Multiple Immunoreactive Molecular Forms of Parathyroid Hormone in Human Serum[1] (36681)

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The radioimmunoassay of parathyroid hormone (PTH) in human serum or plasma has proved difficult, and conflicting results have been reported from various laboratories (1). The existence of immunochemical heterogeneity of circulating PTH was first demonstrated by Berson and Yalow in 1968 (2). Subsequently, Arnaud, Tsao and Oldham (3) and Sherwood, Rodman and Lundberg (4) independently demonstrated that PTH extracted from parathyroid glands and PTH in serum or plasma are immunologically different. More recently, Habener *et al.* (5) demonstrated that PTH is secreted into the circulation as glandular hormone and then converted to a smaller molecular species.

The present study demonstrates the existence of immunoreactive fragments of PTH in human sera. The results explain the discrepancy of data obtained by investigators using different antisera.

Materials and Methods. Peripheral venous blood was collected from hyperparathyroid patients before, during, and after calcium infusion or parathyroidectomy. Calcium was given intravenously as calcium gluconate–glucoheptonate, 4 mg Ca/kg/hr for 4 hr. Serum samples from 5 similar patients were pooled, concentrated, and fractionated, as shown in Fig. 1. Pooling of samples was necessary in order to obtain sufficient amounts of PTH in the eluted fractions. Immunoreactive PTH was measured in the eluted fractions by a sensitive radioimmunoassay using one antiserum, CH-824 (6). This antiserum has been used in all previous human studies from this laboratory. Assay results are expressed in microliter equilvalents per milliliter, an arbitrary unit relating the potency of test samples to that of an arbitrarily selected standard hyperparathyroid serum. PTH in eluted tubes was measured at two widely varying concentrations, and assays were repeated on tubes containing any measurable hormone. The coefficient of variation of samples assayed at different concentrations was 10%.

Results. Fractionation of sera from patients with hyperparathyroidism due to a single adenoma are shown in Fig. 2. Immunoassayable PTH emerged in 3 distinct peaks. From the characteristics of column, the molecular weights for peaks I, II, and III were estimated to be 9500, 7000–7500, and 4500–5000, respectively. Since the elution volume of peak I coincided with that of the ^{131}I-PTH marker, this peak is assumed to represent glandular hormone. In this experiment, the same eluted fractions were assayed for PTH using a second antibody (CH-71). The resulting pattern of immunoreactivity in the eluted fractions was very similar to that shown in Fig. 2, indicating that the two antibodies used possessed similar affinity characteristics for glandular hormone and the smaller fractions.

The three molecular species of PTH separated by gel chromatography were immunologically distinct (Fig. 3). The bound to free hormone concentration (B/F), expressed as

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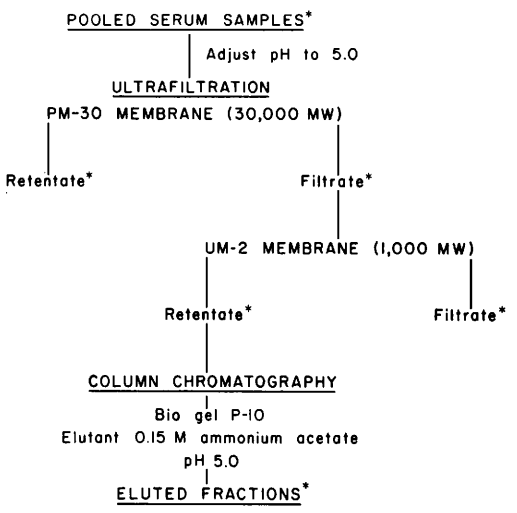

*Immunoassay of PTH

FIG. 1. Procedure for concentration and fractionation of hyperparathyroid sera. The concentrating membranes used were Amifco Diaflo membranes (Amicon Corp., Lexington, MA). A 15–20-fold concentration was attained. Bio-gel was obtained from Bio-Rad Laboratories. The column size was 1.5 × 50 cm. Bovine serum albumin (2%) was passed through the column immediately before addition of serum samples to minimize loss of hormone.

percentage of incubations containing only trace amounts of labeled hormone, is shown as a function of varying amounts of unlabeled material. In this system, unfractionated whole serum yields a function which is indistinguishable from that obtained with glandular bovine or human PTH (6, 7). Adjustment of the abscissa scale made possible superimposition of functions obtained with unfractionated serum and with peak I. A superimposition of functions could not be effected for peaks II and III, no matter how the abscissa scale was adjusted. Such results have been generally interpreted as indicating immunologic differences between the peaks and similarity between peak I and unfractionated serum (3).

