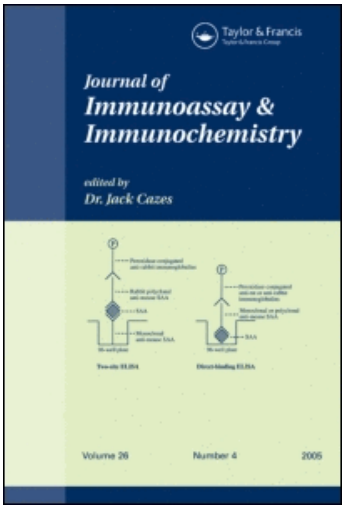

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Immunological Evidences for the Presence of Small Late Carboxylterminal Fragment(S) of Human Parathyroid Hormone (PTH) In Circulation in Man

Pierre D'amour[a]; France Labelle[a]; Rahel Wolde-giorghis[a]; Lucie Hamel[a]
[a] Centre de recherche clinique André-Viallet, Hôpital Saint-Luc, and Département de médecine, Université de Montréal, Québec, CANADA

To cite this Article D'amour, Pierre , Labelle, France , Wolde-giorghis, Rahel and Hamel, Lucie(1989) 'Immunological Evidences for the Presence of Small Late Carboxylterminal Fragment(S) of Human Parathyroid Hormone (PTH) In Circulation in Man', Journal of Immunoassay and Immunochemistry, 10: 2, 191 — 205
To link to this Article: DOI: 10.1080/01971528908053236
URL: http://dx.doi.org/10.1080/01971528908053236

JOURNAL OF IMMUNOASSAY, 10(2&3), 191-205 (1989)

IMMUNOLOGICAL EVIDENCES FOR THE PRESENCE OF SMALL LATE CARBOXYLTERMINAL FRAGMENT(S) OF HUMAN PARATHYROID HORMONE (PTH) IN CIRCULATION IN MAN.

Pierre D'Amour, France Labelle, Rahel Wolde-Giorghis, Lucie Hamel
Centre de recherche clinique André-Viallet, Hôpital Saint-Luc, and
Département de médecine, Université de Montréal,
Montréal, Québec, CANADA, H2X 3J4

ABSTRACT

Two antisera, C-52 and C-97, raised against bovine (b)PTH(1-84) in guinea pigs, were evaluated with ^{125}I-[tyr^{53}] human (h)PTH(53-84) as tracer and intact hPTH(1-84) and synthetic hPTH(39-84), representative of large carboxylterminal («C») fragments found in circulation, as standards. In both assays, hPTH(39-84) was 5-6 times more potent than hPTH(1-84) on a molar basis in displacing the tracer. With both antisera, progressive deletion at the aminoterminal end of large «C» fragments, as in hPTH(53-84) and hPTH(65-84), lead to decreased immunoreactivity, hPTH(69-84) being non-immunoreactive. The mid-carboxylterminal fragments, hPTH(44-68) and hPTH(39-68), did not react in either assay. Each antiserum measured known quantities of pure hPTH(1-84) or hPTH(39-84) standards similarly. Serum PTH values obtained with antiserum C-97 were about 3 times higher in renal failure, 1.75 times higher in normal individuals and those with primary hyperparathyroidism, while similar to values measured with antiserum C-52 in individuals with secondary hyperparathyroidism without renal failure or with pseudohypoparathyroidism. When circulating PTH taken from patients with these disorders was fractionated by gel chromatography, both antisera recognized similar peaks of intact hPTH(1-84) and of large «C» fragments while antiserum C-97 further recognized a peak of smaller «C» fragments. This explained the different clinical behavior of the latter antiserum. Our findings demonstrate the existence of small late «C» fragments in circulation. They further suggest an influence of serum calcium and of renal function on the quantity of these fragments.

