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Utilization of Enzymatically Hydrolyzed Soybean Protein and Crystalline Amino Acid Diets by Rats with Exocrine Pancreatic Insufficiency

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ABSTRACT Exocrine pancreatic-deficient rats (PD) were prepared by cannulation and ligation of the common duct in a way which permitted bile, but not pancreatic juice, to enter the intestine. After surgery the PD rats were fed purified diets containing a high quality nitrogen source in the form of crystalline amino acids (AA), an enzymatic hydrolysate of soybean protein supplemented with essential amino acids (EH) or intact soybean protein with an essential amino acid supplement (SP). PD rats fed SP failed to regain any of their surgically-induced weight loss during the 10-day postoperative period. PD rats fed AA did not resume weight gains until after the sixth postoperative day while those fed diet EH resumed growth by the third postoperative day and grew at a rate which was nearly identical to that observed in sham-operated controls. The three diets afforded almost identical growth rates in weanling rats and in sham-operated rats used as controls for the PD rats. The amino acid compositions of AA and EH were nearly identical. Thus, the better utilization of diet EH in the PD rats was attributed to the fact that approximately 85% of the nitrogen in EH was in the form of oligopeptides. The results indicate that pancreatic enzymes are not required for the terminal stages of protein digestion and suggest that enzymatically hydrolyzed proteins are utilized more efficiently than mixtures of amino acids in the absence of exocrine pancreatic function. J. Nutr. *104:* 793–801, 1974.

INDEXING KEY WORDS pancreatic insufficiency · protein hydrolysates · amino acid diets · peptide absorption · protein digestion

The results of recent studies in man suggest that the end-product of protein digestion within the intestinal lumen is a mixture consisting primarily of oligopeptides with lesser amounts of free amino acids (1, 2). Although the terminal events involved in transporting the peptides into the enterocytes are not fully understood, there is direct evidence from human studies indicating that hydroxyproline peptides (3) and glycylglycine (4) are taken up from the gut lumen intact and are transported as such to the portal blood. While the amount of peptide material actually entering the portal blood is not large, these findings do support the contention that some peptides are absorbed from the intestinal lumen intact (5–8).

The significance of peptide absorption in the nutrition of normal animals and man has yet to be established. However, in patients with cystinuria and Hartnup disease, two inherited diseases in which the absorptive mechanisms for certain dietary essential amino acids in the free form are greatly impaired, the ability to absorb essential amino acids in the form of peptides is considered to be important for survival (9–11).

There is now evidence indicating that defects in amino acid absorption may be found in association with other clinical conditions. Thus, DiMagno et al.[1] have shown that there is a marked impairment in the ability of patients with nontropical sprue to absorb mixtures of essential amino acids. Adibi and Allen (12) reported a reduction in the rates of essential amino acid absorp-

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[1] DiMagno, E. P., Go, V. L. W. & Summerskill, W. H. J. (1973) Duodenal absorption of essential amino acid in health and non-tropical sprue. Gastroenterology *64*, A34 (abstr.).

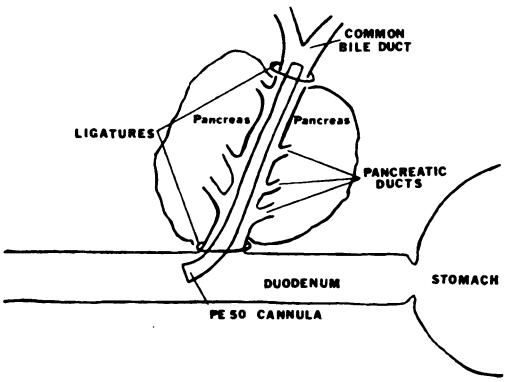

Fig. 1 Diagram of the rat pancreas showing the PE50 cannula within the common duct. Ligatures at both the distal and proximal ends of the common duct prevent pancreatic juice from entering the duodenum while bile is permitted to flow through the cannula. Omission of the proximal ligature (near the duodenum) created the common duct-cannulated sham in which pancreatic juice could flow to the duodenum on the outside of the cannula while bile flowed through the cannula.

tion in starved as well as protein-depleted human subjects. A report by Lis and Matthews (13) demonstrated that the rate of absorption of free amino acids is greatly decreased in the protein-depleted rat while the rate of absorption of an enzymatic hydrolysate was not appreciably affected. These findings suggest that in certain conditions where the normal digestive or absorptive processes are impaired, dietary nitrogen in the form of peptides or partially digested protein may offer some advantage over nitrogen derived from a mixture of crystalline amino acids. The results of the present studies indicate that in the absence of exocrine pancreatic function, a diet containing an enzymatic hydrolysate is better utilized than a crystalline amino acid diet.

