

Immunologic Differentiation of Primary Hyperparathyroidism from Hyperparathyroidism due to Nonparathyroid Cancer

B. LAWRENCE RIGGS, CLAUDE D. ARNAUD, JAMES C. REYNOLDS, and LYNWOOD H. SMITH

From the Mayo Clinic and Mayo Foundation: Division of Endocrinology and Internal Medicine; Department of Endocrine Research; and Division of Nephrology and Internal Medicine; and the Mayo Graduate School of Medicine, University of Minnesota, Rochester, Minnesota 55901

ABSTRACT Serum immunoreactive parathyroid hormone (IPTH) was measured by radioimmunoassay in 54 patients with primary hyperparathyroidism and in 18 consecutive patients with ectopic hyperparathyroidism due to nonparathyroid cancer without apparent skeletal metastasis. Although serum calcium concentration was higher in the group with ectopic hyperparathyroidism, serum IPTH was lower (rank sum test, $P < 0.001$) and was undetectable in eight. A second anti-PTH antiserum also differentiated between IPTH in the two groups, although IPTH was undetectable in only 1 of 14 sera. When IPTH values in serial dilutions were plotted, slopes for the two patients with ectopic hyperparathyroidism who had relatively high IPTH were less ($P < 0.001$) than slopes for standard hyperparathyroid sera. By using differences in either IPTH rank or slope of the dilutional curve of sera, primary hyperparathyroidism could be excluded as a cause of the hypercalcemia in 16 of the 18 patients with ectopic hyperparathyroidism. The data are interpreted as indicating that PTH-like material in the serum of these patients with ectopic hyperparathyroidism is immunologically different from the PTH in the serum of patients with primary hyperparathyroidism.

INTRODUCTION

In patients with nonparathyroid cancer and no apparent skeletal metastasis, a syndrome with all the biochemical

This work was presented in part at the Meeting of the Central Society for Clinical Research, Chicago, Ill., 6 November 1970.

Received for publication 7 December 1970 and in revised form 17 March 1971.

characteristics of primary hyperparathyroidism may develop (1). This syndrome is only slightly less common than primary hyperparathyroidism and is, in all likelihood, due to ectopic synthesis and secretion of an excess of a parathyroid hormone-like (PTH-like) substance by the tumor (2-4).

Increased concentrations of immunoreactive parathyroid hormone (IPTH)¹ in serum have been reported in both primary (5) and ectopic (3, 4, 6) hyperparathyroidism, as well as in some normocalcemic patients with bronchogenic carcinoma (5). Hence, direct measurement of IPTH has been thought to be valueless in separating these two conditions (6, 7).

The antiserum used in our laboratory for the measurement of IPTH in serum (8) appears to distinguish between the immunoreactive material in primary hyperparathyroidism and that in ectopic hyperparathyroidism. Because of this, we have been able to exclude primary hyperparathyroidism as a cause of hypercalcemia in 16 of 18 consecutive patients who had the syndrome of ectopic hyperparathyroidism without apparent skeletal metastasis.

METHODS

We studied 54 patients with surgically proved primary hyperparathyroidism and 18 patients with nonparathyroid cancer. The latter patients had the previously described (1) biochemical characteristics of the syndrome of ectopic hyperparathyroidism including hypercalcemia, low-normal or low serum phosphorus, and no clinical or roentgenographic evidence of bone metastasis. Blood urea nitrogen or serum creatinine concentrations were normal or only slightly in-

¹Abbreviations used in this paper: B/F, bound-to-free ratio; bPTH, ¹²⁵I-labeled bovine parathyroid hormone; IPTH, immunoreactive parathyroid hormone.

TABLE I
Serum Values in Primary and Ectopic Hyperparathyroidism

	Primary		Ectopic		P
	Mean	SE	Mean	SE	
Ca, mg/100 ml	12.1	0.2	13.2	0.4	<0.05
P, mg/100 ml	2.50	0.01	2.60	0.14	NS
IPTH, μ l Eq/ml	392	129	25	9	<0.01
Range	26-6,500		Undet.-110		

creased. In these 18 patients, the tumors were hypernephroma in 5, bronchogenic carcinoma in 5, hepatoma in 4, pancreatic acinar carcinoma in 2, lymphoma in 1, and carcinoma of the penis in 1.

