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# Isolation and Chemical Properties of Porcine Parathyroid Hormone*

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ABSTRACT: Porcine parathyroid hormone was isolated from phenolic extracts of pig parathyroid glands. By solvent and salt fractionation, gel filtration, and ion-exchange chromatography, a preparation that was homogeneous by several criteria was obtained; it contained a single amino-terminal residue.

While the physical properties of the porcine hormone are similar to those of bovine parathyroid hormone, the hormones differ in amino acid composition. The composition of porcine parathyroid hormone is $Asp_5$,$Asn_3$,$Ser_8$,$Glu_6$,$Gln_5$,$Pro_2$,$Gly_5$,-$Ala_6$,$Val_9$,$Met_1$,$Ile_3$,$Leu_{10}$,$Phe_1$,$Lys_9$,$His_5$,$Arg_5$,$Trp_1$. The calculated molecular weight is 9423.

Prominent among the differences in amino acid composition between the hormone from the two species is the presence in the porcine hormone of serine instead of valine at the amino terminus and the lack of tyrosine and one methionine residue. Despite the lack of tyrosine, the peptide can be labeled with $^{125}I$ by standard techniques used in radioimmunoassays. Oxidation of the methionine destroys biological activity. The sequence of the first six residues by Edman degradation was found to be Ser-Val-Ser-Glu-Ile-Gln; residues 2–6 were thus identical with those found in bovine parathyroid hormone.

The most extensively studied forms of parathyroid hormone are those of bovine origin. These are single-chain peptides with 84 amino acid residues (Keutmann *et al.*, 1971). The complete covalent structure of the major form of bovine hormone has recently been determined (Niall *et al.*, 1970; Brewer and Ronan, 1970). Human parathyroid hormone has only been isolated in microgram quantities (O'Riordan *et al.*, 1971). Its charge and size properties are similar to those of bovine parathyroid hormone (BPTH)[1] but chemical and immunological differences from bovine parathyroid hormone have been shown (O'Riordan *et al.*, 1969).

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[1] Abbreviations used are: PPTH, porcine parathyroid hormone; BPTH, bovine parathyroid hormone; CMC, carboxymethylcellulose; MCR, Medical Research Council.

Isolation of porcine parathyroid hormone (PPTH) was undertaken so that further species comparisons could be made, and so that additional structure–function studies would be possible. Another consideration was that, should the porcine hormone be more similar to the human than the bovine hormone, it might be useful in improving radioimmunoassays for human parathyroid hormone.

Littledike (1967) located the parathyroid gland of the pig, making possible collections on a commercial scale. Littledike and Hawker (1967) subsequently reported partial characterization of the porcine hormone. The yield of purified hormone was low and detailed tests of chemical purity and biological potency could not be undertaken. From its ultracentrifuge behavior its molecular weight was estimated to be 11,000; from its amino acid composition a minimum value of 9622 was calculated. The peptide was reported to differ from bovine parathyroid hormone (BPTH) especially in containing two residues of threonine which is absent from bovine parathyroid hormone.

Here we report the isolation of porcine parathyroid hormone using 88% phenol for the initial extraction, followed by solvent and salt fractionation, precipitation with trichloroacetic acid, gel filtration, and ion-exchange chromatography. The product appeared to be homogeneous by multiple criteria. The amino acid composition was found to differ from that reported previously (Littledike and Hawker, 1967).

## Methods

*Isolation of Porcine Parathyroid Hormone: Defatting of Parathyroid Glands.* Porcine parathyroid glands (4.5 kg; obtained from Oscar Mayer & Co., Madison, Wis.), deep frozen from the time of excision, were dehydrated by homogenization in acetone (50 l.) precooled to −20°. The homogenate was filtered through linen and the residue defatted by homogenization in *n*-hexane (25 l.) also at −20°. A further 20 l. of acetone was added and the suspension filtered again. The residue was homogenized in a further 15 l. of acetone and after a final filtration was dried under a partial vacuum to yield 580 g of powder.