(KEY WORDS: Parathyroid hormone, radioimmunoassay, hypercalcemia)

INTRODUCTION

Circulating parathyroid hormone (PTH) is immunoheterogenous (1). It is composed mostly of large carboxylterminal fragments (2-9), which are biologically inactive (10-11), and a small amount of intact hormone (2-9), which is biologically active (10-12). This immunoheterogeneity is not a static phenomenon since the relative amount of intact hormone and of large carboxylterminal fragments appears related to serum calcium concentration (7,9), hypocalcemia stimulating the presence of intact hormone with respect to large carboxylterminal fragments and hypercalcemia the reverse process (7,9). Smaller fragments, either aminoterminal (2,5,12) and biologically active (10-12) or carboxylterminal (2,7,8) and biologically inactive (10,11) have also been described. The smaller carboxylterminal fragment(s) have so far been best revealed by antisera with mid-carboxylterminal specificity (8,13,14) and their control by serum calcium concentration inferred in at least one study (13). We are presenting here immunological evidences for further heterogeneity of smaller carboxylterminal fragments of PTH in circulation in man. These data are derived from the different clinical behavior of two immunologically very similar late carboxylterminal antisera.

MATERIALS AND METHODS

Antisera

Two different antisera, raised against partially purified bPTH(1-84) in guinea pigs, were used for these studies. The first, C-97, was

used at 1/60,000 dilution while the second, C-52, was used at 1/50,000 dilution after saturation of its mid-carboxylterminal component with an excess of hPTH(44-68), as previously described (5). These dilutions gave ~ 30% binding of the tracer in absence of standards.

Tracer

[tyr^{53}]hPTH(53-84) was purchased from Bachem Inc. (Torrance, CA, 90505). Iodination and purification were performed as previously described (13).

Standards and Pools

hPTH(1-84), hPTH(1-34), hPTH(44-68), hPTH(53-84), hPTH(64-84), and hPTH(69-84) were purchased from Bachem Inc. (Torrance, CA, 90505) while hPTH(39-84) and hPTH(39-68) were obtained from Peninsula Laboratories Inc. (Belmont, CA, 94002). The concentration of all standards refers to the initial amino acid content. All preparations were initially dissolved in a small volume of 0.2 mol/L acetic acid. Standards and pools were further diluted to the desired concentration using barbital buffer, 0.05 mol/L, pH 7.6, 1% with bovine serum albumin. Aliquots were stored at -75°C until used.

Clinical Samples

Blood samples were obtained by venipuncture from 26 subjects: 8 normal volunteers (laboratory personnel), 8 patients with renal failure on chronic hemodialysis, 5 patients with primary hyperparathyroidism subsequently proven surgically, 3 patients with secondary hyperparathyroidism without renal failure due to malabsorption, and 2

patients with pseudohypoparathyroidism type I. After centrifugation, serum was stored at -75°C until further processed.

Gel Chromatography of Serum Samples

Four samples of 7.5 ml serum from one normal individual, one patient with primary hyperparathyroidism, one with secondary hyperparathyroidism without renal failure, and one with renal failure, were analyzed using gel chromatography on a 2.5 X 100 cm BioGel P-100 column (Bio-Rad Laboratories, Richmond, CA, 94804). The serum was equilibrated and eluted with 0.1 mol/L ammonium acetate buffer, pH 4.6, with bovine serum albumin 1 g/L. Five ml fractions were collected, freeze dried and eventually reconstituted in 1 ml of PTH assay buffer minus the bovine serum albumin. Appropriate dilutions were assayed with both antisera. Recovery of added iPTH from the column was better than 80% for each run.

hPTH Immunoassays

The two assays were performed in non-equilibrium conditions, standards, pools or samples being preincubated for 24 hours with the antiserum prior to addition of the tracer. After 48 hours of incubation, charcoal-dextran was used to separate bound from free phase (15).

Statistical Analysis

Paired "T" tests were used to compare the results of pools or samples obtained with the two antisera.