METHODS

Animal model. Male Sprague-Dawley rats [2] weighing 180 to 200 g were used. The rats had free access to a commercial rat diet [3] until 5 days before surgery when they were transferred to a fiber-free, water-soluble human diet.[4] The latter is a complete diet containing 1.12% nitrogen in the form of crystalline amino acids (footnote 2 in table 1), maltodextrin, essential fatty acids, vitamins and minerals. Although the nitrogen content of this diet is suboptimal for the growing rat, in several unpublished rat growth studies conducted in our laboratory it provided significantly better growth than a 9% reference casein rat diet (14). Rats in the 180 to 225 g range gain approximately 5 g per day when fed this diet. In the present studies it was fed in the dry form primarily to reduce the bulk in the gastrointestinal tract prior to surgery and to allow the rats a short period of time to adjust to eating a powdered diet.

Exocrine pancreatic deficiency (PD) was produced surgically in rats under ether anesthesia. Access to the pancreas was gained through a ventral mid-line incision and a small puncture wound was made in the intestinal wall at a point opposite the entry of the common bile duct. A PE50 cannula was passed through the wound and up through the papilla of the common duct to the hilus of the liver. The cannula was tied in place above the point at which the distal pancreatic ducts enter the common duct and also at the point where the common duct enters the intestine. The end of the cannula was placed into the gut lumen to permit the entry of bile a few centimeters beyond the papilla (fig. 1).

Two types of sham-operated rats were used. One consisted of traumatizing the pancreas and the duodenum by gentle digital manipulation. The other was a common duct-cannulated sham in which the PE50 cannula was inserted into the common duct as had been done in the PD rats but the proximal common duct ligature was omitted to allow both pancreatic juice and bile to enter the intestine.

Experimental diets. Three purified diets were used (table 1). In diet AA, all the nitrogen was supplied as a mixture of crystalline L-amino acids (table 2) containing nearly 40% essential amino acids in a pattern closely resembling that used by Winitz et al. (15) in man. Diet EH differed from AA in that an enzymatic (bacterial protease) hydrolysate of isolated soybean protein was used as the nitrogen source. The hydrolysate contained 2.4% free

[2] Laboratory Supply, Indianapolis, Ind.
[3] Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.
[4] W-T Low Residue Food, Warren-Teed Pharmaceuticals, Inc., Columbus, Ohio.

α-amino nitrogen, 15% Kjeldahl nitrogen and 8% of the amino acids were in the free form. It did not exhibit any protease activity with casein as the substrate and there was no increase in free α-amino nitrogen when a 1% solution of the hydrolysate was incubated at 37° for 4 hours. The total amino acid composition of the hydrolysate was determined with an automatic amino acid analyzer [5] and was then supplemented with five essential amino acids so that the essential amino acid contents of diets AA and EH were practically identical (table 2). Both diets contained 2.5% nitrogen or the equivalent of 6.25 g nitrogen/1000 kcal. The protein efficiency ratios (PER) of these nitrogen sources were determined in weanling rats by a standard procedure employing 1.44% nitrogen diets (14).

A control diet (SP) contained intact isolated soybean protein in place of the soy hydrolysate and was supplemented with the same essential amino acid premix used in diet EH.