*Extraction of Parathyroid Hormone.* The acetone powder was extracted with 5.5 l. of 88% phenol (Aurbach, 1959) followed by 35 l. of 20% acetic acid in acetone precooled to 4°. β-Mercaptoethanol (0.01 M) was added to prevent oxidation of methionine residues during the extraction process. Sodium chloride (80 ml of 4 M) and Celite (250 g) were added. After 1 hr the suspension was filtered and insoluble material was discarded. Ether (25 l.), precooled to 0°, was added and the mixture allowed to stand overnight. A heavy precipitate was collected by filtration and washed in cold acetone. The washed residue was stirred into glacial acetic acid (320 ml); it was then diluted to give a final concentration of 20% acetic acid, 0.04 M in cysteine hydrochloride. The ether precipitate did not dissolve completely in the acetic acid but formed a thick gel. Gelatinous material was removed by centrifugation at 2000*g* for 30 min and reextracted three times with acetic acid as before. These extracts were fractionated individually. Sodium chloride was added to a concentration of 6%. After centrifuging, the precipitate was taken up in acetic acid as before and NaCl again added to 6%. The supernatant was combined with that of the previous precipitation and filtered through a bed of Celite. One-hundred per cent (w/v) trichloroacetic acid was added to the filtrate to a final concentration of 4%. The third reextraction of the gel yielded no precipitate when trichloroacetic acid was added and so was discarded. After 2 hr at 4°, precipitates were removed by centrifugation at 2000*g* for 30 min and resuspended in 200 ml of 1 M acetic acid containing 0.01 M β-mercaptoethanol. Trichloroacetic acid was again added to a concentration of 4% and after 2 hr the precipitate was collected by centrifugation at 1800*g* for 12 min. This precipitate was suspended in 40 ml of 1 M acetic acid containing a trace of β-mercaptoethanol. Ion-exchange resin (IRA 400) in the acetate form was added until the precipitate dissolved. The solution was then poured down a column of IRA 400 (2 × 8 cm), diluted to 400 ml, and lyophilized. This product was designated trichloroacetic acid–PPTH.

*Final Purification of Trichloroacetic Acid–PPTH.* Trichloroacetic acid–PPTH dissolved in 1 M acetic acid was fractionated on a 2.5 × 100 cm column of Sephadex G-100 (beaded form). The columns were equilibrated with 0.14 M ammonium acetate (pH 4.75) at 4° and pumped at a rate of 12 ml/hr. The elution of protein was monitored by measuring optical density at 280 mμ. The elution position of the hormone was detected by radioimmunoassay and bioassay of fractions.

The product of gel filtration was lyophilized and then dissolved in 0.005 M ammonium acetate (pH 5.3) and subjected to chromatography on a 1 × 5 cm column of carboxymethylcellulose (Whatman CM 52). The column was eluted with a linear gradient of increasing conductivity, made with 0.01 M ammonium acetate (pH 5.3, conductivity 0.55 mmho) and 0.33 M ammonium acetate (pH 7.0, conductivity 14 mmhos). One liter of each buffer (both of which contained 0.0014 M β-mercaptoethanol) was used, and the column was pumped at a rate of 14 ml/hr at 4°. Elution was monitored in the same manner as for the Sephadex G-100 column. The product was designated CMC–PPTH.

*Disc electrophoresis* was carried out using 20–100 μg of peptide in 15% polyacrylamide gels, employing the pH 4.5 buffer system of Reisfeld *et al.* (1962). Urea (8 M) was incorporated in the gel buffers, and protein bands were stained with 1% Amido Black in 7% acetic acid.

To locate immunoassayable hormone, the gels were split longitudinally. One half was stained, the other half sliced into sections and eluted with 8 M urea in 10% acetic acid for immunoassay (O'Riordan *et al.*, 1971).

*Thin-layer chromatography* was performed using Brinkmann 20 × 20 cm glass-backed cellulose plates of 100 μ thickness. The solvent system consisted of butanol–water–pyridine–acetic acid (15:12:10:3, v/v). Plates were stained with ninhydrin, and also with Ehrlich's reagent for detection of tryptophan.

