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Native Human Parathyroid Hormone: an Immunochemical Investigation*

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Abstract. We have produced an antiserum to porcine parathyroid hormone (PTH) which distinguishes between the PTH in human hyperparathyroid serum and that in extracts of parathyroid adenomata. The PTH secreted into the medium by parathyroid adenoma slices in culture reacts with this antiserum in the same way as the PTH in human hyperparathyroid serum, but extraction of the same serum by a conventional procedure used for the extraction of glandular tissue converts the serum hormone to a species which reacts more like hormone extracted from adenomata. The results suggest that we have immunologically identified a native molecular species of PTH, that the antiserum used recognizes primarily this species in human hyperparathyroid serum, and that the PTH extracted from parathyroid adenomata is immunochemically altered.

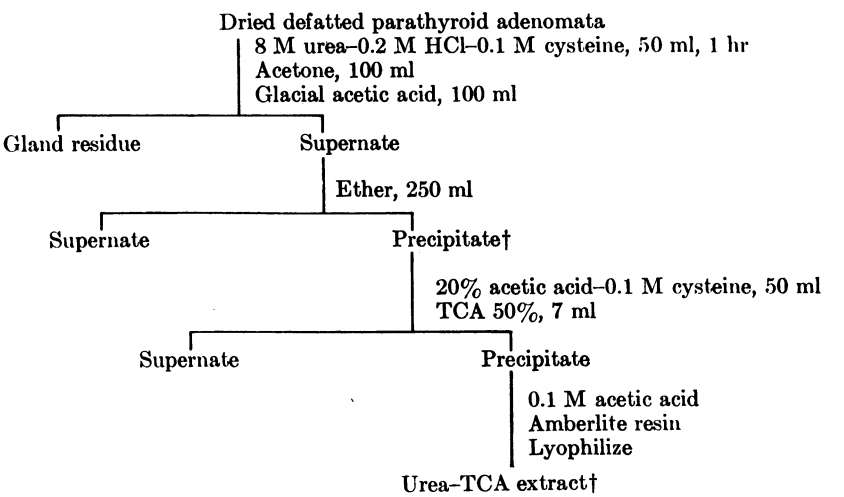
It is likely that the naturally occurring form of a secreted polypeptide hormone is the ideal molecular species to use as a standard preparation in immunoassays of the hormone in serum. It often has been assumed that polypeptide hormones extracted and isolated from glandular tissue are true representatives of the native secreted species, but there is little evidence that, in specific instances, this is true.

Most studies of the biologic and immunologic activities[1–10] as well as the covalent sequence[8,10] and specific molecular configuration[5,8,11] of parathyroid hormone (PTH) have been made with a molecular species extracted and isolated from bovine glandular tissue by procedures[1,8,12] which have the potential for altering the native structure of the molecule. We have now produced an antiserum to PTH which distinguishes between the PTH in extracts of human parathyroid adenomata and the PTH in hyperparathyroid serum. In our immune system, serum PTH cannot be distinguished from the PTH in parathyroid tumor culture medium, but extraction of the serum by the procedures used in the isolation of bovine PTH from glands[1,8,12] converts the serum PTH to a species which reacts immunologically in a manner similar to PTH extracted from adenomata. The results of these experiments suggest that we have immunologically identified a native molecular species of human PTH, that the antiserum used recognizes primarily the native hormone in serum, and that the hormone extracted from parathyroid adenomata is immunochemically altered.

Methods. Sources of human parathyroid hormone: Parathyroid adenomata (50 g) were lyophilized and defatted with chloroform. The resulting 10 g of dry powder was extracted with urea–HCl–cysteine, followed by fractionation with acetone, ether, sodium chloride, and trichloroacetic acid (TCA) as previously described for bovine PTH[13] (Table 1). This extract was further purified by gel filtration on a 1 × 200 cm column of Sephadex G-100 (Fig. 1). Human PTH was measured in the eluate fractions by radioimmunoassay.

Sera were obtained from patients with primary, secondary, or tertiary hyperparathyroidism and from two patients with parathyroid cancer. Serum from one of these latter

TABLE 1. *Procedure for isolation of parathyroid hormone.**



* Entire procedure was performed at 4°C.
† Extracts used in immunochemical studies.