In Fig. 4 are shown results of fractionation of sera from patients with primary hyperparathyroidism before, during, and after parathyroidectomy (PTX) for a single adenoma. At the time of PTX, peak I increased markedly. However, by 30 min after PTX, peak I had disappeared and remained absent thereafter. Peaks II and III persisted. By 90 min, peak II had decreased significantly, and peak III remained as the predominating circulating immunologic moiety.

The results of similar studies on sera obtained before, during, and after calcium infusions are shown in Figs. 5 and 6. Figure 5 shows the elution profiles of immunoreactive PTH obtained during a 4 hr calcium infusion in patients with primary hyperplasia. The base line pattern was similar to that obtained in patients harboring a single adenoma. Peak I decreased during the calcium infusion, being definitely smaller by 1 hr, barely detectable by 2 hr, and definitely absent thereafter. There was a suggestion that peak II also decreased during the course of the calcium infusion while peak III remained essentially stable. In Fig. 6 is shown the same study performed in patients with adenomas. There was a small decrease of peak I throughout the calcium infusion, but this peak remained clearly detectable after 4 hr of infusion. Peak II decreased slowly while peak III actually increased.

Fractionation of sera from patients with severe chronic renal insufficiency (creatinine clearance, < 15 ml/min) is shown in Fig. 7. Peak I again eluted in the same area as the

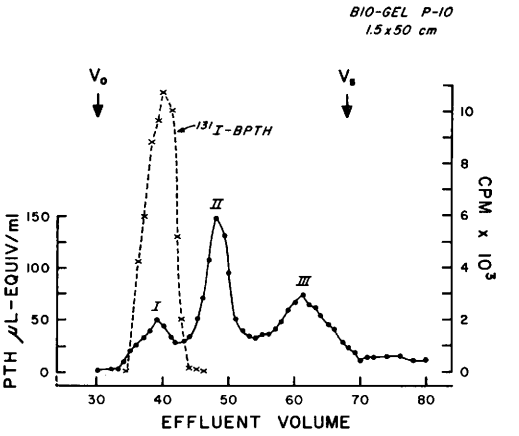

FIG. 2. Fractionation of pooled sera from patients with hyperparathyroidism due to a single adenoma. V_o and V_s indicate the void and salt volumes, respectively. (- -) the elution pattern of ^{131}I-labeled highly purified bovine PTH (Wilson Laboratories). (—) the elution profile of immunoassayable PTH.

^{131}I-labeled bovine PTH marker. Peaks II and III also had the same elution pattern as the corresponding fragments in patients with primary hyperparathyroidism. However, a smaller molecular weight fragment appeared in the salt volume. On Bio-gel P-6, the molecular weight of this fragment approximated 2500.

Control experiments to determine whether concentration of serum samples and the pooling procedure introduced artifacts were performed but are not shown in graphic form. When the entire procedure was applied to highly purified bovine PTH, with and without ^{131}I-label, all immunoreactivity and radioactivity eluted in a single peak, indicating that the experimental procedure does not result in fragmentation of glandular hormone. Concentrating large volumes of serum from a single hyperparathyroid patient and subsequent fractionation yielded an elution profile of immunoreactive PTH identical with that observed with pooled samples. Hence, pooling did not introduce artifacts.

The overall recovery of immunoreactive PTH through the entire concentrating and

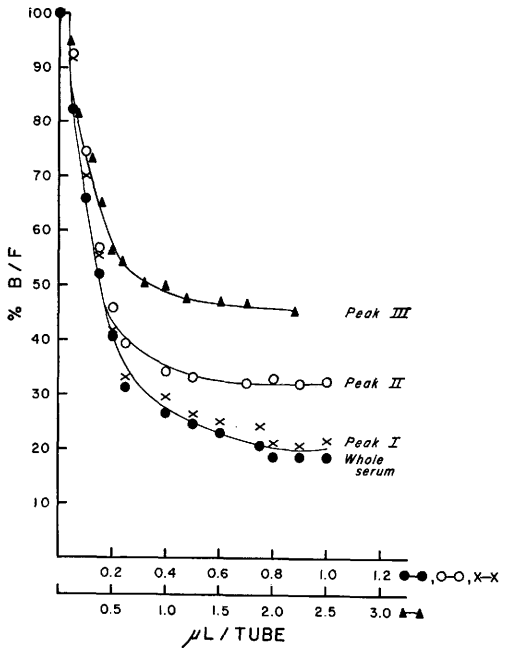

FIG. 3. Bound to free hormone concentration (B/F) as a function of unlabeled hormone or hormone fragments in the incubation mixture. B/F is expressed as percentage of incubations containing only tracer hormone. Peak I, tubes 36–42; peak II, tubes 45–51; and peak III, tubes 59–66 (Fig. 2). Pools were concentrated by lyophilization. Absolute units on abscissa are arbitrary. Failure to achieve superimposition of the functions for the 3 peaks indicates immunologic differences between them.

