Parathyroid Hormone: Radioimmunoassay and Clinical Interpretation

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ABSTRACT

A radioimmunoassay for serum immunoreactive parathyroid hormone (iPTH), which has had widespread clinical use for five years, is described in detail. The iPTH results in large groups of patients are reported, and are discussed in relation to the specificity of the assay and in relation to other assays. The assay has excellent precision and is highly proficient in discrimination of groups of patients. Ninety-three percent of 412 patients with surgically proven primary hyperparathyroidism were confidently separated from normal subjects or patients with hypercalcemia owing to other causes, while 86 percent of 160 patients with chronic renal failure and secondary hyperparathyroidism had iPTH values more than 2 S.D. above the normal mean. Results in patients with ectopic hyperparathyroidism were lower than in primary hyperparathyroidism although these groups showed considerable overlap. The antiserum used in this assay for iPTH appears to be specific for the carboxy-terminal region of the secreted or intact form of PTH but recognizes predominantly the secreted form rather than carboxy-terminal fragments believed to be in the circulation. It does not recognize amino terminal fragments. The assay is useful in selective venous catheterization for preoperative localization of hyperfunctioning parathyroid tissue.

Introduction

The first radioimmunoassay (RIA) procedure for human immunoreactive parathyroid hormone (iPTH) was described in 1963. However, this assay was not able to measure iPTH in all normal subjects tested, and nearly half of a group of surgically confirmed hyperparathyroid patients had normal iPTH levels. Subsequently, Reiss and Canterbury in 1968 and Arnaud and associates in 1971 have reported RIA procedures for iPTH which are highly proficient in differentiation of hyperparathyroidism. The key assay components in this regard have been their respective antiseraums which have better specificity and sensitivity for the predominant forms of circulating iPTH.

Only in the past three to four years has the measurement of iPTH become a routine clinical tool in the differential diagnosis of hyperparathyroidism from other disorders of calcium homeostasis. A number of factors have contributed to this delay in widespread clinical application of the parathyroid hormone RIA. Less than a handful of antisera specific for the predominant forms of circulating iPTH have been prepared and these are of extremely limited supply. There has not been a consistent commercial or institutional source of highly purified PTH for use as standard. Although human PTH has been
isolated from surgically excised tissue and partially characterized, only one or two investigators have had enough for use as an RIA standard. Most workers have employed bovine PTH as standard and as the immunogen for antiserum production. The lack of cross-reactivity between bovine and human species has hindered progress in the accurate measurement of iPTH levels. The generally lower concentration of circulating iPTH compared to many other peptide hormones, the high affinity of PTH for glass and other surfaces as well as the susceptibility of radiolabeled PTH to degradation are additional factors which have hampered the efforts of most workers.

However, the major factor contributing to the delay in widespread clinical use of the PTH assay has been the variation among different assays in antiserum specificity which has expressed itself primarily as variation in assay results and the ability to differentiate hyperparathyroid patients from normal subjects. In recent years, it has become clear that this variation is a result of the immunochemical heterogeneity of PTH in serum first described by Berson and Yalow, and later demonstrated by several groups.

Arnaud and his colleagues have recently described their investigation of the influence of this immunoheterogeneity of circulating PTH on radioimmunoassay results. Their conclusions can be briefly summarized as follows:

1. PTH is secreted by parathyroid glands into the bloodstream primarily as a large molecule (molecular weight 9500 daltons) referred to as intact PTH.

2. This 9500 dalton PTH is thought to have a relatively short half life (less than 0.5 hours) in serum.

3. The major portion of circulating PTH appears to be comprised of one or more smaller peptide “fragments” of intact PTH (6000 to 8000 daltons, 4000 to 5000 daltons).

4. These smaller molecules have only been detected by antisera with specificity directed towards the COOH-terminal region of the intact PTH molecule.

5. These smaller PTH fragments are thought to have relatively longer half-lives in serum (hours to days) and, thus, accumulate to greater concentrations than intact PTH.

6. Antisera with specificity for COOH-terminal PTH fragments are considered to be better in the differential diagnosis of hyperparathyroidism, whereas antisera with specificity for the NH₂-terminal region of intact PTH better reflect acute changes in glandular secretion of PTH.

7. The phenomenon of immunoheterogeneity of circulating PTH is probably the major determinant of the results of serum iPTH measurement with a given RIA. Knowledge of the immunospecificity of each assay used in clinical evaluation of patients is crucial to the interpretation of the test results.

