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Column Chromatgraphy of Human Serum Parathyroid Immunoreactive Peptides[1] (38635)

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(Introduced by F. Grande)

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Recently various reports have appeared attempting to define the size of serum parathyroid hormone (PTH). Canterbury and Reiss have shown that their assay measured, predominantly, a peptide of about 7000 mol wt and less of those peptides with 9500 and 5000 mol wt (1). They show, however, that the 9500 mol wt peptide is sensitive to calcium perturbation and decays rapidly after parathyroidectomy. Habener *et al.* (2) showed evidence that 9500 mol wt PTH is secreted and concluded that peripheral cleavage results in a 7000 mol wt peptide being predominant in peripheral serum. Martin *et al.* (3) have shown in monolayer cultures of human parathyroid glands that a peptide similar to beef PTH is secreted. Arnaud *et al.* (4) showed evidence that both 7000 and 9500 mol wt peptides are present in uremic serum and parathyroid adenomas, and concluded that the former peptide is a better steady state indicator or parathyroid dysfunction while the large form is related to acute changes of parathyroid function.

In this communication we will show evidence that uremic serum has principally a peptide of 10,000 mol wt and that, in contrast, the pattern of serum PTH peptides from patients with parathyroid adenomas can be quite variable. These data support the hypothesis presented previously, relating to the synthesis and secretion of human parathyroid hormone (5).

Methods. Measurement of parathyroid hormone. An antibody to beef parathyroid hormone was prepared in chickens by injecting them for 9 mo with 75% pure beef PTH of 9500 mol wt. The antibody recognizes the intact beef PTH molecule but does not recognize the *N*-terminal 1–34 segment of beef PTH. This antibody is unique in that it is multivalent in its ability to recognize a number of human parathyroid peptides ranging from >25,000 to 3000 mol wt (5). A two-antibody method is used similar to that described for insulin by Morgan and Lazarow (6). Details of the procedure are being published separately.

Highly purified beef parathyroid hormone from Wilson was used for labeling by the Hunter Greenwood method (7) and for standards. The PTH assay is set up as a disequilibrium method following the flow diagram in Table I. All solutions contain 0.13 *M* borate buffer, pH 8.4, with 1% bovine serum albumin except for the diluent used for ^{125}I-bPTH which contained 0.044 *M* EDTA.

The assay characteristic of three human preparations of PTH are compared to beef PTH in Fig. 1. A uremic serum rich in PTH was serially diluted and assayed. A 10,000 mol wt parathyroid peptide from a human parathyroid gland, isolated by column chromatography, was tested (5). PTH was also adsorbed from serum by Quso G-32 and subsequently eluted, brought into assay buffer and tested. Quso G-32 is s finely divided silicate known to adsorb PTH (8). All of these preparations were linear from 6 ng/ml to lower values.

Column chromatography. Serum samples were filtered in a cold room on 1.5 × 60 cm columns of Sephadex G-75 Superfine (Pharmacia) or Bio-Gel P-10 in 0.13 *M* borate buffer, pH 8.4, with 1% bovine serum albumin as the carrier. These columns were fitted with flow adaptors, permitting upward flow chromatography. Buffer was pumped at a rate of 4.5 ml/hr. Results of elution volume

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TABLE I. PROTOCOL FOR THE PARATHORMONE ASSAY

1) 200 μl sample, standard, control.
2) 100 μl chicken anti-bPTH (1/400 dilution); incubate 48 hr.
3) 100 μl ^{131}I-bPTH, 2 ng/ml; incubate 24 hr.
4) 200 μl rabbit anti-chicken plasma (whole) and 200 μl normal chicken serum (1/200 dilution added as a carrier); incubate overnight.
5) Separate precipitate by centrifugation; decant supernate.
6) Count supernate and precipitate

