A Biologically Active Hormonal Fragment Isolated from Bovine Parathyroid Glands (BPTH 1-65)†

Timothy M. Murray,* Syed Ali Muzaffar, John A. Parsons, and Henry T. Keutmann

ABSTRACT: Fresh frozen bovine parathyroid glands were defatted in acetone, then extracted with phenol. Following trichloroacetic acid precipitation, the resultant peptides were chromatographed on Sephadex G-100. Parathyroid hormone (BPTH) characteristically elutes in the fourth peak. However, we also observed significant hormonal activity, both biological and immunological, in the fifth elution peak. The peak V material had potent hypercalcemic activity in the rat and chick, and stimulated adenylate cyclase activity in the rat renal cortex bioassay. This material was further purified by ion exchange chromatography on carboxymethylcellulose in 8 *M* urea. The biological activity of the purified peptide (3700 MRC units/mg) was equivalent to that of the native hormone on a molar basis. Amino acid analysis, carboxypeptidase digestion, and partial Edman sequence analysis identified this material as BPTH 1-65, a hormonal fragment lacking the C-terminal 19 residues of the 84 residue hormone molecule. Several immunoassays using different anti-PTH antisera had variable reactivity toward the BPTH 1-65 fragment, showing that it may be useful for further characterizing antibody recognition sites. The presence of a lysine residue at position 65 suggests a tryptic-like cleavage may be responsible for the genesis of this hormonal fragment. Further investigation will be necessary to determine if this peptide has physiological significance.

The method most frequently used for purification of bovine parathyroid hormone (BPTH)[1] is gel filtration on Sephadex G-100 (Aurbach and Potts, 1964) after extraction of bovine parathyroid glands with phenol (Aurbach, 1959) or acid–urea (Rasmussen et al., 1964). In this system, the hormonal peptide characteristically elutes as the fourth peak (Aurbach and Potts, 1964; Keutmann et al., 1971). In previous work with bovine hormone purification, however, Rasmussen et al. (1964) recognized an active peptide eluting later from Sephadex. In the course of more recent purifications of parathyroid hormone from fresh frozen bovine glands by this method, we observed considerable hormonal activity, both biological and immunological, in the fifth elution peak as well as in the fourth peak. We have isolated and chemically characterized the material from the fifth peak and find it to be a biologically active fragment of bovine parathyroid hormone, lacking the carboxyl-terminal 19 amino acid residues.

Materials and Methods

Hormone Purification. Fresh frozen bovine glands were defatted in acetone as described by Aurbach (1959) and extracted with phenol for 90 min at room temperature. The extracts were processed by precipitation with acetone, acetic acid, and NaCl, and ether fractionation, followed by precipitation with 3% trichloroacetic acid (Cl_3CCOOH) (Aurbach, 1959). The Cl_3CCOOH precipitate was chromatographed on a 5 × 140 cm column of Sephadex G-100 in 0.14 *M* ammonium acetate (pH 4.9) at 4° (Keutmann et al., 1971). Peptide elution was monitored by measuring ultraviolet absorbance at 280 nm and by radioimmunoassay of selected tubes across the profile. The peak tubes were pooled and lyophilized twice, and specific hormonal activity was measured by bioassay and radioimmunoassay. The peak IV and V peptides were rechromatographed on a 1.2 × 40 cm column of Bio-Gel P-100 (Bio-Rad) in 0.1 *N* acetic acid to ensure that their relative elution positions were reproducible.

Peptides from peaks IV and V were further purified by ion exchange chromatography on a 0.9 × 5 cm column of carboxymethylcellulose (CM-cellulose) (CM-52, Whatman). A linear conductivity gradient was established, using ammonium acetate buffers (75 ml each) prepared in 8 *M* urea, as described by Keutmann et al. (1971). The purified parathyroid polypeptide was freed from urea by gel filtration on Bio-Gel P-2 (Bio-Rad).

Chemical Methods

Amino Acid Analysis. Acid hydrolysis was carried out in 5.7 *N* HCl for 24 hr at 110° in the presence of 1:2000 mercaptoethanol (Keutmann and Potts, 1969). Amino acid analyses were performed on the Beckman Model 121 automatic amino acid analyzer, using the 2-hr sodium citrate elution schedule (Hubbard and Kremen, 1965). Residues were normalized into moles per mole of peptide by best fit based on recovery of all stable residues; values for serine and threonine were corrected for loss during hydrolysis. For evaluation of the products of carboxypeptidase digestion, the lithium citrate elution system described by Benson et al. (1967) was also used, to obtain separation of glutamine, asparagine, and serine.

Edman Degradation. Sequential Edman degradations were carried out by the manual method (Edman, 1960), modified as described by Sauer et al. (1974). The dried peptide was coupled using 5 μl of phenyl isothiocyanate in 0.1 ml of dimethylallylamine buffer (0.4 *M*, pH 9.7) for 20 min

† From the Department of Medicine (T.M.M. and S.A.M.), University of Toronto, Toronto, Canada, the National Institute for Biological Standards and Control (J.A.P.), Holly Hill, London N.W.3, England, and the Endocrine Unit (H.T.K.), Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. *Received May 6, 1974.* Supported in part by a grant from the Medical Research Council of Canada (MA 4515), and grants from the National Institute of Arthritis and Metabolic Diseases (AM-04501 and AM-11794).

