PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 588–596 (1981)

Proteolysis of Parathyroid Hormone *in Vitro* by Sera from Acute Pancreatitis Patients[1] (41220)

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Abstract. The degradation of ^{125}I-labeled parathyroid hormone *in vitro* has been investigated in order to test the hypothesis that proteolytic degradation of PTH may be related to the hypocalcemia associated with acute pancreatitis. A polyacrylamide gel electrophoresis technique has been employed to separate bovine PTH (1-84) from lower molecular weight peptides produced by proteolytic cleavage. It has been demonstrated that trypsin bound to the plasma protease inhibitor α_2-macroglobulin rapidly degrades PTH and destroys its biological activity *in vitro* in a kidney cortex adenyl cyclase assay system. Substantial PTH degrading activity was also detected in serum samples obtained from nine patients with severe acute pancreatitis. In contrast, significant PTH degradation could not be detected in normal serum or plasma. Furthermore, no degradation was observed with sera from two patients with mild pancreatitis due to biliary tract stones or four patients with hyperamylasemia but no evidence of pancreatic disease. The PTH degrading activity in patient sera could be blocked by the combination of two peptide chloromethylketones that specifically inhibit the pancreatic endopeptidases trypsin, chymotrypsin, and elastase 2. These results suggest that the PTH degradation assay represents a sensitive test for biologically relevant proteolytic activity in acute pancreatitis, and that it can specifically detect molecular forms of the pancreatic proteases in blood reflecting the intrapancreatic activation of zymogens in the course of pancreatic inflammation.

Severe hypocalcemia is a well-recognized complication of acute pancreatic inflammation, and has been correlated with mortality in this disease (1). Since serum Ca^{2+} levels are mainly regulated by PTH[2] (2, 3), it has been suggested that the hypocalcemia of acute pancreatitis might at least partially be due to degradation of parathyroid hormone by circulating pancreatic proteases (4, 5). Alteration of the biological activity of PTH by serine proteases with tryptic specificity is likely, since the highly trypsin-sensitive sequence $Arg_{25}-Lys_{26}-Lys_{27}-Leu_{28}$ exists within the biologically active *N*-terminal portion of both the human (6) and bovine (7, 8) PTH molecules. Hydrolysis of any of these peptide bonds would result in partial or total loss of biological activity (9).

Enzymatic assays for trypsin and/or other neutral serine proteases in plasma or serum of patients with acute pancreatitis, based on low-molecular-weight synthetic substrates, have not yielded convincing results because of their lack of sensitivity and specificity, as well as the high concentration of protease inhibitors in plasma. For these reasons, in this laboratory the detection of pancreatic proteases in blood has been through the use of radioimmunoassay (10–13). In sera of patients with acute pancreatitis we have identified three molecular forms of immunoreactive cationic trypsin: free zymogen, α_1-PI bound trypsin, and trypsin bound to α_2-M. Proteases bound to the latter inhibitor are potentially of great significance in pathophysiology because of the unique properties of the respective

[1] Supported by the Medical Research Service of the Veterans Administration.

[2] Abbreviations used: PTH, parathyroid hormone; α_1-PI, α_1-protease inhibitor (also known as α_1-antitrypsin); α_2-M, α_2-macroglobulin; Suc-, succinyl-; -CH_2Cl, chloromethyl ketone.

[3] J. W. Brodrick and S. B. Ray, unpublished observations.

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complexes. While α_2-M-bound endopeptidases do not hydrolyze high-molecular-weight proteins (15, 16), they retain activity toward low-molecular-weight ester and amide substrates (17) as well as peptide hormones such as vasopressin, angiotensin II (18), and proinsulin (19). The studies reported here were undertaken in an attempt to demonstrate *in vitro* that the proteolytic degradation of circulating PTH might be responsible for the hypocalcemia observed in severe acute pancreatitis. We provide evidence that the biological activity of PTH, as measured in a chicken kidney plasma membrane adenyl cyclase assay system, can be destroyed by α_2-M-bound trypsin, thus illustrating the potential significance of proteolysis of hormones by enzymes bound to this inhibitor.

