

Immunochemical Localization of Parathyroid Hormone in Cancer Tissue from Patients with Ectopic Hyperparathyroidism

Genaro M. A. Palmieri, ... , Robert E. Nordquist, Gilbert S. Omenn

J Clin Invest. 1974;53(6):1726-1735. <https://doi.org/10.1172/JCI107724>.

Research Article

Immunoreactive parathyroid hormone (PTH) in nonparathyroid malignant tumors associated with hypercalcemia and hypophosphatemia in the absence of demonstrable bone metastases was determined by radioimmunoassay and immunofluorescent techniques. Six of seven tumors contained material with immunological cross-reactivity to bovine PTH by radioimmunoassay and immunofluorescence. The intensity of the immunofluorescent stain varied considerably in the different tumors. From 15 to 90% of neoplastic cells were stained specifically with fluorescein-labeled anti-PTH. In contrast, normal parathyroid glands and parathyroid adenomas showed uniform distribution of immunofluorescence in all parenchymal cells. In one malignant tumor, PTH was localized also by immunoradiography. In every case PTH was detected only in the cytoplasm of parenchymal cells. One patient lacked detectable PTH in his tumor, yet showed regression of the hypercalcemia to normal values after removal of large masses of neoplastic tissue and recurrence of hypercalcemia when new growth occurred.

Dilutional radioimmunoassay curves of nonparathyroid malignant tumors were in most cases different from those obtained with extracts of normal parathyroid glands and parathyroid adenomas. Although both nonparathyroid neoplasms and parathyroid extracts demonstrated immunoheterogeneity by gel filtration, greater heterogeneity was found in nonparathyroid malignant tumors.

In those tumors in which immunological cross-reactivity to PTH was detected, the capability of secreting PTH may be restricted to derepressed cell clones amidst other neoplastic cells, whereas the greater heterogeneity of ectopic PTH may [...]

Find the latest version:

<https://jci.me/107724/pdf>



Immunochemical Localization of Parathyroid Hormone in Cancer Tissue from Patients with Ectopic Hyperparathyroidism

GENARO M. A. PALMIERI, ROBERT E. NORDQUIST, and GILBERT S. OMENN

From the Veterans Administration Hospital, Oklahoma Medical Research Foundation, Departments of Medicine and Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104; and the Department of Medicine, University of Washington Medical School, Seattle, Washington 98105

ABSTRACT Immunoreactive parathyroid hormone (PTH) in nonparathyroid malignant tumors associated with hypercalcemia and hypophosphatemia in the absence of demonstrable bone metastases was determined by radioimmunoassay and immunofluorescent techniques. Six of seven tumors contained material with immunological cross-reactivity to bovine PTH by radioimmunoassay and immunofluorescence. The intensity of the immunofluorescent stain varied considerably in the different tumors. From 15 to 90% of neoplastic cells were stained specifically with fluorescein-labeled anti-PTH. In contrast, normal parathyroid glands and parathyroid adenomas showed uniform distribution of immunofluorescence in all parenchymal cells. In one malignant tumor, PTH was localized also by immunoautoradiography. In every case PTH was detected only in the cytoplasm of parenchymal cells. One patient lacked detectable PTH in his tumor, yet showed regression of the hypercalcemia to normal values after removal of large masses of neoplastic tissue and recurrence of hypercalcemia when new growth occurred.

Dilutional radioimmunoassay curves of nonparathyroid malignant tumors were in most cases different

This work was presented in part at the 4th International Congress of Endocrinology, Washington, D. C., 18-24 June 1972.

Dr. Palmieri is a Clinical Investigator at the Veterans Administration Hospital, Oklahoma City, Okla., and Dr. Omenn is the recipient of Career Development Award 43122 from the National Institutes of Health. Address reprint requests to Dr. Palmieri, Section of Endocrinology, Department of Medicine, University of Tennessee, Memphis, Tenn. 38163.

Received for publication 26 July 1973 and in revised form 16 November 1973.

from those obtained with extracts of normal parathyroid glands and parathyroid adenomas. Although both nonparathyroid neoplasms and parathyroid extracts demonstrated immunoheterogeneity by gel filtration, greater heterogeneity was found in nonparathyroid malignant tumors.

In those tumors in which immunological cross-reactivity to PTH was detected, the capability of secreting PTH may be restricted to derepressed cell clones amidst other neoplastic cells, whereas the greater heterogeneity of ectopic PTH may reflect hormone cleavage by proteolytic enzymes in the tumor that is less specific than the Pro-PTH cleaving enzyme in the parathyroids.

INTRODUCTION

Nonparathyroid malignant tumors in patients without skeletal metastasis may be associated with hypercalcemia, hypophosphatemia, and clinical complications indistinguishable from the syndrome of primary hyperparathyroidism (1, 2). Various immunologic techniques have demonstrated parathyroid hormone (PTH)¹ in extracts of kidney, lung, liver, adrenal, parotid, spleen, and breast tumors (3-6). A venous-arterial gradient for PTH was found in cases due to hepatoma (5) and hypernephroma (7). Some malignancies may produce humoral factors other than PTH capable of inducing hypercalcemia, since it is known that bone resorption is stimulated by a variety of biological agents, including 1,25-hydroxycholecalciferol, vitamin A, and prostaglandin E₁.

¹ Abbreviations used in this paper: HPTH, human parathyroid tissue extract; PBS, phosphate-buffered saline; PTH, parathyroid hormone.

(8). Gordan, Cantino, Erhardt, Hansen, and Lubich (9) related hypercalcemia to the production of a sterol by some breast carcinomas, while Powell, Singer, Murray, Minkin, and Potts (10) found no radioimmunoassayable PTH in extracts of 11 malignant tumors associated with hypercalcemia and hypophosphatemia, five of which produced bone resorption *in vitro*.