[1] Abbreviations used are: BPTH, bovine parathyroid hormone; CM, carboxymethyl; PTH, phenylthiohydantoin.

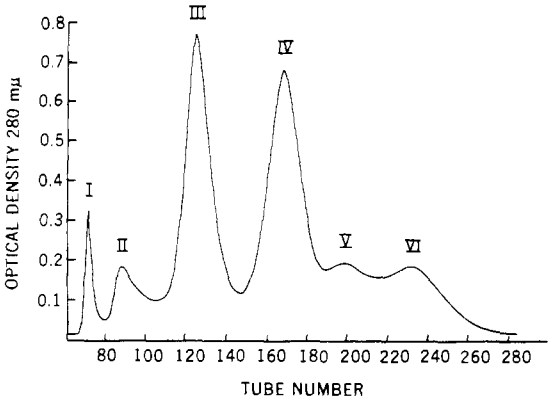

FIGURE 1: Elution profile of the peptides in 664 mg of bovine parathyroid trichloroacetic acid fraction chromatographed on Sephadex G-100 as described in the text; 10-ml fractions were collected. Significant immunological and biological hormonal activity was present in peaks IV and V.

at 54°. A single extraction with 0.2 ml of benzene was used to remove reagents, and the dried aqueous phase was treated with trifluoroacetic acid (0.05 ml, 5 min, 54°). The acid-cleaved thiazolinone derivative was extracted using 0.2 ml of 1-chlorobutane. After drying with nitrogen, the thiazolinone was converted to the phenylthiohydantoin using 1.0 *N* HCl (0.2 ml, 8 min, 80°). The derivative was extracted into ethyl acetate, dried with nitrogen, and redissolved in 20 μl of ethyl acetate for identification.

Phenylthiohydantoins were identified by gas–liquid chromatography (Pisano and Bronzert, 1969) using the Beckman GC-45 DC-560 column (Beckman Instruments) at 180 and 210°, and thin-layer chromatography using the system ethylene dichloride–acetic acid (30:7) with glass-backed silica gel plates (Edman and Begg, 1967).

Disc gel electrophoresis was carried out using a 7% (w/v) acrylamide monomer in the presence of 6 *M* urea at pH 4.4 (Potts et al., 1967). Gels were stained using Amido Black (1% in 7% acetic acid).

Carboxypeptidase Digestion. Carboxypeptidase A and B were obtained from Worthington and further purified by treatment with diisopropyl fluorophosphate as described by Potts (1967). Digestions were carried out at 37° in 0.2 *M* trimethylamine acetate buffer (pH 8.2) using an enzyme–substrate ratio of 1:50 (*M*:*M*).

Preparation of Maleylated Tryptic Peptides. ε-Aminolysine blockade was carried out on 0.5 mg of peptide using maleic anhydride under the conditions used previously for BPTH by Niall et al. (1970). Tryptic digestion was performed using a 1:125 (*M*:*M*) ratio of enzyme–substrate for 45 min at 37°. The tryptic digest was subjected directly to Edman degradation without removal of the maleyl groups. Lysine was identified by thin-layer chromatography as the phenylthiohydantoin of maleyllysine during Edman degradation.

Biological Assays. Assay for parathyroid hormone was carried out in vitro by activation of rat renal cortical adenylate cyclase (Marcus and Aurbach, 1969), and in vivo by measurement of the hypercalcemic response to hormone injected intravenously into the chick (Parsons et al., 1973) or subcutaneously into the parathyroidectomized rat (Munson, 1955, 1961). The results of all assays were expressed in terms of MRC Research Standard A (Robinson et al., 1972). In the adenylate cyclase and intravenous chick assays, a house standard of Sephadex-purified hormone (MRC 72/286) was used, calibrated against MRC Research Standard A by repeated subcutaneous assays in the rat and chick.

Immunoassays. Parathyroid hormone immunoassay was carried out by methods previously described (Berson et al., 1963; Murray et al., 1972). Three anti-PTH antisera were used. Antiserum GP-1 is known to have recognition sites in the amino- and carboxyl-terminal regions of the bovine hormone molecule (Segre et al., 1972; Murray and Keutmann, 1973). Using this antiserum, preadsorbed with synthetic bovine BPTH 1-34 (Potts et al., 1971) and enzymically cleaved natural fragment BPTH 53-84 (Murray and Keutmann, 1973), radioimmunoassays specific for the N-terminal and C-terminal regions of the bovine PTH molecule were done as described by Segre et al. (1972). Assays were also done using antiserum GP-133 and GP-118, both directed toward portions of the molecule C-terminal to position 19 (Segre et al., 1972).