Experimental Procedures. *Materials.* Bovine PTH trichloroacetic acid powder was obtained from Inolex Corp., Park Forest South, Illinois. The hormone was purified to homogeneity by the procedure of Keutmann *et al.* (20). A single band was observed after gel electrophoresis in 8 *M* urea at pH 4.5, by the procedure to be described later in this section. Amino acid analysis demonstrated an amino acid composition very similar to that previously reported for bovine PTH (20) with 0.2 residues of Thr per mole, which indicates that some of the isohormone identified by Keutmann *et al.* (20) was present in the preparation. Suc-$(Ala)_2$-Pro-Leu-CH_2Cl was prepared as described by Largman *et al.* (21), and Phe-Ala-Arg-CH_2Cl was synthesized by the method of Kettner *et al.* (22). Trasylol (bovine trypsin inhibitor of Kunitz isolated from lung tissue) was the generous gift of Bayer A. G. Urea (ultrapure) was obtained from Schwarz-Mann, while acrylamide and *N,N'*-methylenebis acrylamide (electrophoresis grade) were purchased from Bio-Rad, Richmond, California. All other chemicals were of reagent or equivalent grade.

Methods. PTH was iodinated by a modification of the chloramine-T procedure (23) and was purified by gel filtration on Sephadex G-75 equilibrated with 10 m*M* ammonium acetate (pH 4.6) containing 2 mg/ml bovine serum albumin. The $Na^{125}I$ used was diluted threefold with unlabeled NaI. The final specific activity of the product was 0.3–0.4 mCi/nmole.

Adenyl cyclase assay. Chicken kidney cortex plasma membrane fraction was prepared as described previously (24). Cyclic AMP was quantitated according to the method of Salomon *et al.* (25). Adenyl cyclase activity was expressed as picomoles cyclic AMP produced per milligram protein after a 30 min incubation period at 30°. For the experiments reported here, bovine PTH purified in this laboratory was employed as standard. This material exhibited the expected sigmoidal dose–response relationship, and stimulated adenyl cyclase activity sevenfold, with half-maximal stimulation at a PTH concentration of 117 ng/ml, or 1.2×10^{-8} *M*.

Polyacrylamide slab gel electrophoresis was performed by the procedure of Reisfeld *et al.* (26) except that 8 *M* urea was added to the resolving and stacking gels. The resolving gel contained 10% acrylamide and 0.25% *N,N'*-methylenebisacrylamide. Electrophoresis was performed in an apparatus obtained from Bio-Rad Laboratories (Model 220) for 45 min at 50–70 V, until the proteins had entered the running gel. The voltage was then increased to 150–220 V for 3 hr, and the apparatus was cooled by a circulating H_2O bath set at 5°.

Incubations for PTH digestion by plasma or serum samples were performed in a 35-μl reaction volume containing the following components: 40 m*M* *N*-ethyl morpholine acetate buffer (pH 7.4), 14 μl total serum (patient, normal, or a combination), and $4-5 \times 10^5$ cpm of ^{125}I-labeled PTH. Control incubations contained 0.57 m*M* each of Suc-$(Ala)_2$-Pro-Leu-CH_2Cl and Phe-Ala-Arg-CH_2Cl. ^{125}I-labeled PTH was added after 5 min of preincubation of the other components at room temperature. Following incubation at 37° for the times indicated under Results, 15 μl of "stopping" solution containing 1.33 m*M* each of Suc-$(Ala)_2$-Pro-Leu-CH_2Cl and Phe-Ala-Arg-CH_2Cl, as well as 3 μg unlabeled PTH, was added. Following incubation for 5 min at room temperature, 40 mg of urea

was added to each tube to make a final concentration of 8 *M*. The mixtures were then stored at −70° overnight before gel electrophoresis. A 10-μl Hamilton syringe was employed to apply 5 μl of each mixture directly to a sample well in the gel slab.