*Radioimmunoassay.* A radioimmunoassay was used to monitor the initial extraction process and subsequently the elution of immunologically active parathyroid hormone off columns of Sephadex and carboxymethylcellulose. Initially the system used $^{125}$I-labeled bovine parathyroid hormone and a guinea pig antiserum (designated GP-5) produced against bovine PTH and characterized as to immunoreactivity in both bovine and porcine systems (O'Riordan *et al.*, 1969). Using a final antibody dilution of 1:25,000, incubations were carried out for two hours at room temperature, after which unreacted hormone was removed by adsorption onto dextran-coated charcoal. Aliquots of the supernatant containing the antibody-bound fraction were counted in a Nuclear-Chicago Auto-Gamma spectrometer. The relative displacement of [$^{125}$I]BPTH by suitably diluted aliquots of the column eluate was compared with that produced by BPTH standard. Though PPTH has been shown subsequently to differ immunologically from BPTH (Woodhead and O'Riordan, 1970), there is

sufficient cross-reactivity in this system for it to be used to locate PPTH in the eluate.

When CMC–PPTH had been isolated it was labeled with $^{125}I$ by the method of Hunter and Greenwood (1962) at a pH of 7.5 in phosphate buffer. The labeled hormone was freed of excess iodide and damage products by the method of Yalow and Berson (1966) using microfine silica. Antisera were prepared by immunizing guinea pigs with trichloroacetic acid–PPTH in complete Freunds adjuvant. [$^{125}I$]PPTH and anti-PPTH could then be used to locate immunoassayable material eluted from polyacrylamide gels (O'Riordan *et al.*, 1971).

*Bioassay.* Assay of the biological potency of preparations at different stages of extraction were carried out by the method of Amer (1968). Wistar rats (80–100 g) were injected subcutaneously with 0.5-ml samples 5 days after thyroparathyroidectomy. Bovine parathyroid hormone (MRC research standard 67/342; potency, 330 MRC U/mg) was injected at dose levels of 6.7, 13.4, and 26.8 MRC units; 1 MRC unit is approximately 1 USP unit (Robinson *et al.*, 1971). Six animals were used at each dose. Similarly PPTH was assayed at three doses. Changes in serum calcium over a 5-hr period were measured using the method of MacIntyre (1961).

*In vitro* bioassays were performed by the renal cortical adenylylcylcase enzyme activation system as described by Marcus and Aurbach (1969), using either the MRC standard 67/342 or a house standard prepared from CMC purified BPTH and calibrated against the MRC preparation.

*Amino Acid Analyses.* Aliquots (300 $\mu$g) of purified porcine parathyroid hormone were subjected to acid hydrolysis in an evacuated desiccator at 110° for 24, 48, 72, and 96 hr, using 5.7 N HCl containing 1:2000 (v/v) mercaptoethanol (Keutmann and Potts, 1969). Total enzymatic digestion, for determination of the labile residues tryptophan, asparagine, glutamine, and methionine sulfoxide was performed using papain (Worthington) followed by aminopeptidase M (Rohm and Haas, Darmstadt, Germany) (Keutmann *et al.*, 1970). Amino acid analyses were carried out with the Beckman Model 121 automatic amino acid analyzer, equipped for high-sensitivity analyses and 2-hr elution schedules (Hubbard and Kremen, 1965; Hubbard, 1965). Mole fractions and values for moles of amino acid per mole of PPTH were obtained by computer reduction (H. T. Keutmann, unpublished data) of digital integrator readouts (Infotronics Model CRS-12AB), using best fit of all stable residues for normalization. Values for serine were extrapolated to zero time.

*End-Group Analyses.* Amino-terminal end-group analysis and sequence determination was done by the three-stage procedure of Edman (Blomback *et al.*, 1966). Amino acid phenylthiohydantoins were identified by gas–liquid chromatography (Pisano and Bronzert, 1969) and by thin-layer chromatography (Edman and Begg, 1967) in several different solvent systems.