Results

Preparation of Trichloroacetic Acid Precipitate. An initial lot of 400 g of acetone-dried glands yielded 664 mg of Cl_3CCOOH precipitate with a biological potency of 1080 MRC units/mg by the rat kidney adenylate cyclase assay. Extraction of two subsequent lots gave similar yields. The three lots of Cl_3CCOOH powder were purified and analyzed separately; the properties of the active peptides from each lot were found to be identical.

Separation of Active Peptides by Gel Filtration. Gel filtration on Sephadex G-100 of the initial 664 mg of Cl_3CCOOH precipitate (Figure 1) yielded 123 mg of immunoreactive hormonal peptide (designated peak IV), eluting at a K_d of 0.46, a position close to that usually observed for native BPTH. In addition, a smaller but significant amount of immunoreactivity was found in the peptide eluting at a K_d of 0.61, designated peak V (Figure 1). Bioassay potency of the peak IV material after pooling and lyophilization was 2200 MRC units/mg by adenylate cyclase assay

Table I: Biological Activity of Parathyroid Peptides in Eluates from Sephadex G-100.[a]

Gland Extract No.	G-100 Elution Position	Rat Renal Cortical Adenylate Cyclase Assay	Chick Hypercalcemia Assay	Rat Hypercalcemia Assay
I	Peak IV	2200 (1700–3000)	2600 (2000–3400)	1500 (1000–2300)
	Peak V	2400 (1600–3600)	2700 (2100–3500)	1600 (1000–2400)
II	Peak IV		2500 (1600–3600)	
	Peak V	2800 (2400–3400)	3500 (2400–5300)	
III	Peak V	1600 (1400–1900)	2200 (1600–3000)	

[a] Results expressed as MRC units/mg, with 95% confidence limits. Each value based on a combination of at least two independently valid and statistically homogeneous assays.

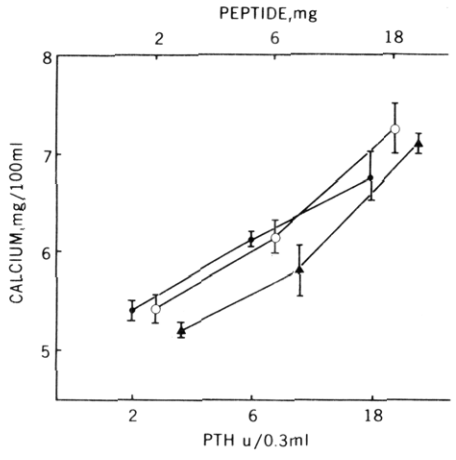

FIGURE 2: The calcemic response of chicks is shown after the intravenous injection of peak IV peptide (○), peak V peptide (▲), and MRC Research Standard A (●) in varying doses. Analysis of variance revealed no significant nonparallelism.

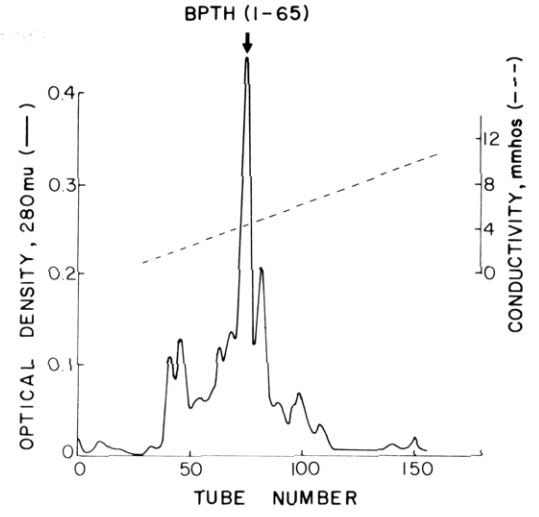

FIGURE 3: Elution profile from chromatography of peak V peptide, obtained from Sephadex gel filtration (Figure 1), on a column of carboxymethylcellulose equilibrated in 8 M urea. The purified peptide (BPTH 1-65) eluted at a conductivity of 4–5 mmho. Native bovine hormone eluted at a conductivity of 3–4 mmho from the same column.

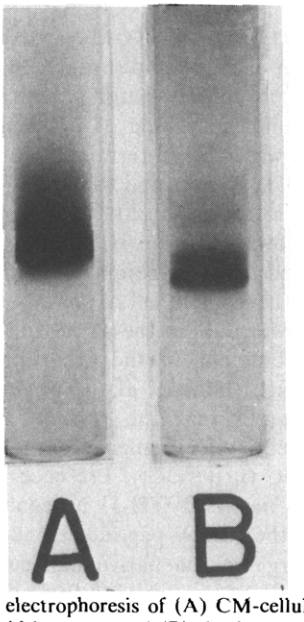

FIGURE 4: Disc gel electrophoresis of (A) CM-cellulose purified native bovine parathyroid hormone and (B) the faster migrating peak V peptide (BPTH 1–65). Appearance of a second, slower band in each preparation reflects presence of the isohormonal form as discussed in the text.

and 2600 MRC units/mg by the in vivo chick bioassay, while the potency of the pooled peak V material was closely similar, 2400 and 2700 MRC units/mg by the two respective methods. Similar gel filtration patterns were obtained for the second and third Cl_3CCOOH preparations. Bioassay data for the three separate purifications are summarized in Table I. Peak IV and peak V materials gave parallel log–dose responses in all bioassay systems used; a typical chick hypercalcemia assay is shown in Figure 2.

