Studies on Human Proinsulin

ISOLATION AND AMINO ACID SEQUENCE OF THE HUMAN PanCREATIC C-PEPTIDE*

(Received for publication, September 8, 1970)

PHILIP E. OYER,† S0OJA CHO, JAMES D. PETERTSON, AND DONALD F. STEINER§

From the University of Chicago, Department of Biochemistry, Chicago, Illinois 60637

SUMMARY

Human proinsulin C-peptide has been extracted from human pancreas with acid-ethanol and purified by means of gel filtration, carboxymethyl-cellulose chromatography, paper electrophoresis, and partition chromatography. The purified material was judged to be about 98% pure by compositional analysis. Amino acid sequence analysis was carried out on the intact C-peptide and fragments derived by hydrolysis with chymotrypsin and thermolysin. On the basis of these results, and in accord with the compositional stoichiometry, the following 31-residue amino acid sequence is proposed: Glu-Ala-Glu-Asp-Leu-Glu-Val-Gly-Glu-Val-Glu-Leu-Gly-Gly-Gly-Pro-Gly-Ala-Gly-Ser-Leu-Gln-Prb-Leu-Ala-Leu-Glu-Gly-Ser-Leu-Gln. Although sufficient human proinsulin has not been available thus far for detailed structural studies, the probable conformation of this pancreatic C-peptide with the corresponding region of human proinsulin is supported by both compositional and immunological studies on human proinsulin. Comparison of the amino acid sequences of the bovine, porcine, and human C-peptides reveals homology but also much greater variability in both length and sequence than occurs among the insulin chains in these species. The conformation and role in peptide chain folding of the connecting polypeptide region of proinsulin is evaluated in the light of these findings.

Proinsulin was discovered in a B-cell adenoma removed from a patient with severe hypoglycemia. Characterization of the small amounts of labeled human proinsulin extracted from this tumor after incubation in vitro with labeled leucine or phenylalanine provided the first evidence for the single chain structure of the precursor and the general ordering of the peptide with the phenylalanine, or B, chain of insulin at the NH2 terminus (1). Subsequently, porcine and bovine proinsulins have indicated that, aside from their very similar insulin moieties which cross-react extensively, the antigenic area or areas located in the connecting polypeptide segments of bovine, porcine, and human proinsulin are nearly species specific. This finding is compatible with the numerous differences in the amino acid sequences of the bovine and porcine connecting polypeptide segments (5). Thus, in order to extend our studies on the secretion, metabolism, and clinical significance of proinsulin to man, it has become essential to know the structure of the connecting polypeptide segment of human proinsulin.

Evidence has been reported previously which indicates that the C-peptide is retained within the beta cells after cleavage of proinsulin by an enzymic process that is probably localized within the maturing secretory granules (6-10). As a consequence of this mechanism considerable quantities of C-peptide can be isolated from fresh bovine and porcine (11) pancreas in equimolar amounts with insulin (8). Similar results were obtained in preliminary studies with human pancreas, as reported earlier (6). Since the quantity of proinsulin in normal pancreas is only a small fraction of the insulin content, and since it is further diminished by autolysis during the long interval between death and postmortem examination, we have chosen to utilize the more abundant human C-peptide for structural studies. This report describes a large scale isolation procedure that yields highly purified human C-peptide. The amino acid sequence of this peptide has been elucidated, and evidence to support the conclusion that it indeed corresponds to the connecting polypeptide region of human proinsulin is provided. A preliminary report of these findings has been presented elsewhere (12).

MATERIALS AND METHODS

Human pancreases were obtained at postmortem examination from subjects without a history of diabetes. They were chilled immediately, trimmed of fatty tissue, and stored at $-20^\circ$ until extraction. When more than 16 hours had elapsed before necropsy the tissue was not used. Crude human proinsulin (6 component) was separated by gel filtration from 1 g of twice

* This work was supported by United States Public Health Service Grants AM-04931 and AM-13014.
† Supported by Public Health Service Training Grant HD-00001 from the National Institute of Child Health and Human Development.
§ Recipient of a United States Public Health Service Research Career Development Award.
crystallized human insulin (prepared by ordinary commercial procedures) and kindly supplied by The Novo Company, Copenhagen. Carboxypeptidase A (disopropyl fluorophosphate-treated) and a-chymotrypsin were supplied by Worthington. Aminopeptidase M was supplied by Henley and Company, New York, New York. The phenylthiobiotinylamines of amino acids used as standards were obtained from The Pierce Chemical Company, Rockford, Illinois. Dansyl\(^1\) amino acid standards were prepared from amino acids supplied by Calbiochem. Dan- syl chloride and Sequanal grades of phenylisothiocyanate, an- hydrous trifluoroacetic acid, pyridine, and triethylamine were obtained from Pierce. Thin layer cellulose (Catalogue No. 6064) and silica gel (Catalogue No. 6060) chromatographic sheets were obtained from Eastman. Polyamide thin layer sheets were supplied by The Cheng-Chin Trading Company (Gallard-Schlesinger Manufacturing Corporation, Carle Place, New York). Formylated cellulose powder (Cellex XF-1) and Bio-Gel P-30 were obtained from Bio-Rad, Richmond, California. Carboxymethylcellulose (CM-28) was obtained from Whatman as was the No. 3MM paper (chromatographic grade) used for preparative electrophoresis. Rabbit antiserum to guinea pig globulin was obtained from The Sylvana Company, Orange, New Jersey.

**Extraction of Human Pancreas**

Frozen human pancreatic tissue was pooled into 500-g lots and extracted in the cold room at 4°C by a modification of the procedure of Davoren (13). To the chopped frozen tissue in a Waring Blender were added 1500 ml of chilled acid-ethanol (7.5 ml of concentrated HCl in 375 ml of 95% ethanol). After blending for 30 sec, 0.2 volume of cold water was added, and the blending was continued 10 to 15 sec longer. The mixture was transferred to a beaker, stirred magnetically for approximately 2 hours and then centrifuged at 600 \(\times\) g for 15 min. The supernatant fluid was collected and the pH adjusted with ammonium hydroxide to pH 8 (Hydron paper). The resulting precipitate was removed by centrifugation at 600 \(\times\) g. The supernatant solution was adjusted with 6 N HCl to pH 5.3 with methyl red as the indicator. One part of 2.0 M ammonium acetate buffer at pH 5.3 per 40 parts supernatant solution was then added, followed by 2 volumes of chilled absolute ethanol and 4 volumes of diethyl ether. One to two drops of 30% NaCl were sometimes added to accelerate precipitation. The precipitate, after standing at 4°C overnight, was removed by centrifugation for 20 minutes at 600 \(\times\) g and the residual ether was evaporated under a stream of compressed air. The supernatant solution was discarded.

