EFFECT OF DIETARY PROTEIN ON PROTEOLYTIC ACTIVITIES IN THE PANCREATIC TISSUE AND CONTENTS OF THE SMALL INTESTINE IN RATS

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Summary The time-courses of proteolytic activities in pancreatic tissue and the contents of the small intestine (the intestinal contents) were determined in rats maintained on a diet containing 30% of various proteins after a switchover from a diet containing 12% casein.

1. The proteolytic activity of the pancreatic tissue quickly responded to change of dietary proteins—within 1 to 6 days—with respect to organ weight, nitrogen content and proteolytic activity, in rats receiving diets containing 30% casein, ovalbumin, lactalbumin, gluten, gelatin or zein.

2. However, the proteolytic activity in the intestinal contents did not necessarily coincide with the pancreatic digestive function; an approximately threefold increase of enzyme activity was demonstrated on the fifth day of feeding in rats receiving gluten.

3. The proteolytic activity in the intestinal contents returned to the initial level on the eighth day in the gluten-fed rats, but those rats maintained on a lysine-supplemented gluten diet exhibited no such elevation of proteolytic activity.

4. No significant difference in pancreatic composition was shown up to the eighth day between the group receiving gluten alone in diet and that receiving the same diet but supplemented with lysine, under the condition of equally restricted food intake. Intestinal trypsin and chymotrypsin levels, however, were higher in the gluten-fed rats, suggesting that the depressed rate of enzyme inactivation in the small intestine might be the principal cause of the finding described under (2) above.

Harper investigated the digestive process of proteins (1,2) and the effect of other food factors on protein digestion (3) primarily by measuring nitrogen content in the alimentary tract in rats (4). Attempts to elucidate the mechanism of protein digestion by way of assays of nitrogen in the contents of the small intestine (the
intestinal contents), however, involve a few intricate problems, since, as has been pointed out by OCHOA-SOLANO and GITLER (5) and by NASSET and Ju (6) the level of endogenous nitrogen in the intestinal contents is normally remarkably high (80 to 90%).

According to NASSET and Ju (6), the amino acid pattern in the intestinal contents is virtually constant irrespective of proteins ingested. They suggest that amino acid absorption from the intestinal tract may take place only after modification of the amino acid composition of the digested protein to a definite pattern with the aid of endogenous nitrogen and that, thereby, protein digestion is affected by the degree of modification. It is a matter of course, nevertheless, that a pattern close to the constant one occurs as long as endogenous nitrogen is sufficient. Published studies demonstrate that the effect of diet on proteolytic activity in the pancreas manifests itself fairly soon after ingestion, and this is generally believed to indicate rapid and sensitive response of the digestive function to diet. For example, effects of saccharides on pancreatic amylase and trypsin were reported by HOWARD and YUDKIN (7) while SNOOK (8) investigated changes in trypsin and chymotrypsin activities in the pancreatic tissue and the intestinal contents following ingestion of casein, egg protein, hydrolysates of these proteins and non-protein diet. Also reported by IMONDI and BIRD (9) are changes of pancreatic weight and enzyme activity observed in association with the alteration of protein content in the diet. All these observations indicate that the dietary influences are reflected very quickly in pancreatic enzyme activity.

In view of these studies, the present authors performed experiments in an attempt to clarify the effect of dietary protein on pancreatic function and on proteolytic enzyme in the intestinal contents by investigating the time-course of changes in proteolytic activity in these viscera following ingestion of various dietary proteins.

EXPERIMENTAL

1. Procedure of animal experiments

Male rats of the Wistar strain were housed individually in wire-bottomed cages and maintained on a preliminary 12% milk casein diet (pre-experimental diet) for 10 days. Rats ranging from approximately 200 to 250 g in weight were divided into groups of 6 each and were then given with 30% protein diets (experimental diet). Nitrogen contents of protein sample used for rat diet were estimated by the Kjeldahl method. Protein concentrations in the pre-experimental and experimental diets were confirmed to be 12% and 30%, respectively. Other ingredients of both diets were a salt mixture (HARPER, 10), 4%; soy bean oil, 5%; a water-soluble vitamin mixture bulked with lactose (HARPER, 10), 1%; choline chloride, 0.15%; vitamin A, 300 I.U.; Vitamin D₂, 0.75 μg; and pregelatinized cornstarch, 100%.
The rats were placed on the following dietary programs:

1) Various protein diets. The rats were given ad libitum either a diet containing 30% protein, i.e., casein, ovalbumin, gelatin, zein, lactalbumin or gluten, or a non-protein diet for 5 to 6 days during which time some of the rats were sacrificed at given intervals.