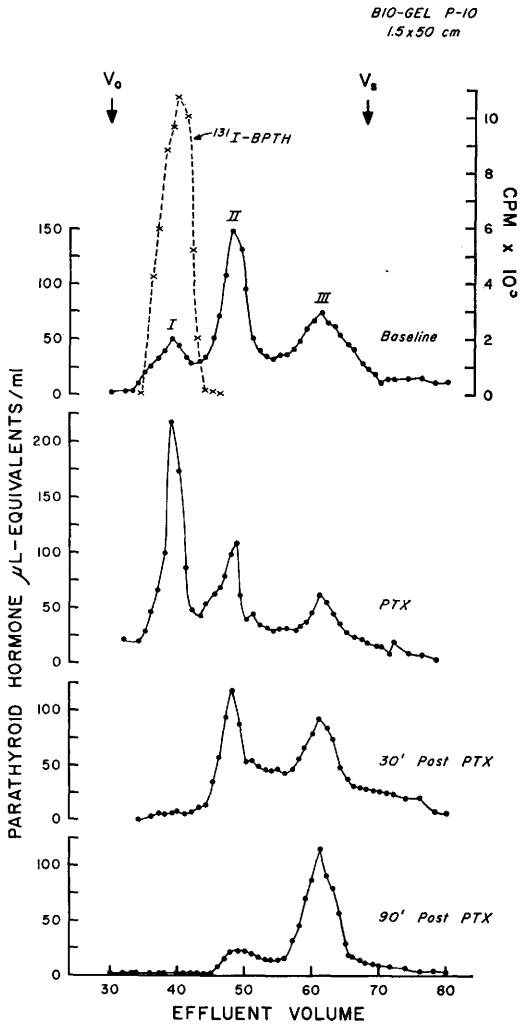

FIG. 4. Fractionation of pooled sera from patients with hyperparathyroidism before, during, and after parathyroidectomy (PTX) for a single adenoma. The top panel is identical with Fig. 2. At the time of PTX, peak I increased markedly, but disappeared rapidly thereafter. The data indicate different kinetic properties of the 3 peaks, with some suggestion of conversion from higher to lower molecular weight species.

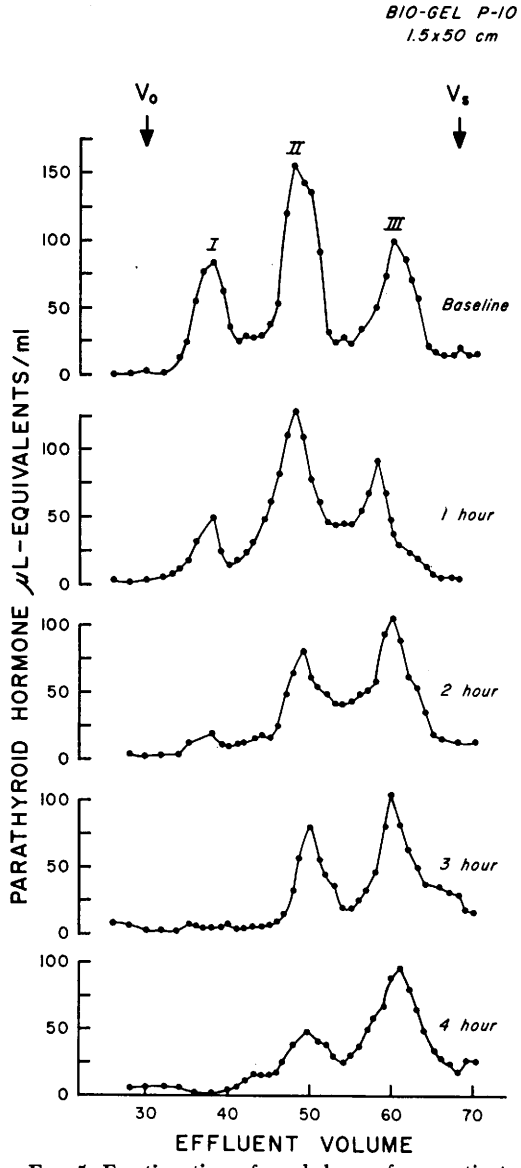

FIG. 5. Fractionation of pooled sera from patients with primary hyperplasia of the parathyroids before and during an intravenous calcium infusion. The base line pattern is similar to that obtained in patients with a single adenoma. Peak I disappeared during the infusion.

fractionating procedure was approximately 40%.