Following electrophoresis, gel slabs were stained for 10–15 min in 0.05% amido black in 20% ethanol, 10% acetic acid and destained partially in 10% acetic acid, 5% glycerol. They were then dried between sheets of dialysis casing in a Bio-Rad gel slab drier and exposed to Kodak X-Omat R film type XR-5 for at least 16 hr. The exact orientation of the gel with respect to the film was determined by use of spots of radioactive India ink. The area in the gel corresponding to the PTH radioactivity band for each sample was then cut out with a razor blade, and the gel chips were counted directly in a gamma counter. These data were converted to percentage of radioactivity applied after determining the counts per minute in a duplicate 5-μl aliquot of each mixture prior to electrophoresis. The coefficient of variation for sample application with the Hamilton syringe was 0.3%, much less than other errors, indicating that the radioactivity in a duplicate aliquot is a good indicator of the amount applied to the gel electrophoresis sample well. Results are expressed as percentage PTH degraded:

$$\left(1 - \frac{\%\ \text{recovered exp}}{\%\ \text{recovered control}}\right) \times 100.$$

Student's *t* statistics was employed to test for significant increases in experimental values in patient versus control groups.

Results. *Degradation of bovine PTH by α_2-M-bound trypsin in vitro.* When bovine PTH (1.25 mg/ml) was incubated with either trypsin or α_2-M-bound trypsin as described under Experimental Procedures, degradation of the hormone could be detected by urea–polyacrylamide gel electrophoresis, as shown in Fig. 1. Trypsin bound to α_2-M, but not α_2-M alone, degrades bovine PTH completely in 1 hr at 37°. The rate of degradation is approximately equivalent to that observed when a sixfold more dilute solution of trypsin is employed.

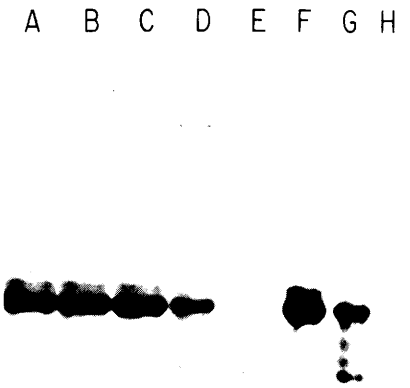

FIG. 1. Polyacrylamide gel electrophoretic analysis of degradation of bovine PTH by trypsin or by α_2-M bound trypsin *in vitro*. All mixtures were prepared and incubated at 37° as described under Experimental Procedures. PTH and α_2-M: lane A, 0 min of incubation; lane B, 60 min of incubation. PTH and α_2-M bound trypsin: lane C, 0 min of incubation; lane D, 15 min; lane E, 60 min. PTH and trypsin: lane F, 0 min of incubation; lane G, 15 min; lane H, 60 min.

A chicken kidney plasma membrane adenyl cyclase assay system, in which cyclic AMP synthesis is strongly stimulated by PTH, was employed to determine the effect of incubation with trypsin or α_2-M-bound trypsin on the biological activity of the hormone. Aliquots of the incubation mixtures whose gel electrophoresis profiles are shown in Fig. 1 were taken such that the PTH concentrations in the adenyl cyclase assays were 30, 300, and 3000 ng/ml, based on the amount of PTH added to the digestion mixtures. All samples were assayed in triplicate. At 30 ng/ml, aliquots of control digestion mixtures containing PTH and buffer or α_2-M stimulated cAMP production somewhat more than the PTH standard. Incubation for 60 min with trypsin or α_2-M-bound trypsin reduced the response, but the reduction was not statistically significant because of the relatively high ratio of

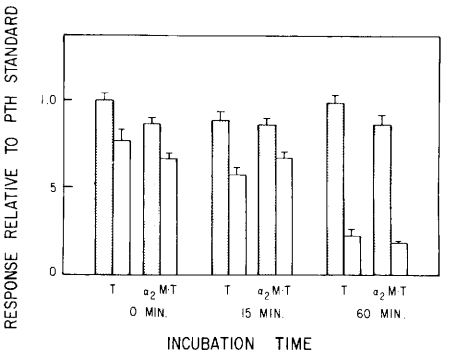

FIG. 2. Effect of incubation of PTH with trypsin or α_2-M bound trypsin on biological activity *in vitro*. The ordinate shows the relative response of various incubation mixtures with respect to standard PTH, as described under Results. All assays were done at a calculated level of 300 ng/ml of PTH. The shaded bars represent controls without trypsin or α_2-M bound trypsin as indicated. Error bars represent standard error of the mean ($n = 3$). All incubated samples had significantly reduced activity with respect to controls ($P < 0.01$ for all comparisons except trypsin versus control at 0 min of incubation, where $P < 0.025$).

error to response at this level. At 3000 ng/ml, aliquots of the control mixtures showed less stimulation than the PTH standard, possibly reflecting interference by other components in the incubation mixtures. In addition, the standard PTH showed maximum response at this level. For these reasons, only results obtained at 300 ng/ml are considered in testing for significant degradation of biological activity. At this level, stimulation of cAMP production by standard PTH is 70–80% of maximum, and the slope of the response curve is near maximum.

