

The effect of age on serum immunoreactive parathyroid hormone in normal and osteoporotic women

J. C. GALLAGHER, B. LAWRENCE RIGGS, CHRISTINE M. JERBAK, and CLAUDE D. ARNAUD,* Rochester, Minn.

Serum iPTH was measured in a large series of normal and osteoporotic women as a function of age, with radioimmunoassays using three antisera: GP-1M, which recognizes primarily a region within the 44-68 amino acid sequence of PTH; CH-12M, which appears to recognize primarily intact hormone; and CH-14M, which recognizes primarily a region within the 1-34 amino acid sequence of PTH. In normal women 20 to 90 years of age, serum iPTH increased significantly with age ($p < 0.001$); the proportional increase was greater when measured with antiserum GP-1M (80%) than when measured with antiserum CH-12M (30%), which suggests that the increase in circulating carboxyl fragments of PTH was greater than the increase in circulating intact PTH. In 40 patients with postmenopausal osteoporosis, the mean value for serum iPTH assayed by antiserum GP-1M did not differ significantly from that for age-matched normal women; however, three osteoporotic patients had elevated values and thus appear to represent a separate population. When these subjects were excluded, mean serum iPTH assayed by antiserum GP-1M also was lower than normal ($p < 0.001$). The mean value for serum iPTH was lower in osteoporotic patients than in normal subjects when assayed by either antiserum CH-12M ($p < 0.001$) or antiserum CH-14M ($p < 0.001$). Values for serum phosphate and renal tubular phosphate resorption, both indices of PTH function, were increased ($p < 0.005$) in the osteoporotic subjects. Although creatinine clearance decreased with age in both normal and osteoporotic subjects, partial correlations with age held constant showed no relationship between creatinine clearance and serum iPTH. This suggests that a decrease in renal function was not the major factor accounting for the rise in serum iPTH with age. We conclude that serum iPTH increases with aging but that for any given age, it is either normal or low in patients with postmenopausal osteoporosis. (J LAB CLIN MED 95:373, 1980.)

Abbreviations: parathyroid hormone (PTH), immunoreactive PTH (iPTH), human PTH (hPTH), bovine PTH (bPTH), calcium-creatinine ratio (Ca/Cr), renal tubular reabsorption of phosphate ($T_{m_{PO_4}}/GFR$)

From the Endocrine Research Unit, Division of Endocrinology/Metabolism and Internal Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minn.

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Reprint requests: J. C. Gallagher, M.D., Creighton University School of Medicine, Omaha, Neb. 68178.

*Present address: Veterans Administration Medical Center and the University of California School of Medicine, San Francisco, Calif. 94121.

Because PTH is the principal regulator of bone resorption, increased PTH secretion could contribute to the pathogenesis of age-related bone loss and to osteoporosis. Although accurate knowledge of the state of parathyroid function is essential for any overall understanding of pathogenesis of osteoporosis and age-related bone loss, reported studies have given conflicting results. Roof et al.¹ found that between ages 20 and 90 years serum iPTH increased in women but decreased in men. Berlyne et al.² compared serum iPTH levels in 10 young adults (18 to 19 years old) and in 16 elderly subjects (65 to 87 years-old) and found a 10-fold increase in serum iPTH in the latter group. In women with postmenopausal osteoporosis, mean serum iPTH has been variously reported to be low (refs. 3 and 4 and D. Baylink, personal communication), normal,^{3, 5} or high.^{6, 7}

Several factors might contribute to these divergent results. First, serum iPTH is immunoheterogeneous and comprises both intact hormone and hormonal fragments.⁸⁻¹¹ Antisera reacting to different immunologic determinants of the PTH molecule might give different results, depending on the proportion of intact hormone and hormonal fragments in the circulation. Second, some of the radioimmunoassay systems used in these studies may not have been sufficiently sensitive to detect small differences in serum iPTH within the normal range. Third, in several of the reported studies, control subjects were not age-and sex-matched with the osteoporotic subjects.

With these problems in mind, we have systematically reevaluated serum iPTH as a function of age in normal and osteoporotic women by use of three sensitive radioimmunoassays of PTH having different immunologic specificities.

Methods

Patients. Seventy-eight normal Caucasian women between 20 and 90 years of age were studied. They were healthy, had no evidence of vertebral fractures on roentgenograms of the spinal column, and were not receiving drug therapy or taking vitamin supplements at the time of the study. Forty postmenopausal Caucasian women who were 52 to 69 years of age and had osteoporosis also were studied. They formed an unselected consecutive series of untreated patients with osteoporosis. All had one or more vertebral fractures that had developed either spontaneously or after minor incidents such as bending or coughing. The patients were ambulatory, were in good health except for osteoporosis, and had no recognizable disease or history of use of drugs known to produce osteoporosis. All control and osteoporotic subjects had had a spontaneous menopause. Iliac crest biopsies were obtained in all patients. These showed only osteoporosis, with no evidence of osteomalacia or any other metabolic bone disorder.

Laboratory studies. Serum samples for determination of calcium, phosphorus, and iPTH values were taken at 0800 after an overnight fast of 10 hr. All normal subjects and osteoporotic patients ate their usual diet on the preceding day and on the day of study. Serum calcium was measured by atomic absorption spectrophotometry. Plasma phosphate and creatinine were measured by standard AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) techniques. Glomerular filtration was assessed by 24 hr creatinine clearance, and each individual value was corrected to a constant surface area of 1.73 m². The amount of phosphate reabsorbed by the kidney was calculated from the plasma phosphate value, and the amount of excreted phosphate per 100 ml of glomerular filtrate was derived from a fasting 2 hr urine collection. Phosphate excretion was calculated from the nomogram derived by Walton and Bijvoet¹² and was expressed as Tm_{Po4}/GFR (mg/100 ml). Urine calcium was determined on the same 2 hr fasting urine collection and expressed as the urine Ca/Cr. This value has been shown to be relatively uninfluenced by absorbed dietary calcium and is an index of net bone resorption.¹³

Serum iPTH was measured by radioimmunoassay procedures described by Arnaud et al.¹⁴ and Flueck et al.¹⁵ Three antisera (GP-1M, CH-14M, and CH-12M) were used, each of which reacts with different determinants in the PTH molecule. This is shown in Fig. 1, in which the reactivities of highly purified hPTH(1-84) (batch CMh21 pool 7, isolated from pooled human parathyroid tumors in collaboration with Dr. Bryan Brewer, Molecular Disease Branch, NIH) and various synthetic frag-