Serum calcium was measured by atomic absorption flame spectrophotometry (normal, 8.9-10.1 mg/100 ml) and serum phosphorus by an automated version of the Fiske and Subbarow technique (9) (normal, 2.5-4.5 mg/100 ml). IPTH was measured by the radioimmunoassay system of Arnaud, Tsao, and Littledike (8) which uses guinea pig anti-porcine-PTH antiserum (GP-1 M, 1:75,000 final dilution), 125 I-labeled bovine PTH (bPTH), and human hyperparathyroid serum as a standard; dextran-coated charcoal is used to separate antibody-bound from "free" PTH- 125 I. The coefficient of interassay variation of replicate determinations was 12%. Dilutional curves with sera from patients with primary hyperparathyroidism with or without renal disease were superimposable on those from patients with secondary hyperparathyroidism due to renal failure or intestinal malabsorption. IPTH was measurable in 94% of the 51 normal sera tested; it correlated inversely with the serum calcium concentration and ranged between undetectable and 37 μ l Eq/ml. Serum IPTH was undetectable in 10 patients with surgical hypoparathyroidism. By using IPTH and serum calcium simultaneously as linear discriminant functions, normal patients could be separated from patients with primary hyperparathyroidism with no overlap.

Sera also were assayed for IPTH with a chicken anti-bPTH antiserum (CH-14 M, 1:3,000 final dilution). In general, this radioimmunoassay was less sensitive than the one using GP-1 M (8).

RESULTS

Serum concentrations of calcium, phosphorus, and IPTH for the two groups are compared in Table I. Hypercalcemia was less severe in primary hyperparathyroidism. Mean serum phosphorus concentrations were similar in the two groups and were significantly ($P < 0.01$) below the normal mean (10). In primary hyperparathyroidism, log IPTH was related to serum calcium ($r = 0.73$, $P < 0.001$). In ectopic hyperparathyroidism, IPTH was undetectable in 8 of 18 sera and did not correlate with serum calcium. Linear discriminant analysis (11), using IPTH and serum calcium simultaneously, separated the two groups (at any serum calcium value, IPTH was lower in the ectopic group) with an overlap of only three patients—two with ectopic and one with primary hyperparathyroidism (Fig. 1).

When the IPTH assay was repeated using CH-14 M anti-bPTH antiserum and sera from 14 patients in the ectopic group and 14 patients in the primary group, the patients were selected so that serum calcium concentrations were similar in both groups. IPTH again was lower in the ectopic group ($P < 0.01$). Antiserum CH-14 M was less sensitive than GP-1 M because it detected IPTH in only 50% of normal sera. However, this antiserum was more sensitive than antiserum GP-1 M in detecting IPTH in ectopic hyperparathyroidism because in only one of the sera was IPTH undetectable (Fig. 2).

In the present study, all sera were examined in duplicate and, when possible, in three dilutions. By plotting the log of serum dilution used in the assay system vs. the corresponding bound-to-free (B/F) ratio, curves could be constructed for all primary hyperparathyroid sera. Each of these curves appeared to be parallel with the curve obtained using standard hyperparathyroid serum (8). In contrast, in sera from patients with ectopic hyperparathyroidism, IPTH was usually too low to permit this test of parallelism; however, the IPTH values were sufficiently high in two patients so that dilutional curves complete enough for statistical testing could be constructed using antiserum GP-1 M. The slopes of fitted straight lines of these two dilutional curves were similar to each other but were significantly ($P < 0.001$)

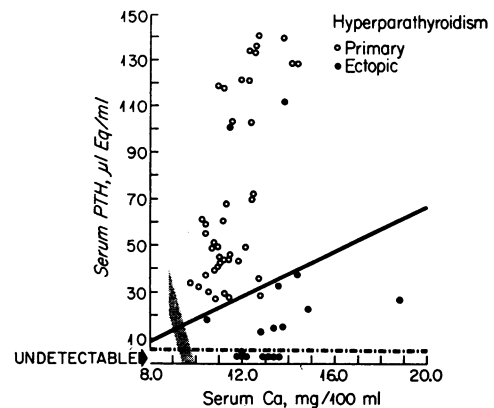


FIGURE 1 Relationship between serum IPTH (assayed using antiserum GP-1 M) and serum calcium in primary (○) and in ectopic (●) hyperparathyroidism. Only patients with primary hyperparathyroidism with serum IPTH values less than 150 μ l Eq/ml are represented although the actual range extended to 6,500 μ l Eq/ml. Serum IPTH is given as equivalent concentration of standard hyperparathyroid serum. For a given level of serum calcium, serum IPTH was lower in ectopic hyperparathyroidism and was undetectable in 8 of the 18 patients. Stippled region indicates where normal values would fall. Linear discriminant function analysis (solid line) separates primary and ectopic hyperparathyroidism with an overlap of only three patients—two with ectopic and one with primary hyperparathyroidism.

