologic and biochemical derangement relative to the activity of the administered hormone. Alternately, transient postcalcitonin hypocalcemia might have occurred with the production of secondary hyperparathyroidism. Such an event would be expected to limit

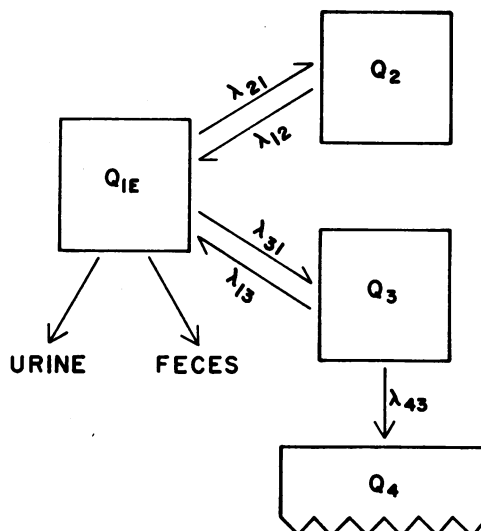


FIGURE 10 Four-compartment open model of calcium exchange utilized for digital computer quantitation of the plasma calcium-specific activity curves. See text for description of the model.

TABLE III  
Computer-Derived Radiocalcium Turnover Data in Patients with Paget's Disease and Osteoporosis

Patient		Compartment size				Calcium flux			Total radiocalcium excretion	
		$Q_{1E}$	$Q_2$	$Q_3$	$\Sigma Q$	$F_{12}$	$F_{13}$	$F_{34}$	Urine	Feces
		<i>mEq</i>				<i>mEq/hr</i>			<i>percent dose</i>	
Paget's disease										
A. W., I	(a)	131	231	1816	2178	142	41.3	21.0	1.8	1.1
	(b)	91.1	145	934	1170	165	44.1	15.9	1.9	0.7
A. W., II	(a)	133	208	1246	1587	124	33.7	16.5	2.5	1.7
	(b)	78.2	192	1015	1285	113	29.1	12.3	2.2	1.3
M. B.	(a)	125	124	550	799	42.2	12.2	5.1	5.4	5.4
	(b)	70.4	112	420	602	62.0	11.1	3.7	6.6	4.9
	(c)	73.0	96	349	518	69.7	12.2	3.7	3.6	6.0
A. V.	(a)	77.5	110	536	724	61.5	15.0	6.8	1.2	2.5
	(b)	67.8	84	344	496	49.2	13.1	4.5	1.5	2.2
C. E. L.	(a)	165	231	1079	1475	95.2	17.4	8.5	2.0	3.5
	(b)	113	185	647	945	168	19.8	7.5	2.2	5.2
F. J. C.	(a)	84.7	121	505	711	66.5	15.9	6.2	5.1	4.6
	(b)	70.4	113	441	624	64.7	14.9	5.8	2.6	4.2
P. K.	(a)	107	122	552	781	57.8	12.9	5.5	4.5	5.3
	(b)	94.4	74	389	557	73.6	15.7	4.8	5.6	6.6
Osteoporosis										
A. D.	(a)	55.7	27.6	78.6	162	35.8	5.5	0.41	16.1	14.6
	(b)	41.4	39.4	66.7	147	46.1	3.7	0.52	13.3	14.9
J. M.*	(a)	43.9	38.4	72.1	154	30.1	2.5	0.32	5.3	18.5

(a) Before calcitonin; (b) After 4-7 wk of calcitonin; (c) After 16 wk of calcitonin.

\* Repeat study not done.

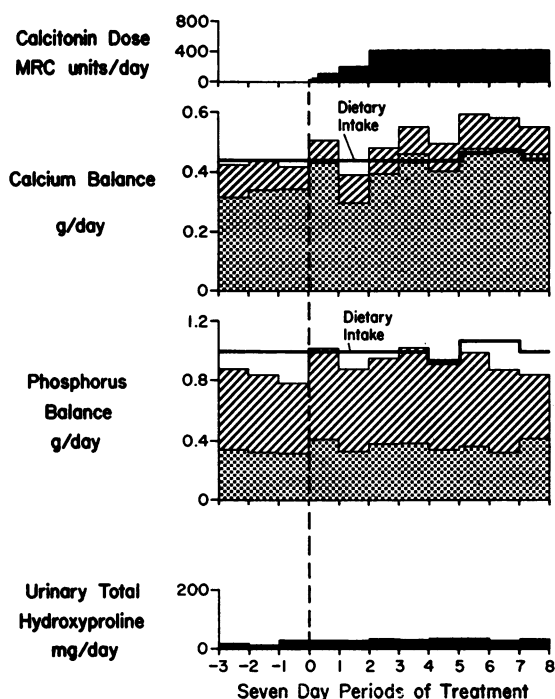


FIGURE 11 Calcium and phosphorus balance and urinary total hydroxyproline in osteoporotic patient (A. D., 56 F, postmenopausal osteoporosis.) Format is the same as Fig. 3. Calcitonin administration resulted in negative calcium and less positive phosphorus balance. The slight increase in urinary hydroxyproline during the last base line period (–1 period) before calcitonin is due to injections of the 16% gelatin vehicle.

the degree of fasting hypocalcemia, positive calcium balance, reduction in serum alkaline phosphatase-activity, urinary hydroxyproline excretion, and radiocalcium turnover induced by calcitonin. The tendency to develop slightly higher fasting serum calcium levels could also have resulted from secondary hyperparathyroidism. No measurements of circulating parathyroid hormone levels were made in these patients. However, elevation of circulating parathyroid hormone levels measured by radioimmunoassay have been found in pagetic patients given calcitonin (20). The additional observation in two pagetic patients that lower doses of calcitonin resulted in as good or better clinical and metabolic effects than the larger dose is also compatible with the production of secondary hyperparathyroidism as an undesirable side effect of high doses. A third possibility for the variable quantitative effect of calcitonin could relate to the “escape phenomenon” observed with calcitonin *in vitro* (21) and in patients with high circulating levels of calcitonin due to medullary carcinoma of the thyroid who do not manifest hypocalcemia (22).