The reproducibility of the gel filtration elution behavior of the respective peptides was tested by a series of rechromatography experiments in the presence of a ^{125}I-labeled BPTH marker. When the biologically active peak IV material from Sephadex G-100 was rechromatographed on Bio-Gel P-100, its elution position coincided with the labeled marker. The peak V peptide consistently eluted later than either the peak IV material or the [^{125}I]BPTH marker. The relative elution positions of these two peptides were not altered by preincubation with 8 *M* urea. The experiment was repeated using ^{125}I-labeled peak V material and unlabeled peak IV material. Again, peak V material eluted later than peak IV. To ensure that peak V material did not result from breakdown of peak IV material during extraction, both peak IV peptide and CM-cellulose purified BPTH-I were exposed to phenol treatment and ether and salt precipitation under identical conditions with those used in the initial extraction. No conversion to peak V peptide was detected by gel filtration on Bio-Gel P-100.

Purification by Ion Exchange Chromatography. For subsequent chemical characterization the active peptides were further purified by ion exchange chromatography on carboxymethylcellulose in 8 *M* urea.

The peak IV material eluted in a pattern and yield comparable to that found for BPTH by Keutmann et al. (1971). Amino acid analysis and end group analyses confirmed that this peptide was the native BPTH [1–84] peptide, as had been suggested by its elution position on gel filtration.

The elution of the peak V peptide is shown in Figure 3. The peptide eluted at a somewhat higher conductivity than is characteristic of BPTH-I. Furthermore, the peak V peptide consistently migrated farther from the anode on disc gel electrophoresis at pH 4.4 than BPTH-I (Figure 4). Both these findings are consistent with a molecule more basic than that of the native 1–84 peptide. After ion exchange chromatography, the product was 97% pure by Edman end group analysis (Figure 5). A total of 2.5 mg of the purified peptide was obtained for chemical, biological, and immunological characterization.

Bioassay of Purified Peak V Peptide. The purified peak V PTH was tested in three separate assays by the in vitro rat renal cortical adenylate cyclase system. The potency was 3700 U/mg (95% confidence limits 2800–5300). Native BPTH possesses a potency of 3000 U/mg (2500–4000) in the same assay system. Insufficient amounts of purified material were available to carry out in vivo bioassays.

Chemical Characterization. The results of the gel filtration studies and biological and immunological assays suggested that the peak V material represented an active fragment of the native parathyroid molecule. Several chemical studies were undertaken to characterize this peptide further.

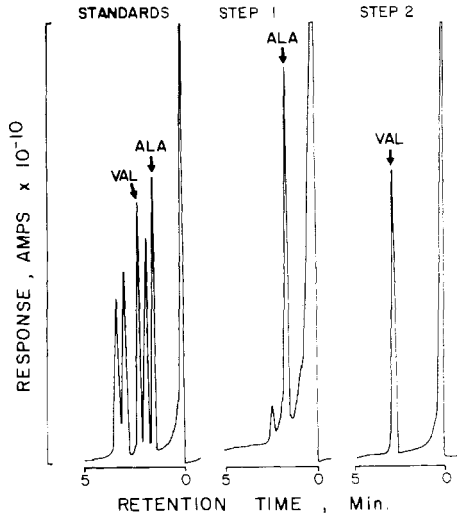

FIGURE 5: Gas–liquid chromatography of phenylthiohydantoins from the first two steps of Edman degradation of peak V peptide from carboxymethylcellulose chromatography (Figure 3). The sequence Ala-Val-, representing residues 1–2 of bovine parathyroid hormone, is seen without other significant contaminating phenylthiohydantoins.

Table II: Amino Acid Composition of Purified "Peak V" BPTH.

Amino Acid	Residues Found[a]	Residues Taken (mole integer)	Residues Expected[b] BPTH 1–65	Residues Expected[b] BPTH 1–84
Asp	5.78	6	6	9
Thr	0.30	0	0	0
Ser	6.65	7	7	8
Glu	7.97	8	9	11
Pro	1.22	1	1	2
1/2-Cys	0.0	0	0	0
Gly	3.10	3	3	4
Ala	4.06	4	4	7
Val	4.87	5	6	8
Met	1.61	2	2	2
Ile	1.76	2	2	3
Leu	5.88	6	6	8
Tyr	1.18	1	1	1
Phe	2.08	2	2	2
Lys	6.13	6	6	9
His	3.85	4	4	4
Arg	5.06	5	5	5

[a] Average of analyses of duplicate 24-hr hydrolyses of urea–CM-cellulose purified peptide, expressed as moles of amino acid per mol of peptide. Tryptophan was not determined. [b] From sequence of native hormone determined by Brewer and Ronan (1970) and Niall et al. (1970). The calculated molecular weight for BPTH 1-65 from this structure is 7532.