Experimental design. Two experiments were conducted. Experiment 1 consisted of a total of 24 PD rats and 24 shams with traumatized pancreas and duodenum. Eight PD and 8 shams were randomly assigned to each of the three experimental diets. The diets were fed ad libitum and individual food consumption and body weights were determined throughout the 10-day postsurgical period.[6] On day 10, the rats were killed by cervical dislocation. The lumen of the small intestine was flushed with 1 mM HCl and the washings were brought to a volume of 25 ml. The spectrophotometric procedure of Schwert and Takenaka (16) was used to assay for chymotrypsin with ATEE as the substrate. The data from any PD rats having detectable chymotrypsin activity were omitted. The number deleted for this reason was approximately 10% of the total number of PD rats prepared. Liver and pancreas were removed and prepared for histological examination by conventional methods with paraffin embedding and hematoxylin and eosin staining.

TABLE 1

Composition of diets

Ingredient	AA	EH	SP
	g/100 g diet		
Basal premix[1]	81.4	81.4	81.4
Complete amino acid premix[2]	18.6	0	0
Essential amino acid premix[3]	0	1.3	1.3
Enzymatic hydrolysate of soybean protein[4]	0	16.9	0
Purified soybean protein[5]	0	0	16.9
Maltodextrin	0	0.4	0.4

[1] The basal premix contains (g/100 g premix): 1.23 vitamin mixture (14), 6.15 salt mixture [USP XVII (1970), p. 885], 6.15 cottonseed oil, 86.5 maltodextrin (Maltrin-10, Grain Processing Corp., Muscatine, Iowa). [2] Contains (g/100 g premix): 4.25 isoleucine, 6.70 leucine, 5.15 lysine, 5.67 methionine, 5.67 phenylalanine, 4.25 threonine, 1.29 tryptophan, 4.77 valine, 4.92 alanine, 8.29 arginine, 10.90 aspartic acid, 17.93 glutamic acid, 8.51 glycine, 1.85 histidine, 3.27 proline, 3.25 serine, 3.32 tyrosine. [3] Contains (g/100 g premix): 51.22 methionine, 17.07 phenylalanine, 14.63 threonine, 10.98 tryptophan, 6.10 valine. All amino acids supplied by R. E. Davis Co., Indianapolis, Ind. [4] Bacterial protease hydrolysate of isolated soybean protein (Grain Processing Corp., Muscatine, Iowa). [5] Nutritional Biochemicals, Inc., Cleveland, Ohio.

TABLE 2

Amino acid composition of the nitrogen component of diets AA and EH

	Diet	
	AA	EH
	g/16 g nitrogen[1]	
Arginine	9.82	7.11
Histidine	2.19	2.44
Isoleucine	5.04	4.89
Leucine	7.93	7.33
Lysine	6.10	6.22
Methionine	6.72	5.78
Cystine	0.00	0.67
Phenylalanine	6.72	6.44
Threonine	5.04	4.67
Tryptophan	1.53	1.56
Valine	5.65	5.11
Alanine	5.84	4.00
Aspartic acid	12.91	11.11
Glutamic acid	21.30	17.78
Glycine	10.08	4.00
Proline	3.88	5.33
Serine	3.84	5.56
Tyrosine	3.94	3.33

[1] Values for AA nitrogen component were derived by calculation using the composition of the "complete amino acid premix" in table 1 which contains 13.5% N. Values for the EH nitrogen component represent the sums of the amino acids added as part of the "essential amino acid premix" in table 1 and those already present in the soy hydrolysate which were determined analytically.

[5] Jeol, Inc., Cranford, N. J.

[6] By administering an indirect pancreatic function test (Imondi, A. R., Stradley, R. P. & Wolgemuth, R. (1972) Synthetic peptides in the diagnosis of exocrine pancreatic insufficiency in animals. Gut *13*, 726–731) to rats at various times after surgery it was found that exocrine pancreatic function in the PD rat returned to normal after 10 days. These findings were confirmed by analysis of gut contents of PD rats for chymotrypsin (ATEE) and trypsin (BAEE). The apparent return of normal exocrine pancreatic function paralleled a return to normal histological appearance of pancreatic acinar tissue. In all cases, the cannula was still present in the common duct although it was not possible to determine whether the proximal ligature was functional.

