Multisite Immunochemiluminometric Assay for Simultaneously Measuring Whole-Molecule and Amino-Terminal Fragments of Human Parathyrin

George G. Klee, Carol M. Preissner, Patricia G. Schryver, Robert L. Taylor, and Pai C. Kao

The immunochemiluminometric assay described uses immobilized anti-human parathyrin (parathyroid hormone, hPTH(1–84)) and anti-hPTH(44–68) antisera and acridinium ester-labeled anti-hPTH(1–34) to simultaneously measure both intact hPTH and its amino-terminal fragments. Results by the assay correlate well with those by a cAMP-based bioassay and the Nichols Allegro immunoradiometric assay. The minimal detection limit is 0.08 pmol/L. The normal range is 1.0–5.0 pmol/L, and values are higher in older women. About 90% of study patients with surgically proven parathyroid adenomas had abnormal preoperative PTH concentrations, whereas patients with hypercalcemia of malignancy had normal or suppressed values. This assay was designed to detect both intact PTH and amino-terminal PTH fragments; however, chromatographic fractionation of pools of primary and secondary hyperparathyroid plasma showed virtually no amino-terminal fragment activity. Nonetheless, the design is important because the absence of carboxyl-terminal binding sites prevents interference by carboxyl-terminal fragments and because bioactive am-ino-terminal fragments will react in the assay if they are present in the patients’ sera or if they are produced by in vitro proteolysis of intact PTH.

Additional Keyphrases: adenoma, hypercalcemia of malignancy, cancer

The measurement of human parathyrin (human parathyroid hormone, hPTH) in peripheral blood is beset with several difficulties related to the heterogeneity of circulating forms and the limited availability of specific high-affinity antisera. PTH is synthesized in the parathyroid glands and secreted mainly as an 84-amino-acid peptide that is degraded in the liver and other sites by cleavage in the 34–37 amino acid region (1). Normally, the amino-terminal fragments are cleared rapidly, whereas the carboxyl-terminal and mid-molecule fragments remain in circulation longer. The circulatory half-life of carboxyl-terminal fragments depends on renal function because they are cleared mainly by glomerular filtration. The net effect of these production and clearance rates is that the circulating concentration of carboxyl-terminal and mid-molecule fragments is greater than the concentration of intact molecule, especially in patients with compromised renal function. However, the functional activity of the PTH molecule is related to the amino-terminal portion of the molecule.

Assays for monitoring the biological activity of PTH should reliably measure both intact and functional amino-terminal fragments without interference from carboxyl- and mid-molecule fragments or other substances. Our functional bioassay (bio-PTH) provides such a monitor by using anti-amino-terminal antisera to immunoextract and concentrate both hPTH(1–84) and the amino-terminal fragments, followed by measurement of their combined activity in stimulating the production of cAMP in cultured osteosarcoma cells (2). Our goals in developing the current assay were to have an assay that would correlate well with this bio-PTH assay and also to have an assay that would be easier to perform and would measure lower concentrations of PTH. (A major problem in the development of reliable assays is the low peripheral blood concentration of intact and amino-terminal PTH.) The functional bioassay achieved a measurement sensitivity of 1.0 pmol/L by extracting and concentrating 2.0 mL of plasma; however, this is not sensitive enough to measure subnormal concentrations of the hormone. Therefore, we wanted to develop an assay capable of measuring PTH concentrations well below the range found in normal subjects.

Key factors in immunoassay sensitivity are the affinity of the antisera, the configuration of the assay, and the specific activity of the detection signal. Brown et al. (3) reported that immunochromatography purified polyclonal antisera were more sensitive than monoclonal antisera for measuring PTH. Miles and Hales (4) found that immuno-metric systems based on a combination of immobilized and labeled antibodies were more sensitive than competitive radioimmunoassays with labeled antigens. Ehrlich and Moyle (5) showed that combinations of antisera with affinities for multiple sites on an antigen can effectively increase the binding affinity. In addition, Weeks et al. (6) showed that acridinium esters can produce immunoassay labels with high specific activities. We have combined each of these observations to develop a highly sensitive immunocomedimunometric assay (ICMA) that uses a combination of three affinity-purified polyclonal antisera. We evaluated the assay analytically and clinically by comparison with our functional bioassay, by comparison with a commercial immunoradiometric assay, and by measuring PTH in plasma from normal subjects and patients with disorders of PTH metabolism.