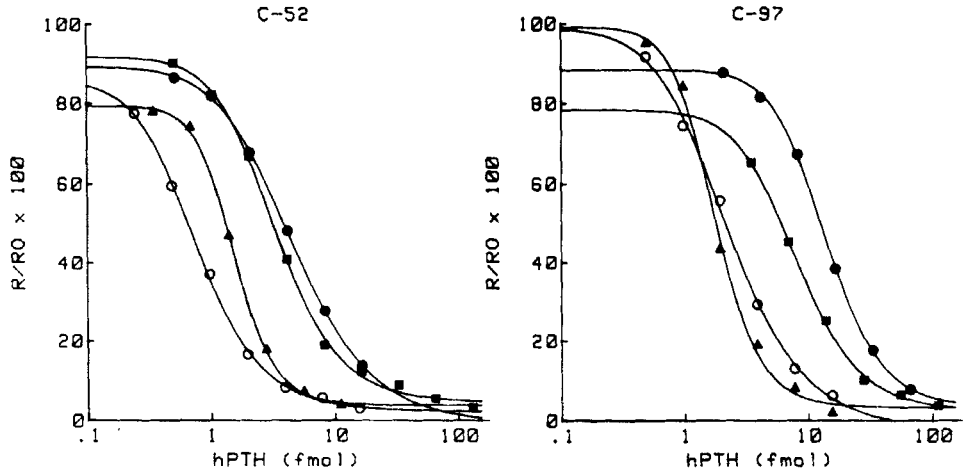

FIGURE 1 Displacement curves with intact hPTH(1-84) (●—●), hPTH(39-84) (○—○), hPTH(53-84) (▲—▲), and hPTH(64-84) (■—■) are illustrated for both antisera. hPTH(1-34), hPTH(44-68), hPTH(39-68), and hPTH(69-84) did not displace the ^{125}I-[tyr^{52}]hPTH(52-84) tracer.

RESULTS

The characteristics of the two antisera used for these studies are illustrated on Figure 1, with dilution curves with standard hPTH(1-84), hPTH(39-84) and various other shorter synthetic carboxylterminal fragments being shown. With both antisera, hPTH(39-84) was most potent on a molar basis in displacing the tracer; 50% displacement was achieved with 0.6 fmol in the case of antiserum C-52 and 1.5 fmol for antiserum C-97, reflecting the greater sensitivity of the former. In both cases, hPTH(1-84) was about 6 times less potent than hPTH(39-84) on a molar basis in displacing the tracer. Detection limits were 1.2 fmol of hPTH(1-84) and 0.2 fmol of hPTH(39-84) with antiserum C-52; these figures were

TABLE 1

Behavior of C-52 and C-97 in Measuring Pure Standards

Antiserum	Standard in RIA	Standard measured: hPTH(1-84) (5.26 fmol)*	Standard measured: hPTH(39-84) (1 fmol)*
C-52	hPTH(1-84)	5.02 ± 0.77 (7)	7.16 ± 1.03 (6)
	hPTH(39-84)	0.75 ± 0.15 (7)	1.13 ± 0.14 (7)
	Molar ratio	6.86 ± 1.41 (7)	6.60 ± 1.38 (6)
C-97	hPTH(1-84)	4.40 ± 0.55 (7)	6.98 ± 1.69 (7)
	hPTH(39-84)	0.71 ± 0.17 (7)	1.13 ± 0.28 (8)
	Molar ratio	6.33 ± 0.88 (7)	6.32 ± 1.23 (7)

( ) = number of assays
* = theoretical values

respectively 5 fmol and 0.8 fmol with antiserum C-97. Progressive deletion of amino acids at the aminoterminal end of hPTH(39-84) as in hPTH(53-84) and hPTH(64-84) led to a slightly more rapid decrease in immunoreactivity for antiserum C-52 when compared with C-97, hPTH(69-84) being non-reactive in both assays. Deletions at the carboxylterminal end of hPTH(39-84) as in hPTH(39-68) and hPTH(44-68) caused loss of immunoreactivity.