The effects of incubation of PTH with trypsin or trypsin bound to α_2-M on its stimulation of cAMP production at the 300 ng/ml level are shown in Fig. 2. The results are normalized to the cAMP production by standard PTH (300 ng) to compensate for the variation in maximum response from one assay set to another. It can be seen that a statistically significant reduction of the activity compared to control values was observed at all times of incubation, and that the reduction is greatest at 60 min of incubation. Significant degradation at 0 min of incubation is probably due to a small amount of proteolysis occurring before all of the trypsin was inactivated; however, this was not seen in control samples after polyacrylamide gel electrophoresis (Fig. 1). When expressed in this way, the response is not proportional to PTH concentration, so that the mean relative response values shown in Fig. 2 do not strictly reflect the fraction of cAMP production activity remaining after incubation of PTH with trypsin or α_2-M-bound trypsin. However, the stimulation observed in the aliquots incubated 60 min is approximately equal to that given by 30 ng/ml standard PTH, indicating that the biological activity in these samples has been reduced by 90% or more. This is consistent with the high amount of degradation shown in Fig. 1.

Assay for Degradation of ^{125}I-Labeled PTH. Following the demonstration of bovine PTH degradation *in vitro* by α_2-M bound trypsin, with concomitant loss of biological activity of the hormone, an attempt was made to detect PTH degrading activity in sera of patients with acute pancreatitis, using the urea–polyacrylamide gel electrophoresis system described above. In this case, ^{125}I-labeled PTH was employed in degradation assay mixtures, permitting quantitative determination of PTH degradation in assay mixtures in which the hormone concentration was approximately 10^{-8} *M*. Bovine PTH migrates significantly faster than plasma proteins in this gel electrophoresis system, allowing easy visualization of unlabeled PTH mixed with plasma by staining with amido schwarz. Autoradiography demonstrated that ^{125}I-labeled PTH migrated as two closely spaced bands, the faster of which corresponded with unlabeled hormone. The nature of the slower component is not known. It may be due to diiodotyrosyl residues or some other side product of iodination; however, no evidence for selective degradation of either form of labeled PTH was observed. Detailed conditions for the assay method are given under Experimental Procedures.

The values for percentage PTH degradation are calculated with respect to the per-

centage recovery of radioactivity in a control incubation containing normal serum or serum preincubated with peptide chloromethyl ketone inhibitors (see Experimental Procedures). The recovery obtained in controls varied between 30 and 55%, depending to some extent on the time elapsed since iodination. Radioactivity in staining and destaining solutions, and in electrophoresis buffer chambers, amounted to 5–10% of the amount applied. Other losses are presumed to be due to surface adsorption and transfer losses. The within-assay coefficient of variation was found to be 5.6% for control incubations and 4.2% for recovery at about 50% degradation. All determinations were done in duplicate to minimize error.

The well-known variability in specific activity of different batches of ^{125}I-labeled proteins necessitates proof that the percentage degradation observed is not dependent on the specific activity of ^{125}I-labeled PTH. The concentration of PTH employed in these studies is probably much less than the K_m for peptide bond hydrolysis. Under these conditions, the rate of substrate hydrolysis is proportional to the substrate concentration. Thus the percentage of the substrate hydrolyzed per unit time would be constant. This was experimentally demonstrated with serum from one patient with acute pancreatitis. The percentage PTH degraded in 1 hr did not vary significantly over a fourfold range of hormone concentration.

Figure 3 shows the amount ^{125}I-labeled PTH degraded after 1 hr at 37° as a function of the amount of serum from a patient with severe acute pancreatitis added to the standard assay system. Figure 3 also shows that with this particular sample, the percentage degradation was a linear function of the amount of pancreatitis serum added up to approximately 60%.