(i) AMINO-TERMINAL SEQUENCE ANALYSIS. Sequential degradation of the peptide from CM-cellulose chromatography was continued through seven cycles. A single sequence was found, consisting of Ala-Val-Ser-Glu-Ile-Gln-Phe, identical with the N-terminal sequence of BPTH-I (Niall et al., 1970; Brewer and Ronan, 1970).

(ii) AMINO ACID ANALYSIS. The results of amino acid analysis are shown in Table II. Several significant differences were noted from the composition known for BPTH-I. In particular, the content of Asp, Ala, and Lys appeared considerably lower than in the native molecule. Since these residues are abundant in the C-terminal region of the BPTH-I sequence (Niall et al., 1970; Brewer and Ronan, 1970), it appeared that the peptide might differ from BPTH-I by deletion of a segment from the C-terminus.

(iii) TRYPTIC DIGESTION. Presence of five residues of arginine (as in native BPTH-I) by compositional analysis suggested that the peak V peptide extended at least through residue 52 (Arg) of the native molecule. This was confirmed by a study of the pattern of tryptic cleavage of arginine residues following ϵ-aminolysine blockade. While the limited amounts of material available prevented separation of the individual peptides from the tryptic digest, additional structural information could be obtained from Edman degradation of the unfractionated digest (Niall et al., 1969). Use of this approach required the assumption that the internal sequence of the peak V peptide be similar to that of the native hormone (suggested in this case by the results of the biological and initial chemical studies described above), in order to permit assignment of a given residue to the appropriate peptide.

The phenylthiohydantoins found at step 1 (Table III) of the degradation (carried out without removal of maleic blocking groups) were identical with those obtained in comparable tryptic digests of native hormone. This finding was consistent with tryptic cleavage at residues 20, 25, 44, and 52, generating peptides comprising residues 21–25, 26–44, 45–52, and 53-carboxyl terminus. The sequence of the amino terminal peptide 1–20 was not seen since the blocking groups remained on the α-amino group as well as the lysine residues. Through six steps of degradation, the phenylthiohydantoins observed continued to correspond to those expected from the internal sequence of native BPTH-I, including the first six residues (Lys-Lys-Glu-Asp-Asn-Val . . .) of the carboxyl-terminal peptide commencing at residue 53 (Table III). These findings provided additional evidence that the peak V peptide resembled native BPTH-I in its internal sequence, as well as further information on the carboxyl terminal extent of the peak V peptide.

(iv) CARBOXYL-TERMINAL ANALYSIS. The nature of the C-terminus of peak V-PTH was studied directly by means of carboxypeptidase digestion. Treatment of the peptide with carboxypeptidase A alone for as long as 3 hr at 37° released no amino acids in significant yield when compared with a control digestion of native BPTH. However, inclusion of carboxypeptidase B with carboxypeptidase A resulted in a significant difference from the control digestion. Lysine (4.1 nmol), histidine (4.0 nmol), glutamine (3.8 nmol), and serine (3.2 nmol) were observed in closely equivalent yields after 30 min of digestion. Treatment with carboxypeptidase B alone released only a single residue, lysine, in a yield comparable to that found with A and B together. These results would be expected if the sequence Ser-His-Gln-Lys was present at the C-terminus of the peak V peptide. This corresponds to residues 62–65 of the native hormone molecule.

When the compositional analysis (Table II) was reexamined, close correspondence between the amino acid composition of the peak V peptide and the theoretical composition of the parathyroid fragment terminating at position 65 was evident. The principal difference was recovery of submolar amounts of threonine and a low valine recovery. This is also found in analysis of BPTH-I purified in the same manner, due to presence in the preparation of a small quantity of the isohormonal form, BPTH-II (Keutmann et al., 1971). The isohormonal form (which may be separated by rechromatography on urea-CM-cellulose) could also be detected in both the native hormone and peak V peptide, as a minor slow component on disc gel electrophoresis (Figure 4).

Table III: Edman Degradation of Peptide Mixture from Tryptic Digest of Maleylated Parathyroid Peak V Preparation.

Cycle Number	Peptide Fragment (theoretical) (21–25)[b]	(26–44)	(45–52)	(53–C term.)	Yield (nmol)
1	Val^{21}	Lys^{26}	Asp^{45}	Lys^{53}	Val-7.3, Asp-4.4, Lys[a]
2	Glu	Lys	Gly	Lys	Glu-3.7, Gly-9.9, Lys[a]
3	Trp	Leu	Ser	Glu	Trp, [a]Leu-8.1, Ser, [a]Glu-8.8
4	Leu	Gln	Ser	Asp	Leu-1.7, Gln, [a]Ser, [a]Asp-4.3
5		Asp		Asn	Asp-4.3, Asn[a]
6		Val		Val	Val-7.1

[a] Identification by thin-layer chromatography. Lysine residues were identified as the phenylthiohydantoin of the maleylated derivative. [b] Theoretical assignment of residues based on structure of native bovine parathyroid hormone.

