

## Parathyroid Hormone: Secretion and Metabolism *In Vivo*

(human/bovine/gel filtration/radioimmunoassay/venous catheterization)

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**ABSTRACT** Gel filtration and radioimmunoassay were used to determine the molecular size and immunochemical reactivity of parathyroid hormone present in gland extracts, in the general peripheral circulation, and in parathyroid effluent blood (obtained by venous catheterization) from patients with hyperparathyroidism, as well as from calves and from cattle. Hormone secreted *in vivo* from normal bovine parathyroid glands and from human parathyroid adenomas is similar in size to the 84-amino-acid peptide (molecular weight of 9500) extracted from the parathyroids. However, much of the immunoreactive parathyroid hormone present in the peripheral circulation of man and cattle is smaller than the extracted or secreted hormone; it elutes from gel columns at a position corresponding to a molecular weight of about 7000. The immunological characteristics of extracted and secreted hormone are identical, while hormone in the general circulation is immunologically dissimilar to extracted and secreted hormone. The results indicate that parathyroid hormone secreted from the parathyroids in man and cattle is at least as large as the molecule extracted from normal bovine glands. However, once secreted into the circulation the hormone is cleaved, and one or more fragments, immunologically dissimilar to the originally secreted hormone, constitute the dominant form of circulating immunoreactive hormone.

Despite recent advances in the structural characterization of parathyroid hormone (extracted from parathyroids) (1-3) and the synthesis of a biologically active peptide consisting of the first 34 amino acids (5), recent findings based on radioimmunoassays have suggested that the biosynthesis and secretion, as well as the metabolism, of endogenous parathyroid hormone in man and animals is quite complex and that, in fact, the exact chemical nature of the active circulating form of the hormone is not yet known (3, 6-9).

Berson and Yalow reported that plasma parathyroid hormone is immunologically different from hormone extracted from tissue, but the chemical basis of this heterogeneity of plasma hormone could not then be determined (7). Other workers have subsequently reported finding immunological differences between the parathyroid hormone extracted from the glands and that found either in plasma or secreted into culture medium during incubation of parathyroid tissue (8, 9). In fact, Arnaud, *et al.* (6) and Sherwood, *et al.* (9) have recently reported that parathyroid hormone secreted from parathyroid tissue during incubation *in vitro* consists of a fragment of the stored 84-amino-acid peptide, and have further suggested that this fragment may also be the form in which the hormone is secreted into the circulation *in vivo*.

The purpose of this communication is to present, for the first time, direct evidence, based on studies *in vivo*, that there

are circulating peptide fragments smaller than the peptide extracted from the gland. However, contrary to the suggestions based on tissue-culture studies, our findings *in vivo*, both in patients with hyperparathyroidism and in normal cattle, indicate that the form of the hormone released from the gland is at least as large as the extracted peptide, and that the smaller circulating form or forms of parathyroid hormone arise from degradation of the secreted hormone during peripheral circulation. The circulating fragments of the hormone react differently in the radioimmunoassay than does the 84-amino-acid polypeptide used as standard; the fragments appear to account for much of the immunochemical difference between plasma hormone and hormone extracted from tissue. These studies point to the need for detailed investigations of the peripheral metabolism of parathyroid hormone and the chemical nature of the circulating molecular species of parathyroid hormone in order to improve the specificity of immunoassay techniques used in physiological and clinical studies.

### MATERIALS AND METHODS

*Source of Samples Containing Parathyroid Hormone.* Heparinized venous blood was obtained, after transfemoral venous catheterization, directly from the inferior thyroid vein or similar small veins draining the parathyroid gland, from 15 patients with primary hyperparathyroidism (10). Concentrations of parathyroid hormone in the plasma of these patients ranged from 15 to 320 ng/ml, concentrations 30 to 50-fold higher than the concentrations of hormone found in plasma obtained simultaneously from the peripheral circulation (0.5-6.0 ng/ml). The details of these catheterization procedures and their usefulness in localizing the site of abnormal parathyroid-hormone secretion have been reported in preliminary form (10)\*. Blood samples were also obtained from two patients with primary hyperparathyroidism, during the period of maximum hypocalcemia induced by an infusion of disodium ethylene-diamine tetraacetate (EDTA) (25 mg per kg per hr), and from nine other patients in the basal state. Blood was also obtained after surgical exposure of the small veins that drain the parathyroid glands of two anesthetized 2-month-old calves, as well as by venipuncture from several cows during the period of spontaneous hypocalcemia that occurs in association with parturition (11). The concentration of parathyroid hormone in these blood samples was

\* Powell, D., and J. L. Doppman, *Proc. 53rd Meeting of the Endocrine Society (J. B. Lippincott, Philadelphia 1971), Abstract.*

20–105 ng/ml. Plasma was immediately chilled, separated from erythrocytes, and frozen for subsequent studies.

