The Proteins of Bovine Pancreatic Juice

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In the years since the classical work of Northrop and Kunitz on the isolation and characterization of the pancreatic proteins, trypsinogen and chymotrypsinogen-α (1), an impressive body of knowledge has accumulated about these and other pancreatic proteins. The proteins which have been isolated in crystalline form from the pancreas gland now include trypsinogen, trypsin, chymotrypsinogens α and β, and the corresponding active enzymes, chymotrypsins α and β, carboxypeptidase, ribonuclease, and deoxyribonuclease (2-5). Each of these proteins has been extensively characterized with respect to its physicochemical properties; the amino acid composition is known for most of them (6-8), and, in the case of ribonuclease, a large segment of the amino acid sequence is known (9). In addition, the chemical events involved in activation of the zymogens, trypsinogen, chymotrypsinogen-α and procarboxypeptidase to their respective enzymatically functional forms have been studied in detail (9-11).

With the exception of crystalline carboxypeptidase and its precursor, procarboxypeptidase, all of the proteins mentioned have been isolated from acid extracts prepared by submerging the freshly collected glands in 0.25 M sulfuric acid. Conditions so far removed from the physiological environment of the cell constituents inevitably raise the question as to whether the crystalline proteins thus prepared do in fact represent the proteins elaborated by the gland. In the present paper, evidence is presented that, at least in the cases of trypsinogen, chymotrypsinogen-α, chymotrypsinogen-B, ribonuclease and procarboxypeptidase, the proteins isolated directly from bovine pancreatic juice by ion exchange chromatography conform closely to their previously isolated counterparts. The present work, which was initiated as part of a broad study of the biosynthesis of the pancreatic proteins, has also led to the establishment of the chromatographic sequence of the major components of bovine pancreatic juice and their relative proportions.

EXPERIMENTAL

Materials and Methods

The choice of bovine pancreatic juice as starting material was based on two considerations: (a) all of the proteins cited are components of the extracellular secretion of the pancreas gland; (b) since the crystalline pancreatic proteins have been prepared routinely from beef glands, the problem of species differences can be circumvented.

Operation upon Animals—With the cooperation of members of the College of Veterinary Medicine at Washington State College, several yearling steers were operated upon. Permanent fistulas were introduced into the pancreatic duct and into the small intestine in such a way as to allow for periodic external collection of the juice. At all times other than the actual collection, the pancreatic juice was simply re-routed to the small intestine. Such animals could thus be maintained for several weeks in good physiological condition. The pancreatic juice was collected in sterile containers at 0° and frozen immediately after collection. Subsequently, the samples were thawed and lyophilized for storage as dry powders.

Crystalline trypsinogen, soy bean trypsin inhibitor and deoxyribonuclease were obtained from Worthington Biochemical Corporation. Chymotrypsinogen-α, 9 times crystallized, was prepared by W. J. Dreyer in this laboratory. Crystalline ribonuclease was purchased from Armour and Company. Chymotrypsinogen-B, 4 times crystallized, was a gift from Dr. M. Laskowski of Marquette University.

Protein concentrations were determined spectrophotometrically at 280 mμ, with the following extinction coefficients for a 1 per cent solution: 20 for chymotrypsinogen-α (6); 18 for chymotrypsinogen-B (12); 13.9 for trypsinogen (13); 10.5 for the soy bean trypsin inhibitor (14); 19 for procarboxypeptidase (11); 23 for carboxypeptidase (13); 7.1 for ribonuclease; and 11.4 for deoxyribonuclease (15). A mean value of 18 was used to estimate the protein concentration of unfractionated pancreatic juice.

Methods of Assay—Tryptic and chymotryptic activities were measured by titration at constant pH, with the use of the specific synthetic substrates BAEE for trypsin and ATEE for chymotrypsin (15). Carboxypeptidase activity was measured against two substrates, viz. CGP for carboxypeptidase-A (15) and BGL for carboxypeptidase-B.4 Nucleolytic activities were measured spectrophotometrically according to the methods of Northrop and Kunitz (6), which differs from Anson’s enzyme in that it acts specifically on basic C-terminal residues. Their respective precursors will be designated as procarboxypeptidase-A and procarboxypeptidase-B.
Dickman et al. (17) for ribonuclease and Kunitz (18) for deoxyribonuclease.