In malignant tumors secreting PTH, the concentration of the hormone, based on dry weight measurements, is 30–300 times lower than in parathyroid adenomas (4). The lower concentration of PTH in these nonparathyroid neoplasms may be due to a slower rate of synthesis or more rapid turnover in most of the cells or to high levels of production by only a small fraction of the neoplastic cells.

We present here studies of the content, intracellular localization, and chromatographic characteristics of PTH in normal parathyroid glands, parathyroid adenomas, and nonparathyroid malignant tumors associated with ectopic hyperparathyroidism.

METHODS

Subjects. Ectopic production of PTH was investigated in patients with malignancies accompanied by hypercalcemia with no evidence of bone metastasis by X ray or at autopsy. In those cases in which a careful exploration of the parathyroid glands was performed at autopsy, the parathyroid glands were found to be normal (Table I). Normal parathyroid glands were obtained at autopsies of patients without a history of metabolic or endocrine diseases. Parathyroid adenomas were obtained during surgical exploration of patients with a diagnosis of primary hyperparathyroidism.

Preparation of tissue extracts. Normal parathyroid glands, parathyroid adenomas, and malignant tumors were obtained during surgery or within 7 h after death. The specimens were placed in liquid nitrogen or dry ice and kept at -20°C . Tissue was homogenized in a VirTis 45 homogenator (VirTis Co., Inc., Gardiner, N. Y.) in a volume of 40% acetone in 1% acetic acid equal to 10 times the fresh weight of the tissue. The homogenized tissue was centrifuged for 10 min at 27,000 *g* in a refrigerated centrifuge. The supernate was lyophilized and resuspended in 2–5 ml of acetone-acetic acid solution or in 0.2 M ammonium acetate buffer, pH 4.6 and centrifuged as indicated above. The extract was stored at -20°C . Protein content in tissue extracts was measured by the method of Lowry, Rosebrough, Farr, and Randall (11).

Radioimmunoassay of PTH. The method of Berson, Yalow, Aurbach, and Potts (12) was used. Samples were incubated for 5 days in 2.5 ml of a 1:5 dilution of human or sheep hypoparathyroid plasma in 0.02 M Veronal buffer pH 8.6 containing Trasylol (FBA Pharmaceuticals, Inc., New York) 400–500 KIU/ml and 0.5% of guinea pig serum. Separation of antibody-bound from free hormone was accomplished with 50-mg talcum tablets (13, 14). Tissue extracts were diluted in hypoparathyroid plasma for radioimmunoassay. "Control" tubes containing the highest concentration of tumor extracts in the absence of antibody were run in every case. Antiserum GP 012-6-23 was used in most tissue studies. This antiserum was obtained in our laboratory by immunizing guinea pigs with partially purified bovine

PTH (TCA-PTH, Wilson Labs., Chicago, Ill.). PTH in plasma of normal subjects, patients with primary hyperparathyroidism, and patients with ectopic PTH syndrome was determined using an identical procedure in 500- μl samples of plasma. Antiserum GP 456-5-6* in a 1:50,000 dilution was also used in determinations of PTH in plasma. This antiserum is more sensitive than GP 012-6-23 for detection of circulating PTH.

Chromatographic fractionation. Lyophilized tissue extracts resuspended in 3 ml of 0.2 M ammonium acetate buffer pH 4.6 were applied to a 2.0×90 cm Bio-Gel P-10 column (Bio-Rad Laboratories, Richmond, Calif.) equilibrated at 4°C with 0.2 M ammonium acetate buffer pH 4.7. The flow rate was approximately 0.18 ml/min. 50–100- μl samples of 1-ml fractions were diluted in 500 μl of hypoparathyroid plasma for radioimmunoassay.

Morphological studies. Immunofluorescent studies were performed on normal parathyroid tissue, parathyroid adenomas, ectopic hormone-secreting tumors, and in other tumors and tissues which contained no detectable parathormone by radioimmunoassay.

The tissues obtained were divided and cryostat sectioned on the face adjacent to the tissue taken for determination of PTH. 7- μm sections were fixed in acetone which was made basic by the addition of enough saturated NaOH in absolute ethyl alcohol to bring the pH to 7.5. After 10 min fixation in this solution the slides were air dried and then washed with phosphate-buffered saline (PBS) at pH 7.2 (0.15 M NaCl, 0.01 M phosphate). Whole guinea pig antiserum against bovine parathormone (GP 012-6-23) was diluted 1:10 with PBS, applied to the sections, and incubated in a humidified chamber at room temperature for 30 min. Increasing dilutions of the anti-PTH serum were applied to sections of all tissues. No detectable immunofluorescence was observed with dilutions greater than 1:100. After incubation, the tissues were rinsed in three changes of PBS for a total of 30 min and postcoupled with a 1:30 dilution of fluorescein-labeled rabbit anti-guinea pig IgG for 30 min. After final rinsing in three changes of PBS for 1 h, the sections were mounted in buffered glycerine, pH 7.2. The finished preparations were viewed in an Olympus UV microscope (Olympus Corporation of America, New Hyde Park, N. Y.) equipped with a sliding bar filter of increasing neutral density for a rapid evaluation of intensity of fluorescein staining and photographed with a Wild automatic camera system (Wild Heerbrugg Instruments, Inc., Farmingdale, N. Y.). Proportion of positive cells was estimated by direct counting at the microscope by two operators. Since fluorescence fades rather rapidly when exposed to ultraviolet light, each microscopic preparation consisted of three serial sections of tissue mounted on one slide. Enough space was left between the sections in order that only one section was exposed to ultraviolet light at any time. One viewer examined the first section, a second viewer the second section, and a photograph was taken of the third section. These studies were repeated at least five times for each tissue. Controls were used to determine the specificity of immunofluorescent stain as follows: (a) normal guinea pig serum was used instead of PTH antiserum; (b) PTH antiserum was absorbed by incubation with increasing concentrations of human parathyroid extract (0–1 mg protein/ml) for 72 h at 4°C . In

* Dr. Palmieri obtained this antiserum while immunizing guinea pigs with TCA-PTH during an associateship with Doctors Solomon Berson and Rosalyn Yalow, Veterans Administration Hospital, Bronx, N. Y.