**Gel Filtration.** Aliquots of 0.1–0.8 ml of plasma were chromatographed at 25°C on a 0.8 × 60 cm column of Bio-gel P-10 (100–200 mesh, Bio-Rad, Inc.). Immediately before each plasma sample was applied to the column, an aliquot of highly-purified <sup>125</sup>I-labeled bovine parathyroid hormone (50,000 cpm, 100 pg) was added to serve as an elution marker.

The Bio-gel columns were repeatedly calibrated by several additional procedures. The <sup>125</sup>I-labeled, synthetic, amino-terminal [1–34] fragment of parathyroid hormone (5) was added as a column marker (elution position; fractions 38–40); <sup>131</sup>Iodine was used to determine the salt volume (elution position; fractions 64–66). The void volume was marked by the elution of the largest plasma proteins (elution position; fractions 15–16). Additional calibrations were also made by filtration and radioimmunoassay of highly purified preparations of bovine parathyroid hormone, synthetic amino-terminal [1–34] bovine peptide, and a partially purified preparation of human parathyroid hormone (4). The elution buffer consisted of 0.05 M veronal (pH 8.4)–0.01 M sodium EDTA–0.05% merthiolate–10% (v/v) human plasma. 0.5-ml fractions were collected (flow rate, 8 ml/hr), and <sup>125</sup>I and <sup>131</sup>I radioactivity were measured simultaneously in a dual-channel gamma-well spectrometer (Packard), with appropriate isotope standards to correct for cross-channel interference. Hormone concentration was determined in 0.1, to 0.4-ml aliquots of each fraction by the radioimmunoassay.

**Radioimmunoassay.** Parathyroid hormone concentrations in plasma samples were measured in multiple dilutions and, in fractions collected from Bio-gel filtrations, measurements were made singly or in duplicate by a sensitive radioimmunoassay based on the bovine hormone (12). <sup>125</sup>I-labeled bovine

parathyroid hormone (300 Ci/g) was used as a tracer and partly-purified human parathyroid hormone (4) was used as a standard. Guinea pig antisera to bovine parathyroid hormone were used at dilutions of 1:250,000 (GP-1) and 1:5,000 (GP-62); thus, a bound-to-free ratio of radioiodinated hormone (B/F) of 0.8–1.2 gave maximum assay sensitivity. Incubations (0.5 ml) were at 4°C in 0.05 M Veronal buffer–0.05% merthiolate–15% (v/v) human plasma, under non-equilibrium conditions, for 6 days (Tracer was added after 3 days of incubation of sample and antiserum, and incubation was continued for an additional 3–4 days). Appropriate incubations of samples without added antiserum were conducted in each assay in order to correct for nonspecific effects due to damage of radioiodinated hormone (16).

## RESULTS

Fig. 1 compares the gel-filtration profiles of immunoreactive parathyroid hormone contained in plasma obtained by (a) venous catheterization of a small vein (inferior thyroid vein, left) draining a parathyroid adenoma to (b) that obtained simultaneously from the peripheral circulation (superior vena cava, center) of the same patient, to (c) a partially purified preparation of human parathyroid hormone extracted from adenomas. The elution patterns of the immunoreactive hormone in the plasma obtained from thyroid venous and peripheral blood were in striking contrast to each other. The hormone in plasma obtained directly from the parathyroid effluent blood (Fig. 1A) eluted slightly earlier than the radioiodinated bovine-hormone marker, whereas much of the immunoreactive parathyroid hormone in the peripheral circulation (1B) eluted later than the marker hormone extracted from the glands. This indicates that the hormone secreted directly from the parathyroid gland is equal to or slightly larger than the 84-amino-acid hormone