*Mild Oxidation with Hydrogen Peroxide.* CMC–PPTH (5 mg) was treated with 0.75 ml of 1:50 (v/v) 30% hydrogen peroxide for 0.5 hr at room temperature (Tashjian *et al.*, 1964). Aliquots were taken before and after bioassay, and for amino acid analysis after acid hydrolysis and total enzymic digestion to quantitate the extent of oxidation.

*Vigorous Oxidation with Performic Acid.* To convert methionine into methionine sulfone for amino acid analysis, a solution of performic acid was prepared by combining 19 ml of formic acid with 2 ml of hydrogen peroxide. CMC–PPTH (2 mg) was added, incubated for 2 hr at 0°, diluted fivefold with water, and lyophilized (Moore and Stein, 1951).

*Carboxypeptidase Digestion.* Aliquots (100 $\mu$g) of peptide were tested with carboxypeptidases A and B (Worthington), respectively, in 1 ml of 0.1 N ammonium bicarbonate buffer (pH 8.5) using an enzyme:substrate ratio of 1:10 (M:M). Digestions were carried out for 1- and 3-hr periods at 35°.

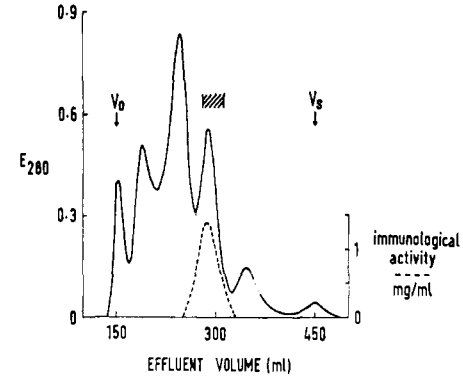


FIGURE 1: Purification of 175 mg of trichloroacetic acid–PPTH on Sephadex G-100. The hatched area indicates the fractions pooled for further purification. $V_0$ represents the void volume of the column and $V_s$ the salt volume, measured with Blue Dextran and sodium chloride, respectively.

*Study of Absorption Spectrum.* CMC–PPTH (2 mg) was dissolved in 0.1 N acetic acid and passed through a Bio-Gel P-2 column (1.2 × 20 cm) to remove all traces of residual salt and mercaptoethanol. After measurement of absorption at 250, 280, and 320 m$\mu$ (using 0.1 N acetic acid as the reference solution) precisely measured aliquots were taken for amino acid analysis. Extinction coefficient was calculated using the net absorption at 280 m$\mu$ of this calibrated solution.

The material was also examined by differential spectroscopy in a Cary Model 15 recording spectrophotometer, using identical aliquots at pH 3 and 10. Similar aliquots of purified BPTH were used for comparison.

## Results

At the stage of precipitation with trichloroacetic acid, the total yield was 1.4 g of powder (trichloroacetic acid–PPTH). The potency of this was 530 MRC units/mg. On disc gel electrophoresis this contained three major and several minor bands.

The elution pattern of trichloroacetic acid–PPTH from Sephadex G-100 is shown in Figure 1 with the location of immunoassayable hormone shown by the broken line. PPTH emerged with a $K_d$ of 0.44 in this system. From 178 mg of trichloroacetic acid—PPTH, 38 mg of immunoassayable hormone was recovered. The biological potency of the peak of immunoassayable material was 2000 MCR units/mg. Although there was good agreement between the estimates of hormone content by immunoassay and the protein content measured by optical density, disc gel electrophoresis still showed that at the peak of elution PPTH was not completely homogeneous.

Material from the peak and descending limb of immunoassayable activity of the eluate off the Sephadex G-100 column was pooled and lyophilized and then subjected to ion-exchange chromatography with a linear ammonium acetate gradient on carboxymethylcellulose. PPTH eluted at a pH of 6.3 and a conductivity of 6.0 mmhos (Figure 2). Eighty per cent of the immunoassayable activity was recovered from the CMC column. The recoveries of hormone at each step were closely comparable to those observed for BPTH (Keutmann *et al.*, 1971). A total of 50 mg of CMC–PPTH was isolated for structural determination and other studies.