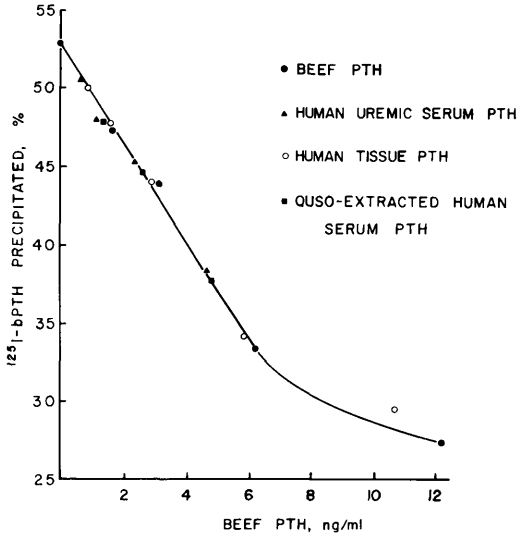

FIG. 1. Dilutional linearity, relative to a beef PTH hormone standard, of several preparations containing human PTH. Human tissue PTH was prepared by methods previously reported (5). For the Quso extracted PTH, 1 ml of uremic serum was extracted with 10 mg Quso. The PTH was eluted with 20% acetone, 1% acetic acid, dried and reconstituted in assay buffer.

are expressed in K_{av} units.[2] The G-75 column was calibrated with various proteins including unlabeled beef PTH and a reference human PTH isolated from parathyroid glands by methods described earlier (5). The line in Fig. 2, is a guide to relating K_{av} units with approximate molecular weight.

[2] $K_{av} = (V_e - V_o)/(V_i - V_o)$

V_o = void volume. V_i = salt elution volume. V_e = elution volume of the unknown.

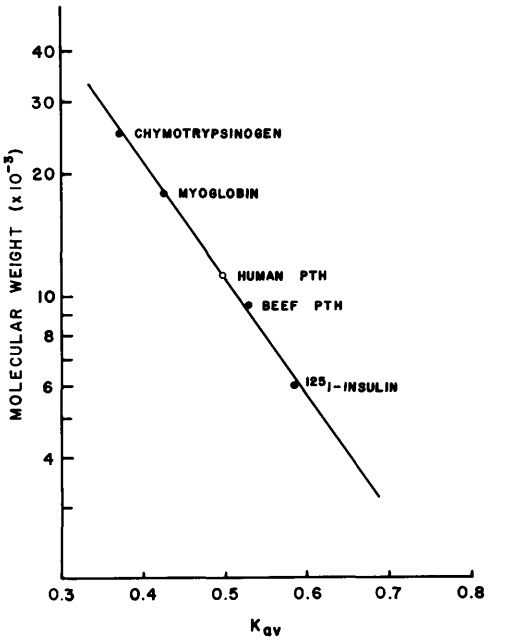

FIG. 2. The relationship between K_{av} and mol wt as determined by gel filtration of five proteins on Sephadex G-75 Superfine.

$$K_{av} = (V_e - V_o)/(V_i - V_o)$$

V_e = elution volume of protein. V_o = void volume. V_i = salt elution volume.

A void marker and salt marker were included with each run.

The molecular weight of the reference beef PTH sample and the human gland reference PTH were estimated by electrophoresis on sodium dodecyl sulfate (SDS)—urea acrylamide gels (9) as shown in Fig. 3. The human preparation has a mol wt of about 10,500 daltons on SDS and a K_{av} of 0.49 on G-75 Superfine, while the beef preparation has a mol wt of 9500 daltons on SDS and a K_{av} of 0.52 on G-75 Superfine (Figs. 2 and 3).

Results. Chromatograms from a uremic serum and beef PTH are shown together in Fig. 4. It is clear that the principal peptides measured in uremic serum are very similar in size to pure beef PTH, assumed to have a molecular weight of 9500 with a K_{av} of 0.52. However, in this serum there is also an appreciable quantity of voided material (K_{av} < 0.2 on G-75 Superfine Sephadex) which behaves in the radioimmunoassay as PTH and represents ^{125}I-bPTH damaging factors.