2) Gluten or lysine-supplemented gluten diet. The rats were supplied daily with equal limited amounts of diet containing 30% gluten or 30% gluten supplemented with lysine. Lysine was supplied to gluten at a ratio of 1:40 (II).

Upon completion of the programmed feeding, all rats were sacrificed by decapitation with exsanguination, followed by evisceration of the small intestine and the pancreas. The contents of the small intestine were then washed out several times with cold physiological saline. The washed-out contents were then diluted up to 25 or 50 ml with saline, homogenized and centrifuged at 20,000 × g, and the supernatant was diluted appropriately to be used as enzyme solution for assays.

2. Enzyme assays

1) Activation of pancreatic zymogen. After homogenization of 25 mg of the pancreatic tissue with 1 ml of cold saline, 0.5 ml of 0.1 M phosphate buffer (pH 5.6) and 1 ml of 0.5% enterokinase (Tokyo Kasei Co., Ltd.) were added to the homogenate and the mixture incubated at 30°C for 30 min to activate the zymogen. The reaction was terminated with 2.5 ml of 0.04 N HCl, and 5 ml of 0.5% CaCl₂ was added to obtain an enzyme solution for assay.

2) Assay for proteolytic activity (12). Assay was carried out using bovine hemoglobin as the substrate. The proteolytic activity was determined by Folin's method and expressed as the equivalent of µg tyrosine per minute.

3) Trypsin and chymotrypsin assays. These enzymes were assayed spectrophotometrically as described by HUMMEL (13), using tosylarginine methylester (TAME) or benzoyltyrosine ethylester (BTEE) as the substrate for trypsin or chymotrypsin, respectively. The enzyme activities were estimated thereby from the absorbance at a wavelength of 247 and 256 nm, respectively.

RESULTS

The time-courses of proteolytic activity in the pancreatic tissue of rats maintained on various dietary proteins are shown in Figs. 1, 2 and 3.

Rats receiving ovalbumin, lactalbumin or casein exhibited a sharp rise in proteolytic activity in units (equivalent of µg tyrosine/min) per mg of tissue in the initial 2 to 3 days, followed by a rather modest change. In the groups receiving gluten, gelatin, zein or non-protein diet, in contrast, the enzyme level fell to degrees corresponding with the quality of respective protein in the initial 1 to 2 days, with a subsequent recovery nearly to the initial level or a slightly higher level, except in the non-protein diet group.

As can be seen from Fig. 1, the differences in pattern between the variously
treated groups are more clearly seen when expressed as total proteolytic activity of the organ in toto.

Fig. 1. Effect of dietary protein on the total proteolytic activity in pancreatic tissue.

Fig. 2. Effect of dietary protein on proteolytic activity in pancreatic tissue (1).

Fig. 3. Effect of dietary protein on proteolytic activity in pancreatic tissue (2).

Fig. 4. Effect of dietary protein on the weight of pancreas.

Further comparisons made in terms of pancreatic weight and nitrogen content are illustrated in Figs. 4 and 5. With ovalbumin, both the weight and nitrogen content of the pancreas increased as in Figs. 4 and 5, respectively; whereas in rats receiving zein or gelatin, the nitrogen content continued to diminish over the first three days as in the case of enzyme activity, with subsequent diminution of pancreatic weight along with a recovery to the initial level of the proteolytic activity per unit weight of tissue. Effects of the various dietary proteins on proteolytic activity in the intestinal contents are shown in Figs. 6 and 7. As evident from
Fig. 5. Effect of dietary protein on pancreatic nitrogen content.

Fig. 6. Effect of dietary protein on proteolytic activity in the contents of the small intestine (1).