**Isolation of C-Peptide from the Crude Pancreatic Extract**

The pancreatic extract was dissolved in approximately 200 ml of 3 M acetic acid and passed through a column, 8 × 100 cm, of Sephadex G-50 or of Bio-Gel P-30 eluted with 1 or 3 M acetic acid. Fig. 1 illustrates a typical result of this purification step. As previous studies had shown that the C-peptide elutes from the Bio-Gel columns almost coincident with insulin (6, 14), the fractions containing insulin were pooled, dried, and dissolved in approximately 10 ml of 0.01 M sodium citrate buffer, pH 5.0, containing deionized water (7 M). This sample was subjected to gradient elution chromatography on a column (1.8 × 30 cm) of carboxymethylcellulose previously equilibrated with this buffer.

The very acidic C-peptide and other acidic peptide material eluted from the column with the breakthrough volume, while less acidic proteins, such as glucagon and insulin, were bound to the cellulose and were eluted with a sodium chloride gradient (Fig. 2). Peak A, containing C-peptide free of insulin and other less acidic peptides, was desalted on a column of Sephadex G-15 eluted with 0.1 M acetic acid. The fractions at the void volume giving a positive ninhydrin reaction were pooled and lyophilized. The dried product, consisting of approximately 50 mg of protein, was dissolved in 75 to 100 ml of 30% formic acid (pH 0.9) and subjected to preparative electrophoresis in this solvent on Whatman No. 3MM paper (15 × 35 cm) at a potential of 5 volts per cm for 16 hours. A typical electrophoretogram obtained at this stage of purification is shown in Fig. 3A. The region of the paper containing C-peptide was eluted (ascending) with 50% acetic acid, dried, and dissolved in 0.5 ml of 1-butanol-pyridine-acetic acid-water (60:40:12:48, v/v). The material was partitioned in this buffer system on a column (0.9 × 55 cm) of formylated cellulose equilibrated with the same solvent system. Fractions collected from this column were assayed for protein content using ninhydrin reagent. As the earliest eluting protein peak contained the C-peptide, these fractions were pooled, dried under a stream of \(N_2\), and dissolved in approximately 50 \(\mu\)l 1.2 M pyridine acetate buffer at pH 6.5. Due to the acidity of the C-peptide, it was necessary to readjust the pH to 6.5 with pyridine. The sample was then applied to Whatman No. 3MM paper moistened with the pyridine acetate buffer and subjected to electrophoresis for 6 hours at a potential of 5 volts per cm. Fig. 3B shows a typical electrophoretogram obtained using the pyridine acetate system.

The total yield from this procedure was approximately 1.5 mg of peptide from 500 g of pancreas. Compositional analysis showed that this material contained less than 2% of amino acids not present in the C-peptide (Thr, Met, Cys, lle, Phe, Tyr, His, Lys, Arg). Fig. 4 summarizes the extraction and purification procedures.

**Chymotryptic Digestion**

Human C-peptide (5 mg per ml) was digested with chymo- trypsin (2%, w/w) in 0.05 M Tris-HCl buffer at pH 8.2. (In one digest the pH was not readjusted to 8.2 after dissolving the peptide, resulting in a final pH of 6.5 for the digestion.) Control digests containing enzyme but no substrate, and C-peptide without enzyme, were also prepared. After incubation of the reaction mixture and controls for 18 hours at 37°C, the reaction was stopped by acidification with glacial acetic acid and the samples were dried under a nitrogen stream.

**Purification of Chymotryptic Fragments**

The dried resedues from the chymotryptic digests were taken up in 30 ml of 1.2 M pyridine acetate buffer at pH 6.5 and subjected to electrophoresis in this solvent on Whatman No. 3MM paper at 5 volts per cm for 6 hours. Five ninhydrin-positive spots, which migrated toward the anode, were resolved by this procedure. The slowest migrating peptide fraction, designated A, consisted of neutral peptides, while Fractions B to E consisted of acidic peptides of increasing mobility toward the anode. Each fraction was eluted separately from the dried paper with 50% acetic acid, dried under a stream of \(N_2\), dissolved in 25 ml of 30% formic acid, and subjected to paper electrophoresis in this solvent at 5 volts per cm for 6 hours. Several of the frac-
Fig. 1. Elution diagram of human pancreatic extract (350 g of pancreas) from a column (8 x 100 cm) of Sephadex G-50 in 1 M acetic acid. Polyacrylamide gel electrophoretograms (pH 8.9, see Reference 1) of material (100 μg) from several of the peaks have been inserted to indicate their composition. The acidic C-peptide does not stain with the Amido schwarz dye used to stain the electrophoretograms. The anode is toward the bottom and the tracker dye trace is seen as a rapidly migrating faint line.

Fig. 2. Gradient elution chromatography of the insulin-containing fractions from gel filtration (Peak B in Fig. 1) on a column of carboxymethylcellulose in 0.01 M sodium citrate buffer, pH 5.0, containing 7 M urea. Polyacrylamide gel electrophoretograms (100 μg each) stained with Amido schwarz have been inserted over the corresponding regions of the chromatogram. Peak A contains the C-peptide and a number of contaminants; Peak C contains glucagon and other peptides; Fractions D and E contain insulin and other peptides. Electrophoretogram O shows the starting material. (See “Materials and Methods” for procedural details.)
FIG. 3. A, photograph of a ninhydrin-stained paper electrophoretogram (30% formic acid) of Fraction A from carboxymethyl cellulose chromatography (CMC) (Fig. 2). Purified bovine and human C-peptide have been spotted as markers. B, photograph of a ninhydrin-stained paper electrophoretogram (1.2 M pyridine acetate, pH 6.5) of Fraction A from carboxymethylcellulose chromatography (material from this step was used to better illustrate the resolution of C-peptide from impurities). Purified bovine and human C-peptide have been spotted as markers. (See "Materials and Methods" for procedural details.)