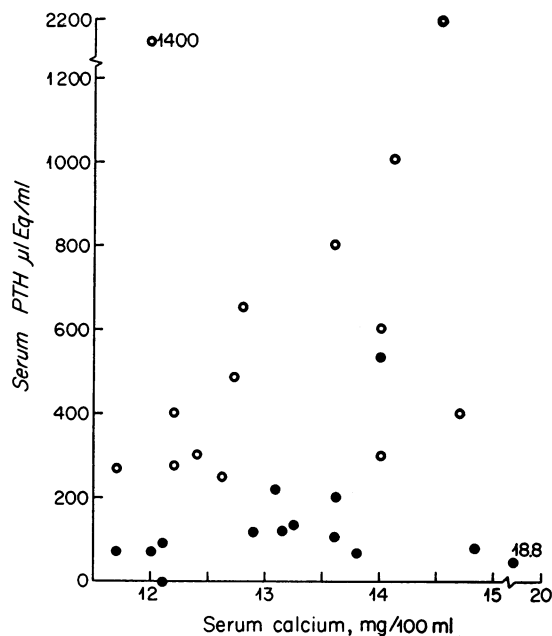


FIGURE 2 Relationship between serum IPTH (reassayed using antiserum CH-14 M) and serum calcium in 14 patients with primary (○) and in 14 patients with ectopic hyperparathyroidism (●). The latter 14 patients were selected so that the serum calcium concentrations in the two groups were approximately the same. IPTH values were lower in the ectopic group. However, this antiserum apparently recognized IPTH in ectopic hyperparathyroidism better than did antiserum GP-1 M since IPTH was undetectable in only one of the sera.

less than the slope of a dilutional curve of standard hyperparathyroid serum (Fig. 3).

Although the apparent IPTH values are empirically useful in diagnosis, the nonparallelism of the dilutional curves indicates that, for patients with ectopic hyperparathyroidism, it probably is not appropriate to report IPTH in terms of equivalent concentrations of standard hyperparathyroid serum. For this reason, we compared sera from patients with primary or ectopic hyperparathyroidism in terms of relative immunoreactivity without reference to a standard preparation. Unknown sera were successively ranked according to their ability to produce a decrease of the B/F ratio at a given dilution of serum. The lowest rank was assigned to that serum which produced the least decrease in B/F in the lowest dilution while the highest rank was assigned to that serum which produced the largest decrease in B/F in the highest dilution that was studied. By this ranking system and the rank sum test (12), IPTH differed significantly between the two groups ($P < 0.001$). 14 of the 18 patients with cancer had ranks lower than those for any of the 54 patients with primary hyperparathyroidism.

DISCUSSION

Our results show that a high proportion of patients with ectopic hyperparathyroidism syndrome without apparent bone metastasis can be differentiated by immunologic means from patients with primary hyperparathyroidism. On the basis of low relative PTH immunoreactivity, all but four of the former could be separated from the latter. Also, two of these four patients appeared to be identifiable immunologically because the slopes of the dilutional curves of their sera were significantly less than the slopes of dilutional curves of standard, primary hyperparathyroid sera and therefore less than the slopes of dilutional curves of the 54 patients with primary hyperparathyroidism.

The biochemical findings in these patients leave little doubt that a biologically active PTH-like material was present in the serum in high concentration. Marked hypercalcemia was observed in the absence of demonstrable bone metastasis and, unlike hypercalcemia in association with bone metastasis, serum phosphorus concentrations were decreased to the level expected in primary hyperparathyroidism. Additionally, PTH immunoreactivity was present in all but one serum from patients in the ectopic group tested with CH-14 M antiserum whereas it was markedly suppressed or undetectable in normal subjects with comparable degrees of hypercalcemia induced with calcium infusions (8).