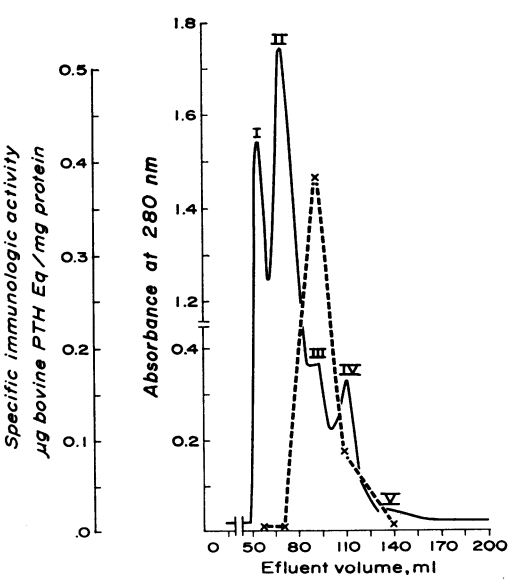


FIG. 1. Elution diagram of gel filtration of 98 mg of urea-TCA extract (from dried defatted parathyroid adenomata) on 1 × 200-cm column of Sephadex G-100. Eluent, 0.2 M ammonium acetate.

Fraction size, 3.0 ml.

Solid line, optical density at 280 nm.

Broken line, specific immunologic activity based on protein and bovine PTH equivalent weight.

patients was extracted in the same manner as that described for parathyroid adenomata to the stage of the TCA precipitate (Table 1).

Thin slices were prepared from a freshly excised parathyroid tumor (adenomatous hyperplasia) and cultured on wire mesh in a defined medium.[14] Medium was collected after 24 hr and frozen (−15°C) until used.

Radioimmunoassay: An antiserum to porcine PTH (G.P. 1 anti-pPTH) was produced by injecting a guinea pig subcutaneously every 2–4 weeks with a 1:1 homogenate of Freund's adjuvant and crude intermediate TCA precipitate (from extracted, dried, defatted porcine parathyroid glands[15]) dissolved in 0.01 M acetic acid. With this antiserum, a radioimmunoassay for human PTH was developed as a modification of that previously described for porcine PTH[16]; the details are being published separately.[17] All preparations of human PTH (serum, adenoma extracts, and culture medium) were added to human hypoparathyroid serum immediately prior to addition to the immune system, and incubations were carried out under identical conditions. A nonequilibrium system was used: incubation of antiserum with unlabeled antigen for 3 days and then with ^{131}I-labeled bovine PTH for 3 days, all at 4°C. Bovine PTH was labeled with ^{131}I by the Berson and Yalow modification[18] of the procedure of Greenwood *et al.*[19] Free and antibody-bound ^{131}I-labeled PTH was separated either by chromatoelectrophoresis[20] or by a modification of the dextran-coated charcoal procedure.[21] Results were analyzed by dividing the value for the bound-to-free ratio (B/F) obtained for a given incubation mixture containing unlabeled human PTH by that obtained for an incubation mixture containing no unlabeled human PTH. Curves were constructed by using these percentages of "trace" values as a function of unlabeled hormone concentration. They were compared by attempting to superimpose them visually as previously described.[9,22] All experiments presented are representative of at least three in which the results were essentially the same. Comparisons were made only within the same experiment.

Results. Seven preparations of PTH extracted from parathyroid adenomata were studied: two intermediate products of the adenoma extraction procedure (Table 1, marked by †) and five fractions obtained from the protein peak of the gel filtration on Sephadex G-100. Most of the immunologic activity was eluted in fraction III (G-100 PTH), and the peak tube of this fraction had a specific immunologic activity of 0.38 based on protein[23] and standard homogeneous bovine PTH, which shows no essential immunologic difference from fraction III when studied in this immune system (Fig. 2). However, all of the other fractions also had immunologic activity (Fig. 3). The specific immunologic activities of these fractions, although markedly less than fraction III, could not be assessed accurately: no suitable standard was available, because each had unique immunoreactivity in the system as gauged by the patterns of their standard curves (Fig. 3).