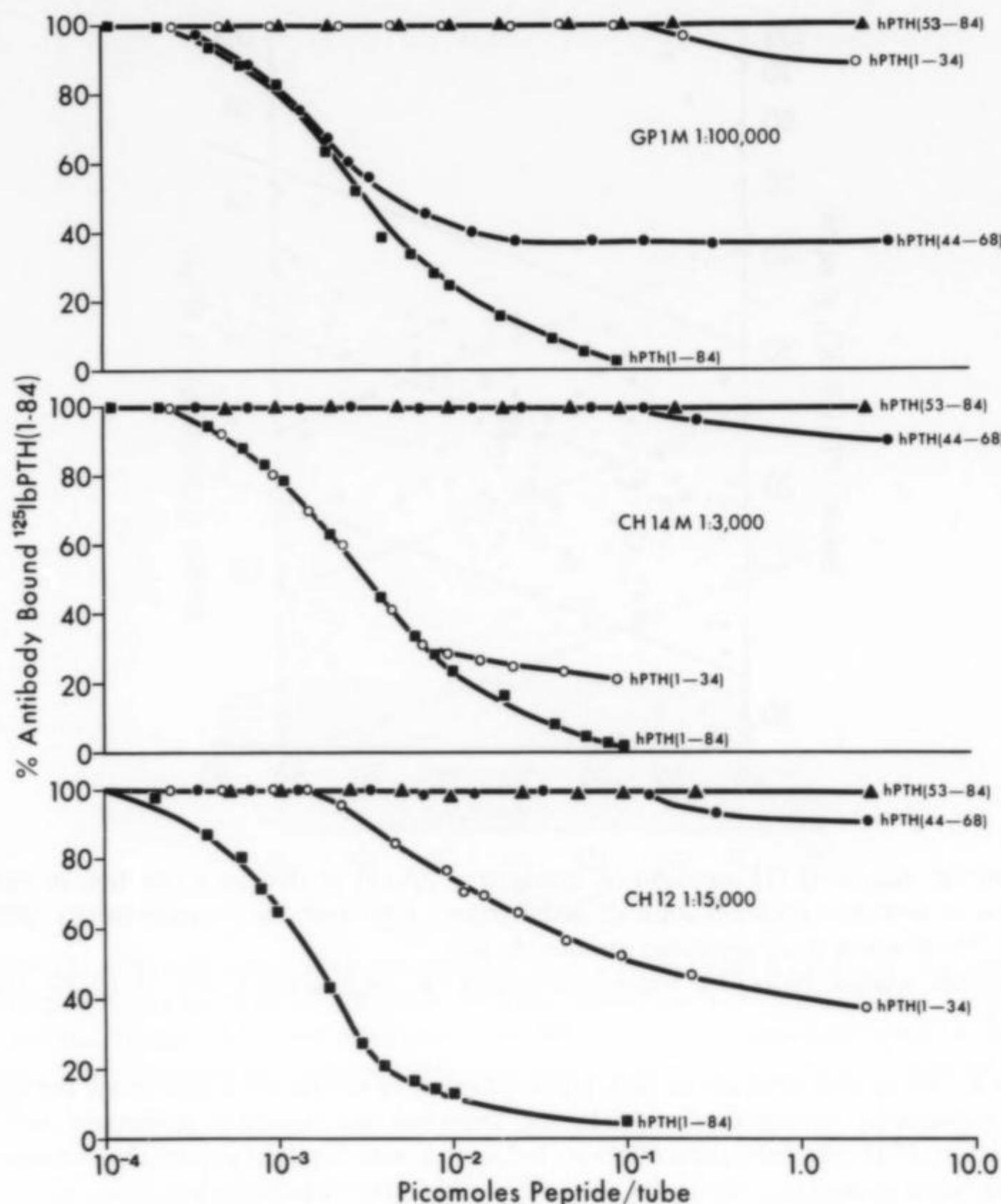
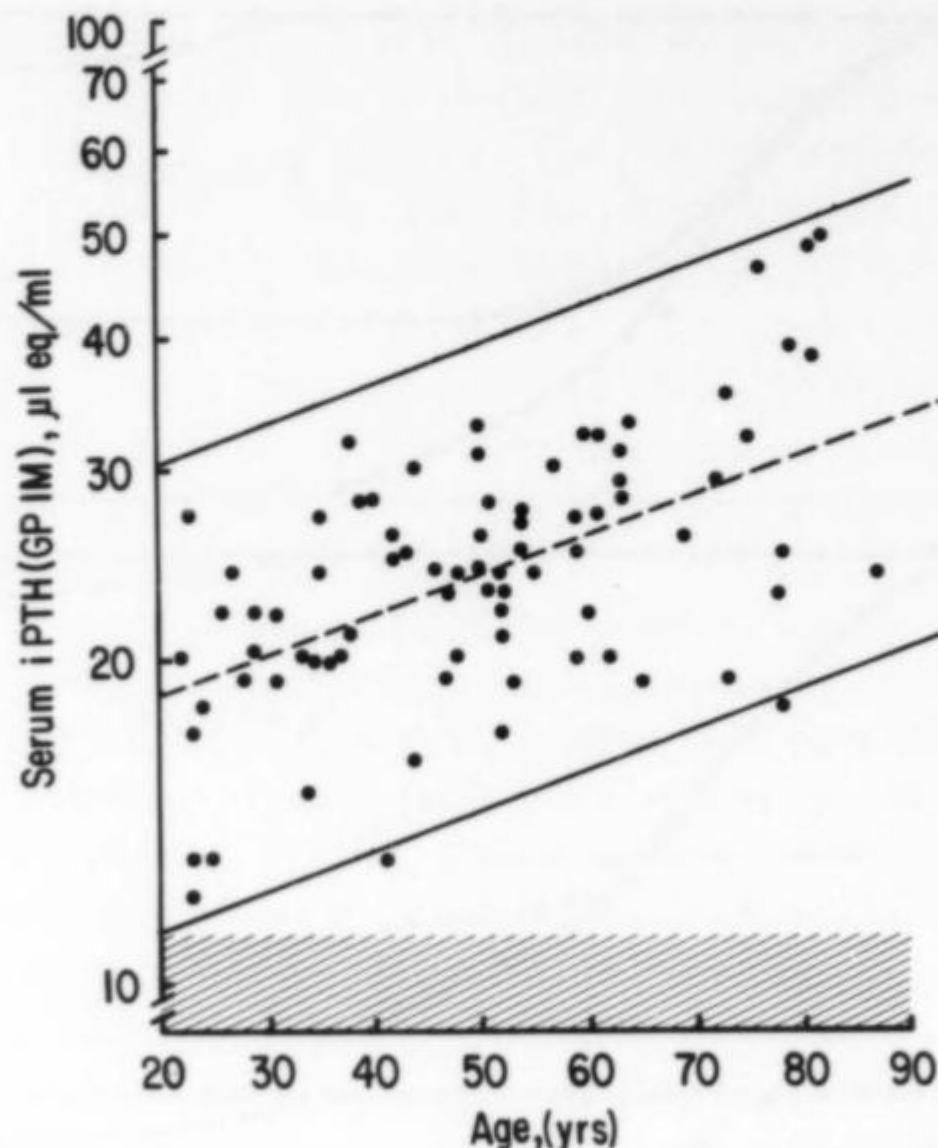


Fig. 1. Reactivities of ■, hPTH(1-84); ○, hPTH(1-34); ●, hPTH(44-68); and ▲, hPTH(53-84) with antisera GP-1M at final dilution 1:100,000 (upper panel), CH-14M at final dilution 1:3,000 (middle panel), and CH-12M at final dilution 1:15,000 (lower panel).

ments of hPTH (1-34, 44-68, 53-84) (kindly provided as a gift from the laboratory of Dr. John Potts, Massachusetts General Hospital, Boston, Mass.) with the three unadsorbed antisera are examined with [¹²⁵I]bPTH(1-84) used as a labeled ligand.

The assay using antiserum GP-1M (upper panel) easily detects as little as 10 pg (10⁻³ pM)/tube hPTH(1-84) and is the one we have routinely used in the clinical evaluation of patients with chronic disorders of parathyroid function. This assay is capable of measuring serum iPTH in >95% of normal subjects. In the region of the standard curve between 100% and 40% antibody binding of [¹²⁵I]bPTH, hPTH fragment (44-86) reacts almost the same as hPTH(1-84), whereas fragment (53-84) shows no reaction at molar concentrations that are 4 orders of magnitude greater than hPTH(1-84) or (44-86), and fragment(1-34) shows minimal reaction only at 3 orders of magnitude greater than hPTH(1-84) or (44-68). In separate studies with the ¹²⁵I-labeled synthetic fragment hPTH(1-34) or bPTH(1-34), we have found that there is a very low affinity population (antiserum dilution 1:10,000 vs. 1:100,000 with [¹²⁵I]bPTH(1-84)) of antibodies to the amino region of PTH in the antiserum, which probably accounts for the failure of fragment (44-68) to completely inhibit the binding of [¹²⁵I]bPTH as well as the minimal reactivity of hPTH(1-34) in this system. Thus it appears that the major immunologic determinant of this antiserum that is operative when concentrations of PTH lower than 1 nM (10 ng/ml) are assayed lies within the amino acid sequence region (44-68). Serum concentrations of iPTH



Figs. 2A and 2B. Serum iPTH assessed by antiserum GP-1M plotted as a function of age. Cross-hatched area at bottom represents limit of detectability. Undetectable measurements (5% of total normal and 7% of osteoporotic patients) are not shown.

Fig. 2A. Normal women ($n = 78$). Individual values (●), regression (----), and 95% confidence limits (—).

less than 8 pM (80 pg/ml) equivalents hPTH(1-84) cannot be evaluated in this assay because of the constraints imposed by the standard curve limits. Therefore serum assays performed with this antiserum measure iPTH concentrations 10 times below that which might permit the measurement of PTH fragments not including amino acid sequences within the (44-68) region.

The assay using CH-14M antiserum (middle panel) is as sensitive as the one using GP-1M antiserum and measures iPTH in >90% of normal sera. The PTH fragment (1-34) reacts almost the same as hPTH(1-84) in the region of the standard curve from 100% to 30% [^{125}I]bPTH(1-84) binding, whereas the fragments (44-68) and (53-84) react minimally, if at all, at molar concentrations 4 orders of magnitude greater than hPTH(1-84) or hPTH(1-34). Thus we cannot positively identify the sequence-region determinant which accounts for the difference in reactivity between hPTH(1-84) and hPTH(1-34) (region of standard curve from 30% to 0%), but we suspect that it is contained within the 10 amino acid overlap region between residues 34 and 44. The fragment (35-43) is not available for us to examine. Also, this sequence is in a region of the PTH molecule which is extremely difficult to manipulate because of its hydrophobicity and insolubility. Thus, for reasons similar to those put forward in the description of the specificity characteristics of the assay performed with GP-1M, it appears that serum assays using CH-14M antiserum would measure circulating iPTH species that contain a region of the hPTH molecule predominantly within the (1-34) amino acid sequence.