This author has previously reported on the application of his radioimmunoassay for iPTH in the diagnosis of ectopic hyperparathyroidism, in evaluation of patients with pseudogout, and in a case of acute alcoholic rhabdomyolysis with acute renal failure and transient hypercalcemia. Other groups have reported on studies of a patient apparently secreting a biologically ineffective form of PTH, and a patient with Albright’s hereditary osteodystrophy and parathyroid hormone deficiency, in which this assay for iPTH was used.

This report details for the first time the RIA method for iPTH developed by the author and studies of the specificity of the antiserum, GP-204, which was used in all of the reports cited. The report also summarizes the data obtained with this assay on patient specimens over a five year period, and attempts to present a unified interpretation of these results and the previous reports based on the specificity of the assay.
Materials and Methods

BOVINE PARATHYROID HORMONE

Bovine PTH was purified to a specific biological activity of 3000 USP units per mg and was assessed to be homogeneous as described. This preparation was used for radioiodination and as the primary assay standard.

ANTI-PTH SERUM

Bovine PTH, purified through the G-100 gel filtration step to a specific biological activity of 300 to 500 USP units per mg, either alone or conjugated to bovine serum albumin as described, was dissolved in 0.15 M NaCl at a concentration of 4 mg per ml and homogenized with an equal volume of complete Freund's adjuvant. One ml (2 mg PTH or conjugate) of homogenate was injected s.c. in multiple sites behind the shoulders of 300 to 400 g female, white guinea pigs. A total of three immunizations were given over a six week period and always followed by bleedings (by cardiac puncture) ten days later. After a three month rest, the animals received s.c. injections (PTH only, no conjugate) in multiple sites in the groin region and were again bled ten days after immunization. Following the first series of injections, none of ten animals showed significant anti-PTH titer. However, three of five animals receiving PTH after the rest period showed markedly high titers after the first such injection. The antiserum which has been routinely used in this PTH assay, GP-204-5 (5 indicating the fifth bleeding of animal 204), was chosen for use after evaluation of all antisera for sensitivity as described. Radioiodinations were performed at two week intervals.

RADIOIODINATED PARATHYROID HORMONE

PTH was labeled with using a modification of previous methods. One mCi Na diluted to 20 μl with 0.5 M sodium phosphate buffer pH 7.45, was reacted with 2.5 μg PTH in the presence of 70 μg chloramine T. After 10 to 20 seconds the reaction was stopped with 192 μg sodium metabisulfite, and 200 μl of normal human serum was added. The contents of the reaction tube were transferred to the top of a dry column of cellulose CTF packed to a height of 2 ml in the bottom half of a 10 ml polystyrene pipet fitted with a glass wool plug. The column was washed three times with 1.5 ml of 0.02 M sodium barbital buffer pH 8.6, and eluted with five 1.0 ml volumes of 20 percent acetone in 1% acetic acid. The eluate with the most 125I-PTH was stored at -76° in 0.1 ml aliquots. Droplets from the eluate and from the original reaction mixture were analyzed by chromatoelectrophoresis as described. Radioimmunoassay procedure

The RIA procedure was similar to that of Arnaud, et al. and employed a non-equilibrium method of incubation. The assay diluent consisted of 2 percent normal guinea pig serum in 0.02 M sodium barbital buffer pH 8.6, and contained 250 KIU per ml of Trasylol as was first described for glucagon. Disposable 12 x 75 mm soft glass culture tubes were numbered consecutively according to a standard protocol, which provided for quadruplicate tubes for total counts, for correction for binding of 125I-PTH to glass, for incubation damage, for serum inhibition of charcoal binding of 125I-PTH, and as zero standards. The protocol also provided for duplicate standards which were pipetted as doubling dilutions of bovine PTH ranging from 4000 pg per tube to 7.8 pg per tube and for unknown specimens and quality control serum pools to be run with 50, 100 and 200 μl of serum.