TABLE I
Immunofluorescence and Radioimmunoassay in Nonparathyroid Malignant Tumors

Patient	Pathology	Parathyroid glands at autopsy	Tumor PTH.		Estimated % of positive Ifluor. neoplastic cells
			RIA*	Ifluor.‡	
Ha	Liver metastasis pancreatic islet cell carcinoma	Normal	Pos.	Pos.	90
Tu	Squamous cell carcinoma of lung	—§	Pos.	Pos.	15
Ro	Squamous cell carcinoma of lung	Normal	Pos.	Pos.	30
Ho	Liver metastasis Gallbladder adenocarcinoma	Normal	Pos.	Pos.	60
De	Renal metastasis Squamous cell carcinoma of lung	Normal	Pos.	Pos.	60
McW	Squamous cell carcinoma of lung	Normal	Pos.	—¶	—
Mo	Malignant melanoma	Normal	Neg.	Neg.	—

* RIA = Radioimmunoassay.

‡ Ifluor. = Immunofluorescence.

§ Still alive 2 yr after lobectomy for carcinoma of the lung.

|| Histologically, adenocarcinoma with distinctive areas of squamous differentiation.

¶ All the tumor sample was used for RIA.

each case, immunofluorescent staining was carried out on sections of parathyroid adenomas and nonparathyroid malignant tissue. The results of the radioimmunoassay and immunofluorescence were analyzed without knowledge of the complementary data on all tumors.

Immuno-autoradiography was performed as follows: the IgG fraction of anti-PTH guinea pig serum (GP 012-6-23) was obtained with a modification of the method of Goldstein, Slizys, and Chase (15). To 0.5 ml of guinea pig serum anti-PTH diluted in 1.5 ml of PBS, 2 ml of saturated ammonium sulfate solution pH 7.0 was added drop by drop in 30 min at 0°C. After centrifugation at 2,000 rpm at 1°C for 20 min, the supernate was decanted, and the pellet was resuspended in 2 ml of PBS. Dialysis was carried out at 4°C against PBS for 16 h. The retentate was concentrated to 1 ml using a Centrifo membrane concentrator (Amicon Corp., Lexington, Mass.).

Immunoglobulins were purified in a DEAE-Sephadex A-50 (Pharmacia Fine Chemicals Inc., Piscataway, N. J.), 60 × 1-cm column with PBS, pH 7.2 as eluent. The Sephadex was prepared as indicated by the manufacturer, and the pH was adjusted to 7.2. A single sharp peak at 280 mμ absorbancy was observed at the void volume corresponding to IgG. The fraction (2 ml) showing the highest absorbancy was concentrated to approximately 3 mg of protein/ml using a Centrifo membrane concentrator.

IgG was labeled with ¹²⁵I by the method of Hunter and Greenwood (16) with minor modifications. To 20 μl of 0.25 M phosphate buffer pH 7.5 were added rapidly, in turn, approximately 800–1,000 μCi of ¹²⁵I, 4–8 μg of IgG, 20 μl of chloramine-T (3.5 mg/ml in 0.25 M phosphate buffer), and 30 μl of sodium metabisulfite (4.8 mg/ml in 0.25 M phosphate buffer). 1 ml of 1% albumin in 0.02 M Veronal

buffer pH 8.6 was added to the iodination mixture and placed in a Sephadex G-75 1.3 × 50-cm column using 1% albumin in 0.02 M Veronal buffer as eluent. 1-ml fractions were collected, and the radioactivity was found to be in two distinct peaks, the first corresponding to the void volume and the second corresponding to the salt peak. The fraction of the first peak with the highest counts was used for autoradiography. [¹²⁵I]IgG was applied to the fixed tissues for 30 min at room temperature. After a thorough washing for 4 h the slides were coated with Kodak NTB-2 Nuclear track emulsion (Eastman Kodak Co., Rochester, N. Y.) and incubated at 4°C for 1–2 wk. Development was accomplished with Kodak D-19 developer and Kodak acid fixer. The finished slides were stained with hematoxylin and eosin.

RESULTS

Table I summarizes our findings in seven malignant nonparathyroid tumors. There was a good correlation between the detection of PTH by radioimmunoassay in tissue extracts and by intracellular localization with immunofluorescence. Fig. 1A shows a typical dilutional curve obtained with an extract of human parathyroid tissue (HPTH). This extract is currently used in our laboratories as standard for human PTH and was obtained from pools of normal glands (15%) and parathyroid adenomas (85%). An extract of a normal parathyroid gland produced identical results. No detectable PTH was observed with more than 100 times higher concentrations of extracts of other normal or malignant tissues