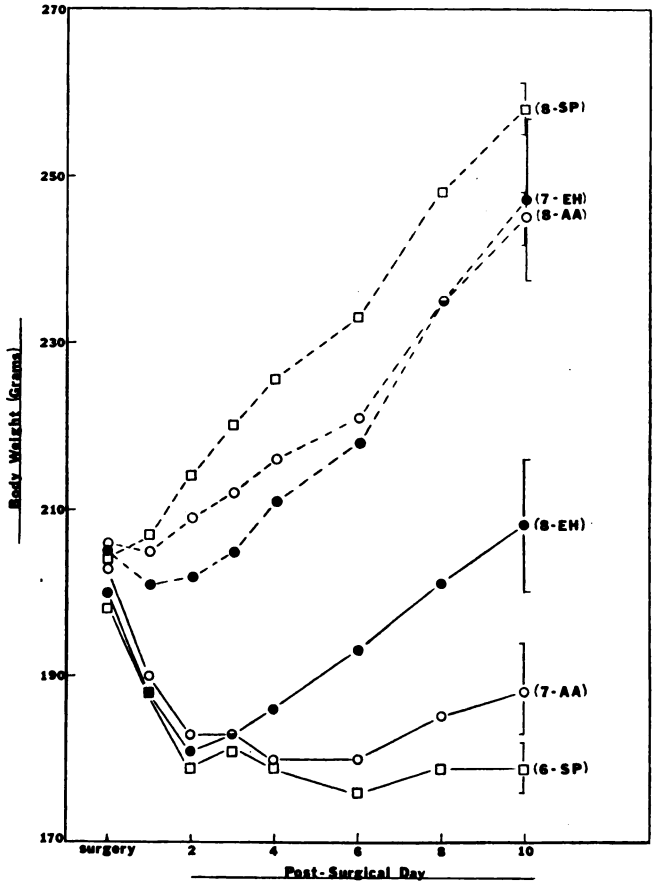

Fig. 2 Effect of diet on body weight gains in exocrine pancreatic-deficient rats (———) and rats subjected to pancreatic trauma (- - - - -) in experiment 1. Each point is the mean of the number of rats shown in parentheses; vertical bars show SEM for the last day. AA, EH and SP denote the diets described in table 1.

Experiment 2 consisted of 30 PD rats and 24 common duct-cannulated shams. The PD rats were divided into two groups —AA and EH—consisting of 15 rats each. The shams were divided into three dietary treatment groups—AA, EH and SP—with eight rats per group. The protocol was essentially the same as that in experiment 1 but a nitrogen balance was determined on each of the PD rats. The nitrogen balance study was divided into two periods—days 3 to 6 (A) and 7 to 10 (B). Urine was collected in beakers containing 1 ml 1 N HCl. Feces were air-dried and ground with a mortar and pestle. Nitrogen was determined by the micro-Kjeldahl method. Histological examinations and intestinal chymotrypsin determinations were carried out as described for experiment 1. Data from PD rats exhibiting detectable levels of chymotrypsin in the gut and data from shams lacking detectable chymotrypsin were omitted. In both experiments, the data from any rats dying within 48 hours after surgery were also omitted on the assumption that the deaths were related to the operative procedure. Significance of the differences between treatment means was determined by Student's t test (17).

RESULTS

The PER of the nitrogen components of diets AA and EH were 3.36 ± 0.11 and 3.62 ± 0.04 (mean $\pm$ SEM with 10 rats/

group), respectively. A repeat assay under identical conditions gave almost identical results and in both cases the EH nitrogen mixture was significantly better ($P < 0.05$). A reference casein standard,[7] spray-dried egg white powder[8] and defatted whole egg powder[9] had PER of 2.32 ± 0.13, 3.68 ± 0.05 and 3.53 ± 0.07, respectively. The mean body weight gains during the 28 days of the PER study period were (g): reference casein, 59; AA, 144; EH, 149; egg white powder, 144; and whole egg powder, 137.

The three experimental diets—AA, EH and SP—were fed ad libitum for 12 days to groups of five weanling rats having a mean body weight of 62 g to determine the adequacy of the complete formulations. The mean ± SEM weight gains during this period were 38 ± 8, 38 ± 5 and 43 ± 8 g, respectively and were not significantly different.