The standard and serum tubes contained

†Eastman Kodak Co.
§H. Reeve Angel and Co.
|| FBA Pharmaceutical Co.
100 μl of antiserum GP-204-5 diluted 1:45,000 with diluent previously described and either 200 μl of standard (diluent for the zero standards) or the indicated amount of serum plus diluent (if necessary) to make the final volume in all tubes 300 μl. The tubes for total counts were left empty. The tubes for glass bind controls and damage controls received 300 μl diluent each, and the tubes for serum inhibition of charcoal binding of 125I-PTH contained either 50, 100 or 200 μl of a normal serum pool plus diluent to a final volume of 300 μl. Antiserum and diluent were added to tubes using an automatic pipette, while all serum specimens and pools were pipetted using precision Lang-Levy micropipets. The racks of tubes and all solutions were placed in ice water baths during the pipetting operations. When the pipetting was finished the racks were covered with Saran wrap and incubated at 4° for two days in a reciprocating shaker.

The racks were uncovered and placed in ice water baths. Using the automatic pipette, 200 μl of diluted 125I-PTH (containing about 2000 CPM of a preparation of 250 to 350 μCi per μg in specific radioactivity) were added to the total counts tubes, and 200 μl diluted 125I-PTH and 500 μl diluent were added to all other tubes. The racks were recovered and replaced in the shaker for three additional days at 4°.

Antibody-bound and free 125I-PTH were separated by a modification of the method of Herbert et al. In an Erlenmeyer flask were placed 13 g charcoal, 1.3 g Dextran T70, 304 ml water, and 7.8 ml normal human serum. The mixture was stirred magnetically overnight at 4° and stored at 4° until used.

The racks were uncovered and placed in ice water baths. The total counts tubes were corked and set aside. Barbital buffer, 0.02 M, pH 8.6 (1.5 ml) was added to the tubes for glass binding controls, and 1.5 ml of the dextran-coated charcoal suspension was added to all other tubes. The suspension was continually mixed in an ice water bath over a magnetic stirrer during the pipetting. The tubes were mixed on a vortex mixer, and the tubes containing charcoal were centrifuged at 3000 rpm at 4° for 10 minutes. All tubes were aspirated, and 2.5 ml water was added to each tube. The tubes were mixed on the vortex mixer and those containing charcoal were centrifuged again. All tubes were aspirated, corked and, together with the total counts tubes, counted in the automatic gamma scintillation spectrometer for five minutes each.

CALCULATIONS

The individual tube counts were subtracted from the total counts average in order to estimate the amount of radioactivity belonging to the antibody-bound 125I-PTH fraction, because charcoal binds intact free 125I-PTH, but not antibody-bound 125I-PTH or damaged 125I-PTH. However, for each tube this calculation also included a correction for the average 125I-PTH bound to glass which was subtracted from the counts for each tube. In addition, the standards were corrected for incubation damage by subtraction of the average of the damage controls from the total counts average, while all tubes containing serum and antiserum were simultaneously corrected for incubation damage and serum interference with charcoal binding of 125I-PTH by subtraction of the average of the latter controls from the total counts average for each serum volume employed.

All standard and serum tubes bound count values thus calculated were divided by the average zero standard bound counts to determine the percent bound in each tube. The standards were subjected to linear regression analysis of the logit transform of the percent bound versus the log of the PTH concen-
From this analysis, the slope and y-intercept of the standard line were obtained. Using the formula for a straight line (where $y = \text{logit of the percent bound}$ and $x = \log$ of the PTH concentration), the quantity of iPTH in each tube for the quality control and unknown sera was computed. These values were then multiplied by the appropriate factors based on the serum volumes per tube to get the amount of iPTH per ml of serum.

**SERUM CALCIUM PROCEDURE**

Serum calcium was determined by atomic absorption spectroscopy on each serum specimen assayed for iPTH and on each quality control pool used for the iPTH assay in order to monitor quality control of the calcium analyses.

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**Results**

**RADIOIODINATED PARATHYROID HORMONE**

In figure 1 are shown the radiochromatogram scanner tracings of the chromatoelectrophoretic analyses of the original reaction mixture and the acetone-acetic acid eluate from the cellulose column purification of one preparation of $^{125}$I-PTH. The specific activity in this instance was 360 $\mu$Ci per $\mu$g as calculated by both planimetry and digital integration of the radiochromatogram tracing of the original reaction mixture. In over 80 consecutive radioiodinations with $^{125}$I-PTH as described above, the specific activity has been consistently greater than 250 $\mu$Ci per $\mu$g. After purification, 98 to 99 percent of the total radioactivity was in the intact $^{125}$I-PTH peak as shown.

Several $^{125}$I-PTH preparations have been tested for ability to bind to antiserum by incubation of trace quantities with excess antiserum. After correction for non-specific counts in the supernate, 80 to 92 percent of the radioactivity was found to be in the antibody fraction using the charcoal system.