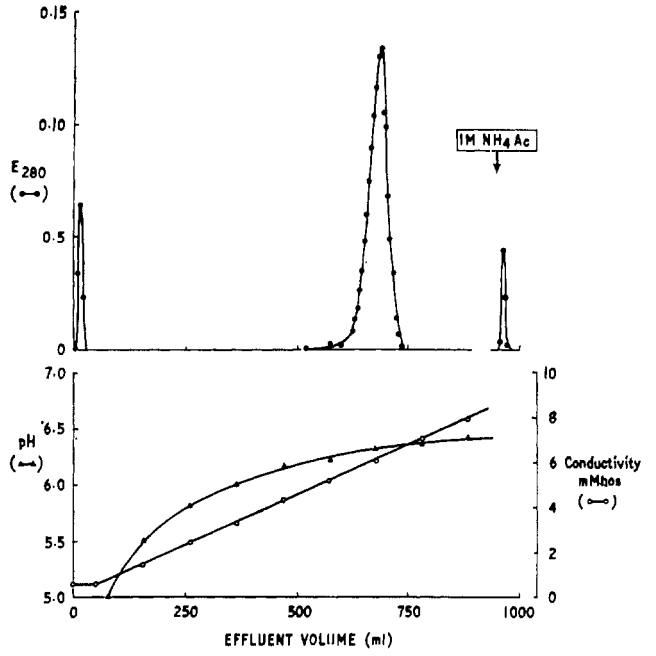


FIGURE 2: Purification of 25 mg of Sephadex G-100-PPTH on carboxymethylcellulose, using a linear gradient of ammonium acetate buffer as described in the Methods section. PPTH elutes at a pH of 6.3 and a conductivity of 6 mmhos.

The log dose–response regression for porcine parathyroid hormone assayed in thyroparathyroidectomized rats was parallel to that for the bovine hormone (Figure 3). Assayed against a bovine standard, the potency of CMC–PPTH hormone was 2400 MCR units/mg (95% confidence limits 1300–3200).

Potency by *in vitro* adenylylcyclase bioassay of the same preparation was 500 units/mg, compared to an activity of 1400 U/mg for purified bovine parathyroid hormones I and II (Keutmann *et al.*, 1971). The two dose-response regressions were again parallel. After mild oxidation with hydrogen peroxide the potency by *in vivo* bioassay was reduced by at least 99%. Amino acid analysis of a total enzymatic digest showed that greater than 95% of the methionine had been converted into methionine sulfoxide; all other residues, including tryptophan, remained intact following oxidation.

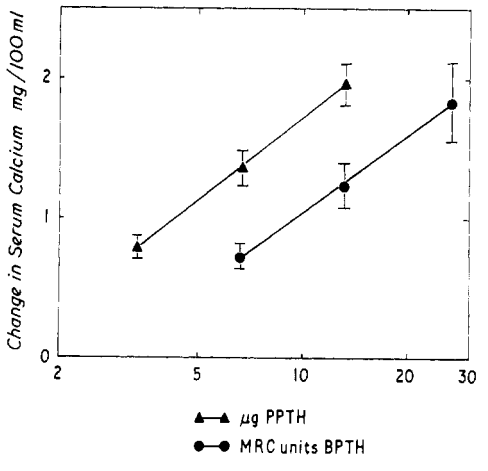


FIGURE 3: Bioassay of CMC–PPTH in thyroparathyroidectomized rats by the method of Amer (1968) at 3 dose levels compared with a standard preparation of bovine parathyroid hormone. The vertical bars indicate the standard error of the responses. The slopes of the regression lines do not differ significantly.