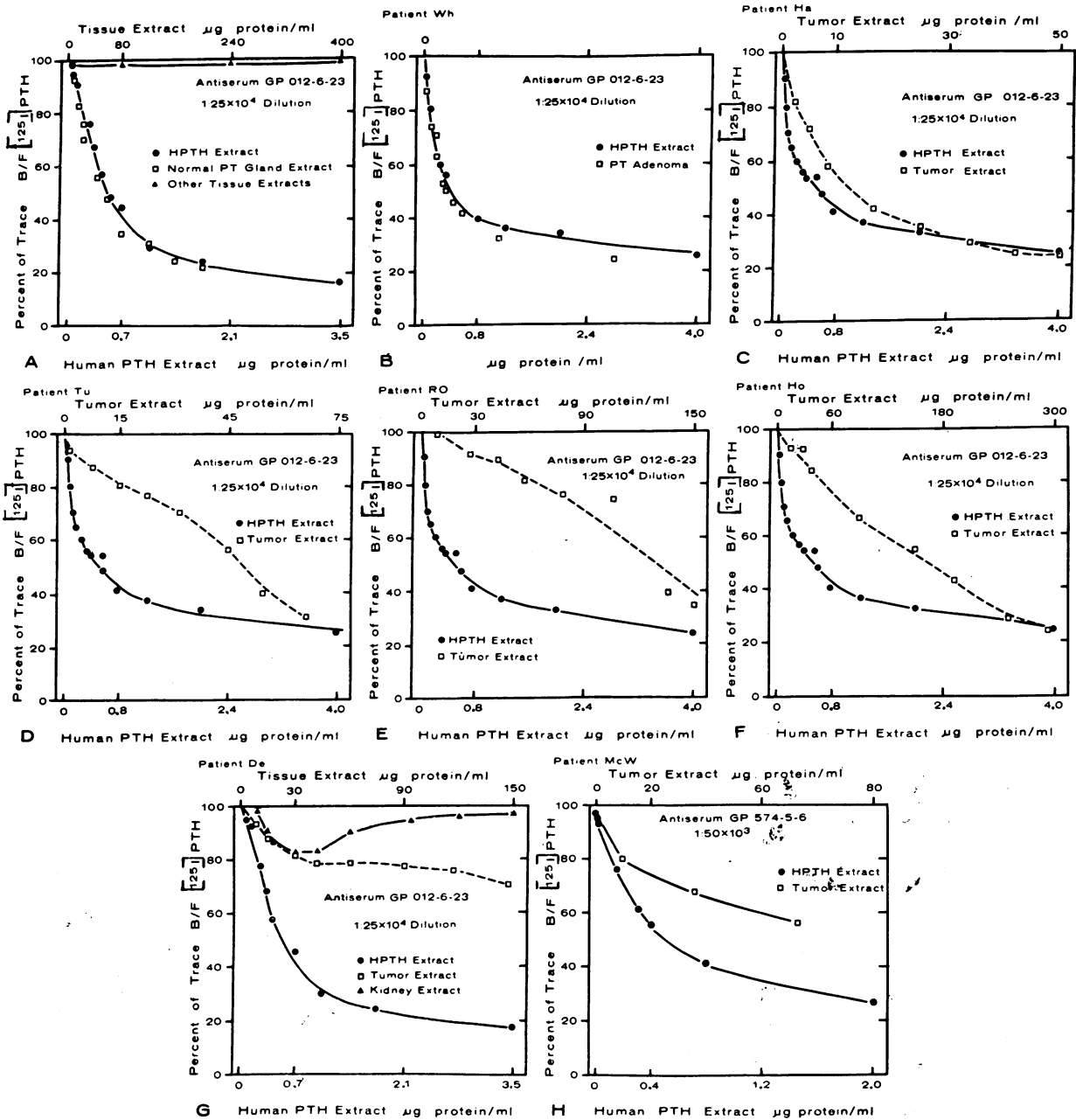
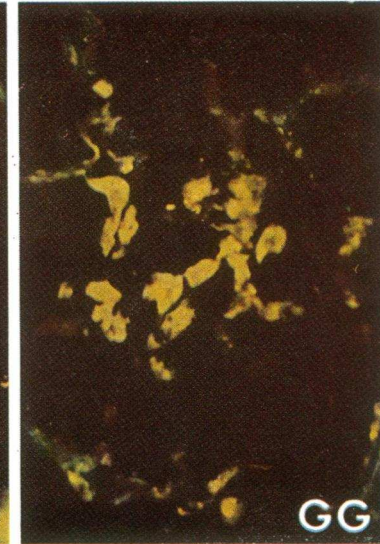
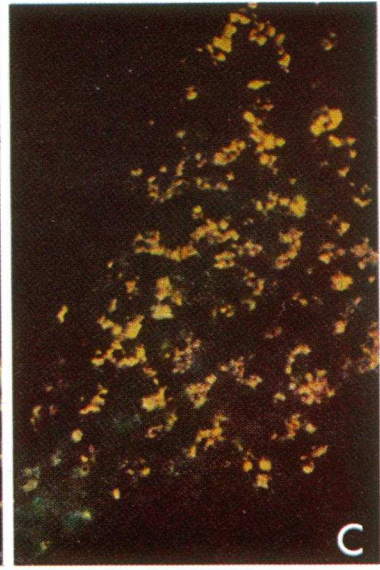
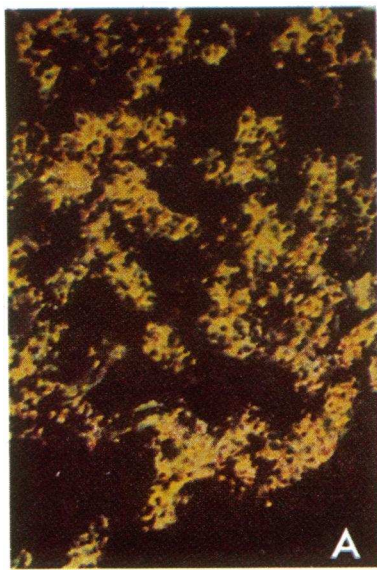