PTH degradation by normal and patient sera. Aliquots of 5 or 10 μl of serum samples obtained from normal, healthy volunteers demonstrated very little degrading activity in the standard PTH assay system. An estimate of the amount of degrading activity in normals was therefore obtained by

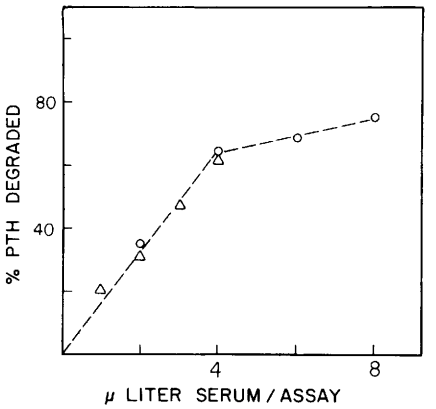

FIG. 3. Degradation of PTH as a function of amount of serum from a patient (No.7 in Table I) with acute pancreatitis. Aliquots of serum as shown were incubated for 60 min at 37° in the standard assay system. The total amount of serum in each tube was adjusted to 14 μl by addition of pooled normal serum having no detectable degrading activity. ○, assay set 1 (12/28); △, assay set 2 (1/2).

incubating 14-μl serum samples from 17 normal volunteers for 2 hr at 37° in the PTH degradation assay. The percentage degradation values obtained were then corrected proportionally to the amount that would be expected for a 1-hr incubation period at 37° with 5 μl of sample. A mean of 1.7% degraded, with an SD of 0.86 and a range of 0.3 to 3.5%, was obtained. In contrast, sera obtained from eight patients with acute alcoholic pancreatitis demonstrated a mean of 30.3% degraded, with an SD of 17.7 under the same conditions (Table I). The mean value obtained for the patient group was significantly greater ($p < 0.005$) than that of the normal controls.

In four patients observed to have clinically elevated serum amylase levels (range 245–445 U/100 ml; upper normal limit 180), in whom no evidence of pancreatic inflammation could be obtained, no significant PTH degrading activity was detected after 1 hr of incubation with 5 μl serum at 37°. Furthermore, in two patients with elevated serum amylase values (9600 and 325 U/100 ml), who were found to have gallstone-induced pancreatitis, PTH degrading activity was in the upper normal limits (3.5%

TABLE I. PTH PROTEASE ACTIVITY IN PATIENTS WITH ACUTE ALCOHOLIC PANCREATITIS

Patient No.	Serum amylase, (U/100 ml)	Total serum immunoreactive cationic trypsin (ng/ml)	Percentage PTH degraded
1	930	1280	46.2
2	1365	915	22.7
3	1005	1210	18.3
4	1065	330	22.7
5	420	404	17.3
6	3800	830	20.9
7	1275	820	68.2
8	305	N.D.	26.5

Note. N.D., not determined. Total serum immunoreactive cationic trypsin was measured as described previously (14).

degraded by 5 μl serum from each patient in 1 hr at 37° in the standard assay).

Comparison of PTH degrading activity and immunoreactive cationic trypsin in blood. As shown in Table I, seven of the acute alcoholic pancreatitis patients included in this study had serum immunoreactive cationic trypsin levels that were greatly elevated above the normal level determined in this laboratory (26 ± 9.3 ng/ml, range 12–41 ng/ml, n = 15, Ref. (14)). The mean value was significantly greater ($P < 0.0005$) than that of the control group. The serum amylase levels for these patients are included to indicate that these were above the upper normal limit in all patients. In one patient who survived an attack of acute hemorrhagic pancreatitis, daily serum samples were obtained during the first week of hospitalization. The PTH degradation activity, as well as the total serum immunoreactive cationic trypsin, is shown as a function of days of hospitalization in Fig. 4. It can be seen that both parameters decreased in an approximately parallel manner, although the profound elevation in immunoreactive cationic trypsin on admission greatly accentuates the magnitude of the fall to ormal levels by Day 6. This type of relationship was found in two other patients.