The results of an analysis of a pool of 5.26 fmol of hPTH(1-84) and of a pool of 1 fmol of hPTH(39-84) assayed against standards hPTH(1-84) and hPTH(39-84) in a miminum of 6 consecutive assays with each antiserum are illustrated at Table 1. Both assays measured

TABLE 2

Clinical Results

Category	No. Patients	No. Samples	C-52 (pmol/L)	C-97 (pmol/L)	C-97/C-52
Normal	8	19	5.42 ± 2.34	8.82 ··· ± 2.69	1.74 ± 0.37
Renal Failure	8	16	98.48 ± 81.54	332.96 ·· ± 324.29	3.28 ± 0.55
PHP	5	6	23.0 ± 14.48	36.47 · ± 19.42	1.78 ± 0.53
SHP	3	6	13.98 ± 1.35	13.86 ± 1.40	1.03 ± 0.15
P_SHP	2	2	29.75 ± 12.8	23.55 ± 8.56	0.97 ± 0.30

PHP = primary hyperparathyroidism; SHP = secondary hyperparathyroidism without renal failure; P_SHP = pseudohypoparathyroidism. $p < 0.0005$, ··· ; < 0.005, ·· ; < 0.05, · .

identical amounts of hPTH(1-84) or hPTH(39-84) using either standard. The difference in molar reactivity between hPTH(1-84) and hPTH(39-84) in both assays caused the hPTH(1-84) pool to be underestimated some 6 times when read on hPTH(39-84) and the hPTH(39-84) pool to be overestimated about 6 times when read on hPTH(1-84), as expected from the difference between the respective dilution curves.

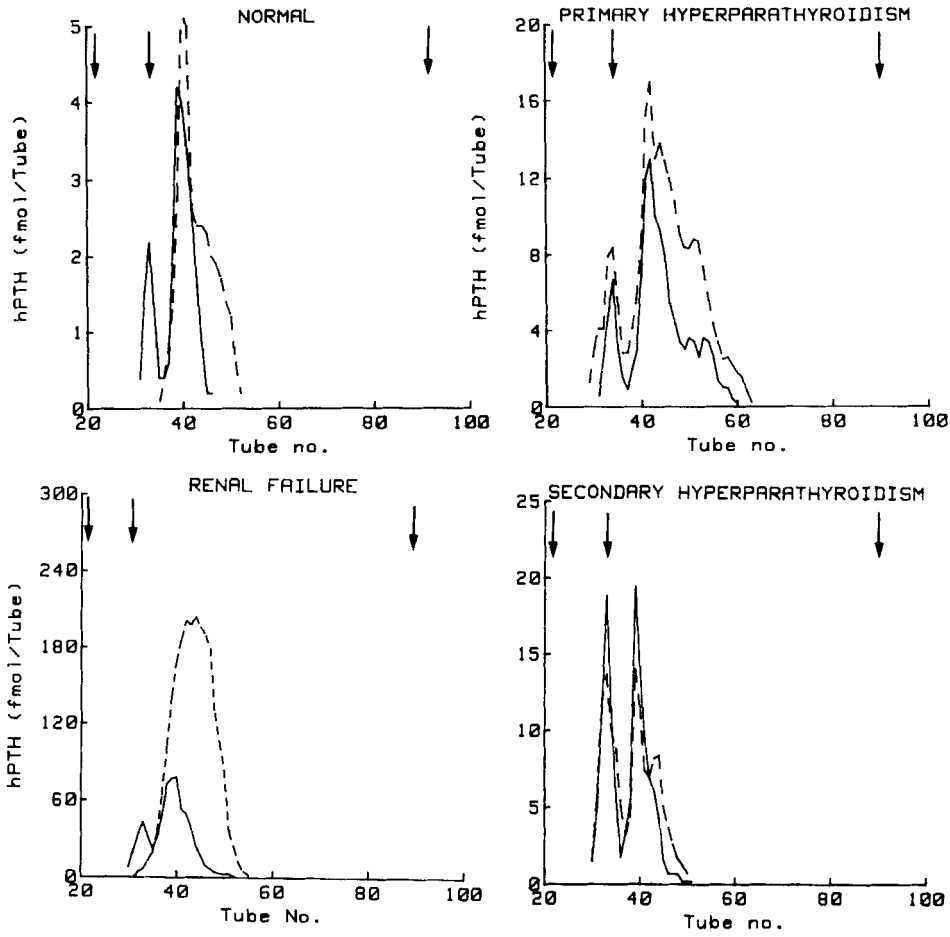

FIGURE 2 Analysis of circulating hPTH by gel chromatography in patients with various phosphocalcic disorders. Results obtained with antisera C-52 (———) and C-97 (------) are illustrated. The position of elution of the void volume (Vo), of ^{125}I-bPTH(1-84) and of ^{125}I are respectively indicated by arrows from left to right.