Fig. 7. Effect of dietary protein on proteolytic activity in the contents of the small intestine (2).

Fig. 8. Effect of supplemented lysine on proteolytic activity of the contents of the small intestine of gluten-fed rats.
are the patterns with the gluten group showing a marked elevation of activity between the second and fifth days of feeding.

In an attempt to explore the cause underlying such a peculiarity of pattern displayed by the gluten group, a group of rats fed the same diet but supplemented with lysine (hence gluten plus lysine) was compared with a group supplied gluten alone in the diet. Fig. 8 illustrates the pattern of proteolytic activity in the intestinal contents shown by the supplemented group as well as by the group fed gluten alone in the diet. The latter group displayed a peak activity on the fifth day (Fig. 8), followed by a return to the initial level three days later, while in the group on the lysine-supplemented diet the proteolytic activity did not fluctuate significantly and remained rather constant throughout the eight-day period.

It follows that no remarkable difference could be observed between the two groups in the effects of lysine supplementation on the proteolytic activity, nitrogen content and weight of pancreatic tissue, all of which evidently increased on the eighth day of feeding.

Specific activities of such pancreatic enzymes as trypsin and chymotrypsin among various proteolytic enzymes in the intestinal contents increased in the gluten-fed group on fifth and eighth days, and were higher as compared with the gluten plus lysine group (Fig. 9).

![Fig. 9. Effect of supplemented lysine on tryptic and chymotryptic activities of the contents of the small intestine of gluten-fed rats.](image)

DISCUSSION

The observed patterns of changes in various pancreatic and small intestinal parameters of rats following a switchover from a diet containing 12% casein to experimental rations containing 30% of various proteins indicated unexpectedly quick changes of the digestive function in response to changes of dietary proteins. Previously published studies by Howard and Yudkin (7) and Snook (8) also suggested this kind of rapid response.
The effects of these proteins were studied in a 30\% protein level in diet rather than a 12\% diet to obtain more remarkable results. Therefore, the 30\% casein group is taken as the control group for examining the effect of protein level on digestive function.

This paper reports the effects of various dietary proteins on digestive function of the pancreas and small intestine, viz. proteolytic activity, organ weights and nitrogen contents in rats maintained on a diet containing ovalbumin, lactalbumin, casein, gluten, zein, gelatin and gluten with lysine supplementation.

The findings noted in these experiments indicate that the digestive function of the above-stated organs in rats generally exhibit a rapid response to dietary proteins or protein level, except for gluten, these changes observed in pancreas and small intestine being roughly correlated to nutritional value of the protein given. Such changes are particularly prominent and quick in the pancreas of rats receiving ovalbumin; an approximately threefold increase of total proteolytic activity in pancreas in toto was noted as early as the third day of feeding. These rats showed pancreatic weight gains by about 60\% on the third day and by 80\% on the sixth day respectively over the initial level. Their pancreatic nitrogen content also increased progressively. The results obviously show that the pancreas of the rats receiving 30\% ovalbumin diet was influenced by the intake of protein of high nutritional value and the amount of ingested protein.

In the group of rats fed zein or gelatin, the proteolytic activity and the nitrogen content of the pancreatic tissue diminished in the initial course, which was followed by a pancreatic weight loss. On the sixth day, both the proteolytic activity level per mg tissue weight and the nitrogen content recovered nearly to the initial values, suggesting a restoration of the balance between pancreatic weight and protein synthesis.

In sum, certain diet-dependent changes of the pancreas were apparently related to the nutritional value of dietary proteins. These changes are brought about by many factors which mainly influence protein or enzyme synthesis.

Results obtained from the previous experiments demonstrate that at least two distinct patterns exist in the pancreatic responses to change of dietary protein. In the first type, changes in enzyme level tend to appear in the same direction if not in magnitude. The second type of pancreatic responses to diet is characterized by the inverse variation among the levels of pancreatic enzymes. As an example of the latter instance, REBOUND et al. (14) demonstrated that rats fed a protein-rich diet incorporated labeled amino acids more rapidly into trypsinogen and chymotrypsinogen, but less rapidly into amylase. SNOOK (15) also proposed that the second type of response occurred in association with changes in carbohydrate nutrition and/or metabolism, and the effect of glucose and insulin were involved in the response.