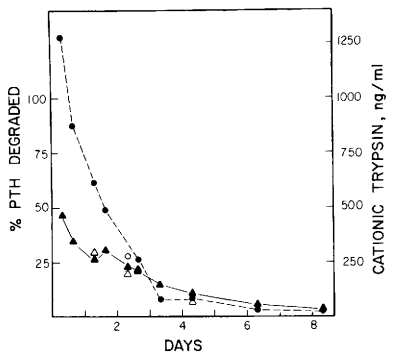

FIG. 4. PTH degrading activity and immunoreactive cationic trypsin in serum from a patient (No. 1 in Table I) with acute pancreatitis as a function of days in hospital. All points represent the amount of degradation observed when 5 μl serum was incubated 1 hr at 37° in the standard incubation mixture. Immunoreactive cationic trypsin was determined as described previously (13). ●---●, cationic trypsin; ▲——▲, PTH degrading activity; △, repeat determinations of PTH degrading activity done on a different day. Two points appearing within the same day represent morning and evening blood samples.

Nature of the PTH degrading activity: Inhibition by peptide chloromethyl ketones. Early in the course of this work it was observed that the PTH degrading activity in several pancreatitis patient sera could be fully inhibited by 5 min of preincubation with Phe–Ala–Arg-CH_2Cl and Suc-$(Ala)_2$–Pro–Leu-CH_2Cl. The former peptide derivative rapidly inhibits bovine trypsin (23), while the latter is a very effective inhibitor of human elastase 2 and bovine chymotrypsin (22). However, neither inhibitor alone was sufficient to fully inhibit the activity, even at higher concentrations. A combination of these peptide derivatives was therefore used routinely to obtain undegraded control values. To further delineate the nature of the proteolytic activ-

ity, inhibition by peptide chloromethyl ketones, as well as by the trypsin inhibitor Trasylol, was examined in more detail in a serum sample that had a high level of PTH degrading activity. Figure 5 shows the percentage inhibition of PTH degrading activity of each compound plotted as a function of inhibitor concentration on a log scale. It can be seen that Suc-$(Ala)_2$-Pro-Leu-CH_2Cl and Trasylol are approximately equally effective inhibitors of PTH degrading activity, while Phe-Ala-Arg-CH_2Cl was significantly less effective. None of the inhibitors alone fully blocked the degrading activity at the concentrations employed. Combinations of two compounds showed slightly less than additive levels of inhibition in most cases. An exception was that 10^{-4} *M* Trasylol with 10^{-5} *M* Suc-$(Ala)_2$-Pro-Leu-CH_2Cl inhibited the activity completely.

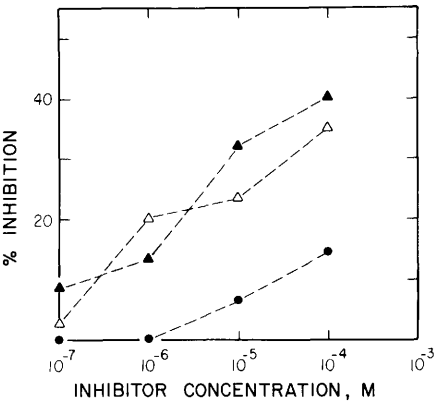

FIG. 5. Inhibition of PTH degrading activity in pancreatitis patient serum by peptide chloromethyl ketones and Trasylol. Inhibitors were added at the final concentrations indicated to 3 μl of serum from a patient (No. 7 in Table I) with acute pancreatitis in the standard incubation mixture without ^{125}I-labeled PTH. The labeled hormone was added after 5 min of incubation at room temperature, and the mixture was then incubated for 1 hr at 37° to determine the amount of PTH degradation. △, Trasylol; ▲, Suc-$(Ala)_2$-Pro-Leu-CH_2Cl; ●, Phe-Ala-Arg-CH_2Cl.

Discussion. The results of this investigation demonstrate that PTH degrading activity is present in sera of patients with severe acute pancreatitis. This is the first demonstration *in vitro* of true proteolytic activity in the sera of such patients. In addition,we have shown that α_2-M-bound trypsin, which may represent a significant fraction of this proteolytic activity in serum in acute pancreatitis, destroys the biological activity of PTH *in vitro* in an adenyl cyclase assay system. We have previously reported the presence of immunoreactive trypsin bound to α_2-M in sera from several patients with acute pancreatitis (14). Previous work has also demonstrated that α_2-M-bound trypsin can convert proinsulin to desalanyl-insulin *in vitro* (19), indicating that proteases bound to α_2-M retain activity toward polypeptides of moderate molecular weight. This finding is confirmed with respect to PTH (M_r = 9100) in the studies reported here. Our results are thus consistent with the hypothesis that degradation of circulating PTH is at least in part responsible for the hypocalcemia observed in severe cases of acute pancreatitis. However, it is possible that stimulation of kidney adenyl cyclase does not fully reflect the biological activity of PTH *in vivo*. In addition, the role of degradation of other relevant peptides (e.g., glucagon and calcitonin) as well as the possible effect of circulating proteases on bone PTH receptors is unknown. It is known, however, that hormone receptors are proteins and are easily destroyed by common proteolytic enzymes. (27).