The assay using CH-12M antiserum is unique for several reasons. First, it is extremely sensitive, detecting as little as 4 pg/tube (4×10^{-4} pM) hPTH(1-84) and measuring iPTH in >97% of normal sera. Second, the only PTH fragment examined with which a clear reaction could be documented was hPTH(1-34). However, at least 30-fold greater molar concentrations of this fragment were required to produce similar inhibition of [^{125}I]bPTH(1-84) binding than of hPTH(1-84). Thus, if a determinant within the 10 amino acid sequence (35-43) (not examined) is not critical to CH-12M antiserum reactivity, it appears that the antiserum may recognize a conformational property of intact hPTH. Whichever of these alternatives is true for CH-12M antiserum (specificity for (35-43) vs. a conformational property), its specificity is distinctly different from that of either assay performed with GP-1M or CH-14M antiserum.

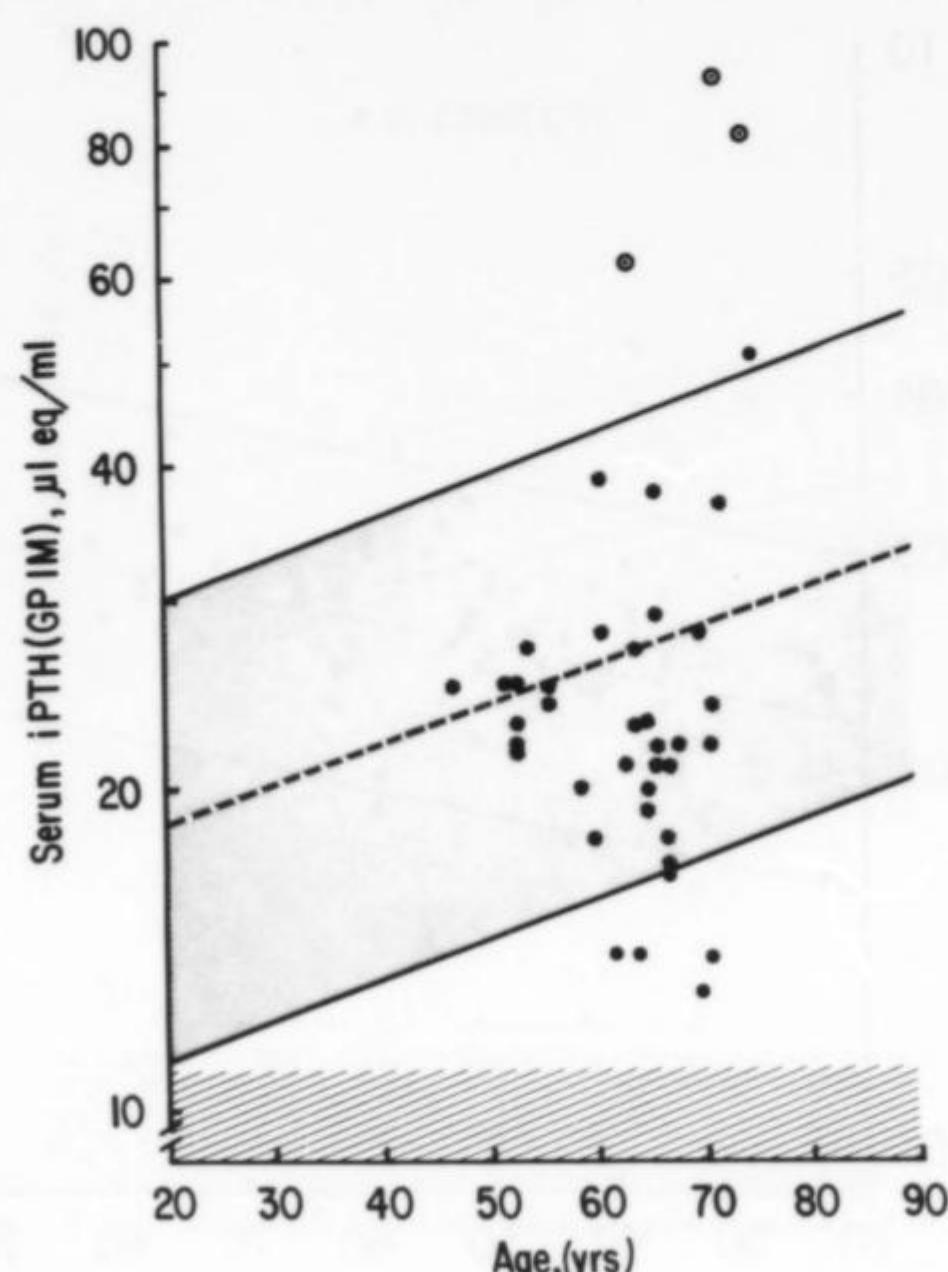


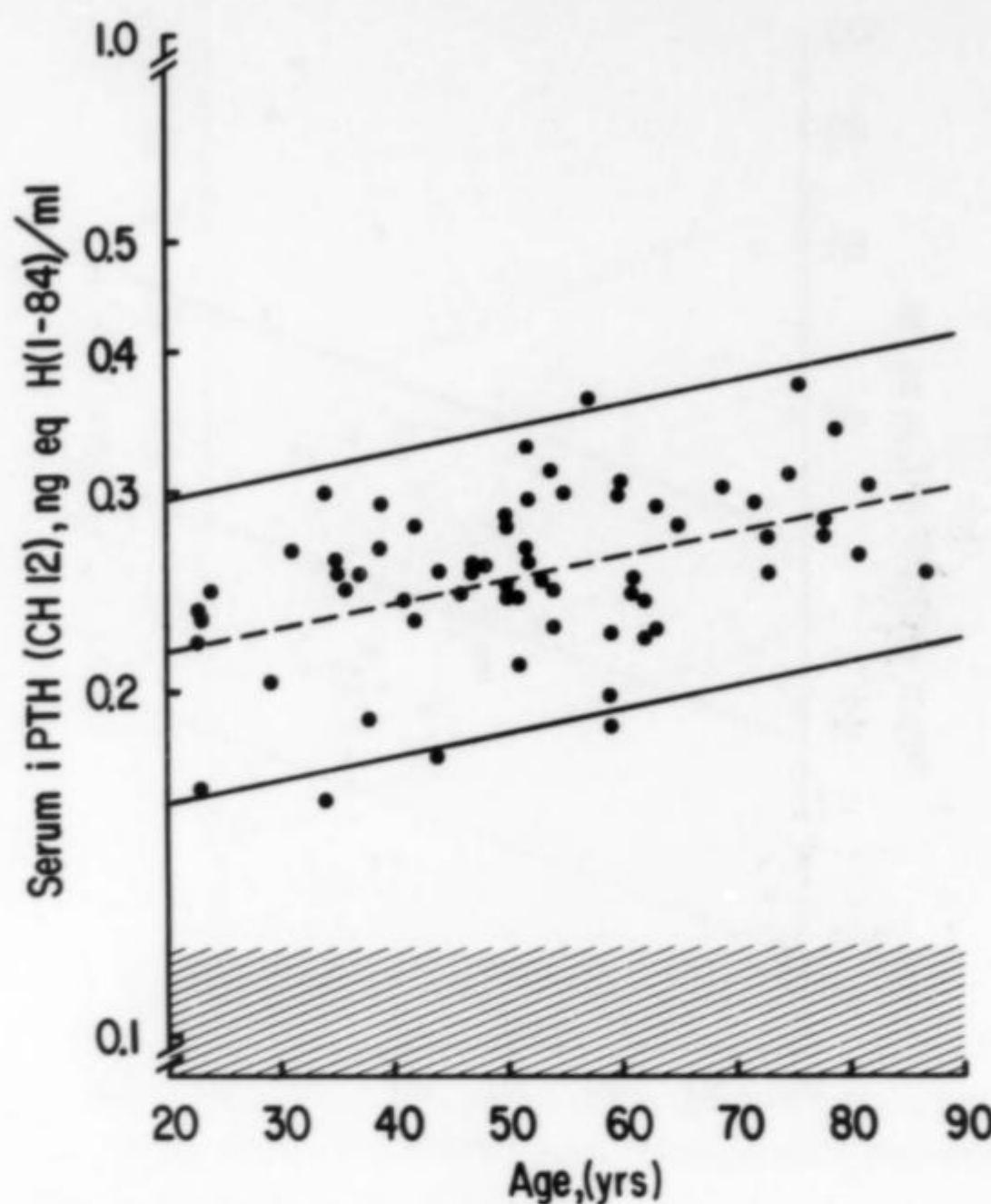
Fig. 2B. Patients with postmenopausal osteoporosis ($n = 40$). Individual values (●) are superimposed on the shaded area representing 95% confidence limits about the mean for regression of serum iPTH on age in normal women. Note that whereas serum iPTH values are generally lower in patients with osteoporosis, three osteoporotic patients had very high values (◎) and may represent a separate population.