**Protein Components of Bovine Pancreatic Juice**

Before attempting resolution of the protein components of bovine pancreatic juice, the concentrations of several enzymes and zymogens in the juice were estimated. Proteolytic activities in freshly thawed samples was usually negligible; accordingly, aliquots of pancreatic juice were activated by the methods which have been described for trypsinogen (13), chymotrypsinogen-α (19) and procarboxypeptidase-A (11). Proteolytic activities in activated pancreatic juice are presented in Table I. Arbitrarily defined units were used to express each enzymatic activity in 1 ml. of the whole juice. This same definition of units was then applied to express the specific activity of the corresponding crystalline pancreatic protein, and the concentration of that protein in the juice was computed. Only in the case of procarboxypeptidase-A have the concentrations of the zymogen, as well as the enzyme, been included, since, in contrast to other pancreatic zymogen-enzyme systems, the zymogen, procarboxypeptidase-A, is a larger molecule (mol. wt. = 96,000) than the enzyme, carboxypeptidase-A (mol. wt. = 34,000) (11). Ribonuclease activity was measured on nonactivated pancreatic juice.

The amylase and lipase activities in bovine pancreatic juice were measured (20, 21) and found to be very low; 1 mg. of protein from unfractionated pancreatic juice contained an amount of amylase activity equivalent to approximately 16 μg. of crystalline hog amylase. Thus, if the specific activities of bovine and hog amylases are equal, amylase constitutes less than 2 per cent of the total protein of bovine pancreatic juice. Unfractionated bovine pancreatic juice contained only 1.2 Borgstrom units of lipase activity per mg. of protein, as compared with 20 to 40 units per mg. of protein from rat pancreatic juice and 59 units per mg. of protein from human pancreatic juice (21).

**Ion Exchange Chromatography**

For resolution of the pancreatic proteins, two ion exchange resins were used. An initial separation into anionic and cationic components was made at pH 8.0 on the DEAE-cellulose resin described by Peterson and Sober (22). The anionic proteins which were adsorbed to this resin were subsequently resolved by a concentration gradient, whereas the cationic components were collected and chromatogrammed on the Amberlite cation exchange resin, XE-64. A typical experiment is described below. Unless otherwise indicated, all operations were conducted at cold-room temperature (approximately 4°).

An amount of lyophilized pancreatic juice corresponding to 335 mg. of protein was dissolved in 75 ml. of potassium phosphate buffer (0.005 M, pH 8.0) containing 9 mg. of soy bean trypsin inhibitor. Diisopropylphosphofluoridate was added to a final concentration of 10⁻³ M and the solution was stirred vigorously for 60 minutes at 0°. The solution of protein was then dialyzed against two changes of 2 liters of potassium phosphate buffer (0.005 M, pH 8.0) containing 10⁻⁴ moles of diisopropylphosphofluoridate per liter.

The DEAE-cellulose resin was equilibrated and packed into a column as has been described (23). The dimensions of the resin column were 1.8 cm. × 70 cm. After application of the protein solution to the column, a continuous flow of potassium phosphate buffer (0.005 M, pH 8.0) was begun and fractions of 10 ml. were collected. The optical density of each fraction was measured at 280 μm.

It was known, from preliminary experiments, that the soy bean inhibitor was held to the resin along with the anionic pancreatic proteins. Trypsinogen, ribonuclease and part of the chymotrypsinogen of pancreatic juice were not adsorbed on DEAE-cellulose at this pH, but emerged as a large protein peak, i.e. the break-through peak, immediately after displacement of the hold-up volume. Accordingly, further precautions were taken against activation of the zymogens in the break-through peak during chromatography. As soon as protein material had appeared in the effluent, use of the automatic fraction collector was discontinued and the effluent was collected directly into a dilute solution of soy bean trypsin inhibitor in the amount of 1 mg./ml. At intervals, aliquots of effluent were collected separately for optical density measurements.