**ANTISERUM SENSITIVITY AND SPECIFICITY**

In figure 2 is shown a typical standard line obtained with pure bovine PTH when plotted by the logit method. The logit transformation, when done by a computer using linear regression analysis and statistical evaluation of significance, is capable of making a confident, reliable estimate of assay sensitivity which is generally at a lower concentration of hormone than might be estimated by visual examination of the plotted data. With this PTH assay, the lower limit of detection has generally been 40–80 pg per ml.

In figure 2 are also shown points for multiple dilutions of serum specimens from a patient with primary hyperparathyroidism and a patient with ectopic hyperparathyroidism owing to a metastatic hyperneph-
Figure 2. Percent antibody-bound $^{125}$I-PTH as a function of the PTH concentration (pg per ml) for the bovine standard PTH (□) or as a function of the serum concentration (ng per ml) for a serum from a patient with a surgically proven case of primary hyperparathyroidism (○) or for a serum from a patient with ectopic hyperparathyroidism (♦). The plot was prepared using the logit transform method (see text) and special graph paper (Codex Book Co., Norwood, MA) which plots the logit of the percent bound as a function of the log of the PTH concentration.

In an effort to assess further the specificity of antiserum GP-204, the synthetic bovine PTH amino terminal peptide of residues 1-34* was tested for cross-reactivity with the purified bovine PTH standard. As shown in figure 3, only 360 pg of bovine PTH standard was required to inhibit 50 percent of the antibody-bound $^{125}$I-PTH, but 2 μg or a 5555-fold greater amount of the synthetic 1-34 peptide was necessary to achieve the same degree of inhibition. On a molar basis, nearly 14,000 times as much of the 1-34 peptide was required. This molar cross-reactivity of 0.007 percent for the synthetic peptide (compared to the bovine standard at 100 percent) indicated that the antigenic determinants recognized by antiserum GP-204 were most likely situated between residues 35 and 84.

PRECISION

Quality control of the assay was monitored through the routine use of three different serum pools in every assay run. Generally, these pools consist of two normal serum pools with mean values in the upper and lower regions of the normal range, respectively, and one hyperparathyroid serum pool. One of the normal pools is replicated at the beginning, middle and end of each run as a check against drift. In figure 4 are shown the results obtained with one hyperparathyroid serum pool (HPTH #1) over a one year period. The line with the longer dashes indicates the time at which several minor changes in methodology were introduced resulting in a marked improvement in precision as shown. The coefficient of variation observed with this pool during this latter period was an excellent 9.24 percent, in contrast to a value of 15.29 percent obtained prior to the improved methodology. Other serum pools currently in use have had similar coefficients of variation: HPTH #2, 9.91 percent (160 determinations); normal pool B-4, 8.89 percent (490 determinations); and normal pool B-5, 9.28 percent (459 determinations.)

Figure 3. Percent antibody-bound $^{125}$I-PTH as a function of the concentration of peptide (pg per ml) for the standard bovine PTH (○) or the synthetic bovine amino terminal peptide (♦). The ordinate was plotted linearly, and the abscissa was plotted as the log of the PTH concentration.
RESULTS IN NORMAL SUBJECTS AND PATIENTS

In the most recent evaluation of iPTH levels in healthy individuals with this assay, 93 company employees who were about equally divided as to sex, whose ages ranged from 20 to 62 years, who had had a physical examination within 18 months and who were known not to have any chronic disorders or to be taking any chronic medications were studied. In this group of volunteers, a mean iPTH value of 255 pg per ml with a standard deviation of 46 pg per ml was obtained; the mean ± 2 S.D. range was 163 to 347 pg per ml. The distribution of iPTH values was normal, with 71 percent of the values within the mean ± 1 S.D. and 97 percent within the mean ± 2 S.D. In a prior study 1.5 years earlier, the mean iPTH value on a different group of 61 normal subjects had also been 255 pg per ml. although the S.D. had been 82 pg per ml. The lower S.D. in the more recent study is thought to be largely due to improvements in methodology resulting in better precision and quality control. In both studies, 100 percent of all the normal subjects tested had measurable iPTH values.