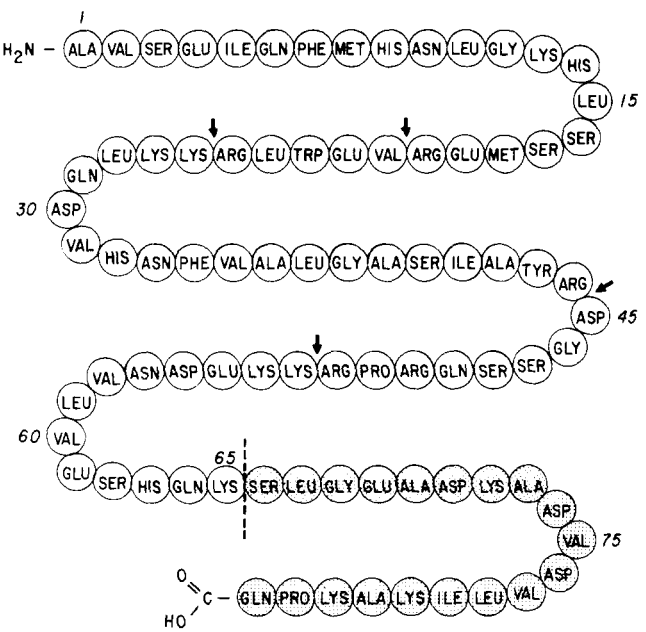

FIGURE 6: Amino acid sequence of bovine parathyroid hormone I. The peak V peptide, terminating at residue 65 (lysine), is shown by the open circles. Shaded circles represent the carboxyl-terminal sequence of the native molecule which is deleted from the 1-65 peptide. Short arrows denote points of cleavage by trypsin after maleic blockade of lysine residues (see text).

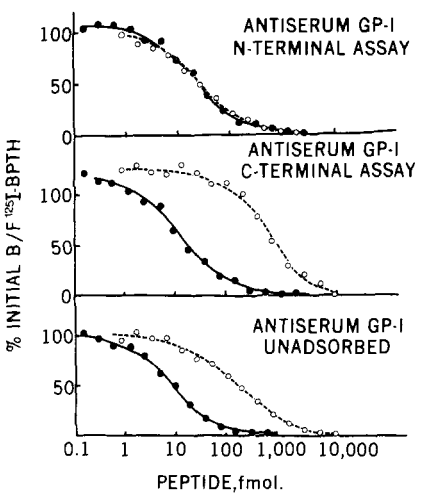

FIGURE 7: Immunoassay of peak IV peptide (●) and peak V peptide (○) with immunoassays specific for each end of the hormone molecule, and with unadsorbed antiserum GP-1 which is directed against both ends. The two peptides are immunochemically identical at the N-terminal end, but very dissimilar at the C-terminal end (see text).

Based on the combined results of these chemical studies, it was concluded that the peak V peptide consisted of a fragment comprising residues 1-65 of the native bovine PTH molecule (Figure 6).

Immunological Studies. The active peptides were studied by radioimmunoassay after CMC purification. When studied with a specific N-terminal immunoassay (antiserum GP-1 preadsorbed with BPTH 53-84), the peak IV peptide and peak V peptide showed virtually identical immunochemical behavior (Figure 5). This suggests that the region within the amino-terminus recognized by this antiserum (Segre et al., 1972) is similar in the two peptides. With the specific GP-1 C-terminal assay (antiserum GP-1 preadsorbed with BPTH 1-34), the peak V response while parallel was very weak; the peak V material contained only 1.7% of the immunoreactivity of native BPTH. We conclude that this C-terminal assay is directed toward portions of the molecule beyond position 65, and that the small amount of immunoreactivity resulted from the presence in our peak V preparation of trace quantities of native hormone undetectable even by highly sensitive chemical means. As expected, the displacement curve obtained with unadsorbed GP-1 antiserum fell between those obtained with the respective preadsorbed antisera (Figure 7). With two other antisera, GP-133 and GP-118, the immunodilution curves of peak V material were not parallel to those of native BPTH, suggesting that these antisera possess significant immunochemical affinity for the portion of the molecule beyond position 65.

Discussion

The presence of active peptides other than the native hormone in eluates from Sephadex G-100 chromatography of bovine parathyroid extracts has been reported by Rasmussen et al. (1964) and Hawker et al. (1966). In the earlier of these studies, material corresponding in elution position to the peak V peptide described here had hypercalcemia and phosphaturic activity in the parathyroidectomized rat, and had different mobility from native BPTH on starch gel electrophoresis (Rasmussen et al., 1964). Our present studies provide further biological and immunological characterization of such a peptide, and for the first time identify it as an active fragment of the bovine parathyroid hormone sequence.

The peak V peptide elutes later than BPTH-I on repeated gel filtration and is more basic than BPTH-I as shown by ion exchange chromatography and disc gel electrophoresis. The small quantities of available purified material precluded the determination of the complete amino acid sequence. However, based upon the amino acid composition, partial sequence analysis by Edman degradation, and carboxyl-terminal analyses by carboxypeptidase degradation, we have

concluded that this peptide consists of residues 1–65 of the BPTH molecule. Its more basic properties are accounted for by deletion of four carboxylated residues (three Asp, one Glu) and three lysine residues, increasing by one the net positive charge.