The three antisera were further characterized by evaluating the circulating molecular forms of PTH in hyperparathyroid serum. When hyperparathyroid sera were gel-filtered on Bio-Gel P-150 (Bio-Rad Laboratories, Richmond, Calif.)¹⁵ columns, the hPTH co-eluting with [¹³¹I] bPTH(1-84) marker was recognized by all three antisera. The major portion of immunoreactivity in hyperparathyroid sera eluted in fractions between [¹³¹I]bPTH(1-84) and [¹²⁵I]bPTH(1-34) markers, and this component was recognized only by antiserum GP-1M.

We have interpreted these immunologic data as follows. Inasmuch as GP-1M recognizes the eluant fractions from gel-filtration of hyperparathyroid sera that are generally considered to be carboxyl region fragments of PTH and it reacted with the synthetic hPTH fragment (44-68) but did not react with (53-84), it can be identified as having mid-region specificity. On the other hand, both CH-14M and CH-12M antisera appear to recognize primarily intact circulating PTH, reacting little, if at all with circulating carboxyl fragments of PTH. However, their specificities are markedly different. In the case of CH-14M antiserum, its major determinant lies within the amino acid sequence region (1-34). In the case of CH-12M antiserum, its major determinant is either a conformational property of intact PTH or an amino acid sequence region (35-43) which, in all probability cannot be examined unless a soluble PTH fragment including this region becomes available to us.

It was necessary, for reasons described previously,¹⁵ to use hyperparathyroid serum as standard in GP-1M assays and highly purified hPTH(1-84) (see above) as standard in CH-14M and CH-12M assays. Theoretically, the use of these different standard preparations should have no influence on the relative differences between serum iPTH values in osteoporotic subjects.

Serum iPTH analyses were performed in assays that were especially designed (low antiserum and [¹³¹I]bPTH(1-84) concentrations) to yield limits of detectability of 3 to 5 pg/tube (sensitivity twice that of routine assays). This permitted the measurement of iPTH in at least two serum dilutions in a region of the standard curve that had the smallest error limits. All sera were studied within 3 months



Figs. 3A and 3B. Serum iPTH assessed by antiserum CH-12M plotted as a function of age in normal women and in women with postmenopausal osteoporosis. The symbols and format are the same as for Fig. 1. Values are significantly lower in the osteoporotic patients.

Fig. 3A. Normal women ($n = 66$).

of collection and stored at -20°C . Each assay run contained age-matched normal and osteoporotic sera so that precise comparisons of raw data could be made without reagent variation.

Results

Radioimmunoassay for PTH

ANTISERUM GP-1M. With use of this antiserum, serum iPTH increased as a function of age in the normal women ($p < 0.001$) (Fig. 2A). The mean increase between ages 20 and 90 years was 80%. Although values for the 40 osteoporotic women generally were lower (Fig. 2B), the mean \pm S.E. ($24.5 \pm 1.5 \mu\text{l eq/ml}$) was not significantly lower than the adjusted mean \pm S.E. for normal women (26.5 ± 1.0). Three osteoporotic patients had very high values that were more than 3 S.D. above the regression line for normal subjects and thus may represent a separate population. When values for these three patients were excluded, mean iPTH ($22.7 \pm 1.1 \mu\text{l eq/ml}$) in the osteoporotic patients was significantly less ($p < 0.001$) than normal. Five percent of the normal subjects and 7% of the osteoporotic patients had undetectable values and were not included in the statistical analysis (analysis of variance).

ANTISERUM CH-12M. There was sufficient serum to assay with antiserum CH-12M only 66 of the controls and 31 of the osteoporotic patients who had had assays with antiserum GP-1M. Serum iPTH assessed by antiserum CH-12M increased as a function of age in the normal women ($p < 0.001$) (Fig. 3A). The mean increase between ages 20 and

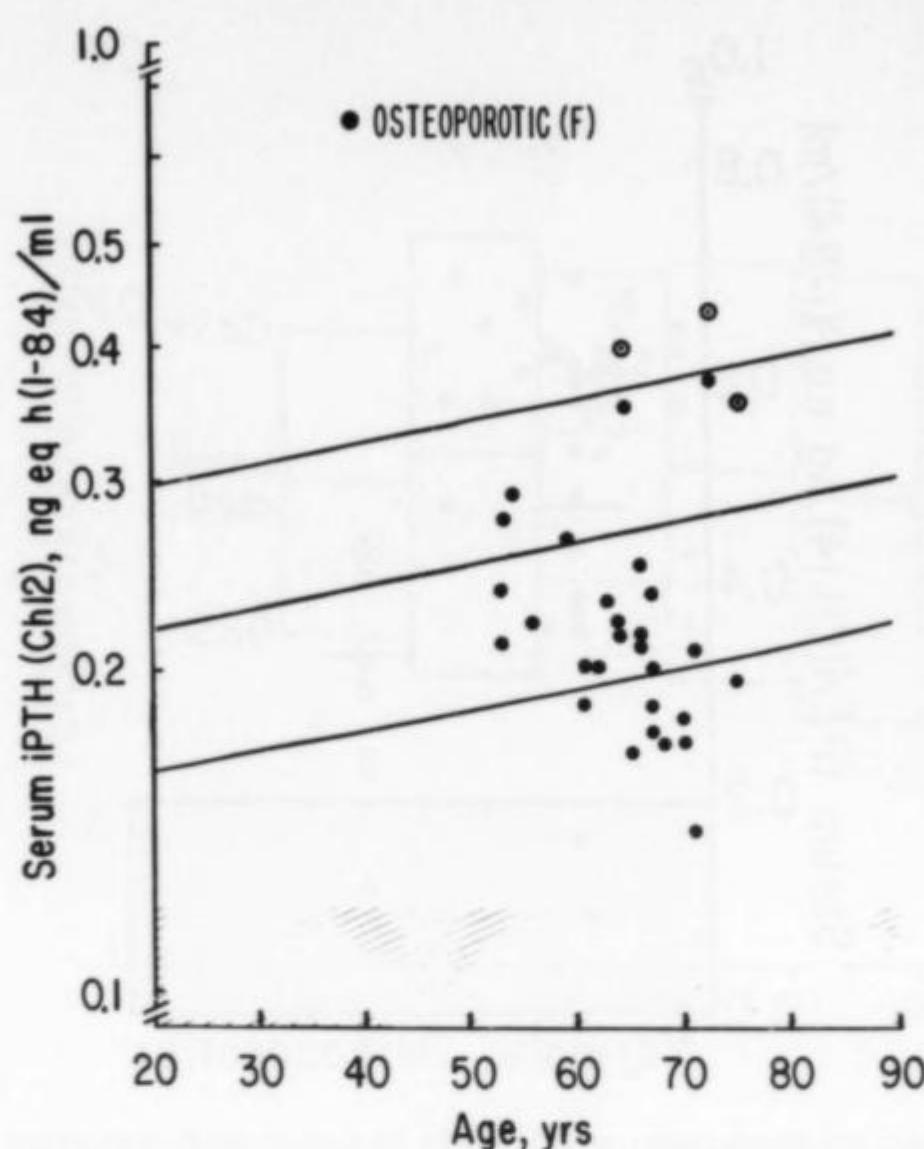


Fig. 3B. Women with postmenopausal osteoporosis ($n = 31$).

90 was about 30%. The mean value for 31 women with postmenopausal osteoporosis (220 ± 15 pg eq of hPTH (1-84) per milliliter) was significantly lower ($p < 0.001$) than the mean value for 66 normal women (268 ± 12) (Fig. 3B). Three percent of the normals and 8% of the osteoporotic patients had undetectable values and were not included in the statistical analysis. Results for serum iPTH assessed by antisera GP-1M and CH-12M were highly correlated in the normals ($r = 0.46$, $p < 0.01$) and in the osteoporotic patients ($r = 0.63$, $p < 0.001$).