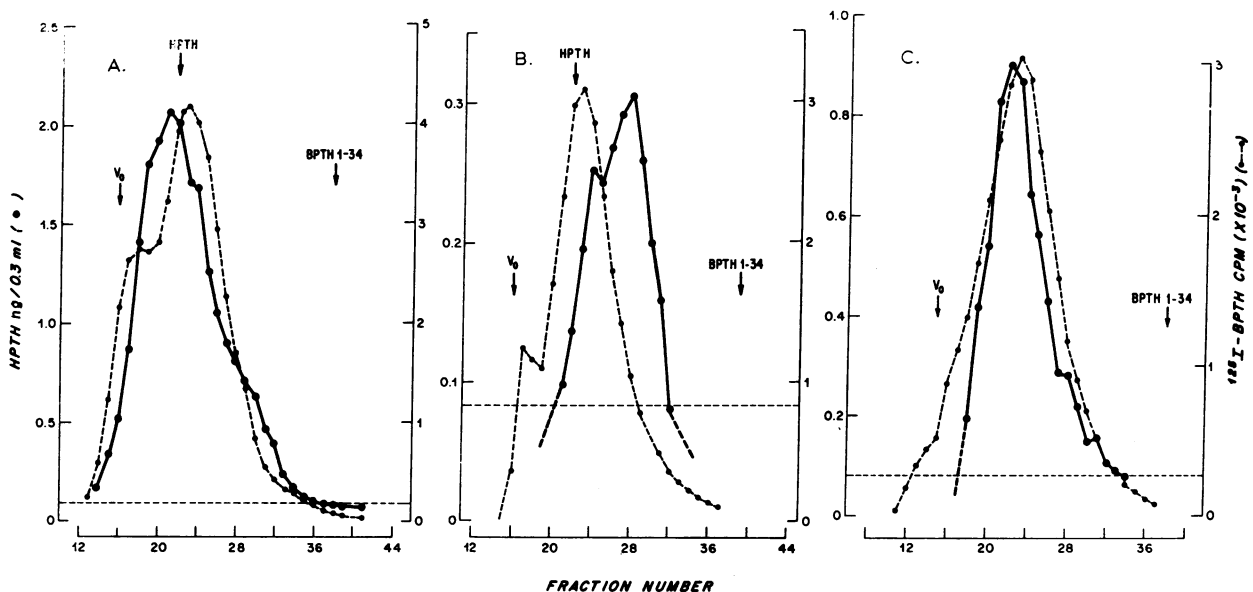


FIG. 1. Bio-gel P-10 filtration of radioimmunoassayable parathyroid hormone obtained simultaneously by venous catheterization from A. Inferior thyroid vein and B. Superior vena cava in a patient with a parathyroid adenoma (●—●). C. filtration of partially-purified hormone, prepared from human parathyroid adenomas (●—●). Parathyroid hormone concentration in A was 150 ng/ml. The indicated hormone concentration in B is only approximate because of nonparallel response in assay. <sup>125</sup>I-labeled bovine parathyroid hormone was cochromatographed as a marker (●—●). Arrows mark elution positions of the first fraction containing plasma proteins ( $V_0$ ), and the additional added markers human parathyroid hormone (HPTH) and synthetic, bovine 1–34 amino-terminal peptide (BPTH 1–34). Dashed horizontal line indicates sensitivity limit of the radioimmunoassay (antiserum; GP-1: dilution 1:250,000).

