*PSEUDOHYPOPARATHYROIDISM: ASSAYS OF PARATHYROID HORMONE AND THYROCALCITONIN**

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Pseudohypoparathyroidism is characterized by developmental abnormalities, hypocalcemia, hyperphosphatemia, and an unresponsiveness to the calcium-mobilizing or phosphaturic effects of injected parathyroid hormone.[1, 2] The parathyroid glands are often hyperplastic;[1, 2] they have not been shown, however, to contain or secrete biologically active parathyroid hormone.

Materials and Methods.—Radioimmunoassay: Human parathyroid hormone was measured in unextracted thyroid venous plasma by radioimmunoassay[3, 4] using guinea pig antibovine parathyroid hormone and carboxymethylcellulose-purified bovine hormone[5] as the I^{131}-labeled marker. Antiserum in this assay was used at a final dilution of 1:150,000 in a nonequilibrium incubation in which the unlabeled hormone or unknown was preincubated at 4°C with antibody for 1–2 days and then with labeled hormone for an additional 4–5 days. The specific biological activity of the bovine hormone by the assay method of Munson[6] was 2600 $\overset{\times}{\div}$ 1.44 USP units/mg. The hormone was labeled with I^{131} by the method of Hunter and Greenwood.[7] All unknowns were determined in duplicate in at least two separate assays. Parathyroid gland extracts were tested at multiple dilutions ranging from 1:75 to 1:80,000. Thyroid venous plasma was tested at multiple dilutions ranging from 1:10 to 1:100. Two standard curves were used in each immunoassay: one curve with unlabeled purified bovine hormone[5] and a second curve with a partially purified extract of human parathyroid glands. Normal human parathyroid glands and parathyroid adenomas were extracted with urea-acetic acid and partially purified to the stage of the trichloroacetic acid precipitate according to the method of O'Riordan *et al.*[8] The specific biological activity of the human extract, by a modification[9] of the assay method of Munson,[6] was 73 $\overset{\times}{\div}$ 1.31 USP units/mg. The results of the immunoassays of human samples are given in terms of units (U) or milliunits (mU) of the human parathyroid extract. The values reported may be subject to revision if, when larger amounts of human hormone become available, it is shown that the slopes of the bioassays for human and bovine hormone are nonparallel. The smallest absolute amount of bovine parathyroid hormone that could be measured by the radioimmunoassay was 100 $\mu\mu$g (0.26 mU).

Preparation and testing of human parathyroid and thyroid glands: Normal human parathyroid glands were obtained at autopsy or at the operating table during surgical exploration of a nontoxic thyroid nodule. Four autopsy glands, one fresh surgical specimen, and a single parathyroid gland from each of two patients (N. B. and P. L.) with pseudohypoparathyroidism were homogenized separately in 0.1 *N* HCl-0.12 *M* cysteine (4 ml/gland) and heated for 10 min in a boiling water bath. Insoluble material was removed by centrifugation (10,000 × *g*, 4°C, 10 min). Two additional autopsy glands were homogenized in cold 0.15 *M* NaCl followed by centrifugation. The supernatant solutions were immunoassayed without further purification.

Until recently, biological assay methods for parathyroid hormone have not been delicate enough to detect the amounts of hormone present in individual human parathyroid glands. In the present study, the HCl-cysteine extract of the parathyroid gland from one of the patients (N. B.) with pseudohypoparathyroidism was tested for calcium-mobilizing activity by a new modification[9] of the Munson bioassay method for parathyroid hormone[6] utilizing thyroparathyroidectomized rats.

A portion of the thyroid gland of the same patient (N. B.) whose parathyroid gland extract was assayed immunologically and biologically was extracted with 0.1 *N* HCl (10 ml/gm) as described by Hirsch *et al.*[10] The extract was assayed biologically for thyrocalcitonin in young male rats fed a low-calcium diet for 4 days.[10]

Statistical method: The data in each biological assay experiment were subjected to an analysis

of variance. The standard errors (SE) given in Tables 1 and 2 were calculated from the residual error term of the analysis of variance.

Results.—Figure 1 shows the standard curve obtained in the radioimmunoassay with the homologous bovine hormone and the less sensitive curve obtained with human hormone. The cross-reaction of human parathyroid hormone with our guinea pig antibovine hormone is considerably less than the essentially complete cross-reaction reported by Potts *et al.*[11] and resembles more closely the weak cross-reaction seen in the complement-fixation assay method.[12] Although the radioimmunoassay reported here was not able to detect as little parathyroid hormone as that reported by Sherwood *et al.*,[4] it was sensitive enough to measure readily the relatively large amounts of hormone in human parathyroid gland extracts and in thyroid venous plasma.