ANTISERUM CH-14M. The amount of this antiserum was sufficient to assay only a few normals and osteoporotic patients. Ten normal female subjects with a mean age of 57 years (range 50 to 70) were compared with 12 women with postmenopausal osteoporosis with a mean age of 62 years (range 56 to 68) (Fig. 4). Mean serum iPTH was significantly lower ($p < 0.02$) in the osteoporotic patients (335 ± 28 pg eq of hPTH(1-84) per milliliter) than in the normal subjects (476 ± 49). Ten percent of the normals and 8% of the osteoporotic patients had undetectable values and were not included in the statistical analysis.

Biochemical findings. Glomerular filtration rate as assessed by creatinine clearance decreased significantly with age from about age 30 years; this decrease was best expressed by a quadratic function in the normal subjects ($r = -0.81$, $p < 0.001$). The decrease was about 50% between 30 and 80 years of age. Creatinine clearance in the osteoporotic patients also decreased with age, and the decrease did not differ significantly from that of the normal subjects. Individual values for osteoporotic patients were distributed equally around the normal regression line.

Serum iPTH assessed in assays using antiserum GP-1M or CH-12M correlated inversely with creatinine clearance (Table I). Sufficient data for statistical testing were not available for the use of antiserum CH-14M to determine serum iPTH. Partial correlations were made to examine the interrelationship of the variables serum iPTH, age, and creatinine clearance. When creatinine clearance was held constant, serum iPTH assessed by

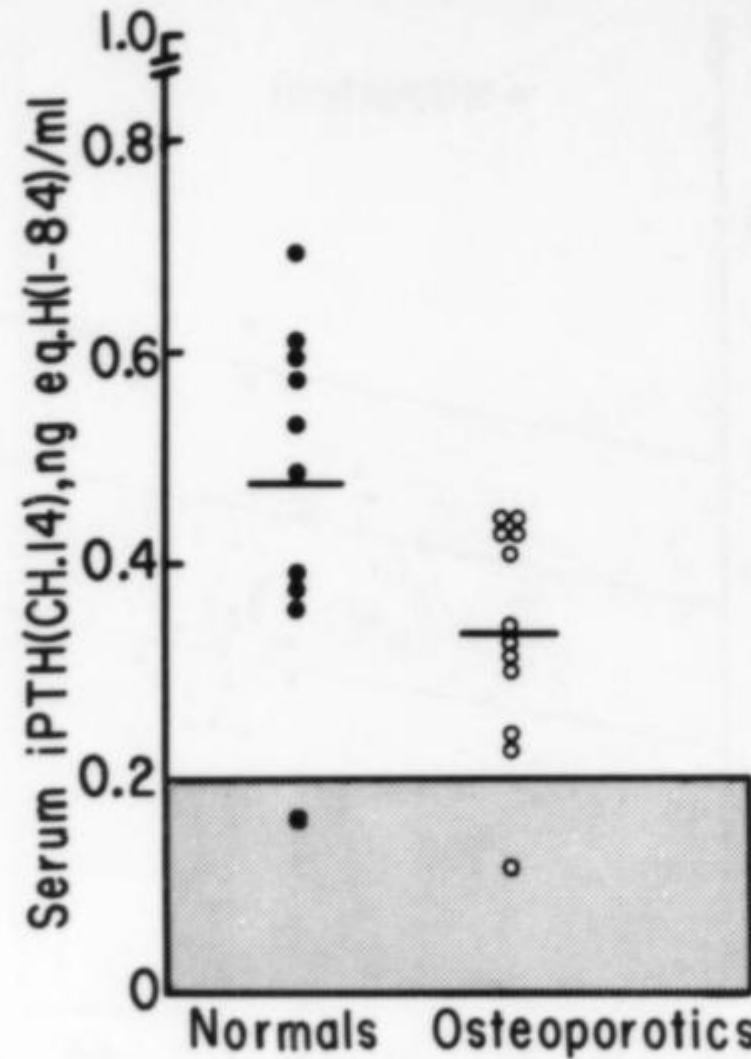


Fig. 4. Serum iPTH as assessed by antiserum CH-14M in age-matched normal subjects ($n = 10$) and in patients with postmenopausal osteoporosis ($n = 12$). Values are significantly lower in osteoporotic patients.

Table I. Interrelationship of age, creatinine clearance, and iPTH in normal subjects^A

	Correlation coefficients	
	Simple	Partial
Age vs. creatinine clearance	$r_{XY} = -0.77^C$	$r_{XY,Z} = -0.77^C$
Age vs. iPTH (GP-1M)	$r_{XZ} = -0.58^C$	$r_{XZ,Y} = 0.39^B$
iPTH (GP-1M) vs. creatinine clearance	$r_{ZY} = -0.46$	$r_{ZY,X} = 0.03$
Age vs. iPTH (CH-12M)	$r_{XZ} = 0.43^C$	$r_{XZ,Y} = 0.25$
iPTH (CH-12M) vs. creatinine clearance	$r_{ZY} = -0.38^B$	$r_{ZY,X} = 0.08$

X = age; Y = creatinine clearance; Z = iPTH.

^AAnalyses based on serum and urine values in 64 of the 78 normal subjects studied. Selection based only on the availability of data. Age range from 70 to 90 years, with approximately equal distribution among decades.

^Bp < 0.01.

^Cp < 0.001.

antisera GP-1M was still significantly correlated with age, whereas with CH-12M the increase was not quite significant ($p = 0.06$). When age was held constant, serum iPTH and creatinine clearance were no longer correlated; this suggests that the decrease in renal function was not the major factor accounting for the increase in serum iPTH with age.

Serum calcium was not significantly different in the normal subjects (9.40 ± 0.06 mg/dl) and the osteoporotic patients (9.42 ± 0.04). Mean fasting urine Ca/Cr in osteoporotic patients (0.11 ± 0.003) was significantly higher than in normals (0.06 ± 0.003 , $p < 0.001$). Mean plasma phosphate (3.83 ± 0.06 mg/dl) in the osteoporotic patients was significantly higher ($p < 0.001$) than in normals (3.49 ± 0.06 mg/dl) (Fig. 5A). Also, mean Tm_{PO_4}/GFR measured in 24 osteoporotic patients (3.80 ± 0.14 mg/dl of glomerular filtrate) was significantly higher ($p < 0.005$) than in 18 age-matched normal subjects (3.16 ± 0.11) (Fig. 5B).

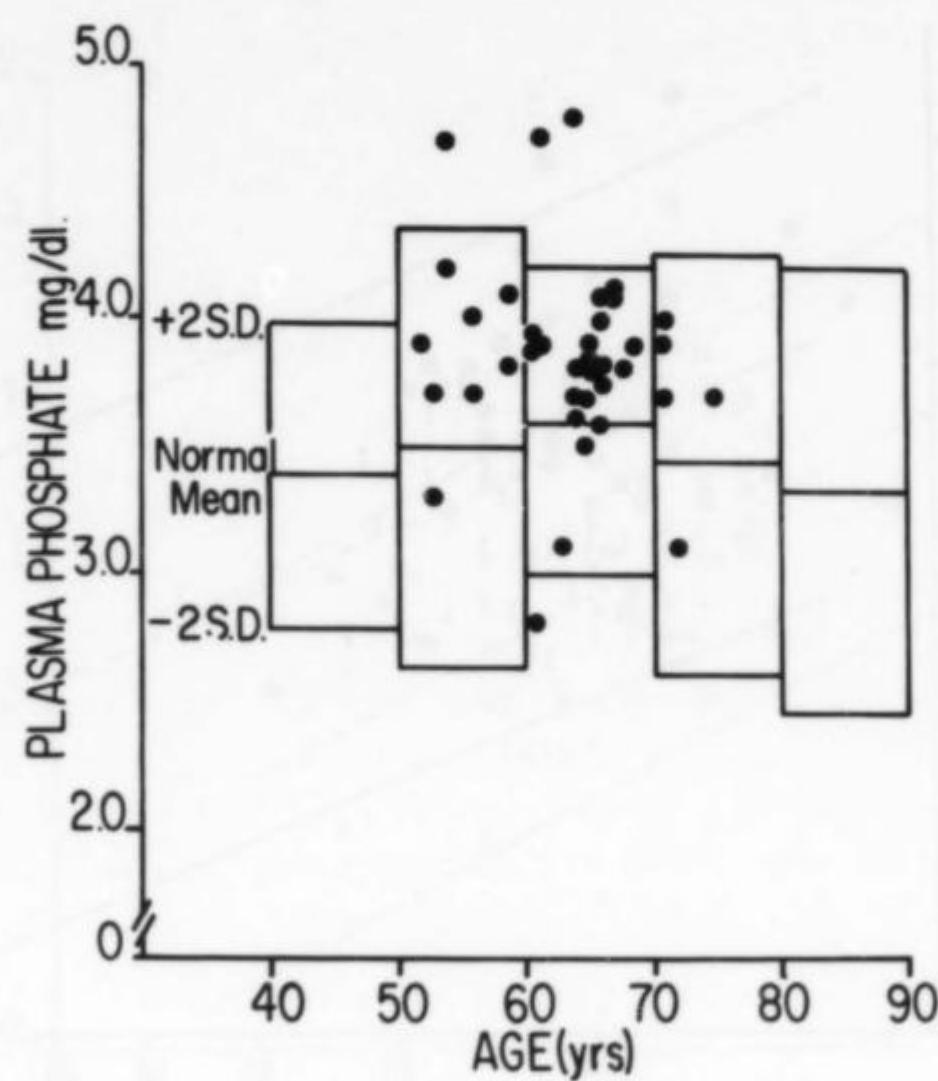


Fig. 5A. Plasma phosphate in patients with postmenopausal osteoporosis ($n = 24$). Bars represent mean ± 2 S.D. for normal female subjects for each decade ($n = 34$). Note that all but five patients had values above normal mean.