The most dramatic clinical effects observed during calcitonin administration related to the neurologic sys-

tem. Spinal cord compression injuries usually affect the large motor fibers first and the sensory fibers later. Recovery occurs in reverse order. In the two patients with thoracic cord impingement but no muscle atrophy, neurologic improvement followed a similar course. Also, in one patient with a corda equina-type lesion and muscle atrophy, complete restoration of sensory modalities occurred despite a minimal return of muscular function. The improved air conduction component of audition, which occurred in three patients, suggests a calcitonin-related change in the pagetic state of the footplate of the stapes. Since most of these neurologic defects have their basis in increased skeletal volume with impingement, the observed reversal of neurologic phenomena probably required a reduction in skeletal volume. Although there is no supporting histologic data, it is tempting to speculate that calcitonin is not simply an inhibitor of bone resorption in Paget's disease. Because of the induced reduction in skeletal turnover, significant reductions in vascularity and in pathologic remodelling of the skele-

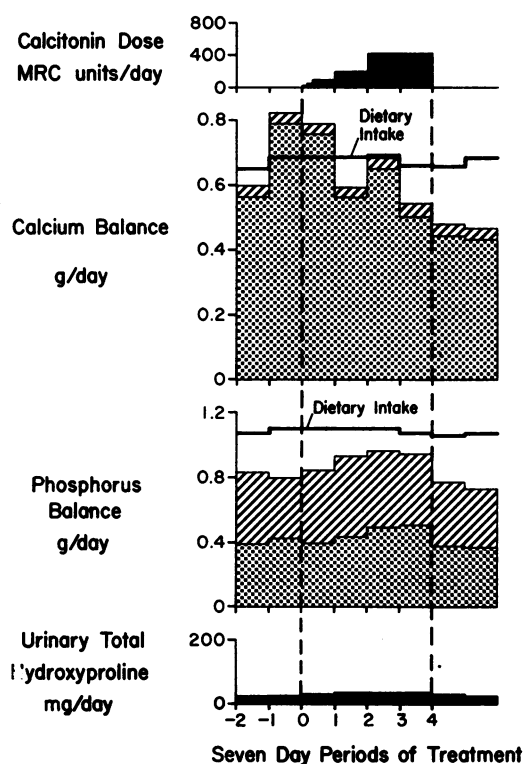


FIGURE 12 Calcium and phosphorus balance and urinary total hydroxyproline in osteoporotic patient (J. M., 80 F, senile osteoporosis) before, during, and after a 4 wk course of calcitonin. Format is the same as Fig. 3. Despite fluctuations in fecal calcium excretion, positive calcium balance is evident during and after calcitonin. Phosphorus balance became less positive during calcitonin and more positive after calcitonin was discontinued. The 16% gelatin vehicle was not administered before calcitonin.

ton occur, which act to reduce the abnormally increased skeletal volume typical of the disease. A minute reduction in skeletal volume in a strategic area, undetectable by X-ray, would suffice to reduce neurologic impairment.

In contrast to the observations in Paget's disease, the results of calcitonin administration in two patients with osteoporosis were not dramatic. Several possible reasons for the disappointing results may be cited. The radiocalcium turnover studies suggest that skeletal turnover was moderately slow in both patients and calcitonin may be relatively inactive in such situations (23). Second, one patient was on estrogen therapy throughout study whereas the other patient had recently discontinued estrogen. In rats, estradiol has been found capable of antagonizing the hypocalcemic action of calcitonin (24; F. Shai, and S. Wallach, manuscript in preparation), suggesting that a similar ability to inhibit calcitonin might occur in humans receiving estrogens. Lastly, the induction of secondary hyperparathyroidism might have completely overridden the positive effects of the calcitonin. Patient A. D. developed sustained hypocalcemia and subsequently developed negative calcium and nitrogen balance, findings which are similar to those observed in hyperparathyroidism (25). The observations in osteoporosis should not be taken as evidence of inefficacy of calcitonin. Lower doses of calcitonin combined with high calcium, magnesium, and/or phosphorus intake might forestall secondary hyperparathyroidism, stimulate bone formation, or enhance the endorgan action of the calcitonin.

The relatively negative data in osteoporosis further highlight the efficacy of calcitonin in ameliorating the clinical and metabolic manifestations of Paget's disease. In studies in progress, it has also been found that outpatient treatment with calcitonin by self-injection, using single daily subcutaneous doses of 100–200 MRC U is equally efficacious. Continuous treatment for more than 6 months has not caused allergic reactions, side effects, or evidence of antibody formation or loss of therapeutic potency. If long term control of this previously untreatable disease can be achieved with calcitonin, the possibility that early therapy might prevent the serious concomitants of the generalized condition becomes an intriguing prospect.

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*Note added in proof:* Since submission of this manuscript, approximately twenty patients have completed 6–12 months of treatment with porcine calcitonin at a single daily subcutaneous dose of 100–200 MRC U. All have maintained the clinical improvement achieved during the first 6 months of treatment. However, in over half the patients, the serum alkaline phosphatase activity, after decreasing during the first 6 months, has remained constant in the range 20–50 KAU. In addition, the urinary total hydroxyproline, after decreasing 50% or more during the first 6 months, has returned to or toward base line levels. Possible explanations include secondary hyperparathyroidism, neutralizing antibody production, and the "escape phenomenon" (21). These possibilities are being explored currently.

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