The postsurgical body weights of the rats in experiment 1 are shown in figure 2. Trauma to the pancreas and duodenum in the sham-operated rats had little effect on body weight and there were no significant differences between the diets. The gain in body weights of the shams during this period was essentially identical to that usually observed in our laboratory for unoperated rats of the same size receiving a commercial laboratory diet.[3]

The PD rats lost approximately 20 g during the first 48 hours after surgery. By day 3, the PD rats fed EH began to show a steady increase in body weight with a rate of gain which was almost identical to that of the shams. During the last 8 days of the study, these rats gained 25 ± 7 g each. The PD rats fed AA gained 6 ± 7 g, while those fed SP remained at a constant weight (0 ± 5 g) for the final 8 days. The weight gains for the EH and SP groups were significantly different ($P < 0.05$) but neither differed significantly from the AA group.

The food intakes for the first two postoperative days tended to be quite variable. Thereafter, the rats appeared to adjust to a more regular pattern of intake and reliable food consumption data for each rat were obtained for the last 7 days of the study (table 3). There were no significant differences in food consumption among the three diets for either PD rats or the traumatized shams although food intake by the shams, which were approximately 30 g heavier, was significantly greater than that of the PD rats ($P < 0.01$).

TABLE 3

Food consumption by pancreatic-deficient and sham-operated rats following surgery (experiment 1)

Diet[1]	Pancreatic deficient	Shams
AA	95± 8 (7)[2]	142±6 (8)
EH	106± 8 (8)	144±9 (7)
SP	92±10 (6)	153±4 (8)

[1] Refer to table 1 for diet composition. [2] Data are mean ±SEM grams of food intake per rat for the last 7 days of the 10-day experimental period. Number of rats in parentheses.

Histological examination of the liver and pancreas of the sham-operated rats revealed no abnormalities. The pancreases of the PD rats lacking detectable chymotrypsin in the gut were found to be fibrotic and atrophic with varying degrees of inflammatory cell infiltration. In those PD rats having chymotrypsin in the gut, the pancreas had histologically normal acini. The livers of all PD rats, with or without chymotrypsin activity and irrespective of dietary treatment, had a mild periportal hepatitis and bile duct proliferation suggesting that the presence of the cannula in the common duct had an effect on bile flow.

Since it was possible that the difference between the shams and the PD rats was due, in part, to the liver pathology, experiment 2 employed a common duct-cannulated sham. Since the poor performance of the intact protein diet (SP) in the PD rats was not unexpected, this diet was not evaluated in the PD rats of experiment 2.

The postoperative body weight changes in experiment 2 are shown in figure 3. In general, the results of this experiment paralleled those of experiment 1 in that there was practically no difference between the three diets in the shams. However, unlike the shams used previously, the common duct-cannulated shams did exhibit a marked weight loss immediately following surgery which suggested that this procedure was more traumatic than that used in the shams of experiment 1. Histological

[7] A.N.R.C. Reference Protein, Sheffield Chemical, Union, N. J.
[8] Marshall Produce, McKenzie, Tenn.
[9] Viobin Corp., Monticello, Ill.