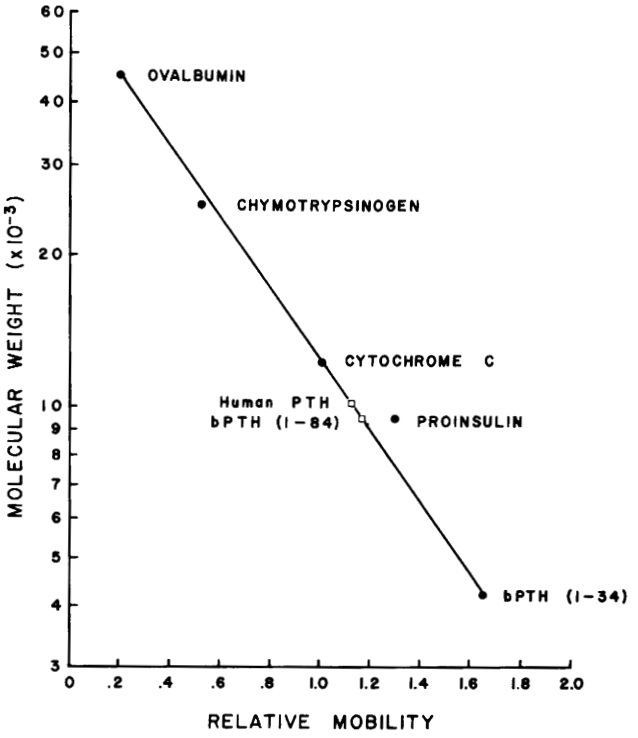

FIG. 3. SDS—urea polyacryamide gel electrophoresis of reference PTH preparation and marker proteins

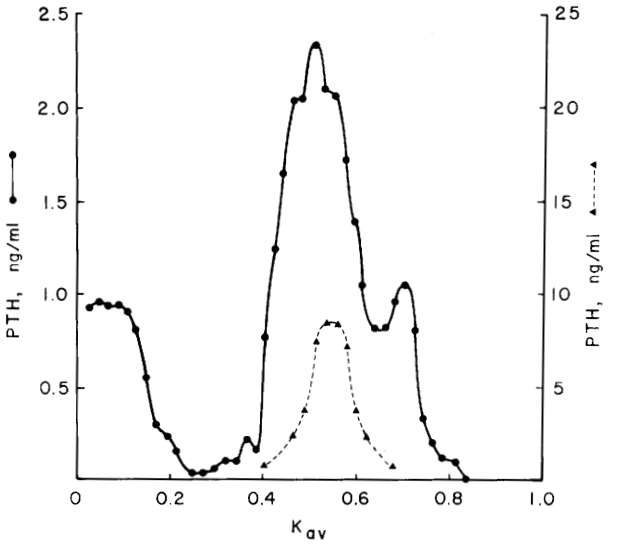

FIG. 4. Parathyroid hormone analysis of eluates from a 1.5 × 60 cm Sephadex G-75 Superfine chromatogram of uremic serum and beef PTH. Solid circles and solid lines: serum. Triangles and dashed line: beef PTH (Wilson).

Some heterogeneity of the iPTH is also present in the uremic serum with a small peak occurring at K_{av} 0.68.

Serum profiles from patients with parathyroid adenomas demonstrate differences from the uremic serum. Two such sera are shown in Figs. 5 and 6. In the first serum, patient A.M., there is a preponderance of