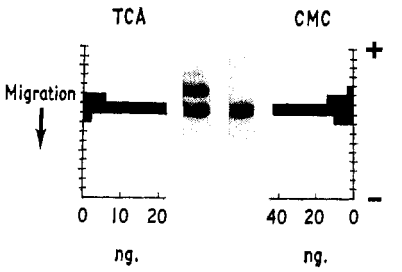


FIGURE 4: Disc gel electrophoresis patterns of trichloroacetic acid–PPTH and CMC–PPTH. Histograms show the distribution of immunologically active hormone, eluted from the gels, and measured by radioimmunoassay.

The CMC preparation of PPTH appeared homogeneous on polyacrylamide gel electrophoresis and had the mobility of the fastest moving band seen in the trichloroacetic acid preparation; immunoassayable activity was confined to the region of that band (Figure 4). The migration of PPTH was similar ($R_F$ 0.47) to that of BPTH-I in the same system. On thin-layer chromatography a single spot ($R_F$ 0.3) was detected after spraying with ninhydrin and with Erlich's reagent. The amino acid composition of this material, based on acid hydrolysis for varying times and on enzymatic hydrolysis, is shown in Table I. The molecule contains 84 residues, and the molecular weight calculated from the amino acid composition is 9423. The extinction coefficient for a 1-mg/ml solution measured at 280 mμ was 0.56 (molar extinction coefficient 5300).

The spectrum, shown in Figure 5, exhibits features characteristic of tryptophan. Differential spectroscopy at pH 11 showed no shift in 290-mμ absorbance at the higher pH, consistent with the findings on amino acid analysis that tyrosine is absent from the molecule. Nevertheless, it could be labeled to a specific activity of 250 μCi/μg with $^{125}I$.

End-group analysis by Edman degradation showed a single amino-terminal residue, serine. Successive manual Edman degradations showed the sequence of residues 2–6 to be Val-

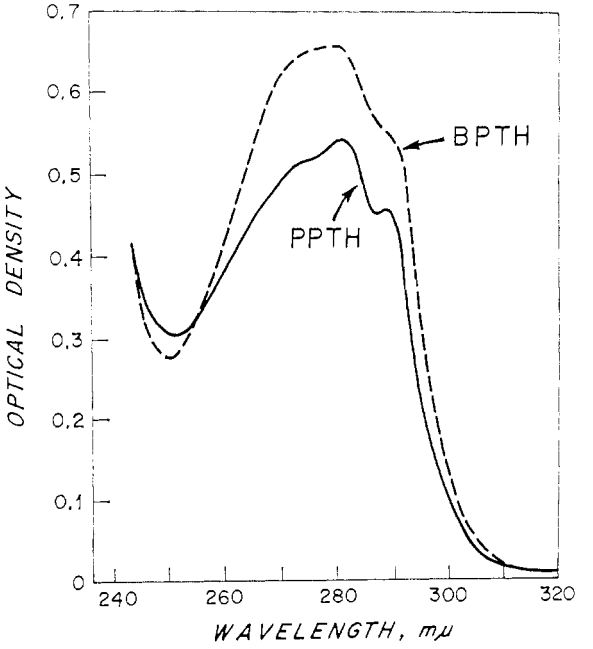


FIGURE 5: Absorption spectrum of porcine PTH compared to bovine PTH-I. Solutions used are 1 mg/ml dissolved in 0.1 M acetic acid.

TABLE I: Amino Acid Composition of Porcine Parathyroid Hormone.[a]