When the material in the break-through peak had been collected and the absorption at 280 μm had returned to a baseline value, gradient elution of the anionic proteins was begun. A linear gradient from 0.005 M to 0.4 M potassium phosphate, pH 8.0, was established. Fig. 1 presents the elution diagram for this experiment. All fractions containing significant amounts of protein were lyophilized as soon as possible and held as dry powders at -20°, for subsequent characterization.

**Anionic Components**

The anionic components of pancreatic juice were tested, before and after incubation with trypsin, against a series of substrates...
TABLE II
Enzymatic properties of anionic components of bovine pancreatic juice

<table>
<thead>
<tr>
<th>Anionic component</th>
<th>BAEE</th>
<th>ATEE</th>
<th>CGP</th>
<th>BGL</th>
<th>DNA</th>
<th>RNA</th>
<th>Tryptic activation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Not required</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Required</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
<td>trace†</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Required</td>
</tr>
<tr>
<td>4</td>
<td>+‡</td>
<td>+‡</td>
<td>+‡</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Not required</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Required</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Required</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Required</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Required</td>
</tr>
</tbody>
</table>

* The conditions of activation, when required, are given in the text.
† Probably due to overlap of Peaks 1 and 3.
‡ Not tested.

TABLE III
Comparison of anionic Component 2 with chymotrypsinogens α and B

The properties of Component 2 were determined as described in the text. The properties of chymotrypsinogens α and B were determined as described elsewhere (25, 26).

<table>
<thead>
<tr>
<th>Property</th>
<th>Component 2</th>
<th>Chymotrypsinogen α</th>
<th>Chymotrypsinogen B</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε∞/c = w</td>
<td>2.53 S</td>
<td>2.49 S</td>
<td>2.53 S</td>
</tr>
<tr>
<td>p I</td>
<td>5.1</td>
<td>5.2</td>
<td>&gt;9.0</td>
</tr>
<tr>
<td>Effluent concentration (phosphate)</td>
<td>0.05 M</td>
<td>0.048 M</td>
<td>Not held</td>
</tr>
<tr>
<td>k*SB, ATEE</td>
<td>1.43</td>
<td>1.43</td>
<td>2.2</td>
</tr>
<tr>
<td>k*SB, ATEE</td>
<td>0.065</td>
<td>0.063</td>
<td>0.64</td>
</tr>
</tbody>
</table>

* Moles of substrate hydrolyzed/liter/min./mg. N/ml.

chosen to reveal specific endopeptidase, exopeptidase or nuclease action. A qualitative summary of the results is presented in Table II.

Components 1 and 3—It will be noted that both Components 1 and 3 have the specificity of carboxypeptidase-B, i.e. they catalyze the release of free lysine from the peptide, BGL (16). Neither of these components is active toward CGP, the specific substrate for carboxypeptidase-A. However, Component 1 was fully active toward BGL without tryptic activation, Component 3 required tryptic activation (0.1 mg. protein N plus 0.01 mg. trypsin N per ml. of pH 8 tris(hydroxymethyl)-aminomethane buffer at 25° for 30 minutes). Accordingly, Component 3 may correspond to procarboxypeptidase-B, as prepared by Folk and Gladner from acetone powder of beef pancreas glands.8

Component 1 cannot be unequivocally identified at this time. Two proteins with enzymatic properties similar to those of Component 1 have been described: (a) carboxypeptidase-B, the activation product of procarboxypeptidase-B (16), and (b) protaminase, as prepared by Weil and Telka (24) from acetone powders of hog pancreas. Both of these enzymes are reported specifically to remove basic amino acid residues from a carboxy-terminal position, but neither of the proteins has been characterized as a physicochemical entity. Hence, Component 1 could correspond either to protaminase or to the product of activation of Component 3 (procarboxypeptidase-B). It is significant in this respect that in this experiment no other zymogen of the pancreatic juice had become spontaneously activated.3 Component 2 accounts for approximately 15 per cent of the total protein of bovine pancreatic juice. As shown in Table II, it is active toward the synthetic substrate ATEE, and requires tryptic activation. It resembles in these respects the pancreatic chymotryptosinogens, chymotrypsinogen-α and chymotrypsinogen-B. Comparison of the physicochemical and enzymatic properties of Component 2 with those of chymotrypsinogens α and B indicated this protein to be identical with Laskowski’s chymotrypsinogen-B (cf. Table III).