Parathyroid hormone was detected immunologically in each of the seven normal parathyroid gland extracts examined. The four HCl-cysteine extracts of autopsy glands contained, respectively, 36, 68, 143, and 89 U/gland. The HCl-cysteine-extracted fresh surgical specimen contained 70 U/gland. Two NaCl extracts of autopsy glands contained only 14 and 2 U/gland. The amounts of hormone in

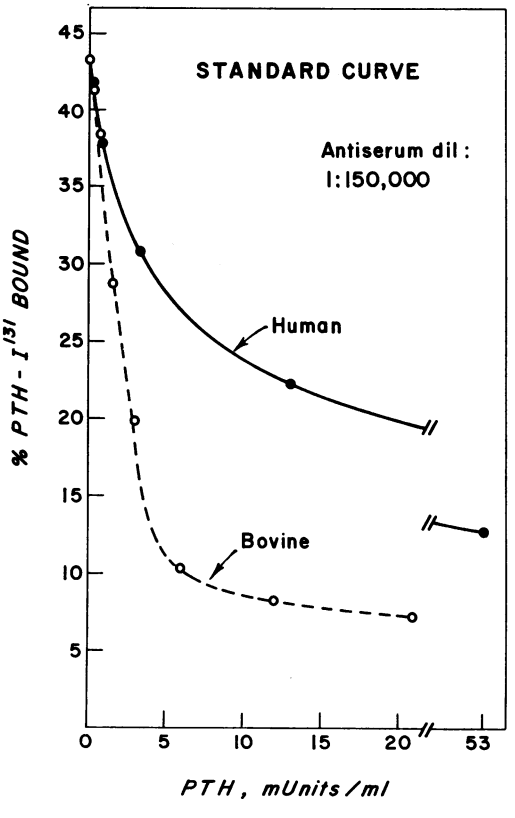

FIG. 1.—Standard curves showing percentage of radioactive tracer bovine parathyroid hormone (PTH-I^{131}) bound to guinea pig antibovine PTH, in the presence of varying amounts of either bovine (*dashed line*) or human (*solid line*) unlabeled PTH. The PTH content of both human and bovine reference preparations was determined by bioassay.

TABLE 1

EFFECT OF AN EXTRACT OF THE PARATHYROID GLAND FROM A PATIENT (N. B.) WITH PSEUDOHYPOPARATHYROIDISM ON THE PLASMA CALCIUM LEVEL OF ACUTELY THYROPARATHYROIDECTOMIZED RATS

Treatment	Plasma calcium* (mg/100 ml)
Control solution†	6.7 ± 0.48
0.5 Units parathyroid hormone‡	7.6 ± 0.40
8.0 Units parathyroid hormone‡	9.6 ± 0.40
Parathyroid extract (patient N. B.)§	8.8 ± 0.48

* Mean values ± SE, 4 or 6 rats/group.
† Dilute HCl-0.12 *M* cystine (0.75 ml per rat).
‡ USP parathyroid extract (Lilly and Co.) injected in dilute HCl-0.12 *M* cysteine, 0.75 ml/rat.
§ Each rat received 0.75 ml of extract equivalent to approximately 1/8 of a single parathyroid gland. The effect was significantly different than that of the control solution ($P < 0.01$). Extracts of liver, salivary gland, adrenal, skeletal muscle, and cardiac muscle had no calcium-mobilizing effect in the assay rats.

HCl-cysteine extracts of the parathyroid glands of the two patients with pseudohypoparathyroidism were 89 and 91 U/gland, respectively, for N. B. and P. L. The shapes of the dilution curves obtained with the two pseudohypoparathyroid gland extracts were not different from those obtained with normal gland extracts.

Parathyroid hormone was measured by radioimmunoassay in high concentration in the thyroid venous plasma of the two patients with pseudohypoparathyroidism and in one patient without parathyroid disease (carcinoma of the tongue). The values in the pseudohypoparathyroid patients were 890 and 990 mU/ml, and in the control patient the level was 123 mU/ml. These results cannot be due to some unknown nonspecific effects of human plasma because no parathyroid hormone has been detected with this assay in the peripheral venous plasma (at similar dilutions) of 13 normal control individuals or in six patients (three were members of one family) with pseudohypoparathyroidism. The inability of our assay to measure human hormone in peripheral plasma greatly limits the clinical usefulness of the method but does not invalidate the findings obtained with thyroid venous plasma or single parathyroid gland extracts.

Since it has been clearly shown that the structural determinants of immunological and biological activity in bovine parathyroid hormone are not identical,[5, 13, 14] the demonstration of immunologically active hormone in the parathyroid glands of patients with pseudohypoparathyroidism does not necessarily show that this hormone has the determinants for hormonal activity. Therefore, we tested for calcium-mobilizing activity the HCl-cysteine extract of the parathyroid gland from one patient (N. B.) with pseudohypoparathyroidism. The results, shown in Table 1, reveal a highly significant parathyroid hormone-like response in the assay rats. Because there was only enough material for testing at a single-dose level, it was not possible to obtain an accurate estimate of the potency of the extract. On the basis of the data available, however, the potency was not significantly different from the value determined on the same sample by radioimmunoassay. We interpret the results of these experiments as showing that the parathyroid hormone extracted from this parathyroid gland has the structural requirements for reaction with antiparathyroid hormone *and* for raising the plasma calcium of parathyroidectomized rats. Since the hormone is also present in large amounts in the thyroid venous plasma, pseudohypoparathyroidism cannot be caused by an absolute lack of parathyroid hormone biosynthesis or release.