Berson and Yalow (13) first reported that PTH in hyperparathyroid plasma is immunoheterogeneous and that different antisera to PTH may have varying affinities for different immunoreactive PTH components. Our experience with the two antisera used in this study

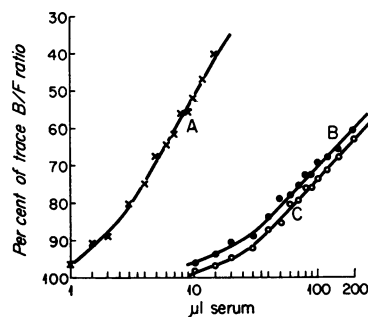


FIGURE 3 Plots of log of dilution of serum used in assay system (antiserum GP-1 M) versus corresponding bound-to-free ratio for standard hyperparathyroid serum (A) and for sera from two patients (B and C) with ectopic hyperparathyroidism who had relatively high PTH immunoreactivity with antiserum GP-1 M. To control possible non-specific effects, sufficient hypoparathyroid serum was added so that all tubes had an equal serum content. Slopes of fitted straight lines for dilutional curves B and C were significantly ($P < 0.001$) less than slope of dilutional curve A ($b = -56.3$, $SE = 3.3$).

support their (13) conclusion. Antiserum GP-1 M recognizes the PTH in serum better than that in adenoma extracts.² With this antiserum, the immunologic characteristics of the sera of all patients with primary and secondary hyperparathyroidism we have studied as well as the PTH in the medium of parathyroid-adenoma slice cultures are indistinguishable (8). For these reasons we believe that GP-1 M recognizes primarily the predominant secretory product of the gland. In contrast, antiserum CH-14 M recognizes the PTH in adenoma extracts better than that in serum.² It also gives variably higher values for IPTH in hyperparathyroid serum than does GP-1 M, suggesting that it measures an additional immunoreactive PTH component(s) which may not be present in serum in a fixed proportion to the component that is primarily measured by antiserum GP-1 M. However, because of serum immunoheterogeneity, the basic problem of interpretation of PTH immunoassay results will not be resolved until all circulating forms of IPTH can be adequately characterized chemically as well as immunologically.

Whereas the development of antisera which permit immunologic differentiation of primary from ectopic hyperparathyroidism must be largely due to chance, we doubt that our antisera are unique in this regard. Sherwood, O'Riordan, Aurbach, and Potts (3) showed that the dilutional curve with serum from a patient with hypercalcemia associated with bronchogenic carcinoma was similar to that obtained with standard gland-extracted bPTH. However, in a study of 26 patients with cancer (20 were normocalcemic; 6 were hypercalcemic and 2 of these had a clear-cut ectopic hyperparathyroidism syndrome) Roof, Carpenter, Fink, and Gordan (14) found that the more sensitive of their two antisera failed to recognize IPTH in the sera of 11 of these 26 patients, but IPTH could be detected in these patients using the other antiserum.

We interpret our data as indicating that the predominant immunoreactive material present in the serum of most patients with primary hyperparathyroidism is similar to but not structurally identical with the PTH present in the serum of patients with primary hyperparathyroidism. It is possible that the primary structure of the "PTH" secreted by cancer cells differs from that of PTH synthesized and secreted by parathyroid glandular tissue. An alternate possibility, and the one favored by us, is that the immunoreactive material in the serum of most patients with ectopic hyperparathyroidism is a precursor or intermediate form of the normally secreted hormone. Considerable evidence now exists that the predominant molecular species of PTH in parathyroid glandular tissue differs from the predominant molecular species in the serum. Berson and Yalow (13) demonstrated that

² Unpublished data.

one of their three anti-PTH antisera reacted differently with gland-extracted and serum PTH. With the same antiserum used in the present study (GP-1 M), Arnaud, Tsao, and Oldham (15) demonstrated that there are various PTH immunoreactive species in urea extracts of parathyroid adenomata and that the PTH extracted from parathyroid adenomata is immunologically different from the PTH present in hyperparathyroid serum or in parathyroid tumor-culture media. Sherwood, Rodman, and Lundberg (16) made similar observations in serum and in bovine glands in tissue culture and Sherwood, Lundberg, Targovnik, Rodman, and Seyfer (16, 17) and Arnaud et al. (18) have shown that the PTH molecule extracted from parathyroid glands has a larger molecular weight than that secreted into parathyroid tissue-culture media. More recently, Oldham, Fischer, Sizemore, and Arnaud (19) produced evidence that porcine parathyroid glandular tissue contains a calcium-dependent enzyme which, in vitro, converts the higher molecular weight glandular molecule into a form that is immunochemically similar to secreted PTH.

These considerations suggest that cancer cells may produce a PTH precursor but lack the ability to convert it to the secreted species of PTH. Proof of this hypothesis will require either the demonstration of immunochemical identity of the circulating immunoreactive material in ectopic hyperparathyroidism with a precursor molecule found in parathyroid tissue or the demonstration in vitro that cultured cancer tissue removed from patients with the syndrome secretes the precursor but not the secreted species of PTH.