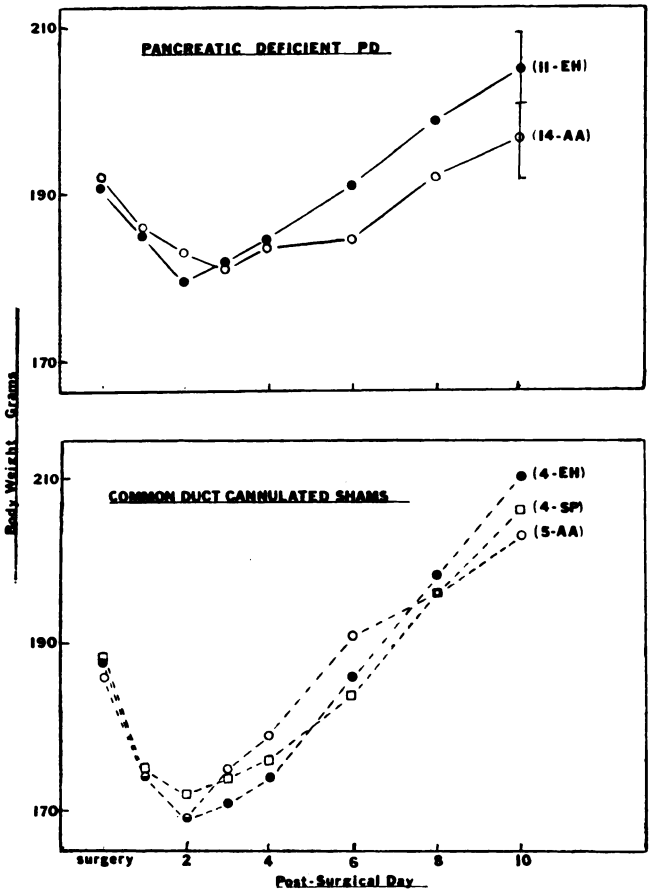

Fig. 3 Effect of diet on body weight gains in exocrine pancreatic-deficient rats and common duct-cannulated shams in experiment 2. Each point is the mean of the number of rats shown in parentheses; vertical bars in the upper figure show SEM for the last day. SEM on day 10 for the shams was 8 g for each of the three diets. AA, EH and SP denote the diets described in table 1.

examination of the livers of these common duct-cannulated shams at the end of the 10-day experimental period revealed periportal hepatitis with bile duct proliferation similar to that observed in the PD rats. The data from five of the common duct-cannulated shams which survived the 10-day experimental period were deleted because no chymotrypsin was present in the intestine at the time of sacrifice.

The results obtained with the PD rats in experiment 2 were also similar to those observed in the initial study in that the rats fed diet EH exhibited a better rate of growth than did those fed diet AA. During the last 8 days of study, the PD rats fed diet AA gained 11 ± 3 g and those fed diet EH gained 25 ± 3 g ($P < 0.01$).

The nitrogen balance data for the PD rats are shown in table 4. There were no significant differences in mean food intakes or fecal excretions between the two groups. The differences between the nitrogen balances of the two dietary groups during either period A or period B were not statistically significant. However, in the AA group urinary nitrogen excretion tended to be greater during period A which resulted in a lower nitrogen balance during period A than during period B ($P < 0.01$). Inspection of the body weight data in figure 3

TABLE 4

Nitrogen balance in pancreatic-deficient rats fed crystalline amino acid or enzymatically hydrolyzed soy protein diets (experiment 2)

Diet	Food intake	Nitrogen intake	Air-dried fecal wt	Fecal nitrogen	Urinary nitrogen	Nitrogen balance
	g	*mg*	*g*	*mg*	*mg*	*mg*
			Period A[1]			
AA	55±4[2]	1384	4.7±0.5	140±20	940±37	329± 78[3]
EH	62±4	1561	5.2±0.4	192±23	809±63	559±113
			Period B			
AA	60±3	1498	4.0±0.4	134±24	718±31	646± 39
EH	60±3	1500	5.1±0.4	182±20	759±41	559± 62

[1] Period A was days 3 to 6, period B was days 7 to 10. [2] Mean ±SEM of 14 AA rats and 11 EH rats. [3] $P < 0.01$ compared to diet AA in period B. These values represent mean nitrogen balances determined on each rat and, therefore, do not agree exactly with nitrogen balance values calculated from the mean intake and excretion data presented in the table.

shows that the PD rats fed diet AA did not resume growth until period B.

DISCUSSION

The cannulation–ligation procedure described in the present report was effective in causing exocrine pancreatic insufficiency for 10 days. During this time, the PD rats fed an intact protein diet (SP) were unable to regain their surgically-induced weight loss. However, nitrogen in the form of either crystalline amino acids (AA) or an enzymatic hydrolysate of soybean protein (EH) did result in positive nitrogen balance and growth.

There were some minor differences in the essential amino acid compositions of diets AA and EH. In diet EH cystine accounted for 10% of the total sulfur amino acid content whereas diet AA lacked cystine. However, total sulfur amino acid content of the two diets was practically identical and was approximately twice that required by the growing rat fed diets devoid of cystine (18). Nevertheless, it is possible that the differences in utilization of these diets by the PD rat were attributable, in part, to the differences in the sulfur amino acid make-up of the two diets.