Materials and Methods

Antisera Production

Three polyclonal anti-hPTH antisera were used in this assay: two attached to the solid-phase capture
system and the third for signal detection (Figure 1). The first antiserum, goat anti-hPTH(1-44), is the same as that used for the immunoaffinity extraction system of the bio-PTH assay (2). It was raised by immunizing a goat with synthetic peptide conjugated to bovine serum albumin and by boosting first with conjugated and then with free unconjugated peptide in incomplete Freund's adjuvant. The second antiserum was also raised in a goat by using hPTH(44-68) conjugated to bovine serum albumin as an immunogen, initially in Freund's complete adjuvant, followed by monthly boosts in incomplete adjuvant. The two goat antisera were affinity-purified with CNBr-activated Sepharose 4B (Pharma- cia, Piscataway, NJ) coupled to the same hPTH peptide fragment as was used for immunization. The third antiserum, which was originally raised in sheep immunized with hPTH(1-34) conjugated to bovine serum albumin, was purchased from Ciba-Corning Diagnostic Corp. (E. Walpole, MA) as affinity-purified antiserum labeled with an acridinium ester: 4-(2-succinimidyloxy-carbonyl)phenyl-10-methylacridinium-9-carboxylate fluorosulfonate.

ICMA Procedure

The assay involves an overnight incubation of 200 µL of EDTA-treated plasma specimens with 100 µL of acridinium-labeled antibody, followed by a 3-h incubation with the two goat antisera coupled to polystyrene beads, washing of the beads three times with 1 mL of distilled water, and quantification of the luminescence. The antisera were coupled to the beads by adding a solution containing each of the affinity-purified goat antisera in 0.1 mol/L phosphate buffer, 1.0 µg per bead, to activated CoBind beads (MicroMembranes, Inc., New- ark, NJ) for 3 h on a horizontal rotator. The beads were washed, blocked with ethanolamine (20 mL/L), and stored dry at 4 °C. The assay was standardized with Asn-76 hPTH(1-84) (Peninsula Labs., Inc., Belmont, CA) prepared in hypoparathyroid plasma collected from a donor with familial hypoparathyroidism. Ten standards (106, 26.5, 10.6, 5.3, 1.6, 0.8, 0.4, 0.2, 0.1, and 0.0 pmol/L) and six controls (three concentrations each at beginning and end) were run with each assay. The controls were made from hypoparathyroid plasma supplemented with hPTH(1-84) in 1-mL portions and frozen at -70 °C before use. All measurements were performed in duplicate. The luminescence was quantified with an automated MLA II luminescent analyzer (CibaCorning), with use of their logit-log data reduction software.

Performance Studies

Detection limit. Five low-concentration pools were made by adding hPTH(1-84) to hypoparathyroid plasma to achieve target concentrations of 0.00, 0.05, 0.1, 0.2, and 0.3 pmol/L. We analyzed eight samples of each in five consecutive assays. The mean, SD, and CV were calculated from determinations in duplicate tubes. We calculated the detection limit both as the concentration corresponding to mean + 2.6 SD above the zero pool and as the concentration that had a 99% confidence range exceeding zero, based on linear interpolation of the means and SDs of the measured pools.