Purification of Thermolysin Fragments

The dried product from the thermolysin digest was taken up in 50 μl of 30% formic acid and subjected to electrophoresis in this solvent on Whatman No. 3MM paper at 5 volts per cm for 6 hours. Five ninhydrin-positive components, designated A to E, which migrated toward the cathode were resolved. Each
was eluted and partially dried under a stream of N₂ to a volume of approximately 30 µl. These solutions were applied to thin layer cellulose chromatographic media and subjected to partition chromatography using 1-butanol-pyridine-acetic acid-water (60:40:12:48, v/v). Each spot from the formic acid electrophorogram was resolved into several discrete spots. The appropriate regions of the cellulose chromatograms were scraped off the backing plate and eluted by suspending the cellulose in 50% acetic acid, centrifuging, and recovering the supernatant containing the dissolved peptide. Aliquots of some of these peptide fragments were hydrolyzed for amino acid analysis or subjected to Edman degradation.

**Edman Degradation**

Stepwise degradation of the purified C-peptide and fragments derived from it by chymotryptic and thermolytic cleavage was carried out by a micro-adaptation of the direct Edman procedure using anhydrous trifluoroacetic acid in the cleavage reaction (2, 17, 18). The phenylthiohydantoin derivatives were detected by thin layer chromatography on sheets of silica gel containing fluorescent indicator as described by Jeppsson and Sjöquist (19).

**Dansylation**

Dansylation reactions were performed on an aliquot of peptide removed before each cycle of Edman degradation. The reactions were carried out on approximately 10 nmol of peptide as described by Woods and Wanz (20). The dansyl derivatives were detected by two dimensional thin layer chromatography on sheets of silica gel containing fluorescent indicator as described by Jeppsson and Sjöquist (19).

**Amino Acid Analyses**

Samples of 30 to 150 µg of protein were hydrolyzed in sealed, evacuated soft-glass tubes containing 0.5 ml of 6 N HCl previously saturated with N₂. The tubes were heated in an oven for 20 to 40 hours at 110°. The acid was evaporated under a stream of nitrogen at 40° after hydrolysis. Analyses were performed on a Beckman-Spinco model 120 C amino acid analyser using a column (0.9 x 55 cm) of Beckman UR-30 resin with pH 3.22 and pH 4.20 sodium citrate buffers for analysis of acidic and neutral amino acids and Beckman PA-35 resin in a column (0.9 x 55 cm) with pH 5.25 sodium citrate buffer for analysis of basic amino acids. Both columns were operated at a temperature of 55°.

A different buffer system was employed in the analysis of carboxypeptidase A and theaminopeptidase M digests in order to separate glutamine and asparagine from other amino acids. This system consisted of lithium citrate buffers at pH 2.80 and 4.16 and was run at a column temperature of 38.8°, as described by Benson, Gordon, and Patterson (21).

**Carboxypeptidase Digestion**

Carboxypeptidase A crystals were washed in water and dissolved immediately before use at a concentration of 5 mg per ml according to the procedure of Ambler (22).

C-Peptide (300 µg) was dissolved in 100 µl 0.05 M Tris containing 0.5% NaCl and the pH was adjusted to 8.5 with 0.1 N NaOH (Hydrion paper). Then 10 µl (100 µg) of the enzyme stock solution were added to give a final enzyme to substrate molar ratio of 1:30. A 20-µl aliquot was immediately removed and quenched with 1 drop of glacial acetic acid. Incubation was carried out at 37°. Further aliquots were removed at 10, 20, 30, and 60 min, lyophilized, and analyzed for free amino acid content. An enzyme control containing no substrate but having all the other constituents described above was prepared and analyzed after 80 min of incubation at 37°. A glutamine control was prepared exactly as was the enzyme control except that glutamine (0.025 pmole) was added to determine whether this amino acid might cyclize to pyrrolidonecarboxylic acid when exposed to these reaction conditions.

**Total Enzymic Hydrolysis of Intact C-Peptide**

**Papain Digestion**—C-Peptide (100 µg) was dissolved in 85 µl of 0.02 M ammonium acetate buffer, the pH adjusted to 5.3, and 5 µl of a 1:32 dilution of mercaptoethanol were added. Then 10 µl of an aqueous solution of papain (0.5 mg per ml) were added to give a final enzyme to substrate ratio of 1:20. The enzyme blank contained all the above constituents except substrate. After incubation for 2 hours at 37°, the reaction was stopped by addition of a drop of glacial acetic acid and the mixture was lyophilized.

**Amino Peptidase M Digestion**—The above samples were taken up in 80 µl of 0.05 M Tris buffer, the pH adjusted to 8.2, and 5 µl of mercaptoethanol (1:32) and 15 µl of a 10 mg per ml aqueous

---

*H. Keutmann and J. T. Putte, personal communication.*
FIG. 5. Gradient elution chromatography on a column (1 X 10 cm) of DEAE-cellulose of human crude proinsulin fraction (14 mg) separated from crystalline human insulin by gel filtration. The fractions (1.75 ml) were pooled as numbered and the buffer and urea removed by gel filtration on Sephadex G-25 in 1 M acetic acid. Amounts of 30 to 50 μg of each fraction were electrophoresed on polyacrylamide gels (pH 8.9) and stained with Amido Schwarz. Gels designated B and H at left are of bovine and human crude proinsulin fractions, respectively, before chromatography. Fraction 1 proteins did not migrate toward the anode on polyacrylamide gels. The heavy band of Fraction 2 was identified as human proinsulin. (See “Materials and Methods” for further procedural details.)

solution of aminopeptidase M were added (enzyme to substrate ratio of 3:2). The enzyme blank from the papain digest was also carried through this step. The reaction tubes were incubated for 3 hours at 37°C; 1 drop of glacial acetic acid was then added, and the mixture was lyophilized. The dry samples were taken up directly in the lithium citrate buffer, pH 2.80, for amino acid analysis.

Double Antibody Immunoassays

Immunoassays were performed by the method of Morgan and Lazarow (23) as modified by Rubenstein et al. (24). Antibodies to human C-peptide were prepared by injection of guinea pigs with the purified peptide coupled to rabbit serum albumin (25). Procedures for preparation and iodination of tyrosylated human C-peptide have been described in detail elsewhere (25).