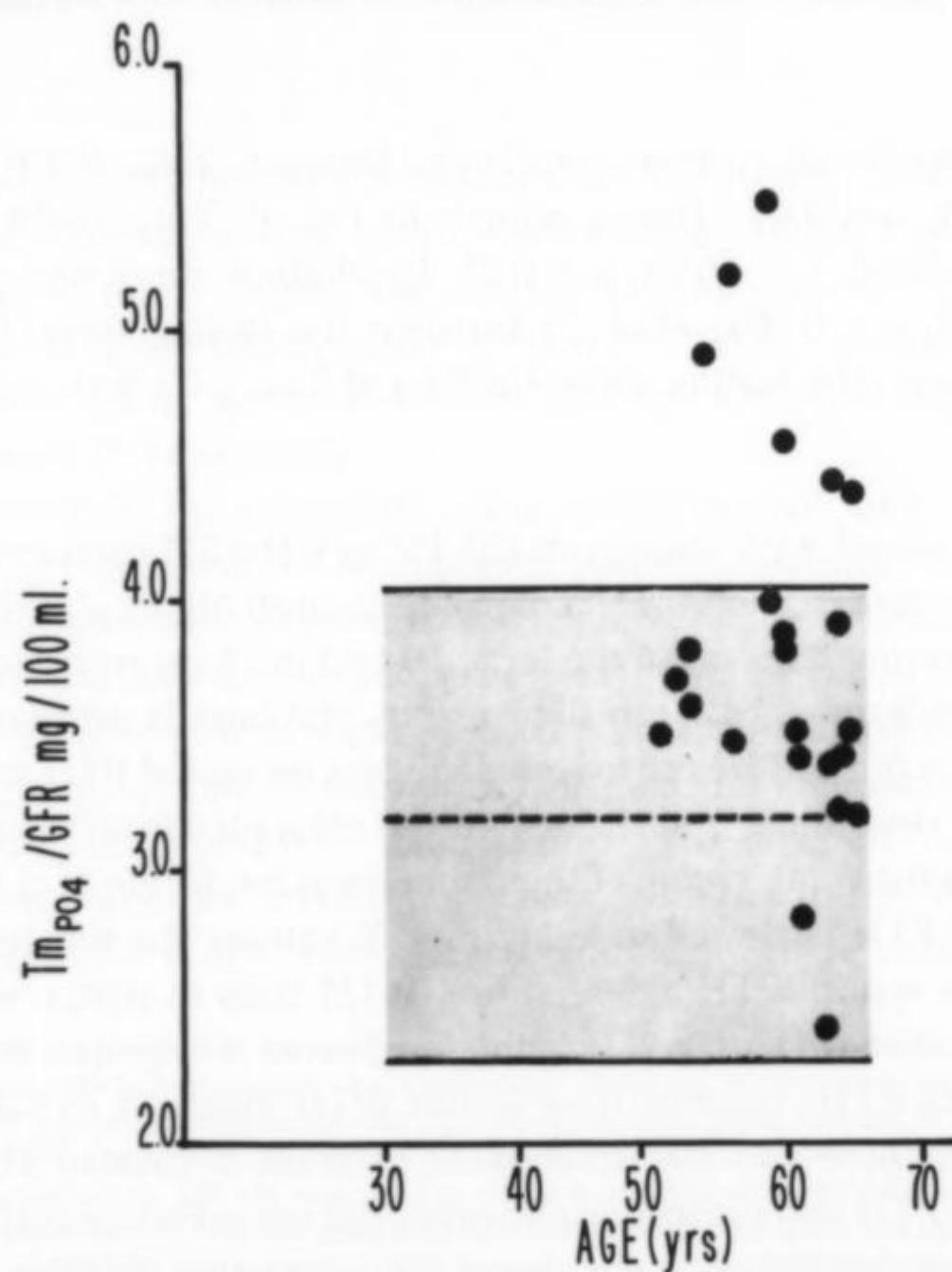


Fig. 5B. Values for Tm_{PO_4}/GFR in patients with postmenopausal osteoporosis ($n = 24$). Parallel lines represent mean ± 2 S.D. for normal subjects ($n = 19$). Note that all but two osteoporotic patients had values above the normal mean.

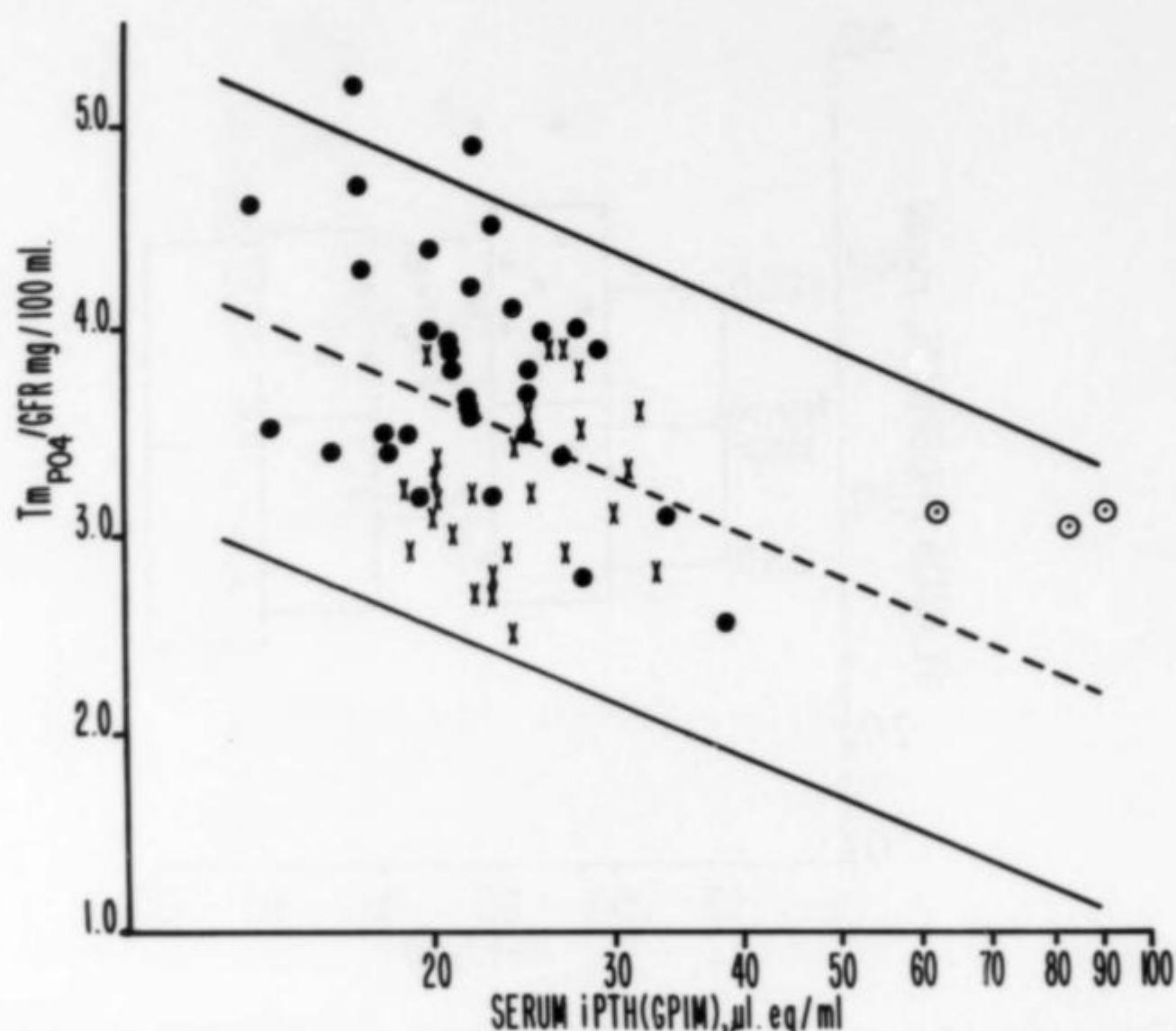


Fig. 6. Relationship between serum iPTH (antiseraum GP-1M) and Tm_{PO_4}/GFR in normal subjects (x), osteoporotic subjects (●), and in the three osteoporotic subjects with elevated values for serum iPTH (○).