Table 2 illustrates the results obtained in measuring clinical samples with both antisera. Values measured with antisera C-97 were significantly higher in normal individuals, those with renal failure or primary hyperparathyroidism, but were similar to those measured by C-52 in patients with secondary hyperparathyroidism without renal failure or with pseudohypoparathyroidism. The ratio of C-97/C-52 values was accordingly greater than 1 in the first 3 conditions but equal to 1 in the two latter.

To gain a better understanding of these differences, the serum of 4 different patients was analyzed by gel chromatography and each profile was analyzed with both antisera (Figure 2). The first peak of immunoreactivity identified by both antisera corresponded to the position of elution of intact hPTH(1-84); the amounts identified by both antisera were about equal, except for the profile of the normal individual where intact hormone was not detected by antiserum C-97, mainly because of its lower sensitivity. The second peak was also identified by both antisera and corresponded to the position of elution of large carboxylterminal fragments of the hormone; again, the amounts detected by both antisera were about equal in all cases. A third immunoreactivity peak, migrating smaller than large carboxylterminal fragments was identified mainly with antiserum C-97, and explained the clinical differences observed between the results obtained with both antisera. This peak was quantitatively greater in the patients with primary hyperparathyroidism and renal failure, and less so in the subject with secondary hyperparathyroidism.

DISCUSSION

The study was designed to see if the immunoreactive behavior of a given antiPTH antiserum could be predicted from the characteristics of dilution curves obtained with hPTH(1-84) and hPTH(39-84), two standards representative of the two main molecular forms of hPTH found in circulation.

Two antisera raised against partially purified bPTH(1-84) in guinea pigs were used for the study. Both demonstrated very similar late carboxylterminal reactivity, recognizing carboxylterminal fragment hPTH(39-84) six times more readily on a molar basis than intact hPTH(1-84); the only difference between the two was that fragments hPTH(53-84) and hPTH(64-84) were slightly more reactive with antisera C-97, although less so than hPTH(39-84). Since hPTH(69-84) did not react with either antisera, we had to conclude that they recognized a dimensional structural aspect maximally expressed in hPTH(39-84), partially expressed in hPTH(53-84) and hPTH(64-84), and absent in hPTH(69-84). Furthermore, neither antisera reacted with synthetic mid-carboxylterminal fragments, hPTH(39-68) and hPTH(44-68).

Based on detection limits, antiserum C-52 could detect 8 pmol/L of serum of hPTH(1-84) and 1.3 pmol/L of hPTH(39-84) while C-97 detected 33.3 and 5.3 pmol/L respectively. Since known concentrations of hPTH(1-84) in the serum of normal individuals are below 8 pmol/L (16,17), both assays appeared relatively insensitive; on the other hand, antiserum C-52 appeared to detect carboxyl-terminal fragments best in view of the lower limit of detection. This

reasoning has justified the initial use of this antiserum in a variety of clinical studies (18,19,20) with very good results.

The ability of each antiserum to measure pure standards of hPTH(1-84) or hPTH(39-84) was similar, taking into account their respective limits of detection. But when we eventually measured PTH in clinical samples with antiserum C-97 and compared the results obtained with those of antiserum C-52 the values obtained were unexpected. PTH values were higher in normal individuals, those with primary hyperparathyroidism or with renal failure when measured with antiserum C-97, while they were similar for both antisera in those with secondary hyperparathyroidism without renal failure and with pseudo-hypoparathyroidism. These differences could be explained by an analysis of the gel chromatography profile of circulating PTH in these clinical conditions. Both antisera were able to detect similar amounts of intact hormone and large carboxylterminal fragments in the various samples, but antiserum C-97 could also recognize smaller carboxylterminal fragments, thus explaining its ability to recognize more PTH in circulation. Since the ratio of PTH C-97/PTH C-52 was greater than one in individuals with normo- or hypercalcemia and equal to one in those with hypocalcemia, it could be postulated that the small carboxylterminal fragments identified by antiserum C-97 were influenced by serum calcium concentration, increasing in line with the serum calcium concentration. Further studies will be required to see if this increase is related to an increased production rate or to a decrease in metabolic clearance. The higher increase seen in renal failure would mainly be a reflection of the kidney's role in metabolic clearance (19).