Purification and Characterization of Human Proinsulin

A crude proinsulin fraction (β component) was separated from 1 g of twice crystallized insulin by gel filtration on Sephadex G-50 as described previously (26). Approximately 14 mg of protein, free of insulin, were obtained which gave the polyacrylamide gel electrophoretogram designated H in Fig. 5. Unlike the bovine or porcine crude proinsulin fraction, which contain a high proportion of intact proinsulin (slowly migrating heavy band in electrophoretogram B, Fig. 5) and intermediate forms, the human preparation consisted mainly of material corresponding to the previously described nonconvertible, or abnormal insulin dimer fraction (26). The crude proinsulin mixture was fractionated by gradient elution chromatography on DEAE-cellulose in 0.04 M Tris-HCl buffer, pH 7.6, containing 7 M urea (Fig. 5). Fraction 2 contained mainly material corresponding to the slowly migrating band of the starting material (electrophoretogram H). The yield of this fraction after removal of urea by gel filtration over Sephadex G-25 was less than 0.5 mg. NH₂-terminal analysis of Fractions 2 through 6 by the dansyl technique yielded mainly phenylalanine only in the case of Fraction 2, the others containing both NH₂-terminal phenylalanine and glycine in about equal amounts, consistent with proinsulin intermediates or insulin dimers. Fraction 2 had the same Rf on polyacrylamide electrophoresis as did labeled human proinsulin prepared by incubation in vitro of slices from an islet cell adenoma.¹

The quantity of proinsulin was limited; it was partially characterized after labeling it with ¹³¹I (4). The iodinated proinsulin was purified on small columns of cellulose and by gel filtration on Sephadex G-50. After sulfhydrylysis in urea (27), ¹³¹I-proinsulin migrated as a single component on paper electrophoresis in 20% acetic acid-8 M urea. After incubation of the labeled proinsulin for 30 min at 37°C with trypsin (2.5 μg per ml, pH 8.5), a product

¹J. L. Clark and D. F. Steiner, unpublished results.
RESULTS AND DISCUSSION

Purification Procedure and Yield of C-Peptide—The extraction and purification procedures used in the isolation of human C-peptide are similar to those used to isolate bovine and porcine C-peptides (6, 8). Because of its small size, the C-peptide elutes near insulin on gel filtration, while most of the contaminating peptides in pancreatic extracts elute nearer the void volume. The C-peptide can then be separated conveniently from insulin, glucagon, and other pancreatic peptides by chromatography on carboxymethylcellulose columns in urea-containing buffer near insulin on gel filtration, while most of the contaminating peptides in pancreatic extracts elute nearer the void volume. The C-peptide can then be separated conveniently from insulin, glucagon, and other pancreatic peptides by chromatography on carboxymethylcellulose columns in urea-containing buffer (Fig. 2). In contrast to the situation with fresh animal pancreas (8), the carboxymethylcellulose fractions containing the human C-peptide are invariably extensively contaminated with a heterogeneous mixture of acidic peptides which evidently arise from postmortem autolysis. The electrophoretic procedure in 30% formic acid eliminates a considerable proportion of these contaminants. In this system the C-peptide migrates slowly toward the cathode due to the presence of only one free amino group, while the contaminants produce a broad ninhydrin-positive smear of more rapidly migrating material (Fig. 3A). The bovine C-peptide migrates slightly more rapidly than the human C-peptide, perhaps due to its smaller size (26 residues versus 31). Compositional analysis of the slow moving C-peptide spot indicated that some contaminants including peptides containing amino sugar residues were still present. Similar glycopeptides had been noted in the bovine pancreatic extracts (8), but these do not appear to be related to the C-peptide.

Electrophoresis of this material at pH 6.5, where carboxy side chains are fully dissociated, cleanly separated most of the residual contaminants (Fig. 3B). The fastest moving of the four major spots contained the C-peptide. Compositional analyses at this stage indicated the presence of some residual material containing threonine and traces of isoleucine, but the glycopeptides were completely eliminated, as in the case of the bovine material (8). As thin layer cellulose partition chromatography removed most of the remaining contaminants, this procedure was adapted to a column system. However, due to the difficulties encountered in completely evaporating the partition chromatography solvent (1-butanol-pyridine-acetic acid-water) it proved more convenient and equally satisfactory to carry out this step prior to the paper electrophoresis at pH 6.5 (as described under "Materials and Methods" and Fig. 4).

The composition of the human C-peptide is shown in Table I. The stoichiometry of the 8 amino acids comprising the C-peptide indicated the presence of 31 residues. The degree of contamination varied from preparation to preparation, but usually amounted to less than 2%, as judged by the amounts of amino acids not present in the C-peptide. The yield from this procedure was generally lower than with other species. Although the insulin fractions from the ion exchange column were saved, they were not purified further nor quantitated; therefore, we have not calculated the relative proportion of C-peptide to insulin extractable from human pancreas. Based on the report of other workers that approximately 30 mg per kg of insulin can be extracted from human pancreas using extraction procedures similar to ours (28), and assuming that the C-peptide is initially present in pancreas in amounts equimolar with insulin (0.8), we can calculate that our yield of 2 to 4 mg per kg represents roughly 13 to 25% of that theoretically possible. Moreover, judging from the yield of human proinsulin from the crystalline insulin preparation of about 0.05%, it is likely that there is more than 200 times as much C-peptide present in the human pancreas at the time that extraction is begun, although this clearly may not accurately reflect the proportion of these components in vivo. Preparation of sufficient human proinsulin for structural studies could therefore require extraction of 500 to 1000 kg of human pancreas.