The peptide stimulates adenylate cyclase activity in rat renal cortex in vitro and has potent hypercalcemic activity in the chick and rat in vivo. The biological activity of the purified fragment on a molar basis, assessed by the in vitro adenylate cyclase assay, was comparable to that of native BPTH. This finding was to be expected since fragments as short as 1–34 have been shown to have activities equivalent to native hormone on a molar basis (Tregear et al., 1973).

The presence of significant immunological activity and full biological activity in the peak V material means that caution should be used in purification of BPTH by gel filtration to ensure that the hormone (peak IV) is properly distinguished from peak V. It should be pointed out, however, that the peak V peptide is not a prominent finding in all Cl_3CCOOH precipitates. For example, this material was not discerned in immunoassay evaluation of column eluates during earlier purifications of BPTH by Aurbach and Potts (1964) and Keutmann et al. (1971). However, subsequent purifications, using Cl_3CCOOH powder from widely differing sources, have demonstrated its presence when the peak V region was examined more critically.

In the purification of native parathyroid hormone from bovine glands, isohormonal forms (BPTH-II and III) have been found in addition to the major peptide (BPTH-I) (Keutmann et al., 1971). In particular, BPTH-II, which contains a residue of threonine (unlike BPTH-I) and only seven valine residues, is found in small amounts in samples of BPTH-I purified as in this study. Presence of submolar amounts of threonine and a low valine recovery in the amino acid composition (Table II) suggest that the 1–65 fragment may be derived from both BPTH-I and BPTH-II. It would appear, however, that the peak V peptide is not itself an isohormone.

The significance of the 1–65 peptide may be considered from two standpoints, firstly, its possible physiological role and secondly, its usefulness as a reagent for immunoassay studies of parathyroid hormone metabolism.

It is not possible to conclude from the studies described here whether the 1–65 fragment originated within the gland in vivo. Approaches other than those we have used would be required to establish this point definitively. In efforts to minimize postmortem autolysis, the glands used in our study were collected under exceptionally rigorous conditions, having been placed on Dry Ice at the moment of removal from the carcass at the slaughterhouse. The results of control treatment of native hormone with the various extraction reagents suggest that the fragment does not result from direct cleavage by these agents.

The presence of a lysine residue at position 65 suggests that this peptide may have resulted from a trypsin-like cleavage. It is of interest that conversion of proparathyroid hormone to parathyroid hormone within the gland (Cohn et al., 1972; Habener et al., 1973) also may involve a cleavage of this type. If present in vivo, this peptide could also appear as a result of early termination of the peptide chain during biosynthesis on the ribosome. The subsequent residue in BPTH (residue 66) is serine, which may mutate through a single base change to the "amber" termination codon (Lehninger, 1970), resulting in completion of the chain at residue 65.

Although the 1–65 peptide could represent one of the hormonal fragments noted in some organ culture systems in vitro (Sherwood et al., 1970; Oldham et al., 1972), it appears not to constitute the dominant circulating fragment in vivo, recently demonstrated to arise from the middle and carboxyl-terminal portion of the molecule (Segre et al., 1974). Proof of a physiological role for this peptide would require its direct demonstration in blood under various controlled conditions.

Regardless of the physiological role of this peptide, it has direct relevance to the further study of parathyroid hormone metabolism in vivo. Studies of hormonal metabolism have been carried out, for the most part, by means of radioimmunoassay techniques, many of which do not discriminate between various hormonal fragments which arise in vivo (Habener et al., 1972; Canterbury and Reiss, 1972). It is of great importance that the processes of hormonal fragmentation and degradation be studied using immunoassays with chemically defined specificity toward different regions of the hormone molecule (Murray and Keutmann, 1973). Although many synthetic peptides from the amino-terminal region have been prepared and used for immunochemical characterization (Segre et al., 1972), no carboxyl-terminal peptides are available for study, with the exception of the natural fragment (53–84) (Segre et al., 1972; Murray and Keutmann, 1973). It is therefore of practical importance that BPTH 1–65, with a specific deletion at the C-terminus, can now be used for the characterization of the regional specificity of antisera to parathyroid hormone. Thus, our present studies (Figure 7) have provided important information regarding the C-terminal specificity of antiserum GP-1, which has been widely used in several laboratories for clinical studies. In this way, further application of well-characterized immunoassays may yield more meaningful information about the complex nature of the metabolism of parathyroid hormone.

References

Aurbach, G. D. (1959), *J. Biol. Chem. 234,* 3179.

Aurbach, G. D., and Potts, J. T., Jr. (1964), *Endocrinology 75,* 290.

Benson, J. V., Jr., Gordon, M. J., and Patterson, J. A. (1967), *Anal. Biochem. 18,* 228.

Berson, S. A., Yalow, R. S., Aurbach, G. D., and Potts, J. T., Jr. (1963), *Proc. Natl. Acad. Sci. U.S.A. 49,* 613.