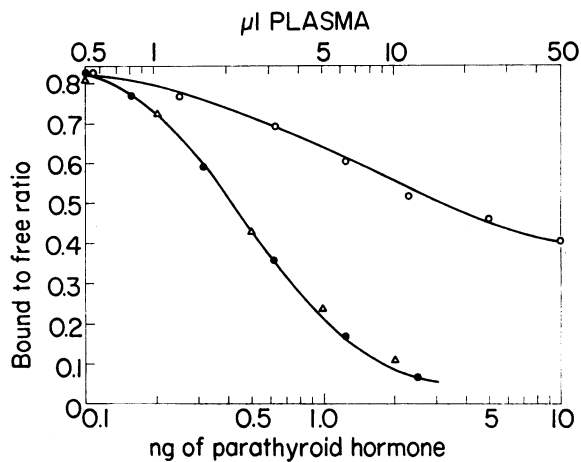


FIG. 2. Demonstration of immunochemical dissimilarity of parathyroid hormone in peripherally-obtained plasma, as compared to plasma from parathyroid gland effluent and to hormone extracted from parathyroid adenomas. The fall in the bound to free ratio of  $^{125}\text{I}$ -labeled bovine hormone is shown for different amounts of plasma or the human parathyroid hormone solution used in the gel filtration study of Fig. 1.  $\circ$ — $\circ$ , superior vena cava;  $\Delta$ — $\Delta$ , inferior thyroid vein;  $\bullet$ — $\bullet$ , human standard.

(marker) and that the hormone in the circulation is principally in the form of one or more smaller, immunologically reactive, fragments.

Human parathyroid hormone, extracted and partially purified from several surgically-obtained parathyroid adenomas (Fig. 1C), eluted at the same position as the radioiodinated bovine-hormone marker. The immunological differences found between the parathyroid hormone present in the peripheral circulation of a patient and hormone either extracted from or secreted directly from the gland of this patient is shown in Fig. 2. The slope defining the change in the bound-to-free ratio of  $^{125}\text{I}$ -labeled hormone produced by additions of successively larger aliquots of plasma from peripheral blood is not parallel to the slopes produced by additions of extracted

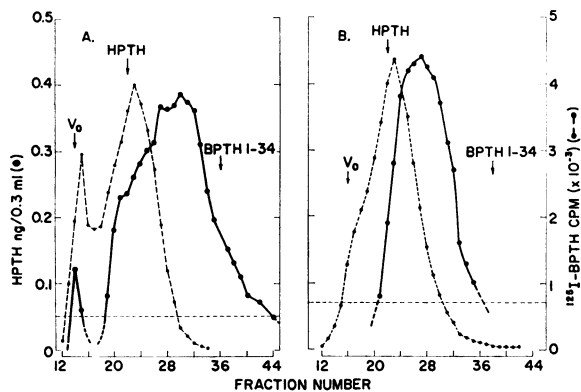


FIG. 3. Bio-gel P-10 filtration patterns of parathyroid hormone contained in the peripheral plasma of two patients with hyperparathyroidism due to A. Parathyroid adenoma and B. Parathyroid carcinoma. Plasma in B was obtained from the inferior vena cava during venous catheterization. Plasma hormone concentrations are approximate, due to nonparallel responses in the radioimmunoassay. Symbols, elution-position markers, and antiserum (GP-1) dilution are the same as in Fig. 1.

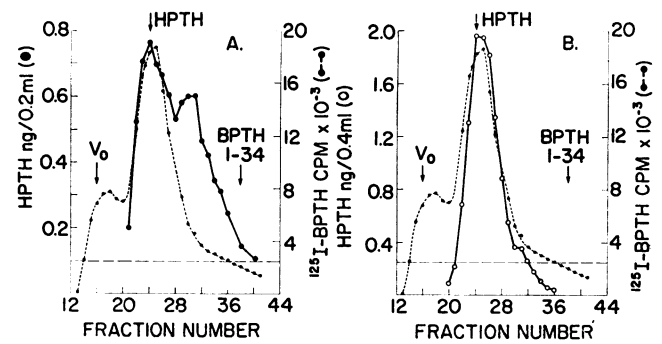


FIG. 4. Bio-gel P-10 filtration patterns of plasma parathyroid hormone measured by radioimmunoassay with two different antisera; A. GP-1 and B. GP-62. The peripheral plasma sample was obtained during an EDTA infusion from a patient with primary hyperparathyroidism (adenoma). Hormone was measured in aliquots of fractions resulting from a single gel filtration. Symbols and elution markers as in Fig. 1. Antisera dilutions: GP-1, 1:250,000; GP-62, 1:5,000.

hormone or hormone secreted directly from the gland. This nonparallel response indicates that the hormone in peripheral blood is immunologically dissimilar to the extracted hormone. On the other hand, the parathyroid hormone secreted directly from the gland, before it enters the general circulation, gives a completely parallel response in the assay to the response, given by different amounts of the standard of extracted hormone. Because of this nonparallel response given by the circulating form of the hormone, it is not possible to accurately measure the absolute concentration of parathyroid hormone in peripheral plasma with the extracted hormone as a standard in the immunoassay. Accordingly, the absolute concentrations of peripheral hormone shown on the ordinate scales of Fig. 1 and subsequent figures are relative, rather than absolute.