These intrapancreatic events may influence the proteolytic activity in the intestinal contents. With an ovalbumin or gelatin diet as well as with a non-
protein diet, the proteolytic activity in the intestinal contents became practically constant within a day or two following the preliminary feeding by the experimental diet. Rats receiving zein exhibited a decline of enzyme activity up to the sixth day. Conversely, rats given gluten, lactalbumin or casein showed a rise in enzyme activity, the level on the fifth or sixth day being as high as about three times the initial level in rats given gluten in particular. This seems to imply that the proteolytic activity in the intestinal contents is not only affected by pancreatic exocrine output, but also by various other factors as well e.g., intestinal secretion and changes of enzymes taking place during digestion and absorption, especially intraluminal activation or inactivation of proteolytic enzymes. The authors take the view that the mechanism of protein digestion may be elucidated by investigating the variety of factors which affect the proteolytic activity in the intestinal contents. A further attempt, therefore, was made to account for the qualitative effects by comparison between two dietary regimens: gluten alone and gluten with lysine supplementation.

The elevation of proteolytic activity in the intestinal contents of rats receiving gluten is rather transient in nature, and the rats in a gluten-plus-lysine group do not display such an elevation. No significant difference in pancreatic tissue constituents was demonstrated between the two groups. However, the gluten-alone group displayed higher levels of trypsin and chymotrypsin which are secreted from the pancreas, thus suggesting their involvement in the enhancement of proteolytic activity in the intestinal contents.

LYMAN and WILCOX (16) also observed that the proteolytic activity in the intestinal contents is higher in rats fed an amino acid mixture deficient in threonine rather than an intact mixture resembling amino acid composition of casein, whereas proteolytic activity in the pancreatic tissue is lower in the same rat group.

Qualitatively and quantitatively, dietary protein may influence the proteolytic activity in the intestinal contents probably by regulating the pancreatic exocrine secretion, the rate of inactivation of proteolytic enzyme, and the secretion from the intestinal mucosa. As observed in man (17) and dog (18), pancreatic exocrine secretion in rats may be augmented by special constituents in the food or breakdown products such as phenylalanine, valine or peptides etc., and it may also be controlled by protein synthesis in the pancreas.

The rate of enzyme inactivation within the intestinal lumen is comparable to the rate of protein digestion. The protein having a high nutritional value might cause an increase in the rate of enzyme inactivation and protein digestion. The observations on gluten-fed rats presented in this report demonstrated that the proteolytic activity in the intestinal contents was considerably influenced by enzyme inactivation. It is presumed that the elevation of proteolytic activity is caused by the low inactivation of enzymes (especially pancreatic enzymes) in the intestinal lumen and the rate of enzyme inactivation is related to the nutritional value of dietary protein. The disparity in the pattern of trypsin and chymotrypsin
activities noted between the gluten-alone group and the gluten-plus-lysine group is supposed to be explained by the difference in the rate of enzyme inactivation.

On the other hand, the authors noted that the proteolytic activity in the intestinal mucosa was negligible in measurements by the Anson method. Therefore, the mucosal enzymes may have little part in the proteolytic activity of the intestinal contents. However, DARGEL and HOCK (19) observed that the peptidase activity in the mucosa of rats receiving various glutes from cereals was roughly correlated to these changes in nutritional value by supplementation of amino acids. The relationship between dietary protein and mucosal peptidase activity must be pursued apart from the proteolytic activity of the intestinal contents in future.

In another experiment which will be published elsewhere, rats aging about 7 weeks and weighing about 200 g used in the present experiment displayed the maximum proteolytic activity in the intestinal contents. Hence, the marked elevation of activity as observed in the gluten-fed rats may be assumed to be caused by simultaneous action of some experimental conditions such as nutritional value of diet, age of rats, food intake, and experimental periods. Further pursuit of protein digestion or dietary regulation of digestive function in the small intestine is considered to be necessary.

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REFERENCES