Cross-reactivity and inhibition of recovery. Synthetic peptides for hPTH(1-34), hPTH(44-68), hPTH(53-84), and PTH-related peptide(1-34) (Peninsula Labs.) were added to hypoparathyroid plasma to yield various concentrations (Table 1). In addition, we made a second set of pools with the same concentrations of PTH peptides plus 26.3 pmol (250 ng) of hPTH(1-84) per liter. The cross-reactivity was calculated as the ratio of the measured concentration to the calculated concentration (molar basis). We calculated the interference in terms of the percentage recovery of the added hPTH(1-84) after subtracting the contribution from the cross-reacting peptide.

Linearity. Plasma samples from four patients with above-normal PTH concentrations of 25-50 pmol/L (by bio-PTH assay) were serially diluted with hypoparathyroid plasma to give five different concentrations. The

| Table 1. Peptide Cross-Reactivities and Inhibition of Recovery of Intact hPTH |
|-----------------------------|-----------------------------|-----------------------------|
| Peptide                     | Concentration, pmol/L       | Cross-reactivity, %          | Analytical recovery of hPTH(1-84), % |
| hPTH(1-34)                  | 12.1                        | 71.1                        | 97                          |
| hPTH(1-44)                  | 48.6                        | 56.0                        | 69                          |
| hPTH(44-68)                 | 9.9                         | 44.0                        | 78                          |
| hPTH(53-84)                 | 39.5                        | 35.4                        | 69                          |
| PTHrP(1-34)                 | 35.3                        | 0.1                         | 99                          |
|                            | 352.6                       | 0.0                         | 83                          |
|                            | 3526.0                      | 0.0                         | 75                          |
|                            | 28.5                        | 0.2                         | 107                         |
|                            | 284.7                       | 0.0                         | 89                          |
|                            | 2847.0                      | 0.0                         | 99                          |
|                            | 28 470.0                    | 0.0                         | 94                          |
|                            | 62.2                        | 0.13                        | —                           |
|                            | 249.0                       | 0.04                        | —                           |

* Relative to PTH(1-84).

Fig. 1. Assay configuration with amino-terminal and mid-molecule affinity-purified antibodies immobilized on polystyrene beads and amino-terminal acridinium-ester antibody used for signal detection.
data were plotted as expected vs measured concentration.

Comparison with bioassay. Plasma from 195 samples that had been analyzed with our bio-PTH assay were reanalyzed by the ICMA. The specimens were stored frozen at -70°C between analyses. These specimens included 49 from normal subjects, 45 preoperative specimens from patients with surgically proven parathyroid tumors, 30 from patients with chronic renal failure, and 71 miscellaneous specimens. The data were cross-plotted, and Deming regression statistics were calculated for the total and each subgroup.

Comparison with an immunoradiometric assay. Plasma from 141 specimens was analyzed with both this assay and the Allegro assay (Nichols Institute Diagnostics, San Juan Capistrano, CA) (7-9). These samples included 37 specimens from subjects without bone disease, 22 preoperative specimens from patients with surgically proven parathyroid tumors, 12 specimens from patients with chronic renal failure, and 70 miscellaneous specimens. The data were cross-plotted, and Deming regression statistics were calculated for the total and each subgroup. In addition, the standards for both assays were analyzed in the comparison assays.

Clinical Evaluations

Specimens from 262 healthy subjects with calcium concentrations =2.52 mmol/L (=101 mg/L) were analyzed to establish a normal reference range. The individuals were determined not to have known disorders of calcium metabolism, based on review of their medical records. In addition, we analyzed preoperative specimens from 110 patients with surgically proven parathyroid tumors, 91 specimens from patients with chronic renal failure, and 10 specimens from patients with hypercalcemia related to malignancy. The data for normal male and female subjects were plotted against subject age, and the 2.5 and 97.5 percentiles were calculated. We determined the distribution of the test results for each group and calculated descriptive statistics.