There was a significant inverse correlation between Tm_{PO_4}/GFR and serum iPTH (GP-1M): $r = -0.34$, $p < 0.01$. This is shown in Fig. 6. Tm_{PO_4}/GFR and serum iPTH (CH-12M) also correlated: $r = -0.31$, $p < 0.05$. In addition, there was a highly significant correlation ($r = 0.62$, $p < 0.001$) (Fig. 7) between the fasting urine Ca/Cr and plasma phosphate and between the fasting urine Ca/Cr and Tm_{PO_4}/GFR ($r = 0.34$, $p < 0.01$).

Discussion

Serum iPTH assessed with antiserum GP-1M or CH-12M increased progressively in normal subjects between 20 and 90 years of age. Although highly significant, the increase that we observed was much less than the large 10-fold increase reported by Berlyne et al.² The reason for the difference between their results and ours is unclear.

The increase of serum iPTH with age could reflect increased PTH secretion, increased production of carboxyl-terminal fragments, reduced clearance from the circulation of PTH or its fragments, or some combination of these mechanisms. Fujita et al.¹⁶ have shown that plasma clearance of PTH is slowed in aging rats. Whatever the mechanism, the greater increase with age in serum iPTH assessed by GP-1M than in that assessed by CH-12M suggests that the increase in carboxyl-terminal fragments was proportionally greater than the increase of intact PTH. Inasmuch as serum iPTH assessed by CH-12M increased, however, presumably there also was an absolute increase in intact PTH.

Because serum iPTH and creatinine clearance did not correlate with each other when age was held constant, our data do not support the suggestion that the increase of serum iPTH with age is entirely a function of progressive renal glomerular failure.² Moreover, it previously has been shown that serum iPTH assessed by antiserum GP-1M does not increase until glomerular filtration rate declines to a level (<30 ml/min) below that found

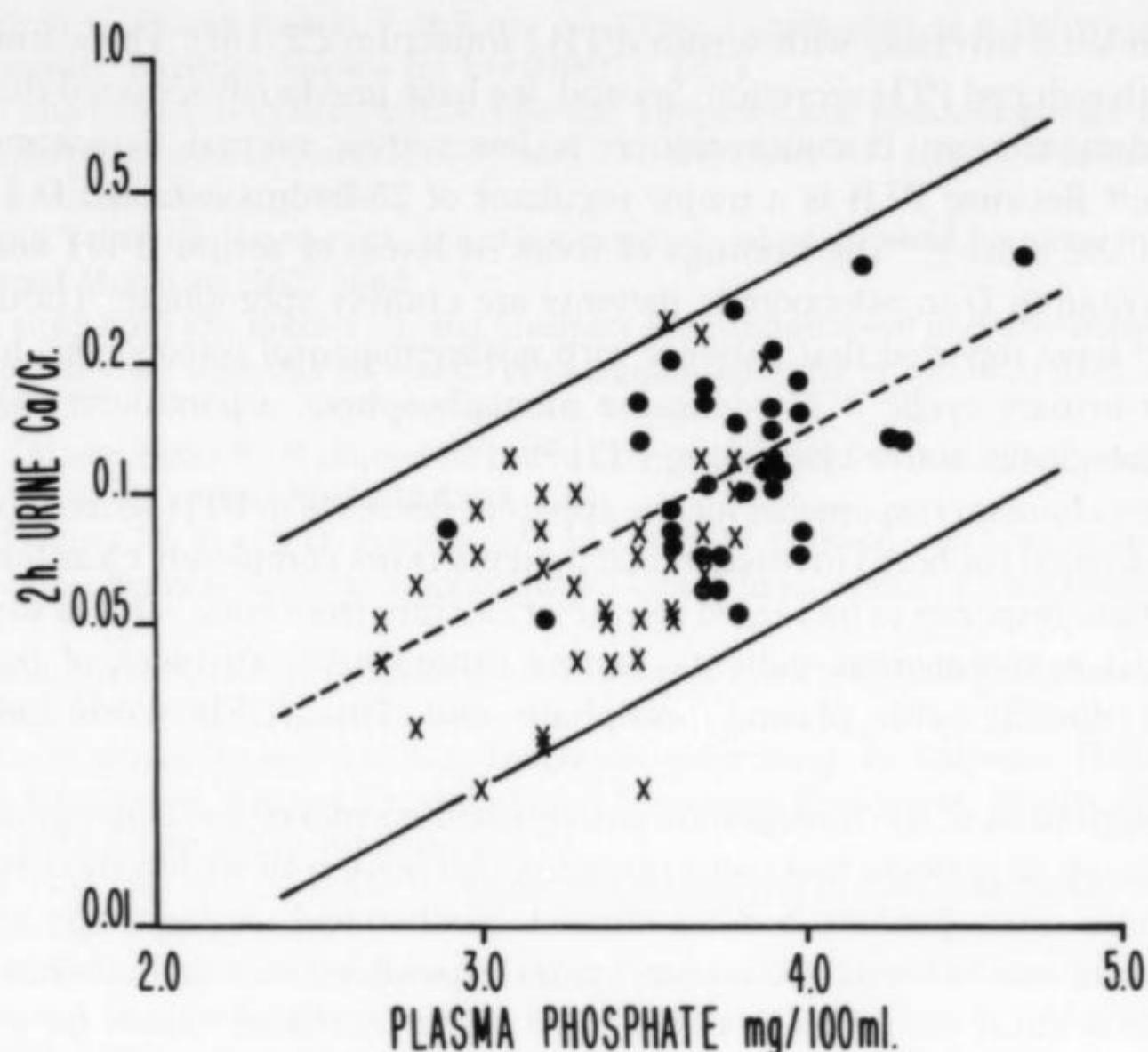


Fig. 7. Relationship between serum phosphate and fasting Ca/Cr in normal (x) and osteoporotic (●) subjects.

in our study subjects.¹⁷ Rather to the extent that the noted increase in serum iPTH represents secondary hyperparathyroidism, additional age-related abnormalities may be involved. In recent studies¹⁸ using the double calcium isotope technique, we have confirmed and extended the observations of others¹⁹⁻²¹ that intestinal absorption of calcium decreases with aging, particularly after age 60, and it is possible that this is a stimulus for increased PTH secretion.

Although serum iPTH increases with aging in both normals and osteoporotic patients, it was lower at any given age in the osteoporotic patients, the mean decrease being about 15% to 20%. The present data, derived by using three radioimmunoassays with different specificities, and the findings in our previous report³ of a series of 146 normal subjects and 87 patients with untreated postmenopausal osteoporosis strongly suggest that parathyroid function in osteoporosis is not increased and, in fact, may be decreased.* Three other observations support this contention. First, as previously reported²² and as also shown in this study, the mean value for plasma phosphate is higher in the osteoporotic subjects. Mean values for Tm_{PO_4}/GFR also were increased, and both these values and plasma phos-

*Recently, one of the co-authors of our earlier report³ independently published a paper which concluded that serum iPTH was generally increased in osteoporosis.⁷ However, we disagree with that author's interpretation because of severe limitations on the data base. In that paper, serum iPTH values from previous publications were merged with new values for osteoporotic patients generated in a routine clinical laboratory. Although antiserum GP-1M was used in these routine assays, (1) they were run under conditions yielding approximately one-half the sensitivity of the assays used in either our earlier³ or our present study, (2) they were performed over a period of at least 5 years, during which time changes in the quality of reagents could not be strictly controlled, and (3) they did not contain simultaneously run age-matched nonosteoporotic sera against which the results obtained with osteoporotic sera could be compared. On the basis of our previous experience, we believe that such assays and this experimental approach are likely to result in erroneously high values when attempts are made to measure concentrations of serum iPTH within the normal range.

phate correlated inversely with serum iPTH (antisera GP-1M). These findings are consistent with reduced PTH secretion. Second, we have previously reported that mean serum 1,25-dihydroxyvitamin D concentration is lower than normal in postmenopausal osteoporosis.¹⁸ Because PTH is a major regulator of 25-hydroxyvitamin D 1 α -hydroxylase activity in the kidney,²³ the findings of reduced levels of serum iPTH and serum 1,25-dihydroxyvitamin D in osteoporotic patients are entirely appropriate. Third, Lindsay and Sweeney²⁴ have reported that patients with postmenopausal osteoporosis have decreased values for urinary cyclic 3',5'-adenosine monophosphate, a parameter that functionally reflects biologically active circulating PTH.²⁵

The mechanism responsible for the apparent decrease in PTH secretion or increase in PTH degradation (or both) in osteoporotic patients is not completely clear but most likely is a homeostatic response to increased release of calcium from bone. This is supported by our finding that in osteoporotic patients, fasting urine Ca/Cr, an index of bone resorption, correlated directly with plasma phosphate and $T_{m_{PO_4}}/GFR$, both indices of PTH function.