Amino Acid Sequence Determination—Sequence studies were performed on a total of 8 to 10 mg of purified human C-peptide. Edman degradation of the intact peptide was carried out initially on 1 mg (0.33 pmole) of the material. With this amount of peptide both phenylthiohydantoin and dansyl derivatives could be identified through residue 12 and dansyl derivatives alone were still detectable through residue 15, as shown in Fig. 6. Since glutamine residues may be hydrolyzed to glutamate in the Edman procedure, it was not certain whether residue 11 was glutamine or glutamic acid. Residues 1, 3, 4, 6, and 9 could be assigned with certainty, however, since PTH-glutamine was clearly detectable at position 9. Appendices A and B summarize the semiquantitative estimates of the amounts of phenylthiohydantoin and dansyl derivatives obtained at each Edman step on the intact peptide and on all proteolytic fragments discussed subsequently. Further studies were performed on peptide fragments produced by the action of chymotrypsin on the human C-peptide. As a result of the absence of aromatic residues in the C-peptide, the major chymotryptic cleavages would be expected to occur carboxy-terminal to each of the 6 leucine residues of the peptide. The peptides isolated from chymotryptic digests are represented schematically in Fig. 6 and compositions are given in Table II. The sequence of Fragments D1 and E was identical with that for residues 1 through 12 of the intact peptide. Moreover, data obtained from degradation of Fragment D1 confirmed that

Table I

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Acid hydrolysis in 5.8 M HCl, 110°, 70 hrs</th>
<th>Amidopeptidase M hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>1.05</td>
<td>1.04</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Serine</td>
<td>1.76</td>
<td>1.91</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>7.90</td>
<td>3.69</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.09</td>
<td>2.80</td>
</tr>
<tr>
<td>Proline</td>
<td>2.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.72</td>
<td>6.20</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.89</td>
<td>3.32</td>
</tr>
<tr>
<td>Valine</td>
<td>2.09</td>
<td>1.60</td>
</tr>
<tr>
<td>Leucine</td>
<td>5.98</td>
<td>6.35</td>
</tr>
</tbody>
</table>

The composition of the human C-peptide is shown in Table I. The stoichiometry of the 8 amino acids comprising the C-peptide indicated the presence of 31 residues. The degree of contamination varied from preparation to preparation, but usually amounted to less than 2%, as judged by the amounts of amino acids not present in the C-peptide. The yield from this procedure was generally lower than with other species. Although the insulin fractions from the ion exchange column were saved, they were not purified further nor quantitated; therefore, we have not calculated the relative proportion of C-peptide to insulin extractable from human pancreas. Based on the report of other workers that approximately 30 mg per kg of insulin can be extracted from human pancreas using extraction procedures similar to ours (28), and assuming that the C-peptide is initially present in pancreas in amounts equimolar with insulin (0.8), we can calculate that our yield of 2 to 4 mg per kg represents roughly 13 to 25% of that theoretically possible. Moreover, judging from the yield of human proinsulin from the crystalline insulin preparation of about 0.05%, it is likely that there is more than 200 times as much C-peptide present in the human pancreas at the time that extraction is begun, although this clearly may not accurately reflect the proportion of these components in vivo. Preparation of sufficient human proinsulin for structural studies could therefore require extraction of 500 to 1000 kg of human pancreas.

Amino Acid Sequence Determination—Sequence studies were performed on a total of 8 to 10 mg of purified human C-peptide. Edman degradation of the intact peptide was carried out initially on 1 mg (0.33 pmole) of the material. With this amount of peptide both phenylthiohydantoin and dansyl derivatives could be identified through residue 12 and dansyl derivatives alone were still detectable through residue 15, as shown in Fig. 6. Since glutamine residues may be hydrolyzed to glutamate in the Edman procedure, it was not certain whether residue 11 was glutamine or glutamic acid. Residues 1, 3, 4, 6, and 9 could be assigned with certainty, however, since PTH-glutamine was clearly detectable at position 9. Appendices A and B summarize the semiquantitative estimates of the amounts of phenylthiohydantoin and dansyl derivatives obtained at each Edman step on the intact peptide and on all proteolytic fragments discussed subsequently. Further studies were performed on peptide fragments produced by the action of chymotrypsin on the human C-peptide. As a result of the absence of aromatic residues in the C-peptide, the major chymotryptic cleavages would be expected to occur carboxy-terminal to each of the 6 leucine residues of the peptide. The peptides isolated from chymotryptic digests are represented schematically in Fig. 6 and compositions are given in Table II. The sequence of Fragments D1 and E was identical with that for residues 1 through 12 of the intact peptide. Moreover, data obtained from degradation of Fragment D1 confirmed that

4 W. S. Rudd and D. F. Steiner, unpublished results.

These data are available on order. Request NAPS Document 01342 from the ASIS National Auxiliary Publications Service, CCM Information Corporation, 909 Third Avenue, New York, New York 10022, remitting in advance (payable to CCM-NAPS) $2.00 for each microfiche or $5.00 for each photocopy.
Fig. 6. Schematic summary of the amino acid sequence analysis of the human C-peptide and of peptide fragments isolated from chymotryptic and thermolysin digests of the peptide. Compositions of some of the peptides are given in Table II. PTH, phenylthiohydantoin; CPase, carboxypeptidase.

Table II
Amino acid composition of peptides isolated from chymotryptic and thermolysin digests of human C-Peptide

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Residues</th>
<th>Amino Acid Compositions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymotryptic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2I (13-24)</td>
<td></td>
<td>Ser 0.9(1), Glu 1.2(1), Pro 2.0(2), Gly 1.0(1), Ala 1.0(1), Leu 1.7(2)</td>
</tr>
<tr>
<td>A3I (13-21)</td>
<td></td>
<td>Ser 1.0(1), Glu 0.1(0), Pro 1.2(1), Gly 1.0(1), Ala 1.0(1), Leu 1.0(1)</td>
</tr>
<tr>
<td>BII (25-31)</td>
<td></td>
<td>Ser 0.9(1), Glu 2.0(2), Pro 0.9(0), Gly 1.2(1), Ala 1.0(1), Leu 1.2(1)</td>
</tr>
<tr>
<td>EII (1-12)</td>
<td></td>
<td>Asp 1.1(1), Glu 5.0(5), Pro 0.2(0), Gly 1.2(1), Ala 1.0(1), Val 1.7(2), Leu 1.5(2)</td>
</tr>
<tr>
<td>EII (1-5)</td>
<td></td>
<td>Asp 1.1(1), Ser 0.2(0), Glu 2.0(2), Gly 0.2(0), Ala 1.0(1), Leu 0.9(1)</td>
</tr>
<tr>
<td>Thermolysin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3 (1-29)</td>
<td></td>
<td>Asp 1.0(1), Ser 1.6(2), Glu 7.1(7), Gly 1.6(6), Val 1.8(2), Leu 4.5(5)</td>
</tr>
<tr>
<td>B3 (24-31)</td>
<td></td>
<td>Ser 0.9(1), Glu 2.1(2), Gly 1.1(1), Ala 0.9(1), Leu 3.0(3)</td>
</tr>
<tr>
<td>C2 (22-24)</td>
<td></td>
<td>Ser 0.2(0), Glu 1.0(1), Pro 0.7(1), Gly 0.2(0), Ala 0.1(0), Leu 0.9(1)</td>
</tr>
<tr>
<td>D2 (26-31)</td>
<td></td>
<td>Ser 1.0(1), Glu 2.0(2), Pro 0.3(0), Gly 1.1(1), Ala 0.3(0), Leu 2.3(2)</td>
</tr>
</tbody>
</table>

Residue 9 was glutamine rather than glutamic acid and suggested that the Glx at position 11 was not the amide.