Elution patterns resulting from gel filtration of plasma obtained from the peripheral circulation of two additional patients with primary hyperparathyroidism are shown in Fig. 3. These plasmas, which contain peripherally circulating hormone in which most of the hormone elutes late from the gel columns, showed severely nonparallel responses when compared with the extracted hormone in the radioimmunoassay. We have observed this immunochemical- and size-heterogeneity in peripheral-blood samples from all of the 11 patients thus far examined. The elution positions of hormone in plasma obtained directly from gland effluent blood from venous catheterization of 14 additional patients (Fig. 6 and others not shown) were similar to that of the patient in Fig. 1. These plasma samples all gave completely parallel responses, and were indistinguishable from extracted hormone, upon dilution in the radioimmunoassay.

We have recently developed a second antiserum to parathyroid hormone in a second guinea pig (GP-62), comparable in sensitivity to our standard antiserum (GP-1). These two antisera recognize different peptide fragments of the hormone with differing affinity. Fig. 4 shows the results of assaying identical aliquots of fractions taken from the same gel-filtration study. The plasma in this study was obtained while EDTA was being infused into a patient with primary hyperparathyroidism (adenoma). The hormone in this plasma is distributed into two fractions when measured with antiserum

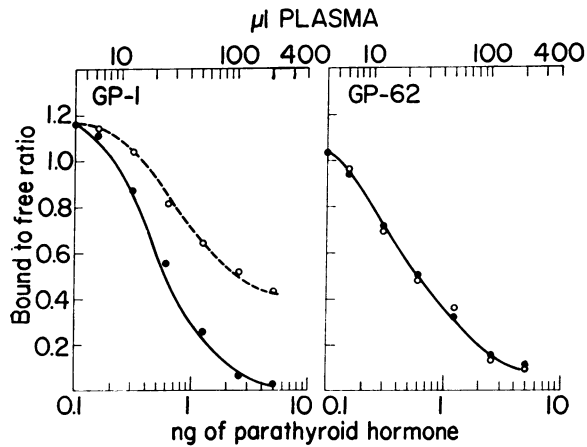


FIG. 5. Contrasting immunoreactive response of parathyroid hormone in peripheral plasma, demonstrated by the use of different antisera (GP-1 and GP-62). A sample of the plasma examined by gel filtration (Fig. 5) was serially diluted and compared to human parathyroid hormone derived from adenoma extracts. Antisera dilutions: GP-1, 1:250,000; GP-62, 1:5,000. O—O, plasma; ●—●, hormone standard.

GP-1, one identical in elution position to the marker of 84-amino-acid hormone and one smaller, a pattern similar to that seen with other peripheral samples. However, when this plasma is assayed with antiserum GP-62, only the hormone that elutes with the marker is seen; the smaller immunoreactive material is not detected.

The immunological behavior of the hormone contained in these plasma samples was compared with extracted hormone by the use of the two different antisera, GP-1 and GP-62. Fig. 5 shows that the plasma hormone is immunologically dissimilar to the extracted hormone when antiserum GP-1 is used, but appears to be similar when antiserum GP-62 is used. These results, which have been confirmed in a similar plasma sample, taken under identical conditions, from a second patient with primary hyperparathyroidism, show that two highly sensitive antisera can differ in their ability to detect immunologically-reactive fragments of the hormone and that this difference affects the slope of the response given by plasma hormone in the assay. Therefore, there could be serious disagreement in the estimation of the concentration of plasma hormone depending on which antiserum is used.

In contrast, when plasma samples from thyroid veins that contain hormone recently released from the gland are examined, the slope of response in the immunoassay is identical whether GP-1 or GP-62 antiserum is used. Furthermore (Fig. 6), only a single peak of immunoreactivity is detected on gel filtration: it appears close to the marker representing the intact [1-84] polypeptide. The two different antisera are in close agreement when used to estimate the quantity of hormone present. With each antiserum, the slope of response is identical for extracted hormone and thyroid-venous-plasma hormone.