Chromatographic Profiles of Plasma Pools

Seven plasma pools representing various forms of PTH were fractionated with a 1.5 x 90 cm P-10 (Bio-Rad, Richmond, CA) column. The PTH in each fraction was measured with both the ICMA and the bio-PTH assays. The void volume of the column as measured with Blue Dextran was 65 mL. We made the first two pools by adding synthetic PTH to aged blood-bank plasma. Pool one contained 24 pmol of hPTH(1-34) per liter, and pool two contained 42 pmol of hPTH(1-84) per liter. Pools three through five were made by combining residual EDTA plasma from patients with renal failure who had above-normal bio-PTH values and increased concentrations of creatinine. Subsequently, we fractionated two additional pools, using a second P-10 column. Pool six was made by adding both 10 pmol of hPTH(1-84) and 5 pmol of hPTH(1-34) per liter. Pool seven was made by combining residual specimens from patients with above-normal bio-PTH values caused by primary hyperparathyroidism. Eluates from the columns were fractionated into 3-mL aliquots, and PTH was measured by ICMA in each aliquot. Aliquots from the prepeak baseline, peak, and postpeak baseline were combined and measured by the bio-PTH assay.

Results

Assay Characteristics

Figure 2 shows a representative composite standard curve. The vertical axis represents the net 2-s luminescent counts after subtracting the background. The average background was 3860 (SD 407) counts per 2 s. The dose-response curve is approximately linear on the log-log scale to 10 pmol/L, after which the slope decreases. The intra-assay detection limits (mean + 2 SD of the zero pool) is 0.08 pmol/L. The 0.1 pmol/L (1 ng/L) pool produced an average signal 3.0 SD above the signal for the zero pool. The interassay CVs were 24% at 0.1 pmol/L, 11% at 0.2 pmol/L, 15% at 0.3 pmol/L, 8.8% at 1.4 pmol/L, 4.6% at 4.2 pmol/L, 5.3% at 10.7 pmol/L, and 3.5% at 30.3 pmol/L.

Table 1 is a summary of the cross-reactivities of the peptide fragments in the ICMA. The amino-terminal peptides react in the assay but are less potent on a molar basis than hPTH(1-84), with their cross-reactivities varying from 35% to 71%. These peptides had minimal effect on the recovery of hPTH(1-84), for which analytical recoveries were 88% to 97%. The mid-molecule peptide, hPTH(44-68), did not react in this assay and only minimally inhibited the recovery of hPTH(1-84), even at very high concentrations. The carboxyl-terminal peptide did not cross-react in the assay and did not interfere with the recovery of hPTH(1-84), even at very high concentrations. PTH-related peptide(1-34) had no immunoreactivity in this assay.

The dilutions of the samples from four patients with above-normal concentrations of PTH paralleled the standard curve. Plots of the expected (x) vs measured (y)
concentrations had regression slopes varying between 0.85 and 1.05 \( (r = 0.9993-0.9997) \).

Comparison with Other Assays

The comparison with the bio-PTH assay is illustrated in Figure 3 and Table 2. Figure 3 displays the 169 data points with values <20 pmol/L in both assays. On the average, the two assays agree well, but there is substantial scatter, particularly at lower concentrations. For normal subjects, the ranges were <1 to 6.3 pmol/L in the bioassay and 0.9 to 6.5 pmol/L in the chemiluminescent assay. When results for samples with very high concentrations were compared, specimens from patients with chronic renal failure gave comparable values in both assays (regression slope of 0.89), whereas some of the specimens from patients with parathyroid tumors gave markedly greater results in the bioassay (regression slope, 0.51). The cause of this difference is not known but may be related to the small number of samples compared or to differences in activity of various forms of PTH in the specimens. The relatively low correlation in the normal range is probably related to the imprecision of the bioassay.

Figure 4 and Table 2 show a comparison of the ICMA with the Nichols Allegro PTH assay. The values for both assays are expressed in the units used in the Allegro assay, ng/L (pg/mL). Figure 4 illustrates that 131 of the 141 samples assayed had values <120 ng/L in both assays. The assays agree well, especially in samples from patients with parathyroid adenomas. However, there appears to be a difference in calibration, with the lower-concentration samples having Allegro values <10 ng/L higher than by the ICMA. We also observed differences when the Nichols standards were measured in the ICMA. Their zero standard was assayed as 0.7 ng/L in the ICMA, whereas the ICMA results for their other standards were approximately one-third of the stated Nichols values. The matrix used in the Nichols standards is not known, but may be different from human plasma and may have caused interference in the ICMA. In the comparisons involving clinical samples, the two assays correlated well, with the ICMA having only slightly lower values.