Solutions of Component 2 were analyzed in the ultracentrifuge (Spinco model E) in sodium acetate-NaCl buffers of ionic strength 0.2, pH 4.0. The protein was ultracentrifugally homogeneous, and sedimentation constants were obtained at four protein concentrations over the range of 0.3 to 1.0 per cent protein. Extrapolation of these data to minute dilution gave the value s20,w = 2.53 Svedberg units for Component 2. Under the same experimental conditions, Smith et al. (25) found the sedimentation constants of chymotrypsinogen-α and chymotrypsinogen-B to be 2.53 and 2.49 Svedberg units, respectively. Component 2 was examined electrophoretically at three pH values in buffers of the composition used by Kubacki, Brown, and Laskowski in their comparison of the physicochemical properties of chymotrypsinogens α and B (26). The sample of Component 2 used for these experiments was not electrophoretically homogeneous, but the electrophoretic mobilities of the major component at pH 5.13, 5.6 and 6.15 with sodium acetate buffers of ionic strength 0.1, were, respectively, -0.05 × 10⁻³, -0.99 × 10⁻³, and -1.20 × 10⁻³ sq.cm./volt sec. The isoelectric point is thus near pH 5.1. Under the same experimental conditions, Kubacki et al. found the isoelectric point of chymotrypsinogen-B to be 5.2, whereas chymotrypsinogen-α is isoelectric at a pH greater than 9.1 (26).

The chromatographic behavior of chymotrypsinogen B and the present anionic chymotrypsinogen were compared on DEAE-cellulose columns, 0.9 cm. × 33 cm., at pH 8.0. Each protein was equilibrated with 0.005 M potassium phosphate, pH 8.0, and applied to a separate column of DEAE-cellulose which had been equilibrated with the same buffer. A linear gradient was established in both cases between 0.005 M and 0.2 M potassium phosphate, pH 8.0. The concentration of phosphate buffer in the eluent fractions containing the maximal concentration of eluted protein was measured, and found to be 0.05 M for Component 2 and 0.048 M for chymotrypsinogen B. Chymotrypsin-α is not adsorbed at this pH on the anion exchange resin, DEAE-cellulose. For study of their enzymatic properties, solutions of the respective chymotrypsinogens were prepared in 0.04 M phosphate buffers, pH 8.0. Trypsin was added to approximately 2 per cent by weight and the activation reaction was allowed to proceed to completion at 0°. Two synthetic substrates were used

8 Arbitrarily, in this manuscript the protein of Component 1 will be designated carboxypeptidase-B, because of its bovine origin.
for comparison of the relative activities of the enzymes: ATEE, 0.01 m in 0.015 m phosphate-0.1 m KCl buffer, pH 7.8; and ATRyEE, 0.01 m in 0.01 m phosphate-0.07 m KCl buffer, pH 7.8, containing 30 per cent methanol. As is shown in Table III, Component 2 corresponds closely to authentic chymotrypsinogen-B in its enzymatic properties. By sedimentation and by chromatographic, enzymatic and electrophoretic analyses, therefore, the anionic chymotrypsinogen of pancreatic juice corresponds to Laskowski's crystalline chymotrypsinogen-B.

Component 4, accounting for approximately 2 per cent of the proteins of pancreatic juice, is the only anionic component which catalyzes the hydrolysis of thymus DNA. At the time of assay, the specific activity of this component was only one-half that of crystalline deoxyribonuclease (18). It seems probable that the lower activity results either from instability of the enzyme in the dilute effluent fractions or from contamination of Peak 4 with adjacent inert protein.

Components 5 and 6 have eluded enzymatic characterization, except in the negative sense portrayed in Table II. Neither component showed a significant level of activity, before or after tryptic treatment, against any of the synthetic substrates which have been tested, nor did they possess significant lipolytic or amylolytic activity. Each component was examined ultracentrifugally at a protein concentration of about 0.5 per cent. The sedimentation constants of Components 6 and 7 at this concentration were 2.65 and 4.5 Svedberg units, respectively.