FIGURE 1 Dilution curves of radioimmunoassays of tissue extracts in comparison to a standardized pool of HPTH. (A) \square , A normal parathyroid gland extract; \blacktriangle , other normocalcemic tissue extracts. (B) Patient Wh: parathyroid adenoma. (C) Patient Ha: liver metastasis from an islet cell carcinoma of the pancreas. (D) Patient Tu: squamous cell carcinoma of the lung. (E) Patient Ro: squamous cell carcinoma of the lung. (F) Patient Ho: liver metastasis from an adenocarcinoma of the gallbladder. (G) Patient De: \square , renal metastasis of a squamous cell carcinoma of the lung; \blacktriangle , extract of normal renal tissue. (H) Patient McW: squamous cell carcinoma of the lung.

from normocalcemic subjects. Sections of the same normal gland used for radioimmunoassay showed localization of fluorescence in the cytoplasm of parenchymal

cells and its absence in connective tissue (Plate 1A). Sections of diverse malignant tumors not associated with hypercalcemia and sections of normal lung, liver, kid-



ney, and lymphatic tissue showed no specific fluorescence. No fluorescence was observed in sections of normal parathyroid gland or parathyroid adenomas when normal guinea pig serum was used instead of the anti-PTH serum. Furthermore, specific staining in sections of parathyroid adenomas and in the tumor of patient Ha could be abolished if the anti-PTH serum was absorbed with HPTH (200 μ g protein/ml) for 72 h at 4°C. All control studies performed clearly indicate that the presence of fluorescence in these studies represents immunoreactive PTH.

Superimposable radioimmunoassay curves were obtained with an extract of a parathyroid adenoma and with the extract of pooled parathyroid tissue (HPTH) (Fig. 1B). Intracellular localization of PTH of an adjacent section of the adenoma is shown in Plate 1B. As in normal parathyroid gland, the adenoma showed fluorescence evenly distributed in all parenchymal cells indicating that PTH was produced by all adenoma cells. Figs. 1C-1F show the different dilutional curves obtained with extracts of nonparathyroid tumors which produced PTH. The extract of a liver metastasis of an islet cell carcinoma of the pancreas in patient Ha (Fig. 1C) showed a dilutional curve similar to that of normal HPTH, although the crossings of the curves at higher concentrations of extract suggests heterogeneity of the hormone. This heterogeneity appears more evident observing the dilutional curves of tumors Tu, Ro, Ho, and De (Fig. 1D-G). The tumor extract of patient McW demonstrated a slope of approximately the same shape as that obtained with HPTH (Fig. 1H). The difference between the two curves does not necessarily indicate heterogeneity and can be explained by the difference in concentration of immunoreactive material.

Immunofluorescence pictures of adjacent areas of these tumors are shown in Plates 1C-G. Not all cells of these tumors were fluorescein positive, in contrast to normal parathyroid glands (Plate 1A) or parathyroid adenomas (Plate 1B). The distribution and intensity of the staining varied considerably. In patient Ha (Plate 1C) most of the tumor cells demonstrated

specific fluorescence, and the general picture resembled normal parathyroid gland (Plate 1A) with the exception that some of the staining had a granular appearance. The granular type of distribution of fluorescein was only observed in tumor Ha (Plate 1C) and was confirmed by immunoperoxidase staining. An homogeneous type of staining was observed in all other fluorescein-positive tissues. Sections of the tumor of patient Tu (Plate 1D) showed zones with intense fluorescence surrounded by negative areas indicating that the capability of producing PTH was probably the property of a minority of cells. Autoradiographs³ of sections of this tumor clearly showed the cytoplasmic localization of the [¹²⁵I]IgG in certain cells (Plate 1DD). Fluorescein positive, typical squamous cells were observed in sections of the squamous cell carcinoma of patient Ro (Plate 1E). Sections of a liver metastasis of an adenocarcinoma of the gallbladder, patient Ho, (Plate 1F) showed almost total loss of the normal liver structure due to severe autolysis; however, immunofluorescent localization was observed in those tumor cells that were spared. The estimated percentage of positive cells in this tumor (Table I) was only approximate and was obtained using a lower magnification than that of Plate 1F.

The study of the renal metastasis of a squamous cell carcinoma of the lung in patient De deserves special consideration. A relatively low concentration of PTH was observed by radioimmunoassay of extracts of the renal metastasis (Fig. 1G). The immunofluorescence studies clearly showed squamous-type cells with specific fluorescence surrounded by nonfluorescent cells (Plate 1G). In normal tissue surrounding carcinomas, no immunofluorescence was detected with the exception of sections of the kidney of patient De in which a distinct localization of immunofluorescent stain was

³ Autoradiographs of sections of other tumors and parathyroid adenomas did not show so clear a picture as in Plate 1DD, due to the variable intensity of background. The occurrence of "damaged" labeled IgG and free ¹²⁵I was relatively high despite being used soon after iodination and purification.

PLATE 1 Morphological localization of PTH by immunofluorescence and immunautoradiography. (A) Normal parathyroid gland, $\times 100$. (B) Patient Wh: parathyroid adenoma, $\times 100$. (C) Patient Ha: liver metastasis from an adenocarcinoma of the gallbladder. A granular distribution of staining is observed in this tumor, $\times 100$. (D) Patient Tu: squamous cell carcinoma of the lung, $\times 100$. (DD) Patient Tu: Immuno-autoradiographic localization of PTH which is distinctively cytoplasmic. The arrows indicate nuclei in which no grains are found, $\times 400$. (E) Patient Ro: squamous cell carcinoma of the lung. Notice the brilliant cytoplasmic fluorescence seen in these typical squamous cells, $\times 400$. (F) Patient Ho: liver metastasis from an adenocarcinoma of the gallbladder. Cellular structure is not well visualized due to severe autolysis, $\times 400$. (G) Patient De: renal metastasis of a squamous cell carcinoma of the lung. Typical squamous cells with cytoplasmic localization of immunofluorescence are demonstrated. The nuclei appear in negative contrast, $\times 400$. (GG) Patient De: normal renal parenchyma with cytoplasmic staining of tubular cells, $\times 200$.