Since these gel filtration studies were all done with plasma obtained from patients with abnormal parathyroid glands, it was important to examine the nature of parathyroid hormone in circulating peripheral and parathyroid venous plasma of normal individuals. Cattle were used. Fig. 7A shows a typical gel-elution pattern of plasma hormone obtained from a small

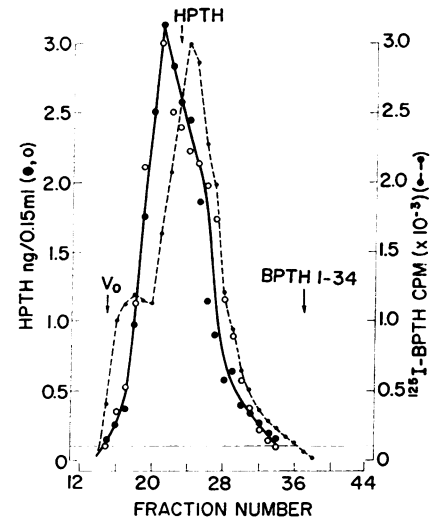


FIG. 6. Bio-gel P-10 filtration pattern of parathyroid hormones in a plasma sample, obtained during venous catheterization, from the right inferior thyroid vein draining a parathyroid adenoma. Hormone in aliquots of Bio-gel fractions was measured by radioimmunoassay with antisera GP-1, 1:250,000 and GP-62, 1:5,000. Conditions and symbols as in Fig. 1. ●—●, GP-1; ○—○, GP-62.

vein draining the superior parathyroid gland in a normal calf. The elution pattern of this secreted bovine parathyroid hormone is identical to that of the  $^{125}\text{I}$ -labeled bovine hormone. However, a peripheral plasma sample taken from a cow with parturient paresis (a hypocalcemia syndrome accompanied by parathyroid hyperplasia), contained late-eluting, small, immunoreactive hormonal fragments (Fig. 7B). Similar results were obtained in the several additional cows and calves examined. Thus, the findings in cattle were identical to those in patients with hyperparathyroidism.

We investigated the possibility that changes in the conformation of the hormone molecule occurring after secretion, rather than cleavage to a fragment, might account for the

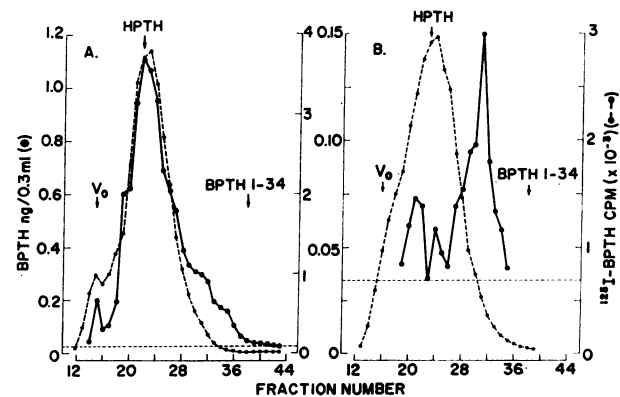


FIG. 7. Bio-gel P-10 filtration patterns of parathyroid hormone in A. plasma obtained from superior parathyroid vein draining normal superior parathyroid gland (histologically confirmed) in a calf, and B. from peripheral blood of a cow with parturient paresis. Hormone concentration in calf plasma, 105 ng/ml; cow peripheral plasma, 5 ng/ml. Conditions and symbols as in Fig. 1.

late elution of the immunoreactive hormone from the gel columns. However, incubation of the plasma samples in 8 M urea prior to gel filtration did not change the elution positions. Thus, changes in molecular conformation alone appear unlikely as an explanation for the late elution.

Some of the immunoreactive hormone in parathyroid effluent blood from certain parathyroid adenomas (3 of 15 patients, e.g., Figs. 1 and 6) eluted from the gel columns earlier than did either intact 84-amino-acid bovine hormone or human parathyroid hormone. Although the explanation for this early elution is uncertain, preliminary evidence also derived from incubation of plasma in 8 M urea before gel filtration suggests that some of the immunoreactive hormone consists of a polypeptide larger than 84 amino acids. Aggregation or protein binding of a smaller hormonal peptide seems to be excluded.