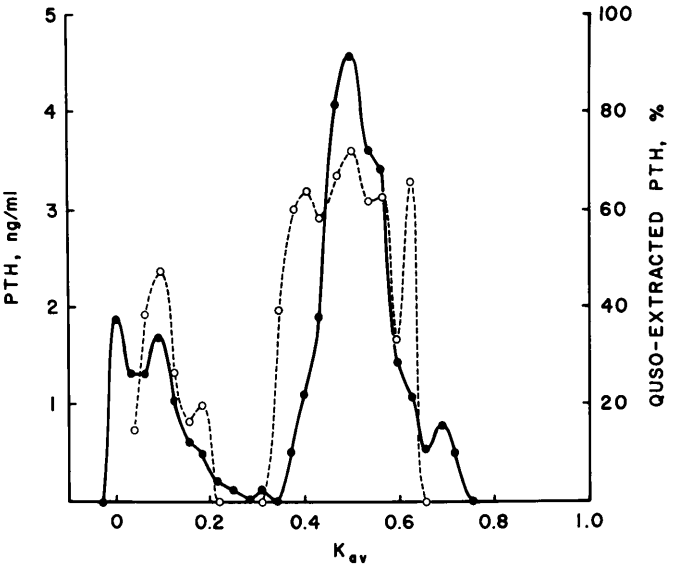

FIG. 5. Parathyroid hormone analysis of eluates from a 1.5 × 60 cm Sephadex G-75 Superfine chromatogram of serum from a patient with a parathyroid adenoma. Solid line: original serum. Dashed line: percent Quso extractibility based on comparison of a similar aliquot of serum extracted with Quso G-32, a finely ground silicone.

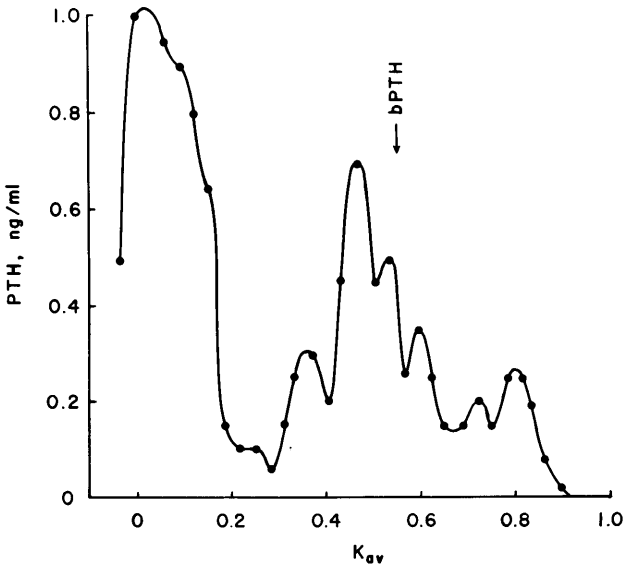

FIG. 6. Parathyroid hormone analysis of eluates from a 1.5 × 60 cm Sephadex G-75 Superfine chromatogram of serum from a patient with a parathyroid adenoma. The position of beef PTH is marked.

iPTH at K_{av} 0.48 with a shoulder at 0.52. Other peptides are represented in smaller quantities. Heterogeneity is seen at the void area of the column representing damage factors. One ml of serum from this patient was chromatographed after being adsorbed with 10 mg Quso G-32 to test the efficiency of Quso to adsorb the various PTH peptides. The percentage of Quso extractibility calculated tube by tube (see dashed line in Fig. 5) demonstrates that material at K_{av} 0.1 is about 25% extractable. Cross-reactive pep-