Regardless of the biochemical nature of this PTH-like material, a means for immunologically differentiating most patients with hyperparathyroidism due to nonparathyroid cancer from those with primary hyperparathyroidism is of great clinical value.

ACKNOWLEDGMENTS

This investigation was supported in part by Research Grants AM-12302 and AM-8665 from the National Institutes of Health, Public Health Service.

REFERENCES

1. Lafferty, F. W. 1966. Pseudohyperparathyroidism. *Medicine (Baltimore)*. 45: 247.
2. Munson, P. L., A. H. Tashjian, Jr., and L. Levine. 1965. Evidence for parathyroid hormone in nonparathyroid tumors associated with hypercalcemia. *Cancer Res.* 25: 1062.
3. Sherwood, L. M., J. L. H. O'Riordan, G. D. Aurbach, and J. T. Potts, Jr. 1967. Production of parathyroid hormone by nonparathyroid tumors. *J. Clin. Endocrinol. Metab.* 27: 140.
4. Knill-Jones, R. P., R. M. Buckle, V. Parsons, R. Y. Calne, and R. Williams. 1970. Hypercalcemia and increased parathyroid-hormone activity in a primary hepatoma: studies before and after hepatic transplantation. *N. Engl. J. Med.* 282: 704.

5. Berson, S. A., and R. S. Yalow. 1966. Parathyroid hormone in plasma in adenomatous hyperparathyroidism, uremia, and bronchogenic carcinoma. *Science (Washington)*. **154**: 907.
6. Reiss, E. 1968. Discussion. *Trans. Ass. Amer. Physicians Philadelphia*. **81**: 114.
7. Potts, J. T., Jr., and L. J. Deftos. 1969. Parathyroid hormone, thyrocalcitonin, vitamin D, bone and bone mineral metabolism. In *Duncan's Diseases of Metabolism*. P. K. Bondy, editor. W. B. Saunders Company, Philadelphia. 6th edition. **2**: 904.
8. Arnaud, C. D., H. S. Tsao, and T. Littledike. 1971. Radioimmunoassay of human parathyroid hormone in serum. *J. Clin. Invest.* **50**: 21.
9. Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**: 375.
10. Keating, F. R., Jr., J. D. Jones, L. R. Elveback, and R. V. Randall. 1969. The relation of age and sex to distribution of values in healthy adults of serum calcium, inorganic phosphorus, magnesium, alkaline phosphatase, total proteins, albumin, and blood urea. *J. Lab. Clin. Med.* **73**: 825.
11. Rao, C. R. 1952. In *Advanced Statistical Methods in Biometric Research*. John Wiley & Sons, Inc., New York. 390.
12. Dixon, W. J., and F. J. Massey, Jr. 1969. In *Introduction to Statistical Analysis*. McGraw-Hill Book Company, New York. 3rd edition. 638.
13. Berson, S. A., and R. S. Yalow. 1968. Immunochemical heterogeneity of parathyroid hormone in plasma. *J. Clin. Endocrinol. Metab.* **28**: 1037.
14. Roof, B. S., B. Carpenter, D. J. Fink, and G. S. Gordan. 1971. Some thoughts on the nature of ectopic parathyroid hormones. *Amer. J. Med.* **50**: 686.
15. Arnaud, C. D., H. S. Tsao, and S. B. Oldham. 1970. Native human parathyroid hormone: an immunochemical investigation. *Proc. Nat. Acad. Sci. U. S. A.* **67**: 415.
16. Sherwood, L. M., J. S. Rodman, and W. B. Lundberg. 1970. Evidence for a precursor to circulating parathyroid hormone. *Proc. Nat. Acad. Sci. U. S. A.* **67**: 1631.
17. Sherwood, L. M., W. B. Lundberg, Jr., J. H. Targovnik, J. S. Rodman, and A. Seyfer. 1971. Synthesis and secretion of parathyroid hormone in vitro. *Amer. J. Med.* **50**: 658.
18. Arnaud, C. D., G. W. Sizemore, S. B. Oldham, J. A. Fischer, H. S. Tsao, and E. T. Littledike. 1971. Human parathyroid hormone: glandular and secreted molecular species. *Amer. J. Med.* **50**: 630.
19. Oldham, S. B., J. A. Fischer, G. W. Sizemore, and C. D. Arnaud. Calcium-dependent enzymatic conversion of glandular to secreted parathyroid hormone. Proceedings of the 4th Parathyroid Conference, Chapel Hill, N. C. 1971. Excerpta Medica Foundation, International Congress Series. In press.