In the same group of 93 individuals described, a mean serum calcium value of 9.3 mg per dl was obtained. The mean ± 2 S.D. range was found to be 8.8 to 10.0 mg per dl, a range in excellent agreement with that used at the Mayo Clinic. In figure 5 are shown the results of the iPTH determinations in the 93 normal subjects as well as 412 patients with surgically confirmed primary hyperparathyroidism, 269 patients with primary hyperparathyroidism (clinically diagnosed, but not operated to the author’s knowledge), 119 patients with ectopic hyperparathyroidism owing to non-endocrine cancers, 160 patients with secondary hyperparathyroidism owing to chronic renal failure, and 73 patients with low iPTH values owing to either idiopathic or surgical hypoparathyroidism or to hypercalcemia not caused by hyperparathyroidism.

Among the group of 412 surgically confirmed primary hyperparathyroid patients (365 or 89 percent with single adenoma, 43 or 10 percent with hyperplasia or multiple adenomata, 4 or 1 percent with carcinoma), 228 (55.3 percent) had iPTH values.
In contrast to the primary hyperparathyroid groups, the patients with ectopic hyperparathyroidism generally had lower iPTH values. The highest result among this group of 119 patients was 876 pg per ml, and the lowest level was 142 pg per ml. Although this group had a mean serum calcium value of 13.0 mg per dl which was higher than the mean serum calcium level of 12.3 mg per dl for the surgically confirmed primary hyperparathyroid group, the mean iPTH value for the ectopic group was only 296 pg per ml compared to 520 pg per ml for the primary group. Only 22 (18.5 percent) of the ectopic hyperparathyroid patients had iPTH levels greater than the mean + 2 S.D., and as shown in Figure 5, the distribution of iPTH results was clearly lower than for the primary hyperparathyroid groups.

The group of 160 patients with chronic renal failure and secondary hyperparathyroidism had iPTH values generally higher than either of the primary hyperparathyroid groups. The highest iPTH value in this group was 9151 pg per ml, and the lowest value was 231 pg per ml. The mean iPTH level for this group was 887 pg per ml compared to 520 pg per ml for the surgically confirmed primary hyperparathyroid group, and the distribution of results was clearly higher than in both primary hyperparathyroid groups. Altogether, 137 (86 percent) of the points in this group had values greater than 2 S.D. above the mean of the normal group.

The group of 70 patients with low iPTH values included 22 with idiopathic hypoparathyroidism and 18 with surgical hypoparathyroidism for a total of 40 hypoparathyroid patients. This low iPTH group also included 30 patients with hypercalcemia due to causes other than hypoparathyroidism. Of these, seven had multiple myeloma, five had milk alkali syndrome, three had hypervitaminosis D, three had Paget’s disease of bone, two had sarcoidosis and 10...
had various cancers with bony metastases and no evidence of ectopic hyperparathyroidism. The highest iPTH value in this group of 70 patients was 281 pg per ml and the lowest iPTH result was 2 pg per ml. The latter value was undoubtedly below the lower limit of detection of the assay, but was the value obtained by the computer on that patient specimen. Only three of the total of 70 patients had iPTH results that could be referred to as "non-detectable." The mean iPTH value of this group was 182 pg per ml, which was about 1.5 S.D. below the normal mean value of 255 pg per ml. As shown in figure 5, the upper half of this low iPTH group roughly overlaps the lower half of the normal group.

In figure 6 are shown the points obtained by plotting serum iPTH as a function of the corresponding serum calcium value for the normal subjects and for all patients in the surgically confirmed primary hyperparathyroid, secondary hyperparathyroid and low iPTH groups whose iPTH points were contained in figure 5, with the exception of one point that exceeded 6000 pg per ml and one point obscured by the bar representing the normal range for serum calcium. The 93 points for the normal subjects showed a highly significant negative correlation (r = -0.412, p < 0.001) between serum iPTH and serum calcium when subjected to linear regression analysis. The solid line through the normal group is the regression line and the dashed lines are parallel lines 2 S.D. above and below the regression line. All but one of the points for the normal group fall within the area encompassed by the dashed lines.

The 412 points for patients with surgically confirmed primary hyperparathyroidism shown in figure 5 are all plotted in figure 6 as the large solid circles. Those points having serum calciums greater than 16 mg per dl were plotted as having serum calciums of 16.0. All but one of the points for patients with hypercalcemia not due to hyperparathyroidism as described above are plotted in figure 6 as the open triangles. Visual inspection of these data in figure 6 showed a markedly improved discrimination between the surgically confirmed primary hyperparathyroid group, the normal group, and the nonhyperparathyroid hypercalcemic group compared to the poor discrimination obtained when only serum iPTH levels are used (figure 5).