The composite of curves shown in Fig. 3 was constructed by adjusting protein concentration scales in such a way as to attempt to superimpose the curves. Except for the two intermediate products of the adenoma extraction procedure, no adjustment of protein concentrations was successful in this regard; this indicates immunologic differences between these PTH preparations. Particularly striking is the difference between the immunoreactivity of serum PTH and the immunoreactivities of adenoma extracts and fractions from the Sephadex G-100 fractionation.

Sera from patients with seven different clinical types of hyperparathyroidism (primary without renal failure, primary with renal failure, secondary associated with renal failure treated and not treated with hemodialysis, secondary associated

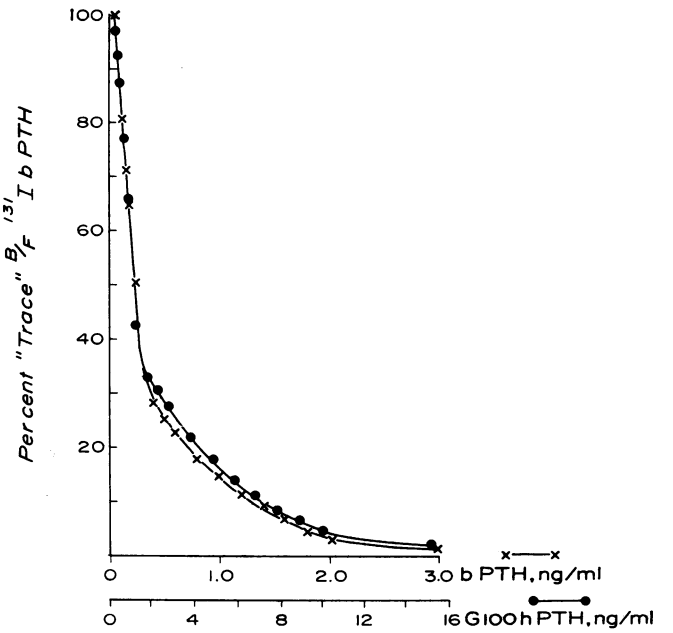


FIG. 2. Comparison of highly purified bovine PTH (bPTH, X) and G-100 human PTH (hPTH, ●) in radioimmunoassay using G.P. 1 anti-pPTH antiserum, 1:75,000.

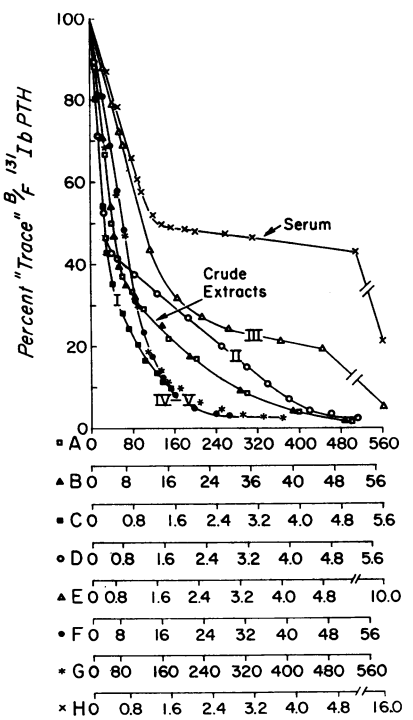


FIG. 3. Comparison of various PTH preparations in radioimmunoassay system using G.P. 1 anti-pPTH antiserum, 1:75,000.

A, crude extract, ether precipitate, parathyroid adenoma, ng/ml.

B, crude extract, TCA precipitate, parathyroid adenomata, ng/ml.

C, fraction I (Fig. 1), μg/ml.

D, fraction II (Fig. 1), μg/ml.

E, fraction III (Fig. 1), ng/ml.

F, fraction IV (Fig. 1), ng/ml.

G, fraction V (Fig. 1), ng/ml.

H, hyperparathyroid serum, μl/ml.

with gastrointestinal malabsorption, tertiary after renal transplantation, and parathyroid cancer) were studied in multiple dilution in the immunoassay system (Fig. 4). Adjustment of the scale of the concentration of each patient's serum in the system allowed the curves produced by all sera to be superimposed. This result suggests that the immunoreactive species of PTH is the same in the serum of all hyperparathyroid subjects.

