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

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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 immunofluorescence. The cross-reactivity 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.

Dilution 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 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) 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-dihydroxycholecalciferol, vitamin A, and prostaglandin E.

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

immunizing were diluted or of 8.6 New Yalow, Farr, centrifuged volume of (VirTis specimens retained without glands and nomas, PTH in neoplastic 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 parathyromone 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 and 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 parathyroid hormone (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 against 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 association with Doctors Solomon Berson and Rosalyn Yalow, Veterans Administration Hospital, Bronx, N. Y.

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

Immunofluorography 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 Gold-
stein, Slizys, and Chase (15). To 0.5 ml of guinea pig
serum the PTH guinea pig serum diluted in 1.5 ml of PBS, 2 ml of satu-
rated 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 car-
ried out at 4°C against PBS for 16 h. The retentate was
concentrated to 1 ml using a Centriflo membrane concen-
trator (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 Sepha-
dex was prepared as indicated by the manufacturer, and
the pH was adjusted to 7.2. A single sharp peak at 280 nm
absorbancy was observed at the void volume corre-
sponding to IgG. The fraction (2 ml) showing the highest ab-
sorbancy was concentrated to approximately 3 mg of pro-
ten/ml using a Centriflo 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 eisin.

RESULTS

Table I summarizes our findings in seven malignant non-
parathyroid tumors. There was a good correlation be-
tween 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 a standard for human PTH and was ob-
tained from pools of normal glands (15%) and parathy-
roid adenomas (85%). An extract of a normal parathy-
roid gland produced identical results. No detectable PTH
was observed with more than 100 times higher concen-
trations of extracts of other normal or malignant tissues

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pathology</th>
<th>Parathyroid glands at autopsy</th>
<th>Tumor PTH.</th>
<th>Estimated % of positive immunofluorescent cells</th>
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<tr>
<td>Ha</td>
<td>Liver metastasis pancreatic islet cell carcinoma</td>
<td>Normal</td>
<td>Pos.</td>
<td>90</td>
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<tr>
<td>Tu</td>
<td>Squamous cell carcinoma of lung</td>
<td>—§</td>
<td>Pos.</td>
<td>15</td>
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<tr>
<td>Ro</td>
<td>Squamous cell carcinoma of lung</td>
<td>Normal</td>
<td>Pos.</td>
<td>30</td>
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<td>Ho</td>
<td>Liver metastasis Gallbladder adenocarcinoma\</td>
<td>Normal</td>
<td>Pos.</td>
<td>60</td>
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<tr>
<td>De</td>
<td>Renal metastasis Squamous cell carcinoma of lung</td>
<td>Normal</td>
<td>Pos.</td>
<td>60</td>
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<td>McW</td>
<td>Squamous cell carcinoma of lung</td>
<td>Normal</td>
<td>Pos.</td>
<td>10</td>
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<tr>
<td>Mo</td>
<td>Malignant melanoma</td>
<td>Normal</td>
<td>Neg.</td>
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* 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.
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-
thyroid, 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 μg 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 suggest 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 fluorescent 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. Autoradiographs of sections of this tumor clearly showed the cytoplasmic localization of the [3H]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 1) 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

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8 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 3H was relatively high despite being used soon after iodination and purification.
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 µg protein/ml) used for radioimmunoassay was incubated for 4-5 days in standard diluent at 4°C in the presence of [³³P]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 postoperatively 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.

Immunassayable 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 immunassayable PTH almost evenly distributed throughout the elution profile. In contrast, fractions of tumor Ha extracts showed immunassayable PTH in two reasonably restricted areas. As shown in Fig. 2, 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 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 antisera 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 immunoochemical 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 \[^{125}\text{I}]\text{PTH}\) was selectively localized in the cytoplasm of proximal tubular cells. These studies confirmed previous investigations on the localization of \[^{125}\text{I}]\text{PTH}\) and \[^{3}\text{H}\]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 IGG 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.

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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.

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