Clinical Correlation Studies

The distributions of PTH values for each of the clinical groups are illustrated in Figure 5. The approximate central 95% range for healthy younger women and men was 1.0–5.0 pmol/L. The median value in 87 women younger than 50 years was 2.6 pmol/L, whereas the median in 82 older women was 3.1 pmol/L; the median in 93 men was 2.1 pmol/L. Eleven of the women ≥50 years had PTH concentrations >5.0 pmol/L, and the lowest value in this age group was 1.6 pmol/L. The age relationships for PTH values in these healthy adults is further illustrated in Figure 6. No age trend was noted for men, but PTH increased progressively with age in women older than 50 years at an approximate rate of 0.5 pmol/L per decade.

The 110 patients with parathyroid adenomas were 29 men and 81 women, mean age 58 years (range, 31–83 years). The mean preoperative calcium and phosphorus concentrations were 2.79 (112) and 0.87 mmol/L (27

![Table 2. Deming Regression Statistics for Method Comparisons](image)

| Group          | n  | Slope | \( S_y | x \) | \( r \) |
|----------------|----|-------|---------|-------|
| ICMA (y) vs Mayo Bio assay (x), in pmol/L |    |       |         |       |
| Normal         | 49 | 0.58  | 0.87    | 0.67  |
| Parathyroid adenoma | 45 | 0.51  | 3.76    | 0.92  |
| Chronic renal failure | 30 | 0.89  | 12.63   | 0.82  |
| Miscellaneous  | 71 | 0.80  | 2.47    | 0.89  |
| Total          | 195| 0.72  | 6.13    | 0.85  |
| ICMA (y) vs Nichols Allegro Assay (x), in ng/L |    |       |         |       |
| Normal         | 37 | 0.78  | 10.73   | 0.91  |
| Parathyroid adenoma | 22 | 1.12  | 21.60   | 0.99  |
| Chronic renal failure | 12 | 0.51  | 85.31   | 0.85  |
| Miscellaneous  | 70 | 0.78  | 11.07   | 0.91  |
| Total          | 141| 0.63  | 35.15   | 0.89  |

Only values <120 pmol/L are plotted.

Fig. 3. Comparison with Mayo bio-PTH assay for 45 patients with hyperparathyroidism (HPT), 49 normal subjects (NML), 30 patients with chronic renal failure (CRF), and 71 patients with miscellaneous disorders (Misc)

Fig. 4. Comparison with Nichols Allegro assay for 22 patients with hyperparathyroidism (HPT), 37 normal subjects (NML), 12 patients with chronic renal failure (CRF), and 70 patients with miscellaneous disorders (Misc)

Only values <120 pmol/L are plotted.
Of the 10 patients with hypercalcemia of malignancy (7 men, 3 women; mean age 65 years), 9 had below-normal PTH values by ICMA and 1 had a value of 2.4 pmol/L. The calcium concentrations in this group ranged from 2.72 (109) to 3.74 mmol/L (150 mg/L) [mean, 3.17 mmol/L (127 mg/L)]. The malignancies consisted of three lymphomas, three squamous cell carcinomas, two ileal cell carcinomas, one prostate cancer, and one multiple myeloma. The patient with a normal PTH concentration was a 68-year-old woman with a diagnosis of ileal cell carcinoma and a calcium concentration of 3.74 mmol/L (150 mg/L).