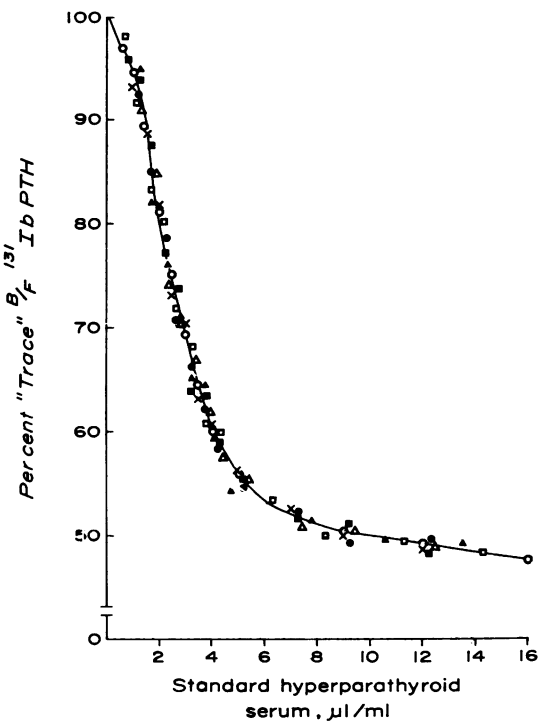


FIG. 4. Comparison of immunoreactivities of seven sera (from hyperparathyroid patients described in the text) in the radioimmunoassay system with G.P. 1 anti-pPTH antiserum, 1:75,000.

Of the several possible explanations of these results, it appeared most likely to us that the PTH in hyperparathyroid serum more closely represented the true secretory product of the gland and that the PTH in preparations of the urea-extracted adenoma tissue were immunochemically altered. This hypothesis was tested by comparing the immunochemical characteristics of human PTH in (1) untreated, lyophilized, and urea–trichloroacetic acid-extracted hyperparathyroid serum, (2) parathyroid tumor slice culture medium, and (3) fraction III from the Sephadex G-100 gel filtration (G-100 PTH). The results (Fig. 5) show that (*a*) lyophilization does not alter immunoreactivity of serum PTH; (*b*) the curve produced by PTH from tumor culture medium can be perfectly aligned with that obtained with serum PTH; (*c*) the curve produced by G-100 PTH cannot be aligned with that obtained with serum PTH; and (*d*) extraction of serum by the urea–TCA method converts the immunoreactive species of PTH in serum to one which reacts more like G-100 PTH.

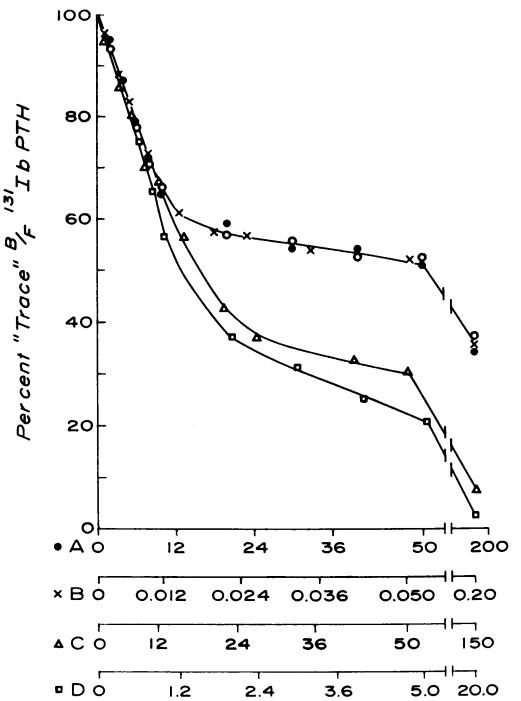


FIG. 5. Comparison of immunoreactivities in radioimmunoassay system using G.P. 1 anti-pPTH antiserum, 1:75,000.
A, hyperparathyroid serum, unlyophilized and lyophilized, μl.
B, parathyroid tumor culture medium, μl.
C, urea–TCA extract of hyperparathyroid serum shown in *A*, μg.
D, fraction III (Fig. 1), ng/ml.

Discussion. Our experiments show that at least two immunologic species of human PTH can be distinguished—one in hyperparathyroid sera and parathyroid tumor culture medium and the other in various preparations of human PTH extracted with urea from parathyroid adenomata.