observed in some cells which appeared to be normal components of tubular parenchyma (Plate 1GG). We have been unable to find a similar picture in kidneys from normal subjects. No kidney tissue was examined from patients with primary hyperparathyroidism. Extracts of kidney of De without evidence of malignancy gave a very peculiar dilutional curve in radioimmunoassay (Fig. 1G). These experiments were repeated three times with very similar results. The possibility that acetone-acetic acid extracts contained hydrolytic enzymes capable of destroying PTH during incubation with higher concentrations of extracts was examined. A test substrate for proteolytic enzymes (Azocoll, 50-100 mesh, Calbiochem, San Diego, Calif.) gave a negative result. When the highest concentration of tissue extracts (150 μ g protein/ml) used for radioimmunoassay was incubated for 4-5 days in standard diluent at 4°C in the presence of [¹²⁵I]PTH, there was no significant "damage" of the labeled hormone.

In patient Mo, four masses of tissue obtained upon surgical removal of thoracic melanoma were frozen immediately in liquid nitrogen. PTH could not be demonstrated by radioimmunoassay of extracts or by immunofluorescence of sections. Nevertheless, hypercalcemia and hypophosphatemia returned to normal post-operatively and recurred with recurrence of tumor 2 mo later.

As shown in Fig. 1, dilutional curves of extracts of nonparathyroid tumors were obtained with protein concentrations of 3-40 times higher than those needed to obtain an appreciable reduction of bound/free ratio with extracts of a pool of normal glands or parathyroid adenomas (HPTH). Most tumor extracts gave a different dilutional curve when compared with HPTH standard (Fig. 1). These findings prompted us to study the chromatographic fractionation of tumor extracts and HPTH.

Immunoassayable PTH was detected in a large number of fractions upon gel filtration of HPTH, but most of the PTH activity occurred in a narrow region within fractions 63-72 (Fig. 2). Minor amounts of PTH activity followed in subsequent fractions. It is highly probable that this activity represents immunoreactive fragments of PTH. Gel filtration fractions of tumor Tu extracts contained immunoassayable PTH almost evenly distributed throughout the elution profile. In contrast, fractions of tumor Ha extracts showed immunoassayable PTH in two reasonably restricted areas. As shown in Fig. 2, the elution of a labeled marker of highly purified bovine PTH during gel filtration of tumors Tu and Ha was delayed when compared with HPTH, probably due to the larger amount of lyophilized tissue extract from nonparathyroid tumors used to obtain measurable amounts of immunoreactive PTH. The volume of the samples applied to the column was

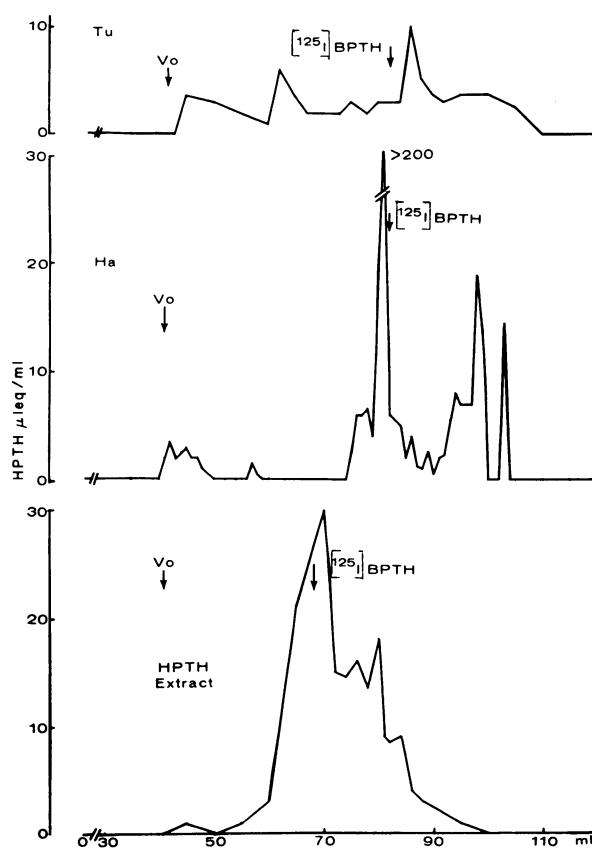


FIGURE 2 Bio-Gel P-10 filtration patterns of radioimmunoassayable PTH from extracts of a squamous cell carcinoma of the lung (patient Tu), a liver metastasis from an islet cell carcinoma of the pancreas (patient Ha), and a standardized pool of HPTH. [¹²⁵I]BPTH: marker of labeled bovine PTH added to each sample before applying to the column. Vo: void volume. Salt appeared at 155 ml. Antiserum GP 012-6-23 was used.

identical. Displacement of the marker was also observed during elution of extracts of tumor Mo that lacked immunoreactive PTH by immunofluorescence and radioimmunoassay. The marker was eluted before a major immunoreactive component in tumor Tu and just after the major component in tumor Ha. In contrast, the marker was eluted with the major immunoreactive component of HPTH. Both tumors and HPTH contained a relatively minor component eluted with the void volume. The relative quantities of different immunoreactive components cannot be compared accurately since the antiserum may react less well with some than with other PTH fragments or precursors. Although immunoreactive heterogeneity appeared likely from the elution profile of glandular PTH extracts (HPTH) (Fig. 2), it was more striking in extracts of the nonparathyroid malignant tumors, especially

tumor Tu. Such increased heterogeneity was also observed in dilutional curves of radioimmunoassays of tumor extracts (Fig. 1).