Chromatographic Profiles

Figure 7 shows the distribution of ICMA PTH concentrations according to the elution fraction number. The three pools of plasma from patients with chronic renal failure each had immunoactivity that eluted in the

mg/L), respectively. The mean creatinine concentration was 97.2 μmol/L (11 mg/L), and all but three patients had creatinine concentrations <176.8 μmol/L (<20 mg/L). The mean preoperative PTH concentration was 12.1 pmol/L, and the median value was 8.8 pmol/L. All but 10 patients (91%) had preoperative plasma PTH concentrations >5.0 pmol/L.

The 91 patients with chronic renal failure were subclassified into three groups, according to the activity on our bio-PTH assay. As previously shown (10), bio-PTH values ≤5.0 pmol/L had a sensitivity of 100% and a specificity of 93% for the presence of either low-turnover bone disease or osteomalacia, whereas values ≥23.4 pmol/L had a 92% sensitivity and 83% specificity for secondary hyperparathyroidism, by comparison with bone biopsy studies. As Figure 5 shows, all but five of the patients with renal failure were classified the same way by the bio-PTH assay and ICMA at the 5 pmol/L decision value, but more overlap was found at the 24 pmol/L decision value. Three patients with bio-PTH concentrations <24.0 pmol/L had PTH concentrations of 36.5, 38.5, and 48.2 pmol/L by ICMA, whereas six patients with bio-PTH assay values >24.0 pmol/L had lower ICMA values, ranging from 17 to 25 pmol/L.

Fig. 5. Distribution of PTH values in 87 healthy women younger than 50 years, 82 healthy women older than 50 years, 93 healthy men, 110 patients with hyperparathyroidism (HPT), 91 patients with chronic renal failure, and 10 patients with hypercalcemia of malignancy

Dotted lines represent normal reference range

Fig. 6. Distribution of PTH with age in 93 men and 168 women

Dotted lines represent normal reference range for women, and solid lines represent normal reference range for men

Fig. 7. Chromatographic profiles of elution of PTH pools from Bio-Rad P-10 column

The void volume is 65 mL, and each fraction is 3 mL. (Top) The dotted line represents PTH(1–84); the dashed line represents PTH(1–34). The three solid lines represent pools of plasma from patients with secondary hyperparathyroidism. (Bottom) The dashed line represents the combination pool of 10 pmol/L PTH(1–84) and 5 pmol/L PTH(1–34). The solid line represents the pool from patients with primary hyperparathyroidism

632 CLINICAL CHEMISTRY, Vol. 38, No. 5, 1992
same fractions as hPTH(1-84). None of the pools contained evidence of immunologically active smaller molecular-mass fragments eluting in the fractions corresponding to hPTH(1-34). For each of the renal failure studies, pools of the three fractions with highest immunoreactivity showed substantial activity on the bio-PTH assay, whereas pools of fractions corresponding to hPTH(1-34) elution had undetectable bio-PTH activity. Figure 7 (bottom) shows the elution profile of a combination of hPTH(1-84) and hPTH(1-34), compared with the elution of pooled plasma from patients with primary hyperparathyroidism. Substantial bioactivity was found in pools of fractions 31, 32, and 33, corresponding to hPTH(1-84), and fractions 39, 40, and 41, corresponding to hPTH(1-34) in the standard preparation; however, bioactivity was found in only fractions 31, 32, and 33 of the hyperparathyroid plasma pool. Thus we found no substantial concentration of circulating hPTH(1-34) in the plasma of patients with renal failure or patients with primary hyperparathyroidism.

Discussion

PTH, like many circulating peptide hormones, has multiple metabolic forms. Both intact PTH and various fragments are found in venous blood. The fragments are secreted directly by the parathyroid glands and are derived from renal and hepatic cleavage of intact PTH (11). The composition of the fragments appears to vary with metabolic activity and disease state. Carboxyl-terminal fragments are most prevalent, and their relative concentration increases with both hypercalcemic conditions and renal failure (12). The amino-terminal fragments are cleared rapidly and generally are found in very low concentrations or are nonexistent in peripheral blood (1, 11, 13), although some investigators have found substantial concentrations in patients with renal failure (14-16).