Fragment A1 (Fig. 5) was found to be a peptide from the central portion of the C-peptide, but was contaminated with free leucine or a peptide having amino-terminal leucine. Degradative data on this fraction could not be interpreted with certainty until a second chymotryptic digestion of the intact peptide was carried out at a higher pH. From the peptides released by this procedure Fragments A2-I and A3-I were isolated. Fragment A2-I was degraded and found to overlap Fragment D1, thus allowing the ordering of the 24 amino-terminal residues of the human C-peptide. Fragment A3-I consisted of the 9 amino-terminal residues of Peptide A2-II (Fig. 5). Although some phenylthiohydantoin-glutamine as well as phenylthiohydantoin-glutamic acid was found at position 10 in Fragment A2-II, further confirmation of the identity of this residue (position 22 of the intact peptide) was considered necessary.

The sequence of the remaining carboxy-terminal region of the C-peptide was established by degradation of chymotryptic Fragment B and confirmed by data from degradation of the mixture of fragments designated C2 (Fig. 6). The phenylthiohydantoin derivative at the carboxyterminus of Fragment B was identified as glutamine.

No fragment could be found in the chymotryptic digests which overlapped the bond at position 24–25. At least two explanations for this are possible. First the chymotryptic cleavage at this point may have been rapid relative to the other cleavages so that the split here was relatively complete after 18 hours of incubation. Chance, Ellis, and Broner (2) have shown that the cleavage at the analogous position in porcine proinsulin indeed is rapid relative to other cleavage sites. An alternative explanation could be that although chymotryptic cleavage may have occurred carboxy-terminal to the leucine at position 21 to yield a fragment overlapping the Leu-Ala bond at position 24–25, cyclization of residue 22 (glutamine) to pyrrolidonecarboxylic acid during the long period of digestion may have rendered such a peptide invisible with the ninhydrin stain.

Thus, from chymotryptic fragments alone, the amino acid...
residues of the entire C-peptide could be ordered, but three features of the primary structure remained to be elucidated; i.e., a fragment overlapping position 24-25 was needed, and the assignment of residues 11 and 22 as glutamine or glutamic acid was necessary.

As thermolysin cleaves peptide bonds in which the amino group is donated by amino acids having bulky hydrophobic side chains, such as leucine, valine, phenylalanine, and others (15), it should produce small fragments containing the regions surrounding residues 11 or 22 and the needed overlap at residues 24 and 25. Fig. 6 illustrates some of the fragments obtained by a limited digestion of the human C-peptide with thermolysin. The amino acid compositions of these are given in Table II. Fragment C2, a tripeptide, was found on Edman degradation to contain glutamine at position 2, thus establishing residue 22 of the C-peptide as glutamine. Another thermolysin peptide, Fragment B3, overlapped the Leu-Ala bond at position 24-25, in accord with the ordering of chymotryptic peptides as shown in Fig. 6.

The only residue remaining uncertain was Glx at position 11. As the intact peptide contained 8 Glx residues, and 7 of these 8 had already been assigned as either glutamine or glutamic acid, the 5th could be determined by difference using analytical data from a total enzymic hydrolysis of the intact peptide. This hydrolysis was performed using a combination of papain and aminopeptidase M and yielded the analytical data reproduced in Table I. In this method all bonds are cleaved except those of the type X-Proline. As shown in the table, no free proline appeared in the enzymic hydrolysate, as expected, whereas 4 glutamic acids and 3 glutamines, a total of only 7, were found. The absence of 1 Glx residue in the enzymic digest is explained by the Gln-Pro sequence occurring at residues 22 and 23. This glutamine therefore did not enter into the analytical stoichiometry. Since 3 of the 7 remaining residues were already known to be glutamic acid and 3 were glutamine, the unknown residue at position 11 was established by difference as glutamic acid. This assignment is in accord with all the degradative data at this position, none of which indicated the presence of phenylthiolydantoin-glutamine in addition to the observed phenylthiolydantoin-glutamic acid. In earlier reports the amide of position 9 was incorrectly assigned at position 11 (12, 25).

The carboxy-terminal region of the human C-peptide was studied further with carboxypeptidase A. Digestion of the intact peptide with this enzyme released glutamine, leucine, and serine as shown in Fig. 7, confirming that chymotryptic Fragment B contained the carboxyl terminus of the peptide. The release of glutamine and leucine was very rapid relative to the rate of serine release, in keeping with the known characteristics of this enzyme. However, the molar ratio of glutamine to leucine released never reached unity, and a small amount (approximately 15%) of alanine was released which did not appear in the reaction blank (Fig. 7). Several possible explanations for these findings can be suggested. First, a contaminating protein unrelated to the C-peptide may have been present; this seems unlikely, however, since compositional analysis of the starting material indicated the presence of only those amino acids that occur in the C-peptide. A more plausible explanation may be that small amounts of partially degraded forms of the C peptide which were not separated in the purification procedure were present in the preparation. Low levels of impurities of this kind could not be detected readily by compositional analysis. Two possible degradation products that might arise during autolysis by chymotryptic-like cleavages and account for the presence of nonstoichiometric amounts of leucine and glutamine and also for the presence of the small amount of alanine are: (a) the entire C-peptide sequence lacking only the carboxy-terminal glutamine, and (b) the C-peptide sequence through residue 26, lacking only the carboxy-terminal pentapeptide (see Fig. 6). Impurities of this kind would not be readily detected, as these would not interfere with sequence analysis studies. A third and less probable explanation is that microheterogeneity occurs in this portion of the C-peptide.