The concentration of PTH in peripheral venous blood was elevated in two of the four patients tested in whom PTH was demonstrated in tumor extracts. Using the same techniques and same antiserum, plasma PTH was elevated in 14 of 17 cases of surgically proved primary hyperparathyroidism.

DISCUSSION

Ectopic hormone production by malignant neoplasms was postulated 40 yr ago by Leyton (17) and later by Albright (18). It was not until the last decade, with the development of immunologic techniques for the determination of polypeptide hormones, that a more systematic exploration of this field began (19, 20).

Immunoreactive PTH has been described in extracts of at least a dozen nonparathyroid malignant tumors from patients presenting with a clinical picture resembling primary hyperparathyroidism (3-7). In most cases the concentration of PTH in tumors was 30-300 times lower than in parathyroid adenomas (4-6), suggesting a relatively lower rate of synthesis, accelerated secretion, or abnormal cleavage of PTH by nonparathyroid malignant tissue.

In this study the presence of ectopic immunoreactive PTH was established in six nonparathyroid tumors by radioimmunoassay, by immunofluorescent localization, and in one case by autoradiographic techniques. The concentration of PTH and the proportions of positively stained cells varied considerably among the tumors. PTH was demonstrated in all parenchymal cells of normal parathyroid glands and parathyroid adenomas. In nonparathyroid malignant tumors between 15 and 90% of cells were positive. The intensity of the fluorescent stain was variable. In general, the immunofluorescent-positive cells appeared brighter and more intensely stained in tumors with ectopic PTH than in cells of normal glands or parathyroid adenomas.

There is no evidence that this difference of staining can be attributed to quenching because the difference in brightness persisted as more dilute antiserum was used. It seems quite possible that upon immunofluorescence of tissue sections, the antiserum detects immunoreactive species of PTH that were not extracted by the acetone-acetic acid procedure for radioimmunoassay. Silverman and Yalow (21) have demonstrated that different extraction procedures for glandular tissue yield different immunoreactive components. The gel filtration studies strongly suggest that extracts of glandular tissues have immunochemical heterogeneity as demonstrated by Silverman and Yalow (21) and that this heterogeneity according to molecular size is more pronounced in nonparathyroid malignant tumors than in

parathyroid extracts. Marked differences were found among the tumors. Immunologic differences between circulating PTH in primary hyperparathyroidism and in ectopic hormone production by nonparathyroid neoplasms have been reported by Riggs, Arnaud, Reynolds, and Smith (20).

The presence of immunoreactive PTH in apparently normal kidney tissue in patient De prompted us to more detailed studies (22). After intravenous infusions of partially purified PTH to dogs, immunoreactive PTH was localized in renal tubular cells by immunofluorescence. The fluorescent stain had a homogeneous type of distribution similar to that observed in all tissues reported in this study with the exception of tumor Ha (Plate 1C). No fluorescent stain was detected in sections of liver and lung nor in kidneys of control dogs. Administered [¹²⁵I]PTH was selectively localized in the cytoplasm of proximal tubular cells. These studies confirmed previous investigations on the localization of [¹²⁵I]- and [³H]PTH in the kidney (23, 24). It is not clear whether the PTH found in renal cells is performing a physiological role, undergoing degradation, or simply being stored. Whatever the answer may be, all the information available indicates that exogenous PTH is rapidly localized in the kidney and to a minor degree in other soft tissues, i.e., muscle, liver, and lung. Therefore, it is likely that Plate 1GG illustrates a similar event occurring in a patient with an increased production of endogenous PTH by a nonparathyroid neoplasm.

The clinical picture resembling hyperparathyroidism in patient Mo, who lacked detectable immunoreactive PTH, has strong similarities to those described by Powell et al. (10). These authors did not detect immunoreactive PTH, using several antisera, in blood and tumor extracts of 11 patients with nonparathyroid malignancies and hypercalcemia. Remission of hypercalcemia occurred in nine patients after antitumor therapy, and extracts of five tumors produced active calcium resorption from bone *in vitro*. Interest in the possibility that prostaglandins accounted for the calcium-mobilizing effect is high because of the availability of indomethacin as a potential therapeutic agent (25). Bioassay of tumor extract was not performed in patient Mo of our series, but this patient showed remission of hypercalcemia after the removal of large tumor masses and its recurrence with renewed tumor growth. It appears, therefore, that the hypercalcemia occurring in malignancies is related, in some cases, to a humoral substance other than PTH. Although this may be the case, we feel that it is rather premature to reject the possibility that fragments of PTH with biological but without detectable immunological activities are the cause of the hypercalcemia, since most antisera have been obtained immunizing with whole bovine PTH.

Earlier studies from our laboratory (26) demonstrated the presence of polypeptides in extracts from human urine with biological PTH-like activity but lacking immune cross-reactivity with PTH. In patients with primary hyperparathyroidism and with hypercalcemia associated with cancer, higher excretion of those polypeptides was found. Therefore, it is possible that biologically active fragments of PTH are present in primary and ectopic hyperparathyroidism which are undetectable by current antisera. Due to striking heterogeneity of the hormone in the nonparathyroid neoplasms, components may or may not be detected immunologically. On the other hand, in parathyroid adenomas in which heterogeneity is less pronounced, immunoreactive PTH is readily detected in tissue and frequently in blood. In view of these considerations, it is our impression that in nonparathyroid malignant tumors associated with hypercalcemia and lacking immunoreactive PTH, the presence of fragments of PTH with biological activity cannot be ruled out. This problem will not be answered until the minimum structural requirements for biological activity of human PTH are established or other calcium-mobilizing substances are isolated from such tumors and characterized in detail.

These studies clearly demonstrate that certain nonparathyroid tumors contain substantial amounts of immunoreactive PTH in many or most of the neoplastic cells. However not all cells contain detectable immunoreactive PTH at a given time. It is conceivable that the synthesis of PTH occurs intermittently and asynchronously; alternatively, in each of these tumors there may be several cell clones growing simultaneously only one of which had undergone the genetic derepression presumably responsible for the synthesis of PTH.