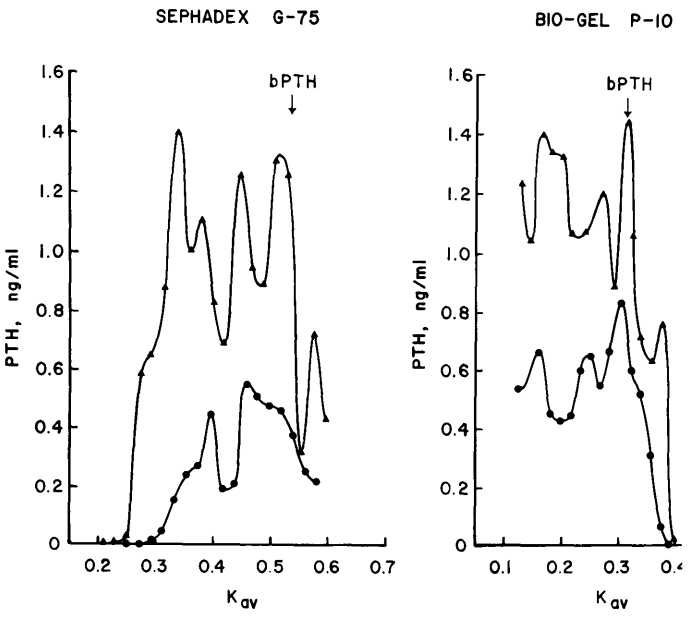

FIG. 7. Comparative chromatograms of the sera from peripheral (solid circles) and juxtaglandular (triangles) sites in a patient with a parathyroid adenoma. Both columns are 1.5 × 60 cm, and the conditions are identical. The position of highly purified beef PTH (Wilson) is shown as a comparative column marker.

tides of less than 4000 mol wt did not extract but the region of interest from K_{av} 0.3 to 0.7 is largely Quso extractable (60–70% in this column). In serum from the second patient with an adenoma, R.S. (Fig. 6), the major peak is at K_{av} 0.47 with other relatively large quantities of iPTH at K_{av} 0.37, 0.52, 0.59 and 0.67. The latter serum is highly heterogeneous both in the PTH region, from K_{av} 0.3 to 0.7, and at the voided region of the column.

Serum from still another patient, C. B., with a parathyroid adenoma, was obtained both from the region of the gland, and from the peripheral circulation. The gland was localized prior to surgery by venous catheterization and found to be substernal in location; the gradient of iPTH was largest in the superior vena cava. Aliquots of serum were chromatographed on both Sephadex G-75 Superfine and Bio-Gel P-10, the latter being used for chromatography of serum by most investigators. Both ^{125}I-bPTH and unlabeled bPTH were run in separate studies as markers. The results are shown in Fig. 7. It is clear that this gland was secreting peptides of K_{av} 0.34, 0.38, 0.45, 0.52 and 0.58 as well as a number of small fragments. This largest peptide would have a molecular weight of >25,000. The peripheral serum, however, showed mainly K_{av} 0.40, 0.46 and 0.52 peptides. There, evidently, was rapid clearance or conversion of the other peptides. With Bio-Gel P-10 the same peptides could be localized; however, the peptides are found nearer the void region where they overlap with damaging factors. Bio-Gel P-10 may resolve the smaller peptides better than Sephadex, but it is a less desirable tool to study the larger forms. The peptide at K_{av} 0.32 on Sephadex is far more concentrated in the superior vena cava serum than in the peripheral serum.

Discussion. The synthesis and secretion of parathyroid peptides is a complex process. In our studies over the past 3 yr, a measure of the complexity is unfolding. Based on our studies of gland homogenates (5), the protein synthetic apparatus of the parathyroid cell appears to have a large form of immunoreactive PTH (>25,000 mol wt). In most cases we have found the principal peptide in the secretion granule fraction to be about 10,000 mol wt.

The question of which peptide is normally secreted has been controversial. In our

experience, the uremic patients have circulating principally, a peptide of about 10,000 mol wt. Serum from patients with adenomas can be a complex mixture of PTH immunoreactive peptides. Some patients have, in their sera, representatives of almost all glandular peptides. In one such case, where we studied the iPTH peptides in the venous effluent of the gland, it was clear that the large form of iPTH that we had previously seen in parathyroid gland microsome fractions was being secreted but was being disposed of peripherally. This observation is somewhat like the observation of Habener *et al.* (2) who found that 9500 mol wt or larger PTH was coming from parathryroid gland effluents while 7000 mol wt forms were present peripherally. They concluded that peripheral conversion was occurring. Our data would not allow selection of the hypothesis of peripheral conversion over that of rapid clearance of large iPTH peptides. Results in our laboratory and others depend on the specificity of the antibodies against human PTH which we have shown to be very heterogenous. Quantitation of the secretion of the various forms of PTH is not possible until all of these peptides have been purified.

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