Discussion. The present studies demonstrate the existence of at least three distinct immunoreactive species of PTH in peripheral blood. These differ in molecular size, immunologic characteristics, and kinetic properties.

Peak I (mol wt 9500) was identified as glandular hormone by its elution in a pattern identical with that of highly purified bovine PTH. Its reaction with our antiserum was the same as that previously observed with glandular bovine and human hormone (7). In addition, it had the kinetic characteristics of glandular hormone, as previously reported by others (8).

The data suggest that the small fragments (peaks II and III) represent metabolic degradation products of glandular hormone. This interpretation is based on the striking increase of glandular hormone at the time of parathyroidectomy when physical manipulation of the parathyorid glands releases preformed hormone into the circulation (9, 10). Habener *et al.* (5) have recently demonstrated directly by collection and assay of parathyroid effluent blood that PTH is secreted

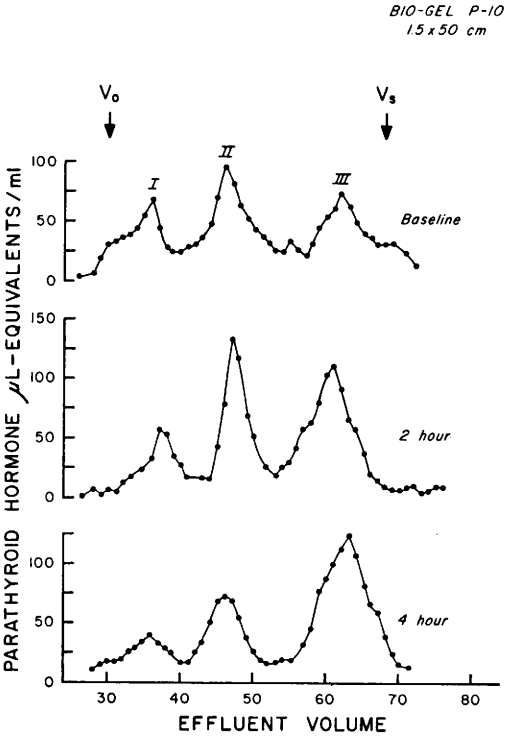

FIG. 6. Fractionation of pooled sera from patients with parathyroid adenoma before and during an intravenous calcium infusion. Although peak I decreased during the infusion, the rate of decrease was small and distinctly different from that observed in patients with hyperplasia (Fig. 5).

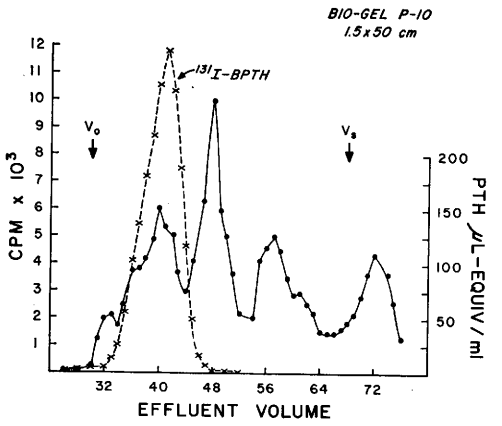

FIG. 7. Fractionation of sera from chronically uremic patients. The first 3 peaks emerged in the expected pattern. Note presence of a fourth peak in the salt volume.

in the form of glandular hormone. They also demonstrated the presence of smaller fragments in the peripheral circulation but, using unconcentrated plasma, their fractionation did not yield discrete fragments as reported here. The alternative explanation, that cleavage of PTH occurs at the time of secretion, is therefore not supported by Habener and coworkers' or our data.