However, there was still a residual amount of overlap evident in figure 6, and it was necessary to define a minimum confidence limit for the primary hyperparathyroid group in order to make the figure useful on a routine basis. Because the dashed lines parallel to the regression line effectively enclosed the normal group, the author arbitrarily extended the upper dashed line down and to the right to a serum calcium of 11.1 mg per dl, and then parallel to the abscissa at an iPTH level of 225 pg per ml which is one coefficient of variation (based on a mean value of 9.5 percent for current quality control pools) above the highest point (206 pg per ml) in the non-hyperparathyroid hypercalcemic group. This line which is the dotted line in figure 6 separated 382 (93 percent) of the total of 412 points in the primary hyperparathyroid group from the region of overlap. An additional 16 points (4 percent) are above the highest points in the nonhyperparathyroid hypercalcemic group, making a total of 97 percent of the 412 points that are not overlapping, and only 3 percent of this surgically confirmed primary hyperparathyroid group that are overlapping the non-hyperparathyroid hypercalcemic group.

The 412 points for the surgically confirmed primary hyperparathyroid group showed a highly significant positive correlation (r = 0.367, p < 0.001) between serum iPTH and serum calcium. A weaker, but significant correlation (r = 0.275, p < 0.001) was obtained between serum iPTH and adenoma weight for 142 patients for
Figure 6. Serum iPTH concentration (pg per ml) as a function of serum calcium concentration (mg per dl) in normal subjects (•), patients with surgically proven primary hyperparathyroidism (○), patients with hypercalcemia not due to hyperparathyroidism (△), patients with chronic renal failure and secondary hyperparathyroidism (■), and patients with idiopathic or surgical hypoparathyroidism (○). The mean iPTH value in the normal subjects was 255 pg per ml, and the S.D. was 46 pg per ml. The solid diagonal line was determined by regression analysis of the normal points (r = -0.412, p < 0.001). The dashed lines are parallel to and 2 S.D. above and below the regression line. The dotted line is an extension of the upper dashed line to a serum calcium of 11.1 mg per dl and thence parallel to the abscissa at an iPTH value of 225 pg per ml which is one C.V. (9.5 percent) above the highest point (206 pg per ml) for a patient with hypercalcemia not due to hyperparathyroidism. The solid bar indicates the normal range for serum calcium.
whom this weight was known; however, there was not a significant correlation ($r = 0.131, p < 0.2$) between serum calcium and adenoma weight in these patients.

Also shown in figure 6 are the points for the 40 patients with idiopathic or surgical hypoparathyroidism. All but one of these points would fall below the lower dashed line if it were extended up and to the left.

SELECTIVE VENOUS CATHETERIZATION

Preoperative selective venous catheterization of patients suspected of primary hyperparathyroidism was performed according to generally described procedures. To date, more than 150 patients have had this technique at various medical centers in the U.S. and Canada with the collected serum specimens being assayed for iPTH in the author's laboratory. In 110 (72 percent) of these groups of specimens, iPTH values significantly elevated above the level in at least one other specimen were obtained, suggesting the potential helpfulness of the technique. Lack of follow-up information on most of these patients prevents a comprehensive evaluation of the usefulness of the procedure, but the findings demonstrate that this radioimmunoassay for iPTH is capable of detecting increased parathyroid hormone secretion.

Typical results with this technique are shown in figure 7. This patient was found at surgery to have moderate hyperplasia of the parathyroid glands of the upper right, upper left and lower left poles of the thyroid, and all three glands were completely excised. The lower right parathyroid gland was judged to be grossly normal and was not removed. The iPTH levels were significantly elevated above baseline in two left superior thyroid vein specimens as well as specimens from the left middle thyroid vein, the left inferior thyroid vein, the right middle thyroid vein, the lower right internal jugular vein and the lower right innominate vein. These elevations in serum iPTH were consistent with the finding of hyperplasia, and the low level of 199 pg per ml in the right inferior thyroid vein was consistent with the apparent lack of hyperplasia in the lower right parathyroid gland.