Various preliminary studies have been performed on a 31-residue peptide isolated from green monkey pancreas (Cercopithecus aethops) by a procedure essentially identical with that used in this work. Compositional data indicates that this peptide differs from the human C-peptide only by the presence of one more residue of proline and one less residue of leucine. Carboxypeptidase A digestion of the intact monkey peptide also liberated glutamine, leucine, and serine and a very small amount of alanine, as in the case of the human C-peptide. In this case the carboxypeptidase treatment released less alanine from the monkey peptide than it had from the human peptide and the glutamine to leucine ratio more closely approximated unity, in support of the above postulate that partly degraded forms of the human C-peptide may contaminate our preparations. This is a reasonable assumption since the period between death and postmortem examination, during which time the C-peptide may be exposed to exocrine proteases, was generally much longer in the human than in the monkey preparations.

On the basis of the data derived from the direct Edman degradation studies and the results of the carboxypeptidase and total enzymic analyses, the following 31 residue sequence of the human C-peptide can be proposed: Glu-Ala-Glu-Asp-Leu-Glu-Val-Gly-Gln-Val-Glu-Leu-Gly-Gly-Gly-Pro-Gly-Ala-Gln-Val-Gly-Glu-Leu-Ala-Leu-Glu-Gly-Ser-Leu-Gln-Pro-Leu-Ala-Leu-Glu-Gly-Ser-Leu-Gln.

The molecular weight of this peptide is 3021. This sequence is in agreement with the composition of the peptide (Table I) and with compositional data on human proinsulin. In subsequent experiments we have confirmed it by degradation through the

---

Fig. 7. Release of free amino acids from purified human C-peptide by carboxypeptidase A. The quantity of peptide represented by each point was approximately 0.014 μmole. (See "Materials and Methods" for procedural details.)
Human Proinsulin C-Peptide

Vol. 246, No. 5

Amino acid composition of human proinsulin

This analysis was performed on approximately 30 μg of human proinsulin from Preparation IDE-2. Hydrolysis was carried out at 110° for 30 hours in 6 N HCl. The valine and isoleucine values are low due to the resistance to hydrolysis of the Ile-Val bond at position 2-3 of the insulin A chain. The half-cystine content of the proinsulin was not determined.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Insulin</th>
<th>C-Peptide</th>
<th>Proinsulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>3</td>
<td>1</td>
<td>4.73</td>
</tr>
<tr>
<td>Threonine</td>
<td>3</td>
<td>2</td>
<td>3.29</td>
</tr>
<tr>
<td>Serine</td>
<td>3</td>
<td>2</td>
<td>5.75</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>7</td>
<td>8</td>
<td>14.80</td>
</tr>
<tr>
<td>Proline</td>
<td>1</td>
<td>2</td>
<td>2.20</td>
</tr>
<tr>
<td>Glycine</td>
<td>4</td>
<td>7</td>
<td>11.50</td>
</tr>
<tr>
<td>Alanine</td>
<td>1</td>
<td>3</td>
<td>4.50</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>4</td>
<td>2</td>
<td>4.86</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2</td>
<td></td>
<td>1.29</td>
</tr>
<tr>
<td>Leucine</td>
<td>6</td>
<td>6</td>
<td>11.30</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4</td>
<td></td>
<td>4.02</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3</td>
<td></td>
<td>2.93</td>
</tr>
<tr>
<td>Lysine</td>
<td>1</td>
<td></td>
<td>1.81</td>
</tr>
<tr>
<td>Histidine</td>
<td>2</td>
<td></td>
<td>2.15</td>
</tr>
<tr>
<td>Arginine</td>
<td>1</td>
<td></td>
<td>3.50</td>
</tr>
</tbody>
</table>

FIG. 8. Proposed partial amino acid sequence of human proinsulin based on the known structures of human insulin (29) and of the human C-peptide. The four open circles indicate the probable locations of the four additional basic residues present in human proinsulin (Table III), based on the known structures of bovine and porcine proinsulins.

Species Differences in Proinsulin C-Peptides—The primary structures of the C-peptides from the three species now known are reproduced in Fig. 9. Several interesting features of these peptides can be compared. Most striking are their variations in length by a total of 5 residues; the bovine peptide is the shortest with 26 residues and the human peptide the longest with 31 residues, while the porcine C-peptide of 29 residues is intermediate in length. In addition, the amino acid sequences vary considerably among these three species, differing over-all in approximately 50% of their residues. This is in marked contrast to the respective insulins which differ in only 3 to 4% of their amino acid residues. Despite the numerous structural differences, the peptides exhibit considerable homology throughout their entire lengths, and analysis of the minimum number of nucleotide changes required for their interconversion bears this out. Thus, all the amino acid substitutions could be accomplished by a single nucleotide change per codon, except in two instances (residues 4 and 9) in which two nucleotide changes would be required to interconvert the three peptides. The average frequency per codon of nucleotide changes has been calculated for all three species and this number is well below the level that would be encountered in comparing unrelated peptides having little or no homology (30).
The high degree of homology also provides additional evidence to support the conclusion that the pancreatic peptide that we have isolated is indeed the human proinsulin C-peptide, for it is unlikely that an unrelated peptide would exhibit such similarities with the C-peptides of the other two species. Moreover, the identity of the bovine proinsulin and pancreatic C-peptides has been established (8). It is particularly noteworthy that the terminal residues of the C-peptides are identical in all three species, providing evidence that the human sequence indeed comprises all of the C-peptide, and not just a fragment of it.

The high variability among these peptides is similar to that seen in the mammalian fibrinopeptides, which have been compared from a large number of species (31). These peptides also vary in length as well as in having many amino acid substitutions, but the residues at the sites of proteolytic cleavage by thrombin are preserved among all species.

Certain properties, such as net charge, appear to be well conserved in the proinsulin connecting segment. Thus all three peptides have 4 glutamic acid residues while the human also has an additional acidic residue of aspartic acid. The presence of this single difference in negative charge could account for the more rapid migration toward the anode of human than bovine (or porcine) proinsulin on polyacrylamide gel electrophoresis (Fig. 5). The difference in mobility on polyacrylamide gel electrophoresis is similar to that noted between insulin and mono-deamido insulin (14). The carboxy-terminal residue of the insulin A chain, asparagine, has been shown to be much more rapidly deamidated in acid solutions as a result of the presence of the free ε-carboxyl group (32). Because the insulin obtained in both cases was not significantly deamidated at position A-21, it is unlikely that either the human proinsulin or the isolated C-peptide had undergone significant deamidation during the acid isolation procedures.