Components 7 and 8 correspond, in all respects, to preparations of procarboxypeptidase previously studied by the authors (23). As is shown in Table II, these two components have identical enzymatic properties, e.g., an endopeptidase action on ATEE and an exopeptidase action on CGP. Tryptic activation is required for both activities. As was observed with procarboxypeptidase-A, prepared from acetone powders of beef pancreas, the two enzymatic activities appear at different rates. Thus, activation of the zymogen to the endopeptidase proceeds rapidly at low temperatures and low concentrations of trypsin, whereas conversion to the exopeptidase requires incubation at 37°, with relatively high concentrations of trypsin and involves, in addition, participation of the ATEE-hydrolyzing enzyme. These relationships are being further investigated. The sedimentation constants of Components 7 and 8 were measured at a single protein concentration of approximately 1 per cent and found to correspond to the sedimentation constant of procarboxypeptidase-A (5.86 Svedberg units). Moreover, the behavior of these components on DEAE-cellulose is the same as the behavior of previously isolated procarboxypeptidase (23).

**Cationic Components**

Portions of the breakthrough peaks from DEAE-cellulose columns were again subjected to chromatography, with use of the Amberlite cation exchange resin XE-64. For comparison, mixtures containing known proportions of several crystalline pancreatic proteins were prepared and analyzed under the same conditions. Fig. 2 presents the results of a typical experiment. A solution containing 2.3 mg of soy bean trypsin inhibitor, 4.3 mg of chymotrypsinogen-α and 8.5 mg of trypsinogen in 1 ml of potassium buffer (0.2 m, pH 6.0) was prepared. Another solution was prepared, containing 11 mg of lyophilized break-through proteins in 1 ml of the same buffer. Each solution was chromatographically analyzed on XE 64, according to the procedure of Hirs, Stein, and Moore (7), except that the experiment was conducted at 4° rather than at room temperature. It will be noted (cf. Fig. 2) that protein peaks appeared at the same effluent volumes in the two diagrams. Moreover, enzymatic assays have shown that in both patterns Peak 1 is the added soy bean trypsin inhibitor, Peak 2 is trypsinogen, and Peak 3 is chymotrypsinogen-α. Upon activation, the zymogens of pancreatic juice attained the same specific activities as did their crystalline counterparts.

In subsequent experiments, crystalline ribonuclease was added to the synthetic mixture of proteins, and chromatography was performed at pH 6.47. Trypsinogen and chymotrypsinogen-α were poorly resolved at this pH, but a component corresponding to ribonuclease-A was found to exist in the pancreatic juice. Although no component corresponding to ribonuclease-B was seen (28), it cannot be said in the light of these experiments that this protein is not present in pancreatic juice.

In personal communications we have learned that Dr. Harris Tally has developed a similar method for the chromatographic analysis of trypsinogen on XE-64 at 4°, wherein the effluent volume is approximately 20 ml; Dr. Stanford Moore informed us that he has also observed chymotrypsinogen-a to emerge at about 44 ml when chromatography was carried out in the cold.
pancreatic juice. Ribonuclease-B may be present at a concentration too low to have been detected, or could conceivably have been masked by the relatively larger peaks of trypsinogen and chymotrypsinogen-a.

A composite diagram of the anionic and cationic components of pancreatic juice which were resolved by the two resins is presented in Fig. 3. For purposes of presentation, data from the cation exchange column (XE-64) were adjusted to conform to the scale of the anion exchange column, DEAE-cellulose. The components are arranged, in order of decreasing basicity, from ribonuclease-A to procarboxypeptidase-A. The relative proportions of the respective components, as estimated from optical density measurements, are presented in Table IV. In general, these data are in agreement with the earlier estimates (cf. Table I) made from enzymatic activities of unfractionated pancreatic juice. It is noteworthy, moreover, that the chromatographic spectra of different samples of bovine pancreatic juice have been quite similar, qualitatively and quantitatively. Although the absolute concentration of protein in the secretion have been quite similar, qualitatively and quantitatively. Table IV) made from enzymatic activities of unfractionated pancreatic juice. It is noteworthy, moreover, that the chromatographic spectra of different samples of bovine pancreatic juice have been quite similar, qualitatively and quantitatively. Although the absolute concentration of protein in the secretion have been quite similar, qualitatively and quantitatively.