| Amino Acid | Total Enzymic Digestion | Timed Acid Hydrolysis (5.7 N HCl, 110°) 24 hr | 48 hr | 72 hr | 96 hr | Residues/ Mole (Combined Results) | Comparison to Bovine PTH-I Bovine | Difference |
|---|---|---|---|---|---|---|---|---|
| Aspartic acid | 5.20 | 8.05 | 8.00 | 8.20 | 8.15 | 5 | 6 | −1 |
| Asparagine | 3.00 | | | | | 3 | 3 | |
| Threonine | 0.10 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Serine | 6.85 | 7.15 | 6.45 | 6.05 | 5.40 | 8 | 8 | |
| Glutamic acid | 6.10 | 11.25 | 11.20 | 11.25 | 11.25 | 6 | 6 | |
| Glutamine | 4.70 | | | | | 5 | 5 | |
| Proline | 2.10 | 1.95 | 2.05 | 1.90 | 1.90 | 2 | 2 | |
| Glycine | 5.00 | 4.90 | 4.85 | 4.95 | 4.95 | 5 | 4 | +1 |
| Alanine | 6.35 | 6.05 | 6.00 | 6.00 | 6.00 | 6 | 7 | −1 |
| Valine | 7.60 | 8.50 | 8.65 | 9.05 | 9.05 | 9 | 8 | +1 |
| Methionine[b] | 0.75 | 0.80 | 0.70 | 0.70 | 0.70 | 1 | 2 | −1 |
| Isoleucine | 2.90 | 2.50 | 2.65 | 2.75 | 2.75 | 3 | 3 | |
| Leucine | 9.70 | 10.00 | 9.90 | 10.05 | 10.15 | 10 | 8 | +2 |
| Tyrosine | 0 | 0 | 0 | 0 | 0 | 0 | 1 | −1 |
| Phenylalanine | 1.30 | 1.00 | 1.05 | 1.00 | 1.00 | 1 | 2 | −1 |
| Tryptophan[c] | 0.85 | | | | | 1 | 1 | |
| Lysine | 9.30 | 8.95 | 8.90 | 8.95 | 8.90 | 9 | 9 | |
| Histidine | 3.95 | 4.95 | 5.05 | 5.10 | 5.05 | 5 | 4 | +1 |
| Arginine | 5.05 | 5.20 | 5.10 | 4.95 | 5.05 | 5 | 5 | |
| | | | | | Totals | 84 | 84 | |

[a] All values expressed as moles of amino acid per mole of PTH, normalized as described in text. [b] One mole of methionine sulfone per mole of PTH recovered after performic acid oxidation. [c] One mole of tryptophan per mole of PTH found by spectral determination.

Ser-Glu-Ile-Gln. The average repetitive yield for the degradation was 95%. The serine phenylthiohydantoin derivative was recovered in lower yield than the others, as expected from its tendency to undergo breakdown through a $\beta$-elimination reaction of the side chain (Ilse and Edman, 1963). The identification of each residue was confirmed by thin-layer chromatography (Edman and Begg, 1967). Incubation of PPTH with both carboxypeptidases A and B, each for 1 and 3 hr, released no amino acids.[2]

## Discussion

It is now possible to examine the properties of porcine parathyroid hormone and compare them with those of the hormones of bovine and human origin. In their size and charge properties the three hormones appear to be similar. Thus, they behave similarly during the initial fractionation process and on gel filtration using Sephadex G-100; the $K_d$'s of porcine, bovine, and human parathyroid hormones are 0.44, 0.50, and 0.45, respectively. In addition, the three elute under similar conditions from the ion-exchange resin, carboxymethylcellulose. In the *in vivo* bioassay system with thyroparathyroidectomized rats as well as in the *in vitro* adenylylcyclase bioassay, the responses to porcine and bovine parathyroid hormones are parallel. However, the potency of PPTH by *in vitro* bioassay was much lower than by the *in vivo* assay method, whereas the potencies for the bovine hormone by the two methods are closely equivalent. The reason for this difference in potencies is being studied further; such a result could reflect the use of different target tissues in the two assay systems: bone for the *in vivo* assay, renal cortex for the *in vitro* adenylylcyclase assay.

By use of the radioimmunoassay, it has been shown that there are immunological differences between porcine, bovine, and human parathyroid hormones (Woodhead and O'Riordan, 1970; O'Riordan and Woodhead, 1970). These differences were demonstrated by examining the ability of these three peptides to desplace antisera raised against BPTH. Evidence was also obtained, however (using [$^{125}$I]PPTH instead of [$^{125}$I]BPTH as tracer), that parts of the molecule from all three species compete equally and thus appear immunologically to be similar.