Functional Aspects of Proinsulin C-Peptides—One of the most important functions of the single chain proinsulin, if not its sole function, must be to facilitate the formation of the native structure of the insulin molecule by influencing the folding of the polypeptide chain to insure the correct pairing of cysteine residues necessary for the formation of the characteristic disulfide bonds. This property of proinsulin, which was originally predicted by Anfinsen and coworkers on the basis of studies of the stability of insulin in the presence of disulfide exchange enzymes (30), has been demonstrated in experiments in vitro. When the fully reduced and unfolded intact polypeptide is allowed to reoxidize in the presence of 1 mM thiol, it regains native structure with yields as high as 70 or 80%, as judged by immunological assays (34). Under the same conditions the reduced chains of insulin recombine only to an extent of 1 or 2%. Similarly, the intermediate fraction of bovine proinsulin, which consists of material cleaved at either the A chain or B chain junctions of the connecting segment, shows no greater refolding ability than insulin chains. These results indicate that the integrity of the proinsulin polypeptide chain is a necessary prerequisite for efficient folding. The results, however, do not allow a distinction to be drawn between the possibilities, that, on the one hand, the connecting segment may specifically interact with regions in the A and B chain portions of the molecule to align these appropriately, or on the other, that the segment merely provides a highly flexible connector that converts the process of chain combination from a bimolecular to a more efficient monomolecular reaction. The large species variations observed between the connecting segments of porcine, human, and bovine proinsulin would tend to support the latter view. For example, due to the distribution of proline residues throughout the peptides (Fig. 9) it is unlikely that extensive α-helix could exist. Indeed, optical studies of porcine proinsulin have failed to detect significant α-helix beyond that attributable to the insulin portion (35).

On the other hand, as noted above, acidic residues are conserved to a considerable degree, both with respect to number and position. All three peptides have 2 glutamic acids very near the amino-terminal region, and 1 glutamic acid located 5 residues from the carboxyl end, and an additional glutamic acid residue located about a third of the way along the peptide chain (Fig. 9). This distribution suggests the possibility that these residues may interact with specific side chains in the insulin portions of the molecule and thus play a role in directing the folding of the peptide chain in a more specific manner.

Another remarkable feature of the connecting region in proinsulin is the similarity and restriction in composition which it exhibits among all of the mammalian species that have been examined, including monkey and rat (26), and extending to more primitive vertebrate species, such as cod (37) and angler (38) fish. None of these connecting peptides contain any aromatic residues, histidine, or cysteine. Clearly this must indicate the existence of some specific requirements with regard to the properties of the side chain groups in this portion of the molecule, perhaps with respect to the maintenance of an appropriate balance between hydrophilicity and hydrophobicity in the molecule as a whole (39). On comparing the peptides with regard to the polar and nonpolar characteristics of their amino acid side chains, it is clear that these are preserved when interspecies amino acid substitutions occur. At either end of the connecting peptide are regions having a high proportion of polar residues and just beyond these regions in proinsulin there are 2 additional polar basic residues. In contrast, the central portion of the connecting peptide contains a high concentration of glycine residues surrounded by residues that are largely nonpolar. This composition suggests that the central part of the molecule is more flexible and may bend back upon itself through interactions of the nonpolar side chains to produce a compact hydrophobic micelle which may interact with some hydrophobic area on the surface of the insulin moiety during peptide folding. The polar regions at both ends of the peptides may contribute to the over-all balance of hydrophilic and hydrophobic character, but perhaps more importantly, they may tend to keep these portions of the proinsulin in contact with the external solvent. This distribution might influence the folding process, and it might also help to maintain these regions on the outside of the molecule where they would be readily accessible to the proteolytic cleavage enzymes that convert the proinsulin to insulin. Clearly much further study of the conformation of the connecting region and its functional role in folding are in order before any definitive conclusions can be drawn. It seems probable, however, from these considerations that specific structural requirements do exist and that the C-peptide plays a more complex role in folding than simply acting as a string holding the insulin chains in close proximity.

Little information has yet accumulated regarding possible extrapancreatic functions of the C-peptide. It has been demonstrated that C-peptide is secreted by isolated rat islets into the incubation medium (7), and it can be detected by immunological methods in both bovine and human serum (7, 26). Some ex-
periments have indicated that the C-peptide does not affect the action of insulin upon certain tissues in vitro (40); however, these limited studies were done using bovine or porcine C-peptide in rat tissues. Due to the large sequence variation among species, these may not be expressed in all heterologous species, as in the case, for example, of growth hormone; thus any study of the action of insulin upon certain tissues in vitro (40); however, these experiments have indicated that the C-peptide does not affect the biological activity and metabolism of the C-peptide and proinsulin in man. They would also be useful as antigens for the development of specific antisera for the detection of these proteins in physiological fluids by immunoassay procedures.

Knowledge of the amino acid sequence of the human C-peptide also provides the basis for a search for mutations involving this region of the proinsulin gene in man. Certain mutations might alter the folding properties of the proinsulin molecule, especially if they involved the substitution of hydrophobic or aromatic residues for hydrophilic ones, and thus reduce the efficiency of production of insulin in the β-cells. Likewise loss or addition of basic residues through mutation could alter the susceptibility of the proinsulin to the proteolytic conversion mechanism and might lead to the retention of portions of the connecting region in the resultant insulin. Such modified insulin molecules might have lower or altered biologic activities. If the C-peptide is eventually shown to possess extrapancreatic activity, mutations in this region could have even more far reaching effects.

Acknowledgments—We are grateful to Dr. J. Schlichtkrull and coworkers at The Novo Company (Copenhagen) for provision of the crude human proinsulin fraction and for some supplies of human and monkey pancreata. We are indebted to Dr. Seymour Glasgow of the pathology department of the University of Chicago and Dr. Nathan J. Averick of the pathology department of St. Francis Hospital (Blue Island, Illinois) for collection of most of the human pancreata used in this work. We wish also to thank Dr. A. H. Rubenstein, Dr. F. Melani, and W. P. Welbourne for assistance in various aspects of this work and Mrs. H. Watson for her assistance in the preparation of the manuscript.

REFERENCES
Studies on Human Proinsulin: ISOLATION AND AMINO ACID SEQUENCE OF THE HUMAN PANCREATIC C-PEPTIDE
Philip E. Oyer, Sooja Cho, James D. Peterson and Donald F. Steiner


Access the most updated version of this article at http://www.jbc.org/content/246/5/1375

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/246/5/1375.full.html#ref-list-1