### DISCUSSION

Several conclusions can be drawn from these studies about the physical and immunochemical properties of endogenous parathyroid hormone circulating in peripheral blood, as well as the hormone released from the gland both in normal animals and in patients with primary hyperparathyroidism. The hormone circulating in peripheral blood is smaller than the 84-amino-acid hormonal polypeptide extracted from glands, whereas the hormone released from the parathyroids, *in vivo* (either adenomas or normal glands) is similar in size to the hormone extracted from the glands and is larger in size than the hormone found in peripheral plasma. Therefore, cleavage of the secreted hormone to smaller fragments must occur in peripheral sites after release from the gland, and not, as has been proposed by Sherwood, *et al.* (8) and Arnaud, *et al.* (6) from their *in vitro* studies, by cleavage in the parathyroid gland concomitant with release. We have also found that immunoreactive hormone secreted by parathyroid tissue *in vitro* is smaller than the stored hormone (3); thus, we must conclude that parathyroid glands, maintained as organ cultures, do not appear to be adequate models for the study of hormone biosynthesis and secretion *in vivo*.

The present findings have practical implications for the applications of radioimmunoassay studies, in that they may explain the conflicting results reported by different investigators who used immunoassays that employed different antisera. Variable results have been reported concerning the quantity of parathyroid hormone found in plasma either in normal subjects or in patients with hyperparathyroidism. Furthermore, there are apparent disagreements with respect to control of hormone secretion in primary hyperparathyroidism (10, 14, 15). When one considers that the hormonal fragment or fragments present in blood may be detected by one antiserum but not by a second and, in addition, the fragment(s) may be cleared from the circulation at rates that are different than for the intact, recently-secreted hormone, then entirely different impressions would be gathered concerning not only absolute concentration of hormone, but also rates of hormone disappearance. Therefore, interpretations of data that even concern the control of hormone secretion may depend upon the characteristics of the particular antiserum used.

The presence of hormonal fragments appears to explain the nonparallel responses produced in the radioimmunoassay by hormone in peripheral plasma. We have observed such non-

parallel slopes with one or more antisera in the radioimmunoassay with most of the peripheral-plasma samples tested, while hormone in blood directly from the parathyroid glands has given, without exception, slopes completely parallel with those of hormone extracted from the glands. One explanation for the nonparallel slopes seen in the radioimmunoassay of parathyroid hormone in peripheral plasma may be the presence in the assay antiserum of two or more antibodies with different antigenic determinants, resulting in different slopes for, and sensitivities to, the intact hormone and hormonal fragment(s) (17). Alternatively, cleavage of the intact hormone at an important antigenic site of the molecule might change the displacement slopes.

Cohn, *et al.* have recently reported a high molecular weight precursor of parathyroid hormone (18). Our studies are consistent with this possibility, in that certain adenomas appear to secrete an immunoreactive peptide larger in size than the 84 amino acids. However, further work will be required to prove the existence of such a precursor and to define its metabolic importance or contribution to immunoreactive parathyroid hormone in plasma.

At the present stage of our knowledge of the complex nature of immunoreactive parathyroid hormone in plasma, the issue of greatest practical and theoretical interest is the exact chemical nature and metabolic activity of the circulating hormonal fragments. It is not possible to decide from these studies whether the late-eluting immunoreactive hormone detected by Bio-gel filtration of peripheral plasma consists of a single hormonal peptide fragment or of a mixture of fragments. The peak corresponding to hormonal fragment(s) invariably occupied an elution position about mid-way between the 1-84 intact hormone and the 1-34 synthetic fragment (Figs. 1, 4, 5, 8). Calculations based on elution positions on Bio-gel P-10 estimate a molecular weight for the fragment of about 7000. One would expect that cleavage of the hormone of molecular weight 9500, to a fragment of molecular weight 7000, should have generated a fragment of molecular weight 2500. However, little immunoreactive material was detected by either antiserum that eluted from the gel after the position marked by the synthetic peptide which consisted of residues 1-34. Failure to detect such a smaller fragment might mean that the region of the molecule constituting this putative fragment is not detected by antiserum, or that the smaller fragment is cleared rapidly from the circulation.

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