Segre et al. (13), using region-specific antisera and column fractionization techniques, showed that 5-25% of immunoreactive PTH in peripheral circulation is intact PTH; they detected no amino-terminal fragments. Roos et al. (14) found a moderate amount of M, 3400 amino-terminal fragment in parathyroid extracts but relatively low amounts in immunoextracts of peripheral plasmas. Goltzman et al. (15), using a cytochemical bioassay, found no biologically active amino-terminal fragments in three nonuremic patients with hyperparathyroidism, whereas three uremic patients with secondary hyperparathyroidism and severe skeletal involvement and undergoing chronic hemodialysis had approximately half of their bioactivity as amino-terminal fragments. Nissenson et al. (16), using a renal adenylate cyclase assay, found two peaks of bioactivity in parathyroid venous effluents from each of five patients with hyperparathyroidism. The first peak corresponded to PTH(1-84), and the second peak was intermediate between PTH(1-84) and PTH(1-34). The variation in these findings may be related to the specificity of the assay systems and the severity of the disease states.

The current assay measures intact PTH best and reacts with amino-terminal fragments at approximately half potency on a molar basis, but it does not detect mid-molecule or carboxyl-terminal fragments. It is generally agreed that measurement of intact PTH is the best diagnostic method for parathyroid disorders (1, 7-9, 17-25). Each of the published assays uses a combination of an amino-terminal antibody with either mid-molecule or carboxyl-terminal antibodies, which therefore do not measure amino-terminal fragments. The addition of the second solid-phase antibody against PTH(1-44) in this assay provides for the detection of amino-terminal fragments in addition to intact PTH. This enhanced detection probably is not necessary in most patients, but it could be important in some patients, especially those with renal failure. The enhanced detection also should make the assay more robust by ensuring detection of PTH even if in vitro proteolysis cleaves the intact molecule into amino-terminal and carboxyl-terminal fragments (although this premise has not been experimentally proven).

Newman and Ashby (8) showed that sera from patients with pancreatitis significantly degrade PTH(1-84), which causes decreased values on the Nichols Allegro intact assay. Although this stability issue has not been formally studied with this assay, theoretically we would expect stability to be less of a problem because amino-terminal fragments are detected. In addition, the use of antisera to a mid-molecule fragment, hPTH(44-68), instead of carboxyl-terminal antisera, provides further assay robustness by preventing interference by carboxyl-terminal fragments, which may be present in large concentrations in the plasma of patients with renal failure.

Sandwich assays based on antisera against hPTH(53-84) show significant negative interference in the measurement of intact hormone when carboxyl-terminal fragments are present (8). Theoretically, an assay could be designed with only the amino-terminal antisera; however, the hPTH(44-68) region of PTH is a strong immunological binding site, and inclusion of antisera to this site provides greater analytical sensitivity. In addition, the use of affinity-purified polyclonal antibody instead of monoclonal antibody provides greater assay sensitivity (3). The Ciba-Corning ICMA, which uses monoclonal anti-hPTH(44-68) as the capture antibody on paramagnetic particles and the same hPTH(1-34) signal system as this assay, has a detection limit of only 0.15 pmol/L (1.4 ng/L) (23), approximately twice the amount detectable by this assay.

The reference range for healthy subjects reported in this study is similar to that for other intact PTH assays (7, 17-25). Our comparison with the Nichols Allegro assay shows that the two assays produce similar values, although the stated upper limit of normal of their assay is 65 ng/L compared with 5.0 pmol/L (47 ng/L) for this assay. Both Newman and Ashby (8) and Endres et al. (9) questioned the stated upper limit of the Allegro assay and reported limits of 50 and 44 ng/L, respectively.