The data obtained by studying different Sephadex G-100 fractions in the immune system might be construed as evidence in favor of the existence of PTH isohormones in life. However, in view of our observation that the immunochemical characteristics of serum PTH can be converted, by urea–TCA extraction of serum, to those of PTH extracted from adenomata, we consider that the results are more likely explained on the basis of artifactual, extraction-induced changes of the PTH molecule, possibly involving its tertiary structure. Furthermore, on the basis of chance alone, it is unlikely that the extraction procedure used could have selected isohormones of PTH which reacted in the immune system so differently from serum PTH or tumor culture medium PTH. Also, there were no essential differences in the immunochemical characteristics of any of the seven hyperparathyroid sera studied, in contrast to what one might have expected if different quantities of isohormones of PTH were being secreted by different types of pathologic parathyroid tissue. The data cannot completely exclude the possibility that isohormones of PTH might have contributed to some of the immunochemical differences we have observed, but we consider their role to have been minor.

Berson and Yalow,[22] who reported studies with an antiserum (to bovine PTH) with qualities similar to those of our G.P. 1 anti-pPTH, reached similar con-

clusions regarding the existence of isohormones of PTH. In the same paper, they gave convincing evidence for immunoheterogeneity of PTH in plasma. While two antisera to bovine PTH (272 and 273) could not distinguish between the PTH in a 20% acetone–1% acetic acid extract of a normal parathyroid gland and the PTH in hyperparathyroid serum, another antiserum (329) could. Moreover, the rate of disappearance of PTH from the serum after parathyroidectomy in patients with hyperparathyroidism seemed to be much greater when it was assayed with antiserum 329 than with 271 or 272. We have confirmed this work with another antiserum to bovine PTH which cross-reacts with human PTH.

These observations lead to difficult problems in the interpretation of assays of human PTH in serum and seem to us to be related to (1) the identification of the native, unaltered, secreted PTH and its use as a standard in radioimmunoassays and (2) the selection of an antiserum which recognizes this molecular species primarily and not others which are chemically modified by metabolic processes. Our experiments with the antiserum G.P. 1 anti-pPTH, which compared the immunochemical characteristics of serum PTH, tumor culture PTH, and G-100 PTH, have direct bearing on these problems. It is unlikely that the PTH molecule secreted into the defined medium (no serum) of the isolated tumor culture system *in vitro* was altered by any metabolic process. Yet immunochemically it was indistinguishable from the PTH in serum but markedly different from the PTH in adenoma extracts at all levels of purity and the PTH in a urea–TCA extract of hyperparathyroid serum. These observations strongly suggest that the PTH in the tumor culture medium is a true representation of a native secreted species of PTH, that G.P. 1 anti-pPTH primarily recognizes this molecule in hyperparathyroid serum, and that the molecular species of PTH in our adenoma extracts are chemical modifications of the native species. Hence, the radioimmunoassay system which we have developed for human PTH, using G.P. 1 anti-pPTH as antiserum and hyperparathyroid serum or tumor culture medium as a standard (details to be published), and which is extremely sensitive (capable of measuring the PTH in 94% of normal sera) may have surmounted the problem of immunoheterogeneity of PTH in serum.

The observations presented in this paper have raised serious concern in our minds regarding our present knowledge of the chemistry and biology of PTH. Most, if not all, studies of this important hormone have used a molecule which is probably altered chemically from that which exists in life. The immunochemical changes we have demonstrated may reflect only minor alterations in the structure of the native molecule and may not influence its biologic potential or its metabolic handling. However, we think that answers to these questions can be obtained only after the native secreted molecular species of PTH is isolated, characterized chemically, and studied both biologically and immunologically.

In a more general way, our work emphasizes the importance of exercising great care in selecting antisera and standard preparations when radioimmunoassays are developed for the measurement of proteins in biologic fluids. As is already clear from the work of Berson and Yalow,[22] the demonstration of im-

munologic similarity between a standard hormone preparation and an immunologic species present in serum is not sufficient proof that a given antiserum recognizes an unaltered secreted hormone. At present, it appears that certainty can be ensured only if the standard hormone is a true representation of the native secreted species and if the antiserum used recognizes this species and not others present in serum.

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Abbreviations: PTH, parathyroid hormone; TCA, trichloroacetic acid; G.P. 1 anti-pPTH, an antiserum to porcine PTH.

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