Discussion

In five years of use, this radioimmunoassay for PTH has achieved a consistent record of performance and reliability. Several factors have contributed to this record. One has been the ability to make preparations of $^{125}$I-PTH of nearly constant specific activity and with high reactivity for the antiserum. Great care has been taken in the aliquoting and storage of all assay reagents, serum pools, etc., to protect against loss of activity and, as a result, the antiserum dilution has not been altered during this five year period. Perhaps most important has been the ample use of
quality control serum pools of varying iPTH concentration to monitor inter-assay and intra-assay variation.

One example of the consistent performance of this assay is the excellent coefficient of variation on each of several quality control pools. These values, examples of which have been given, compare very favorably with values reported by other workers. Another example was the finding of identical mean iPTH values of 255 pg per ml for two different large groups of normal subjects despite an interval of 1.5 years.

The data contained in figures 5 and 6 represent all of the data on patients in those categories on whom diagnostic follow-up information had been obtained at the time of this writing. Owing to the nature of the author's laboratory, this amounts to less than 10 percent of the specimens actually assayed. However, to our knowledge, this is the largest group of data on iPTH levels in patients reported to date.

This assay was found to measure iPTH levels in 100 percent of the healthy subjects that have been tested. The distribution of values (figure 5) has a Gaussian curve indicating that the range of iPTH values in normal subjects did not overlap the lower limit of detection of the assay which would tend to give a skewed, non-Gaussian distribution. Most other workers have reported normal ranges with a lower limit of "non-detectable." Other than the assay described here, the only assay for iPTH reported to detect iPTH in 100 percent of the normal subjects tested is that of Reiss and Canterbury.

Appreciable overlap was observed in iPTH values between normal subjects and patients with surgically confirmed primary hyperparathyroidism (figure 5), an observation which has also been made by nearly every other group, the lone exception again being the assay of Reiss and Canterbury. Arnaud and associates were the first group to demonstrate that by consideration of serum calcium values along with serum iPTH values better discrimination between the hyperparathyroid and normal groups could be obtained. This separation is made possible by their observation that in normal subjects serum iPTH and serum calcium are inversely correlated, a finding that to date has been confirmed only by Roof et al. and with our assay.

By plotting serum iPTH as a function of serum calcium (figure 6) discrimination between the primary hyperparathyroid and normal groups with the assay described here is markedly improved to a value of 93 percent, and 97 percent of the 412 hyperparathyroid patients in the group were free from overlap with either normal subjects or patients with hypercalcemia not due to hyperparathyroidism. This degree of differentiation of patients with primary hyperparathyroidism is excellent for such a large group of unselected patients.

It is perhaps disturbing that 45 percent of the patients with surgically proven primary hyperparathyroidism have iPTH values within normal limits, but this observation only serves to emphasize that a true definition of "normal" for iPTH must be based on serum calcium. It is also likely that the iPTH levels in these patients are indicative of the presence of "mild" or early parathyroid disease. Indeed, the statistically significant correlations between serum iPTH and serum calcium and between serum iPTH and adenoma weight in the surgically proven patients suggest that the iPTH levels measured with this assay are correlated with the degree of parathyroid disease.

There has been considerable discussion regarding the existence of normocalcemic hyperparathyroidism. The serum calcium procedure used in our laboratory in conjunction with the PTH assay has had a mean coefficient of variation of 2.5 percent for several recent quality control serum pools.
and has proven to be a very precise procedure. Among the 412 patients with surgically proven primary hyperparathyroidism, the lowest observed serum calcium was 10.0 mg per dl, the exact upper limit of normal. Only 14 (3.4 percent) patients had a serum calcium of 10.3 mg per dl or lower, a value that is within one coefficient of variation of the upper limit of normal. Thus, this assay was unable to confirm even one case of normocalcemic hyperparathyroidism and even allowing for laboratory error in the serum calcium technique, the number of patients with possible normocalcemic hyperparathyroidism is exceedingly low.

The observation of measurable iPTH levels in the 40 patients with idiopathic or surgical hypoparathyroidism raises the question of non-specificity of the assay. It is likely that all or much of the iPTH detected in these patients is due to background or blank in the assay. Other workers have employed hypoparathyroid serum to dilute standards and unknown serum specimens, but this was not done with this assay because of the scarcity of such serum and possible variations among patients. The Gaussian distribution of iPTH values in the normal group, the lowered iPTH values in patients with hypercalcemia not due to hyperparathyroidism, and the suppressibility of this iPTH in normal subjects during calcium infusions all support the contention that the assay is specific. Nevertheless, these levels in hypoparathyroidism do not present a practical problem in the interpretation of assay results because, as shown in figure 6, all but one of these patients had iPTH values too low for their respective serum calcium values.