There is no uniform agreement in the literature.
concerning the age dependency of PTH values. Most investigators have found higher PTH values in older women (26-29). Two studies report an increase in PTH in older men as determined with an amino-terminal assay (26, 28). Sokoll et al. (30) reported mean values of 30 and 30.3 ng/L in the fifth and sixth decades, respectively, with mean values increasing to 33.5 and 36.4 ng/L in the seventh and eighth decades, respectively, in women with use of an assay for intact PTH; however, they found that these changes were significant only at \( P = 0.06 \). Bouillon et al. (23) reported that 45 healthy elderly men (ages 78 ± 5 years) had intact PTH concentrations not significantly different from those in 94 younger adults (ages 38 ± 11 years). Our data support the increase of PTH concentration with age in women after age 50 and the constant values of PTH in men, although we did not test healthy men older than 70.

The published performance of assays of intact PTH in the diagnosis of primary and secondary hyperparathyroidism has been exceedingly good, with reported detection rates of 97% to 100% (7-9, 18-20, 22-25). The detection rate found with this assay (91%) is lower than that in other reports, but this difference probably reflects the patient distribution used for evaluation rather than the assay performance. The Nichols Allegro assay reportedly gives very high detection rates, yet in comparison with the Mayo ICMA, 5 of 20 patients with surgically proven hyperparathyroidism had normal values in the Allegro assay. Even when the upper limit of normal is adjusted to 5.0 pmol/L, three patients would have had normal values reported by the Allegro (the same three had normal values by the Mayo ICMA). Endres et al. (9) also reported above-normal values in only 21 of 29 cases of hyperparathyroidism with use of Nichols Allegro assay, in which the upper limit of normal was set at 65 ng/L. In a recent editorial in this journal, Nusbaum (31) reported that the National Institutes of Health consensus conference on asymptomatic hyperparathyroidism yielded agreement that immunometric assays measure above-normal concentrations of PTH in 90% of hyperparathyroid patients, and that the remaining 10% of patients have serum PTH (1-84) values near the upper limit of normal.

This assay has been in clinical use at the Mayo Clinic for the past three years and has had an overall sensitivity of 88% in 361 cases of surgically proven primary hyperparathyroidism (32). Statistically, the sensitivity of the Mayo ICMA is only slightly better than that found with our mid-molecule/carboxyl-terminal PTH radiomimunoassay (32), which had a sensitivity of 86% in 1061 patients with surgically proven parathyroidism (32, 33). However, the Mayo ICMA is easier to interpret clinically because the carboxyl-terminal fragments in renal failure do not cause above-normal values. High values in the mid-molecule/carboxyl-terminal radiomimunoassay could be caused by renal failure or hyperparathyroidism, and because some patients have both disorders, it is difficult to assess the pathogenesis of increased PTH concentrations. However, the mid-molecule/carboxyl-terminal assay measures the time-averaged metabolic products of PTH, whereas the Mayo ICMA measures only the short-lived intact and amino-terminal fragments.

Minisola et al. (34) reported falsely normal intact PTH values in patients with mild primary hyperparathyroidism caused by episodic secretion and short half-life of intact PTH. When Kitamura et al. (35) studied this episodic fluctuation, they found 23 discrete PTH pulses per day, which caused an average increase of PTH above nadir of 32%. Harmes et al. (36) used spectral analyses to show that the peaks of intact PTH are concordant with peaks in PTH (44-68), both having maximal spectral power at 45-min periods, but the spectrum for intact PTH lasted sevenfold longer. These reports illustrate the disadvantage of measuring the rapidly changing biologically active components of PTH rather than the more stable carboxyl-terminal waste products; however, this did not decrease the overall sensitivity for detection of hyperparathyroidism and is substantially offset by the enhanced assay specificity, especially in patients with renal failure.

We deeply appreciate the expert assistance of Ms. Ione Schloegel in performing the chromatographic separations. We are also indebted to the clinicians and surgeons of the Mayo Clinic for the patients' studies included in this report.

References
13. Segre GV, Habener JF, Powell D, Tregear GW, Potts JT Jr. Parathyroid hormone in human plasma: immunochemocharac-