ACKNOWLEDGMENTS

The authors thank the kind cooperation of members of the Departments of Surgery, Medicine, and Pathology, University of Oklahoma Health Sciences Center, the University of Washington, Seattle, Wash., and Dr. Edward R. Munnell, Presbyterian Hospital, Oklahoma City, Okla. Pure bovine PTH for iodination was a gift of Dr. John T. Potts, Jr., Massachusetts General Hospital, Boston, Mass. Mrs. Nancy Volling, Miss Josephine Hawrylko, and Mr. Monte Camp provided able technical assistance. Mrs. Fredda Schafer and Miss Shirley Wells provided expert editorial assistance.

This study was supported in part by research funds of the Veterans Administration Hospital, Oklahoma City, the Oklahoma Medical Research Foundation, and U. S. Public Health Service Grant G.M. 15253.

REFERENCES

1. Lafferty, F. W. 1966. Pseudohyperparathyroidism. *Medicine (Baltimore)*. **45**: 247.
2. Omenn, G. S., S. I. Roth, and W. H. Baker. 1969. Hyperparathyroidism associated with malignant tumors of nonparathyroid origin. *Cancer*. **24**: 1004.

3. Tashjian, A. H., Jr., L. Levine, and P. L. Munson. 1964. Immunochemical identification of parathyroid hormone in non-parathyroid neoplasms associated with hypercalcemia. *J. Exp. Med.* **119**: 467.
4. 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.
5. 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.
6. Mavligit, G. M., J. L. Cohen, and L. M. Sherwood. 1971. Ectopic production of parathyroid hormone by carcinoma of the breast. *N. Engl. J. Med.* **285**: 154.
7. Blair, A. J., Jr., C. D. Hawker, and R. D. Utiger. 1973. Ectopic hyperparathyroidism in a patient with metastatic hypernephroma. *Metab. (Clin. Exp.)*. **22**: 147.
8. Wener, J. A., S. J. Gorton, and L. G. Raisz. 1972. Escape from inhibition of resorption in cultures of fetal bone treated with calcitonin and parathyroid hormone. *Endocrinology*. **90**: 752.
9. Gordan, G. S., T. J. Cantino, L. Erhardt, J. Hansen, and L. Lubich. 1966. Osteolytic sterol in human breast cancer. *Science (Wash. D. C.)*. **151**: 1226.
10. Powell, D., F. R. Singer, T. M. Murray, C. Minkin, and J. T. Potts, Jr. 1973. Nonparathyroid humoral hypercalcemia in patients with neoplastic diseases. *N. Engl. J. Med.* **289**: 176.
11. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265.
12. Berson, S. A., R. S. Yalow, G. D. Aurbach, and J. T. Potts, Jr. 1963. Immunoassay of bovine and human parathyroid hormone. *Proc. Natl. Acad. Sci. U. S. A.* **49**: 613.
13. Rosselin, G., R. Assan, R. S. Yalow, and S. A. Berson. 1966. Separation of antibody-bound and unbound peptide hormones labelled with iodine-131 by talcum powder and precipitated silica. *Nature (Lond.)*. **212**: 355.
14. Palmieri, G. M. A., R. S. Yalow, and S. A. Berson. 1971. Adsorbent techniques for the separation of antibody-bound from free peptide hormones in radioimmunoassay. *Horm. Metab. Res.* **3**: 301.
15. Goldstein, G., I. S. Slizys, and M. W. Chase. 1961. Studies on fluorescent antibody staining. I. Non-specific fluorescent-coupled sheep anti-rabbit globulins. *J. Exp. Med.* **114**: 89.
16. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature (Lond.)*. **194**: 495.
17. Leyton, O. 1934. Multiglandular disease. *Lancet*. **1**: 1221.
18. Case Records of the Massachusetts General Hospital. Case #27461. 1941. *N. Engl. J. Med.* **225**: 789.
19. Omenn, G. S. 1973. Pathobiology of ectopic hormone production by neoplasms in man. In *Pathobiology Annual 1973*. H. L. Joachim, editor. Appleton-Century-Crofts, New York. 177.
20. Riggs, B. L., C. D. Arnaud, J. C. Reynolds, and L. H. Smith. 1971. Immunologic differentiation of primary hyperparathyroidism from hyperparathyroidism due to nonparathyroid cancer. *J. Clin. Invest.* **50**: 2079.
21. Silverman, R., and R. S. Yalow. 1973. Heterogeneity

- of parathyroid hormone. Clinical and physiological implications. *J. Clin. Invest.* **52**: 1958.
22. Nordquist, R. E., and G. M. A. Palmieri. 1974. Intracellular localization of parathyroid hormone in the kidney. *Endocrinology*. In press.
23. de Kretser, D. M., T. J. Martin, and R. A. Melick. 1970. The radioautographic localization of ¹²⁵I-labelled bovine parathyroid hormone. *J. Endocrinol.* **46**: 507.
24. Zull, J. E., and D. W. Repke. 1972. The tissue localization of tritiated parathyroid hormone in thyroidectomized rats. *J. Biol. Chem.* **247**: 2195.
25. Tashjian, A. H., Jr., E. F. Voelkel, P. Goldhaber, and L. Levine. 1973. Successful treatment of hypercalcemia by indomethacin in mice bearing a prostaglandin-producing fibrosarcoma. *Prostaglandins*. **3**: 515.
26. Palmieri, G. M. A., L. P. Eliel, and J. Hawrylko. 1968. Human urinary polypeptides with parathyroid-like activity. *J. Clin. Endocrinol. Metab.* **28**: 1571.