Brewer, H. B., Jr., and Ronan, R. (1970), *Proc. Natl. Acad. Sci. U.S.A. 67,* 1862.

Canterbury, J. M., and Reiss, E. (1972), *Proc. Soc. Exp. Biol. Med. 140,* 1393.

Cohn, D. V., MacGregor, R. R., Chu, L. L. H., Kimmel, J. R., and Hamilton, J. W. (1972), *Proc. Natl. Acad. Sci. U.S.A. 60,* 643.

Edman, P. (1960), *Ann. N.Y. Acad. Sci. 88,* 602.

Edman, P., and Begg, G. (1967), *Eur. J. Biochem. 1,* 80.

Habener, J. F., Kemper, B., Potts, J. T., Jr., and Rich, A. (1973), *Endocrinology 92,* 219.

Habener, J. F., Segre, G. V., Powell, D., Murray, T. M., and Potts, J. T., Jr. (1972), *Nature (London), New Biol. 238,* 152.

Hawker, C. D., Glass, J. D., and Rasmussen, H. (1966), *Biochemistry 5,* 344.

Hubbard, R. W., and Kremen, D. M. (1965), *Anal. Biochem. 12,* 593.

Keutmann, H. T., Aurbach, G. D., Dawson, B. F., Niall, H.

D., Deftos, L. J., and Potts, J. T., Jr. (1971), *Biochemistry 10,* 2779.

Keutmann, H. T., and Potts, J. T., Jr. (1969), *Anal. Biochem. 29,* 175.

Lehninger, A. I. (1970), Biochemistry, The molecular basis of cell structure and function, New York, N.Y., Worth Publishers, pp 722–723.

Marcus, R., and Aurbach, G. D. (1969), *Endocrinology 85,* 801.

Munson, P. L. (1955), *Ann. N.Y. Acad. Sci. 60,* 776.

Munson, P. L. (1961), in The Parathyroids, Greep, R. O., and Talmage, R. V., Ed., Springfield, Ill., Charles C. Thomas, p 94.

Murray, T. M., and Keutmann, H. T. (1973), *J. Endocrinol. 56,* 493.

Murray, T. M., Peacock, M., Powell, D., Monchik, J. M., and Potts, J. T., Jr. (1972), *Clin. Endocrinol. 1,* 235.

Niall, H. D., Keutmann, H. T., Copp, D. H., and Potts, J. T., Jr. (1969), *Proc. Natl. Acad. Sci. U.S.A. 64,* 771.

Niall, H. D., Keutmann, H. T., Sauer, R., Hogan, M., Dawson, B., Aurbach, G. D., and Potts, J. T., Jr. (1970), *Hoppe-Seyler's Z. Physiol. Chem. 351,* 1586.

Oldham, S. B., Fischer, J. A., Sizemore, G. W., and Arnaud, C. D. (1972), in Calcium, Parathyroid Hormone and the Calcitonins, Talmage, R. V., and Munson, P. L., Ed., Amsterdam, Exerpta Medica, p 213.

Parsons, J. A., Reit, B., and Robinson, C. J. (1973), *Endocrinology 92,* 454.

Pisano, J. J., and Bronzert, T. J. (1969), *J. Biol. Chem. 24,* 5597.

Potts, J. T., Jr. (1967), *Methods Enzymol. 11,* 648.

Potts, J. T., Jr., Reisfeld, R. A., Hirsch, P. F., Wasthed, A. B., Voelkel, E. F., and Munson, P. L. (1967), *Proc. Natl. Acad. Sci. U.S.A. 58,* 328.

Potts, J. T., Jr., Tregear, G. W., Keutmann, H. T., Niall, H. D., Sauer, R., Deftos, L. J., Dawson, B. F., Hogan, M. L., and Aurbach, G. D. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 63.

Rasmussen, H., Sze, Y. L., and Young, R. (1964), *J. Biol. Chem. 239,* 2852.

Robinson, C. J., Berryman, I., and Parsons, J. A. (1972), in Calcium, Parathyroid Hormone and the Calcitonins, Talmage, R. V., and Munson, P. L., Ed., Amsterdam, Exerpta Medica, p 515.

Sauer, R. T., Niall, H. D., Hogan, M. L., Keutmann, H. T., O'Riordan, J. L. H., and Potts, J. T., Jr. (1974), *Biochemistry 13,* 1994.

Segre, G. V., Habener, J. F., Powell, D., Tregear, G. W., and Potts, J. T., Jr. (1972), *J. Clin. Invest. 51,* 3163.

Segre, G. V., Niall, H. D., Habener, J. F., and Potts, J. T., Jr. (1974), *Am. J. Med. 56,* 774.

Sherwood, L. M., Rodman, J. S., and Lundberg, W. B. (1970), *Proc. Natl. Acad. Sci. U.S.A. 67,* 1631.

Tregear, G. W., van Rietschoten, J., Greene, E., Keutmann, H. T., Niall, H. D., Reit, B., Parsons, J. A., and Potts, J. T., Jr. (1973), *Endocrinology 93,* 1349.