The distribution of nonessential amino acids in each of the two diets was also similar with the only major difference being the higher glycine and lower serine content of diet AA. Although it has been shown that increasing the levels of these two nonessential amino acids, either together or singularly, in diets devoid of glutamic acid depresses growth of young rats (19), it is unlikely that the levels of glycine and serine in the present diets, which did contain glutamic acid, were critical. Moreover, despite the statistically better PER of the nitrogen component of diet EH, both nitrogen sources resembled the egg proteins in terms of protein quality and the experimental diets—AA and EH—afforded identical weight gains in weanling rats. Hence, the major difference between diets AA and EH appears to be the form in which the amino acids were present, oligopeptides (EH) or free amino acids (AA).

The growth rates of sham-operated control rats fed diets AA and EH were nearly identical. These results suggest that in rats with normal digestive functions, the utilization of a mixture of oligopeptides derived from enzymatic hydrolysis is equivalent to that obtained with a mixture of crystalline amino acids when the amino acid composition of each mixture is similar. However, in the absence of exocrine pancreatic function, the enzymatic hydrolysate appears to offer some advantage in terms of nitrogen utilization.

Although it is possible that the soy hydrolysate underwent some degradation in the stomach before entering the small intestine, results of an in vitro experiment [10]

[10] Gastric juice was obtained from a fasted rat which had undergone esophageal and pyloric ligation. One ml of the undiluted juice (pH 1.6) was incubated at 37° with 25 ml of either hemoglobin or the soy hydrolysate (2% in 0.1 M citric acid adjusted to pH 2.2). Aliquots from each reaction vessel were mixed with an equal volume of 10% TCA at timed intervals for up to 1 hour. The absorbance of the TCA-soluble material liberated from the hemoglobin was determined at 280 nm and was found to increase at a rate exceeding 0.16 O.D. units per minute indi-

which showed that the soy hydrolysate was resistant to digestion by rat gastric juice argues against this possibility. The ability of the PD rat to absorb nitrogen from the enzymatic hydrolysate indicates that pancreatic enzymes are not required in the terminal stages of protein digestion and supports the view that, under normal conditions, oligopeptides are major end-products of intraluminal protein digestion (2, 13, 20–22).

The reasons for the apparently better utilization of the enzymatic hydrolysate by the PD rats are not entirely clear. The present studies do not permit any conclusions to be drawn concerning peptide absorption per se and no attempt was made to compare the rates of nitrogen absorption from the two diets. It has been suggested that the more rapid rate of absorption of certain peptides as compared to their constituent amino acids is due to the presence of separate transport systems for amino acids and peptides (9, 10, 23). Thus, when amino acids are present in the gut in the free form there would be competition for a limited number of transport sites which could delay the absorption of certain amino acids (24). On the other hand, a mixture of small peptides and amino acids utilizing different transport sites will allow transport systems to participate in the absorptive process during the same period of time and would tend to provide a more uniform mixture of amino acids to the portal blood. Since it is generally recognized that optimal utilization of dietary nitrogen is achieved when all the amino acids are available for protein synthesis at the same time (25), it follows that the processes by which dietary nitrogen is absorbed can play an important role in protein utilization. While these same principles would be expected to apply in animals with normal exocrine pancreatic function, they may have been of greater significance to the PD rats which had pancreatic damage in addition to the surgical stress imposed on the sham-operated controls. It is possible that the PD rats, especially during the first few postoperative days, resembled to some extent protein-depleted rats in which absorption of amino acids but not enzymatically hydrolyzed protein was impaired (13). Indeed, this hypothesis is supported by the fact that in both of the present experiments, the PD rats fed diet EH began to exhibit weight gains by the third postoperative day, while those fed diet AA did not resume growth until after day 6.

The importance of prompt restoration of body tissues following severe trauma is well known and great progress has been achieved in this area through the use of parenteral feeding (26) and oral feeding of elemental diets composed of amino acids and simple sugars (27). The results of the present studies indicate that an enzymatic hydrolysate containing small peptides may be utilized with greater efficiency than a mixture of crystalline amino acids during catabolic states. This possibility has important clinical implications and deserves further attention.

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