In spite of these findings, the primary cause of pseudohypoparathyroidism remains unknown. A number of different abnormalities might explain the pathophysiology.[1, 2] Among them is the possibility of excessive secretion of a hypocalcemic agent such as the thyroid polypeptide, thyrocalcitonin.[2] Indeed, the thyroid gland of one of our patients (N. B.) contained at least 50 times more thyrocalcitonin than normal human thyroid glands (Table 2).

Discussion.—The importance of the data obtained in these studies is that they show unequivocally the presence of immunologically and biologically active parathyroid hormone in the parathyroid glands of patients with pseudohypoparathyroidism. The data do not exclude the possibility that the hormone in pseudohypoparathyroidism may differ qualitatively from normal hormone. The finding of high levels of parathyroid hormone in the thyroid venous plasma of these patients also shows that the hormone in the glands is being released into the circulation. Although the values in the pseudohypoparathyroid patients are higher than the value in the control patient, further observations will be necessary to confirm this elevation because of the small number of observations and the variability between individuals of the venous drainage of the thyro-parathyroid glands.

The extremely high level of thyrocalcitonin in the thyroid gland of patient N. B. requires special consideration. The idea that hypocalcemic substances from the parathyroid or thyroid glands might play a role in the pathophysiology of pseudohypoparathyroidism arose in part from observations by Drs. S. Krane, A. Forbes, J. Raker, and O. Cope of another patient (R. M.) with this disease whose case has been followed for many years at the Massachusetts General Hospital. This idea gained added significance when Aliapoulios, Voelkel, and Munson discovered that this patient's thyroid gland contained approximately 100 times more thyrocalcitonin than normal human thyroid glands.[15] It is not yet known, however, whether the high levels of thyrocalcitonin in the thyroid glands of these two patients with pseudohypoparathyroidism is a primary event in the disease process, is secondary to treatment (both patients had received large doses of vitamin D although administration of the vitamin to N. B. was discontinued 3 weeks before surgery), or represents

TABLE 2

HYPOCALCEMIC EFFECT OF AN EXTRACT OF THE THYROID GLAND FROM A PATIENT (N. B.) WITH PSEUDOHYPOPARATHYROIDISM

Treatment	Plasma Calcium (mg/100 ml)[a] Expt. 1	Expt. 2
Control solution[b]	9.4	9.1
2 Hirsch units thyrocalcitonin[c]	8.0	8.4
8 Hirsch units thyrocalcitonin[c]	7.0	7.4
N. B. thyroid extract, 0.08 ml/rat[d]	Nt[e]	6.4
" 0.04 ml/rat	7.0	Nt
" 0.02 ml/rat	Nt	7.7
" 0.01 ml/rat	7.7[f]	8.2[f]
" 0.005 ml/rat	Nt	8.9
" 0.0025 ml/rat	9.3	Nt

[a] Mean values, 4 or 8 rats/group. The standard errors were 0.16–0.22 and 0.18–0.25 for expts. 1 and 2, respectively.
[b] Dilute HCl (0.5 ml per rat).
[c] Reference standard for thyrocalcitonin.[10, 15] All solutions, standards, and diluted unknowns were injected subcutaneously in a volume of 0.5 ml. The rats were bled by cardiac puncture 1 hr after injection. Plasma calcium was determined by the method of Copp.[16]
[d] Similar extracts of 11 normal human thyroid glands required doses of at least 0.4–2.0 ml of extract per rat to obtain hypocalcemic effects which were significant at $P < 0.05$.[15]
[e] Nt = not tested.
[f] Significantly different from control values $P < 0.001$ and $P < 0.05$ for expts. 1 and 2, respectively.

accumulation of thyrocalcitonin in the thyroid gland due to chronic hypocalcemia, a possibility for which there is some experimental support.[17]

Summary.—Parathyroid hormone was measured by radioimmunoassay in thyroid venous plasma obtained from two patients with pseudohypoparathyroidism. An extract of a parathyroid gland from one patient produced a significant rise in the plasma calcium level of thyroparathyroidectomized rats. These results show that the parathyroid glands in pseudohypoparathyroidism can synthesize and secrete a substance which has the structural requirements for the immunological and biological activities of parathyroid hormone. The thyroid gland of one patient contained at least 50 times more thyrocalcitonin than normal human thyroid glands.

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