Although most of the osteoporotic patients had normal or low concentrations of serum iPTH, three of 40 patients had concentrations that were well within the hyperparathyroid range. These three patients had no clinical, biochemical, or histologic evidence of osteomalacia. In one of them, gross parathyroid hyperplasia was demonstrated surgically.²⁶ In a previous study from this laboratory, we found increased values for serum iPTH in seven of 47 patients.³ Also, in a highly selected group, Teitelbaum et al.²⁷ found increased serum iPTH in six of 16 osteoporotic patients; these patients also had higher osteoclast counts in bone biopsy specimens. Thus there appears to be a relatively small subgroup of osteoporotic patients (probably about 10% of the total) in whom increased PTH secretion may be of etiologic importance. The cause or causes of the increased PTH secretion in these patients is presently unknown and is a subject of continued investigation. The most likely causes are secondary hyperparathyroidism due to intestinal calcium malabsorption and normocalcemic primary hyperparathyroidism.

Addendum

Subsequent to submission of this manuscript, Wiske et al.²⁸ reported a doubling of serum iPTH with age in normal men and women, using an assay specific for the carboxyl-terminal portion of the PTH molecule. Their results are similar to those that we are reporting using antisera GP-1M.

The antisera specificity studies could not have been performed without the generous gifts of hPTH(1-84) and synthetic fragments of hPTH from Drs. Brian Brewer and John Potts, Jr. We express our sincere thanks to them.

REFERENCES

- Roof BS, Piel CF, Hansen J, and Fudenberg HH: Serum parathyroid hormone levels and serum calcium levels from birth to senescence. *Mech Ageing Dev* **5**:289, 1976.
- Berlyne GM, Ben-Ari J, Kushelevsky A, Idelman A, Galinsky D, Hirsch M, Shainkin B, Yagil R, and Zlotnik M: The aetiology of senile osteoporosis: secondary hyperparathyroidism due to renal failure. *Q J Med* **44**:505, 1975.
- Riggs BL, Arnaud CD, Jowsey J, Goldsmith RS, and Kelly PJ: Parathyroid function in primary osteoporosis. *J Clin Invest* **52**:181, 1973.
- Franchimont P and Heynen G: Parathormone and Calcitonin in Radioimmunoassay in Various Medical and Osteoarticular Disorders. Philadelphia, 1976, J. B. Lippincott Co., p. 101.
- Bouillon R, Geusens P, Dequeker J, and DeMoor P: Parathyroid function in primary osteoporosis. *Clin Sci* **57**:167, 1979.

6. Fujita T, Orimo H, Okano K, and Yoshikawa M: Clinical application of parathyroid hormone radioimmunoassay. *Excerpta Medica Int Ser* **270**:274, 1973.
7. Jowsey JOM and Offord KP: Osteoporosis: juvenile, idiopathic and postmenopausal. In *Mechanisms of Localized Bone Loss*, Horton JE, Tarpley TM, and Davis WF, editors. Washington D.C., 1977, Information Retrieval, p. 345.
8. Berson SA and Yalow RS: Immunochemical heterogeneity of parathyroid hormone in plasma. *J Clin Endocrinol Metab* **28**:1037, 1968.
9. Arnaud CD, Goldsmith RS, Bordier PJ, and Sizemore GW: Influence of immunoheterogeneity of circulating parathyroid hormone on results of radioimmunoassays of serum in man. *Am J Med* **56**:785, 1974.
10. Canturbury JM and Reiss R: Multiple immunoreactive molecular forms of parathyroid hormone in plasma. *Proc Soc Exp Biol Med* **140**:1393, 1972.
11. Segre GV, Habener JF, Powell D, Tregebar GW, and Potts JT Jr: Parathyroid hormone in human plasma: immunochemical characterization and biological implications. *J Clin Invest* **51**:3163, 1972.
12. Walton RJ and Bijvoet OLM: Nomogram for derivation of renal threshold phosphate concentration. *Lancet* **2**:309, 1975.
13. Nordin BEC, Horsman A, and Aaron J: Diagnostic procedures. In *Calcium, Phosphate and Magnesium Metabolism: Clinical Physiology and Diagnostic Procedures*, Nordin BEC, editor. Edinburgh, 1976, Churchill Livingstone, p. 477.
14. Arnaud CD, Tsao HS, and Littledike T: Radioimmunoassay of human parathyroid hormone in serum. *J Clin Invest* **50**:21, 1971.
15. Flueck JA, Di Bella FP, Edis AJ, Kehrwald JM, and Arnaud CD: Immunoheterogeneity of parathyroid hormone in venous effluent serum from hyperfunctioning parathyroid glands. *J Clin Invest* **60**:1367, 1977.
16. Fujita T, Ohata M, Tanimoto T, Hanano Y, Funasako M, and Uezu A: Aging and parathyroid hormone. *Excerpta Medica Int Congr Ser* **421**:118, 1977.
17. Arnaud CD, Wilson DM, and Smith LH: Primary hyperparathyroidism, renal lithiasis and the measurement of parathyroid hormone in serum by radioimmunoassay. In *Urinary Calculi: Recent Advances in Aetiology, Stone Structure and Treatment*, Cifuentes Delatte L, Rapado A, and Hodgkinson A, editors. Basel, 1973, S Karger, p. 346.
18. Gallagher JC, Riggs BL, Eisman J, Hamstra A, Arnaud SB, and DeLuca HF: Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients. Effects of age and dietary calcium. *J Clin Invest* **64**:729, 1979.
19. Avioli LV, McDonald JE, and Lee SW: The influence of age on the intestinal absorption of ^{47}Ca in women and its relation to ^{47}Ca absorption in postmenopausal osteoporosis. *J Clin Invest* **44**:1960, 1965.
20. Bullamore JR, Gallagher JC, Wilkinson R, Nordin BEC, and Marshall DH: Effect of age on calcium absorption. *Lancet* **2**:535, 1970.
21. Alevizaki CC, Ikkos DG, and Singhalakis P: Progressive decrease of true intestinal calcium absorption with age in normal man. *J Nucl Med* **14**:760, 1973.
22. Albright F and Reifenstein EC Jr: *The Parathyroid Glands and Metabolic Bone Disease: Selected Studies*. Baltimore, 1948, The Williams & Wilkins Co.
23. DeLuca HF: Recent advances in our understanding of the vitamin D endocrine system. *J LAB CLIN MED* **87**:7, 1976.
24. Lindsay R and Sweeney A: Urinary cyclic-AMP in osteoporosis. *Scottish Med J* **21**:231, 1978.
25. Chase LR, Melson GL, and Aurbach GD: Pseudohypoparathyroidism: defective excretion of 3'5'-AMP in response to parathyroid hormone. *J Clin Invest* **48**:1832, 1969.
26. Riggs BL, Gallagher JC, DeLuca HF, Edis AJ, Lambert PW, and Arnaud CD: A syndrome of osteoporosis, increased serum immunoreactive parathyroid hormone, and inappropriately low serum 1,25-dihydroxyvitamin D. *Mayo Clin Proc* **53**:701, 1978.
27. Teitelbaum SL, Rosenberg EM, Richardson CA, and Avioli LV: Histological studies of bone from normocalcemic postmenopausal osteoporotic patients with increased circulating parathyroid hormone. *J Clin Endocrinol Metab* **42**:537, 1976.
28. Wiske PS, Epstein S, Bell NH, Queener SF, Edmondson J, and Johnston CC: Increases in immunoreactive parathyroid hormone with age. *N Engl J Med* **300**:1419, 1979.