Our data suggest that the PTH degrading activity is probably due to two or more pancreatic serine proteases, for the following reasons. First, inhibition by peptide chloromethyl ketones is a property limited to serine and sulfhydryl proteases among the known classes of endopeptidases (28). Of the known sulfhydryl proteases, only cathepsins B1 and L could potentially degrade PTH. Each of these proteases has been shown to be unstable at pH values above 7; however, we cannot rule out the possibility that a cathespin stabilizing factor is present in blood. An indication that at least part of the activity is a serine protease of trypsin-like specificity is suggested by the partial inhibition by Trasylol. This inhibitor has no effect on thiol proteases, and is much more potent as an inhibitor of trypsin than of chymotrypsin (29, 30). In addition, inhibition of PTH degrading activity by Suc-$(Ala)_2$-Pro-Leu-CH_2Cl suggests

that a substantial part of the proteolytic activity is due to human chymotrypsin and/or elastase 2. Finally, the demonstration of an association of PTH protease activity with acute pancreatitis supports the hypothesis that the degradation is due to serine proteases originating in the pancreas.

Two recent reports have shown that increased levels of benzoyl-arginine ethyl ester hydrolase activity are present in plasma of patients with acute pancreatitis (31, 32). However, the amount of esterase activity in normal plasma was such that only 2- to 3-fold elevations were observed in patient samples. In contrast to the studies on esterase activity, the PTH degradation assay yields substantially increased proteolytic activity (up to 20-fold) in sera of patients with acute pancreatitis. Furthermore, we observed no difference in percentage PTH degradation when pancreatitis plasma and serum were compared. It is possible that the higher esterase activity found in normal serum as compared to plasma could make insignificant the increase observed by these authors in acute pancreatitis (31, 32).

Clinical laboratory studies on the relationship between the levels of circulating PTH, as determined by radioimmunoassay, and the degree of hypocalcemia have been contradictory. This can at least partially be explained by the measurement of a variety of PTH degradation fragments which are devoid of biological activity but retain immunoreactivity. Thus immunoreactive PTH might not correlate well with biological activity in the analysis of individual serum samples. An additional factor in the interpretation of results obtained with PTH radioimmunoassays in plasma of patients with acute pancreatitis deserves special consideration. Artifactually high values of plasma immunoreactive PTH could be obtained due to the proteolytic degradation of PTH tracer in the radioimmunoassay. This potential artifact might be especially relevant in the studies of Imrie *et al.* (33) who reported increased plasma PTH levels in a series of patients with acute pancreatitis. Radioimmunoassays of PTH in plasma or serum of patients with acute pancreatitis should include appropriate control incubations to demonstrate that no such degradation is occurring. Protease activity in the incubation mixture could be inhibited by a combination of specific active site inhibitors and Trasylol, but Trasylol alone is probably not sufficient.

Our results demonstrate that the PTH degradation assay, despite the fact that it is cumbersome and time consuming, constitutes a much more sensitive and biologically meaningful index of proteolytic activity in serum than the previously reported esterase assays. We are currently developing a modification of the PTH degradation technique in which fluorescein isothiocyanate-labeled PTH is employed. This method is based on the measurement of fluorescence polarization, which is sensitive to changes in the molecular weight of a specific fluorescent molecule in a complex system (34, 35). With this new assay technique an extensive study will be undertaken concerning the relationship of serum PTH degrading protease activity to both the hypocalcemia and the severity of pancreatic inflammation.

We wish to thank Dr. Robert A. Nissensen, Veteran's Administration Medical Center, San Francisco, for performing the adenyl cyclase bioassays.

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Received December 3, 1980. P.S.E.B.M. 1981, Vol. 167.