The amino acid composition of the homogeneous preparation of porcine parathyroid hormone reported here is different from that previously reported by Littledike and Hawker (1967). The results suggest that their preparation was heterogeneous. Highly purified PPTH does not contain threonine,

[2] Earlier work in our laboratory had shown release of several amino acids from bovine parathyroid hormone during 72-hr digestion with DFP-treated carboxypeptidase A. It subsequently became evident that these amino acids were originating from near the amino terminus, apparently consequent to an unusual cleavage within the peptide chain. The current study was carried out using a different preparation of carboxypeptidase. While no amino acids were released from PPTH or BPTH, control peptides released several residues after as short a period as 20-min digestion.

nor does it contain tyrosine; it has nine and not seven residues of valine. The estimates of the content of proline, glycine, alanine, and phenylalanine have each been reduced by one.

Compared to bovine parathyroid hormone I (Table I) the amino acid composition of porcine parathyroid hormone shows interesting similarities and differences. The contents of serine, glutamic acid, proline, isoleucine, lysine, arginine, and tryptophan are the same. Neither hormone contains threonine or cystine. The porcine hormone has an additional residue of glycine, valine, and histidine and two more residues of leucine. In addition to lacking a tyrosine residue, it has one fewer residue of aspartic acid, alanine, methionine, and phenylalanine. The absence of tyrosine was established after analyses of acid and enzymatic hydrolysates and confirmed by spectral analysis. Despite the lack of tyrosine, porcine parathyroid hormone can be iodinated readily at pH 7.5 in 0.3 M phosphate buffer. Presumably the iodine is reacting with one or more of the five histidine residues.

The sequence of the first six amino acid residues of porcine parathyroid hormone is identical with that found in bovine parathyroid hormone except for serine instead of alanine at position 1. The failure of any amino acids to be released during incubation with carboxypeptidase suggests that the PPTH molecule could contain a penultimate proline residue, as exists in the bovine molecule. At this stage in the examination of the chemical and biological properties of parathyroid hormones, comparisons of the hormone from the two species are helpful in several respects. The demonstration of similar chemical properties, including an identical sequence of residues 2–6 of the proposed biologically active region of the two species constitutes strong validation of the premise that the active principles from both species have been isolated in pure form. Extensive sequence analyses of PPTH are therefore in progress using the material prepared as described.

Examination of the structural requirements for biological activity in parathyroid hormone is now possible. In the bovine hormone, amino-terminal fragments of 29 amino acids prepared by dilute acid hydrolysis of the native molecule (H. T. Keutmann *et al.*, in preparation) and 34 residues prepared by peptide synthesis (Potts *et al.*, 1971) are biologically and immunologically active. Porcine parathyroid hormone does not contain tyrosine, and this amino acid is not included within the active fragments (1–29 or 1–34) of BPTH-I. Thus, tyrosine is not an essential structural feature of parathyroid hormone even though earlier structure–activity studies had suggested that this residue might be important for biological activity (Potts *et al.*, 1966). Another difference between composition of parathyroid hormone of bovine and porcine origin is the methionine content. There are two methionine residues in BPTH, both in the amino-terminal half of the molecule in the region of the active site; oxidation of these residues to methionine sulfoxide or methionine sulfone results in loss of biological activity (Tashjian *et al.*, 1964; Potts *et al.*, 1966). It was suggested that oxidation of only one of these methionines might be responsible for loss of biological activity of the bovine molecule (Rasmussen and Craig, 1962). Now it is clear that oxidation of the single methionine in the porcine polypeptide causes inactivation; identification of the sequence position occupied by the methionine in PPTH may thus help to establish the location of the methionine in the bovine molecule whose oxidation results in loss of biological activity. Furthermore, analysis of the comparative structure of the bovine and porcine polypeptides should be helpful in the current program (Potts *et al.*, 1971) of study of structure–activity relationships in parathyroid hormone through peptide synthesis.

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