As previously described, this antiserum, GP-204, has been studied for its specificity by testing with the synthetic NH₂-terminal bovine PTH peptide of residues 1-34. This synthetic peptide was not able to inhibit the binding of [¹²⁵I]PTH to this antiserum except at very high and nonphysiologic concentrations. Arnaud has described the results of his own studies of antiserum GP-204 in comparison to his antiserum, GP-1M, using both the bovine and human NH₂-terminal peptides. With each antiserum, the same result was obtained as described, and Arnaud reasoned that by exclusion of the NH₂-terminal region, therefore, both antisera were primarily directed at the COOH-terminal region of the PTH molecule, and that each antiserum should recognize both intact PTH and COOH-terminal fragments in serum. However, the results with our assay in patients with ectopic hyperparathyroidism compared to the results with the Arnaud assay, and the results with these two assays on a patient with hypoparathyroidism producing a biologically ineffective parathyroid hormone, clearly show that the respective antisera have widely different specificities. Neither antiserum has been studied with synthetic COOH-terminal peptides, and their absolute specificities are therefore not known. The available evidence, however, suggests that our antiserum GP-204 predominantly recognizes intact PTH by means of antigenic determinants somewhere between residues 35 and 84, whereas the Arnaud antiserum, GP-1M, predominantly recognizes COOH-terminal fragments also by means of antigenic determinants somewhere between residues 35 and 84. The most likely explanations for this difference in specificity are (1) one or more of the antigenic determinants for GP-204 present in intact PTH are absent in the COOH-terminal fragments which may be smaller than the 35-84 sequence, or (2) one or more of the antigenic determinants for GP-204 present in intact PTH are altered in the COOH-terminal fragments as a result of changes in tertiary structure when intact PTH is cleaved to the smaller fragments. Antiserum GP-204 may have some affinity for COOH-terminal fragments as well as intact PTH, but it is clearly much less than with antiserum GP-1M, because the latter assay has
shown higher results in patients with primary and secondary hyperparathyroidism relative to the results in normal subjects. Nevertheless, the evidence presented here clearly demonstrates the clinical usefulness of our PTH assay, which accurately reflects the status of parathyroid hormone secretion.

In ectopic hyperparathyroidism, the quantity of circulating COOH-terminal fragments is greatly decreased as compared to primary hyperparathyroidism, whereas the quantity of circulating intact PTH is similar in these two groups of patients. If antiserum GP-204 is predominantly measuring intact PTH and is also recognizing COOH-terminal fragments to a lesser extent as discussed above, then it is understandable why our assay gives lower iPTH levels in ectopic hyperparathyroidism than in primary hyperparathyroidism, but gives generally higher values in the former group than the Arnaud assay. Although only 18.5 percent of our group of 119 patients with malignancy had iPTH values greater than 2 S.D. above the normal mean, 82 (69 percent) of these patients have iPTH values too high for their serum calcium levels and would be above the dotted line if plotted in figure 6. Thus, in our assay it is only possible to rule out ectopic hyperparathyroidism as a diagnostic possibility in patients with iPTH values greater than 1000 pg per ml, although 90 percent of the ectopic hyperparathyroid group had iPTH values below 500 pg per ml. This value could probably be used as a criterion for separation of these groups of patients.

Selective venous catheterization of the veins draining the thyroid-parathyroid region is a technique which is rapidly growing in use. Because antiserum GP-204 predominantly measures intact PTH, our assay is well suited for use with this application. As reported, over 70 percent of the groups of specimens assayed by our laboratory have shown a gradient of iPTH values, that is, at least one specimen in the group had an iPTH level significantly higher than one or more of the other specimens. Owing to a lack of follow-up information, it is not known how well the iPTH results have correlated with surgical findings except in a few instances. In those cases, the correlations have been excellent. Attention has been called to the possibility of obtaining gradients of iPTH concentrations in venous specimens from normal individuals by positioning the catheters too close to the parathyroid glands. However, Eisenberg et al. have discussed this possibility in relation to their own findings with selective venous catheterization and have concluded that incorrect diagnosis of hyperfunctioning parathyroid tissue in normal individuals is not likely if the technique is properly performed and a sufficient number of specimens are obtained.

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