The present study is limited in four respects: (a) The existence of circulating molecular species of PTH larger than glandular hormone cannot be excluded since the void volume was not examined systematically. The existence of a prohormone in parathyroid glands has recently been documented by two groups of investigators (11, 12). (b) All of our sera were derived from patients with abnormal parathyroid function, and it is therefore not clear whether PTH fragments are also present in normal sera. Several attempts to fractionate large pools of concentrated normal serum failed to yield sufficient amounts of immunoreactive PTH for assay. (c) The quantitative relationship between PTH fragments is only approximate because of their immunologic differences. (d) We have no indication yet about the biologic activity of the fragments.

The demonstration of immunoreactive fragments of PTH in the peripheral circulation could readily explain the conflicting immunoassay results reported from various laboratories. The antiserum used in these studies reacts well with circulating fragments but does not appear to possess strong affinity for glandular human PTH. Since different antisera probably have different affinities for glandular hormone and its fragments, divergent results should be expected. In view of the kinetic differences between glandular hormone and the fragments, an antiserum possessing high affinity for smaller molecular weight species of PTH should have a high capacity for discriminating between normal and abnormal sera. Our antiserum appears to have such affinity characteristics. By contrast, an antiserum having a high affinity for glandular hormone would be particularly well suited for studies involving secretory rates and preoperative localization of abnormal parathyroid glands by means of differential venous catheterization of the parathyroids.

For standardization of the immunoassay technique in the future, it is likely that each antiserum will have to be characterized with respect to its affinity characteristics to various hormone fractions. Ideally, antiserum should be produced to specific fragments. With the rapid progress in the chemistry of the hormone now being made, including synthesis of various fragments, it is likely that the production of specific antiserum will be possible (13, 14). Individual test sera will then have to be evaluated with a variety of antisera, the choice of antiserum depending on the study to be performed.

Summary. Sera from patients with various forms of hyperparathyroidism were concentrated and fractionated on Bio-gel P-10 columns. Eluted fractions were assayed for immunoreactive PTH. In addition to glandular PTH, two smaller fragments were identified (mol wt 7000–7500 and 4500–5000, respectively). Glandular PTH and fragments differed in their immunologic and kinetic properties. The results appear to explain divergent results reported from various laboratories in the application of the immunossay of PTH to clinical investigation.

J. M. C. has submitted these data in partial fulfillment of the research requirements for the PhD

degree in the Department of Biology, Illinois Institute of Technology, Chicago, IL.

1. Forscher, B. K., and Arnaud, C. D., Amer. J. Med. **50**, 557 (1971).

2. Berson, S. A., and Yalow, R. S., J. Clin. Endocrinol. **28**, 1037 (1968).

3. Arnaud, C. D., Tsao, H. S., and Oldham, S. B., Proc. Nat. Acad. Sci. U.S.A. **67**, 415 (1970).

4. Sherwood, L. M., Rodman, J. S., and Lundberg, W. B., Proc. Nat. Acad. Sci. U.S.A. **67**, 1631 (1970).

5. Habener, J. F., Powell, D., Murray, T. M., Mayer, G. P., and Potts, J. T., Jr., Proc. Nat. Acad. Sci. U.S.A. **68**, 2986 (1971).

6. Reiss, E., and Canterbury, J. M., Proc. Soc. Exp. Biol. Med. **128**, 501 (1968).

7. Reiss, E., and Canterbury, J. M., Amer. J. Med. **50**, 679 (1971).

8. Melick, R. A., and Martin, T. J., Clin. Sci. **37**, 667 (1969).

9. Reiss, E., and Canterbury, J. M., N. Engl. J. Med. **280**, 1381 (1969).

10. Berson, S. A., and Yalow, R. S., N. Engl. J. Med. **277**, 640 (1967).

11. Cohn, D. V., MacGregor, R. R., Chu, L. J. H., and Hamilton, J. W., *in* "Proceedings of IV Parathyroid Conference" (R. V. Talmage, ed.). Excerpta Medica, Amsterdam, 1972.

12. Kemper, B., Habener, J. F., Potts, J. T., Jr., and Rich, A., Proc. Nat. Acad. Sci. U.S.A. **69**, 643 (1972).

13. Brewer, H. B., Jr., and Ronan, R., Proc. Nat. Acad. Sci. U.S.A. **67**, 1862 (1970).

14. Potts, J. T., Jr., Tregar, G. W., Keutmann, H. T., Niall, H. D., Sauer, R., Deftos, L. J., Dawson, B. F., Hogan, M. L., and Aurbach, G. D., Proc. Nat. Acad. Sci. U.S.A. **68**, 63 (1971).

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