The smaller fragments detected by this study appear to retain their carboxylterminal structure intact, if we take into account the immunochemical characteristics of antisera C-97. This would be compatible with progressive cleavage of larger carboxylterminal fragments at their aminoterminal end as outlined in the rat with ^{125}I-bPTH(41-84) injected in vivo (21). Since smaller fragments with mid-carboxylterminal reactivity have also been outlined in various studies (13,14,22), this makes circulating carboxylterminal fragments of PTH even more heterogenous. It is possible that metabolism at both ends of the large fragments could account for this immunoheterogeneity, but this remains to be proved.

Although our approach to evaluate the antisera was theoretically correct, it failed because it did not take into account all the molecular forms of PTH found in circulation and their theoretical reactivity in both assays. This emphasizes the fact that, until we know the molecular structure of all these circulating fragments, and can make synthetic ones to use as standards, serum analysis by means of gel chromatography will remain the best test to verify the immunoreactivity of a given antiserum.

ACKNOWLEDGMENTS

We wish to thank Ms. Manon Cordeau for secretarial help, and Mr. Robert Boileau for the statistical analysis of the data and computer drawing of the various figures. This investigation was supported by grant 7643 of the Medical Research Council of Canada. Reprint requests should be addressed to Doctor Pierre D'Amour,

Centre de recherche clinique André-Viallet, Hôpital Saint-Luc, 264, René-Lévesque Blvd East, Montreal, Quebec, Canada, H2X 1P1.

REFERENCES

1. Berson, S.A., and Yalow, R.S. Immunochemical heterogeneity of parathyroid hormone in plasma. J. Clin. Endocrinol. 1968;28:1037-47.

2. Canterbury, J.M., and Reiss, E. Multiple immunoreactive forms of parathyroid hormone in human serum. Proceedings of the Society for Experimental Biology and Medicine, 1972;140:1393-98.

3. Segre, G.V., Habener, J.F., Powell, D., Tregear, G.W., and Potts, J.T. Jr. Parathyroid hormone in human plasma. Immunochemical characterization and biological implications. J. Clin. Invest. 1972;51:3163-72.

4. Goldsmith, R.S., Furszyfer, J., Johnson, W.J., Fournier, A.E., Sizemore, G.W., and Arnaud, C.D. Etiology of hyperparathyroidism and bone disease during chronic hemodialysis. III. Evaluation of parathyroid suppressibility. J. Clin. Invest. 1973;52:173-80.

5. Silverman, R., and Yalow, R.S. Heterogeneity of parathyroid hormone. Clinical and physiological implications. J. Clin. Invest. 1973;52:1958-71.

6. Arnaud, C.D., Goldsmith, R.S., Bordier, P.J., Sizemore, G.W., Larsen, J.A., and Gilkinson, J. Influence of immunoheterogeneity of circulating parathyroid hormone on results of radioimmunoassays in man. Amer. J. Med. 1974;56:785-93.

7. Dambacher, M.A., Fisher, J.A., Hunziker, W.H., Born, W., Moran, J., Roth, H.R., Delvin, E.E., and Glorieux, F.H. Distribution of circulating immunoreactive components of parathyroid hormone in normal subjects and in patients with primary and secondary hyperparathyroidism: The role of the kidney and of the serum calcium concentration. Clin. Sci. 1979;57:435-43.

8. Gautvik, K.M., Gautvik, V.T., and Halvorsen, J.F. Characterization of the molecular forms of immunoreactive parathyroid hormone in primary hyperparathyroidism and in hyperparathyroidism due to renal failure. Scand. J. Clin. Lab. Invest. 1983;43:553-64.