The relative roles of the two pancreatic chymotrypsinogens remain unknown. Bovine pancreatic juice contains the basic chymotrypsinogen-a and the acidic chymotrypsinogen-B in equivalent concentrations. Although qualitatively similar in their specificities (33), chymotrypsins from the α and β families hydrolyze their substrates at different rates (34), and the relative reaction rates are influenced by the conditions of assay as well as the nature of the substrate (35). In the present case, the activities of chymotrypsin-a and chymotrypsin-B were studied under a limited set of conditions primarily for the purpose of comparison. More data would be required to evaluate the significance of the different ratios of activity observed with the two synthetic substrates, ATEE and ATryEE (cf. Table III).

**DISCUSSION**

By means of ion exchange chromatography, approximately 90 per cent of the protein of bovine pancreatic juice can be recovered as discrete components, and about 76 per cent of the protein can be related to known pancreatic proteins. By the criteria applied, which have included chromatographic behavior, enzymatic activities, and in some cases physicochemical properties, five components of bovine pancreatic juice were found to be identical to their previously isolated counterparts. These are trypsinogen, chymotrypsinogen-a, chymotrypsinogen-B, procarboxypeptidase-A, and ribonuclease-A. Although the comparative study was not extended to the molecular level, for identification of end groups, activation-peptides, etc., the chromatographic identity of these five proteins of pancreatic juice with the corresponding crystalline proteins obtained from acid extracts of the pancreas gland provides strong evidence for their identity.

A large proportion of the total proteins of bovine pancreatic juice is composed of proteolytic enzymes or their respectivezymogens. The proteolytic components which have been identified account for 72 per cent of the total protein of bovine pancreatic juice, whereas only 4 per cent of the protein has nucleolytic activity. The proteolytic activity is composed of several endopeptidases including trypsin, chymotrypsins of the α and β family, and two exopeptidases, both of which are directed toward release of carboxyl-terminal residues. No amino peptidase activity was detectable in unfractionated pancreatic juice.

The low levels of amylase and lipase activities in bovine pancreatic juice are consistent with the digestive habits of the ruminant animal (30). Probably a variety of enzymes, other than those cited herein, are present in the pancreatic secretion in low concentrations, but possibly with high enzymatic activity.

No attempt was made to identify the enzyme or enzymes responsible for elastolytic action, since, although the preparation of crystalline elastase from beef pancreas gland has been reported (31), no properties of the crystalline protein were given. Moreover, crystalline preparations of elastase from other sources (32) have been shown to be complex in nature and to exhibit at least two activities. It has not been ruled out that one of these activities is due to the well known pancreatic protein, chymotrypsin-a.

The relative activities of the two pancreatic chymotrypsinogens remain unknown. Bovine pancreatic juice contains the basic chymotrypsinogen-a and the acidic chymotrypsinogen-B in equivalent concentrations. Although qualitatively similar in their specificities (33), chymotrypsins from the α and β families hydrolyze their substrates at different rates (34), and the relative reaction rates are influenced by the conditions of assay as well as the nature of the substrate (35). In the present case, the activities of chymotrypsin-a and chymotrypsin-B were studied under a limited set of conditions primarily for the purpose of comparison. More data would be required to evaluate the significance of the different ratios of activity observed with the two synthetic substrates, ATEE and ATryEE (cf. Table III).

**SUMMARY**

A chromatographic system has been developed for the quantitative separation of the protein components of bovine pancreatic juice, obtained from steers with a permanent pancreatic fistula. With the use of cellulose ion exchange columns, eight anionic protein components were separated from one another, six of
which could be identified, respectively, as carboxypeptidase-B,
procarboxypeptidase-B, chymotrypsinogen-B, deoxyribonuclease
and the two forms of procarboxypeptidase-A. Resolution of the
cationic components on XE-64 yielded, respectively, trypsinogen,
chymotrypsinogen-α, ribonuclease-A, chymotrypsinogen-B
and procarboxypeptidase-A were found to correspond chromato-
graphically and enzymatically to the forms previously isolated
from beef pancreas glands.

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