9. D'Amour, P., Labelle, F., Lecavalier, L., Plourde, V., and Harvey, D. Influence of serum Ca concentration on circulating molecular forms of PTH in three species. Amer. J. Physiol. 1986;251:E680-87.

10. Canterbury, J.M., Levey, G.S., and Reiss, E. Activation of renal cortical adenylate cyclase by circulating immunoreactive parathyroid hormone fragments. J. Clin. Invest. 1973;52:524-27.

11. Gautvik, K.M., Gordeladze, J.O., Moxheim, E., Gautvik, V.T. Peripheral metabolism of parathyroid hormone in patients with primary hyperparathyroidism as judged by immunological and biological studies. Eur. Surg. Res. 1984;16(Supp. 2):41-54.

12. Goltzman, D., Henderseon, B., and Loveridge, N. Cytochemical bioassay of parathyroid hormone. Characteristics of the assay and analysis of circulating hormonal forms. J. Clin. Invest. 1980;65:1309-17.

13. Roos, B.A., Lindall A.W., Aron, D.C., Orf, J.W., Yoon, M., Huber, M.B., Pensky, J., Ells, J., and Lambert, P.W. Detection and characterization of small midregion parathyroid hormone fragment(s) in normal and hyperparathyroid glands and sera by immunoextraction and region-specific radioimmunoassays. J. Clin. Endocrinol. Metab. 1981;53:709-21.

14. Marx, S.J., Sharp, M.E., Krudy, A., Rosenblatt, M., and Mallette, L.E. Radioimmunoassay for the middle region of human parathyroid hormone: Studies with a radioiodinated synthetic peptide. J. Clin. Endocrinol. Metab. 1981;53(1):76-84.

15. D'Amour, P., Labelle, F., and Lazure, C. Comparison of four different carboxylterminal tracers in a radioimmunoassay specific to the 68-84 region of human parathyroid hormone. J. Immunoassay 1984;5:183-204.

16. Brown, R.C., Aston, J.P., Weeks, I., and Woodhead, J.S. Circulating intact parathyroid hormone measured by a two-site immuno-chemiluminometric assay. J. Clin. Endocrinol. Metab. 1987;65:407-14.

17. Nussbaum, S.R., Zahradnik, R.J., Lavigne, J.R., Brennan, G.L., Nozawa-Ung, K., Kim, L.Y., Keutmann, H.T., Wang, C.A., Potts, J. Jr, and Segre, G.V. Highly sensitive two-site immuno-radiometric assay of parathyrin, and its clinical utility in evaluating patients with hypercalcemia. Clin. Chem. 1987; 33:1364-67.

18. D'Amour, P., Gilbert, F., Gascon-Barré, M., Boutin, J.M., Havrankova, J., Bélanger, R., and Matte, R. Late increase in serum 1,25-dihydroxyvitamin D one month after surgery for adenomatous hyperparathyroidism. Clin. Endocrinol. 1986;24:349-58.

19. Lepage, R., D'Amour, P., Boucher, A., Hamel, L., Demontigny, C., and Labelle, F. Clinical performance of a parathyrin immunoassay with dynamically determined reference values. In press. Clinical Chemistry.

20. Boucher, A., D'Amour, P., Hamel, L., Fugère, P., Gascon-Barré, M., Lepage, R., and Ste-Marie, L.G. Estrogen replacement decreases the set-point of parathyroid hormone stimulation by calcium in normal postmenopausal women. J Bone Min Res 1988;3(supp.1):S162, Abstract #373.

21. D'Amour, P., Lazure, C., and Labelle, F. Metabolism of radioiodinated carboxylterminal fragments of bovine parathyroid hormone in normal and anephrin C rats. Endocrinology 1985;117:127-34.

22. Kubler, N., Krause, U., Wagner, P.K., Beyer, J., and Rothmund, M. The influence of parathyroid hormone and its fragments on results from midregion and C-terminal specific radioimmunoassays. Exp. Clin. Endocrinol. 1987;89(1):61-69.