Changes in the Intraluminal Protein Digestion of Pancreatic Duct-Ligated Rats

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Summary After ligature of the pancreatic duct (PDL), the body weight of rats decreased for several days, but began to increase from day 7, returning to that at the time of the operation on day 14. In these PDL animals, the weight gain was not due to improved digestion resulting from duodenal leakage of pancreatic enzymes or a compensatory increase of proteolytic enzyme activities in the intestinal mucosa. There was no significant difference in pepsin activities in the gastric contents and mucosa of control and PDL rats. However, acidic proteolytic activity, with a pH range between 1 and 4 and an optimum at pH 2.8, was found to be extremely high in the intestinal contents of PDL rats. Furthermore, the intraluminal pH of PDL rats was maintained below 4.0, especially in the upper small intestine, because of the absence of pancreatic bicarbonate secretion, suggesting that compensatory digestion by acidic proteolysis accounted in part for the growth of PDL rats. The transit time of orally administered material through the gastrointestinal (GI) tract in PDL rats was longer than that in control rats on days 7 and 14. These results suggest that the weight gain of PDL rats was caused by compensatory digestion by acidic proteolysis in the small intestine and prolongation of the transit time through the GI tract.

Key Words pancreatic duct ligature, pepsin, acidic proteolytic activity, trypsin, transit time

In general, after pancreatectomy or pancreatic duct ligature, animals should be placed on a diet containing pancreatic enzyme preparations such as pancreatic (1,2) or Viokase (3). However, there are reports that animals with pancreatic exocrine insufficiency or with pancreatic duct ligature maintain (4–7) or even gain (8–12) body weight when no pancreatic enzyme preparation is added to their
diet, even though the activities of pancreatic enzymes in their intestinal contents were extremely low. Hypersecretion of gastric acid (4,13–16) or increased activities of intestinal disaccharidases (11,17,18) were observed in these animals. Moreover, balance studies showed improvement of digestibility of dietary protein in these animals (9,12,19) with time.

In the present study, we examined how the digestibility of dietary protein in the gut lumen was improved in rats after ligature of the pancreatic duct.

EXPERIMENTAL

Animals and diet
Male Sprague-Dawley rats weighing about 170 g were housed in individual stainless steel cages. Animals were kept in an air-conditioned room at 23 ± 2°C with a 12-h lighting schedule from 08:00 h to 20:00 h. Rats were allowed free access to the semisynthetic diet shown in Table 1 until their body weight reached 180–190 g (control group) or 200–210 g (PDL group). Water was given ad libitum throughout the experiment.

Ligature of the pancreatic duct
The pancreatic duct was ligatured in rats that had been starved overnight by the modification of the procedure of Lambert (20) shown in Fig. 1. Rats were anesthetized with Nembutal (40 mg/kg BW) and the abdomen was opened by a midline incision from below the xyphisternum. The common bile duct was ligated at the points shown by arrows and cut between arrows 1 and 2, 3 and 4. A polyethylene tube (OD 0.97 mm, ID 0.58 mm, Intramedic PE50) was inserted into the upper part of the common bile duct and bile was introduced into the duodenum through the tube. Control rats received the sham operation of simple laparotomy. PDL and sham operated rats were given water ad libitum on the day of the operation and allowed access to semisynthetic diet and water from the day after the operation (feeding schedule 1).

Table 1. Composition of semisynthetic diet (%).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein*</td>
<td>20</td>
</tr>
<tr>
<td>Starch b</td>
<td>45</td>
</tr>
<tr>
<td>Sucrose c</td>
<td>23</td>
</tr>
<tr>
<td>Oil d</td>
<td>5</td>
</tr>
<tr>
<td>Salt mixture a</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin mixture a</td>
<td>1</td>
</tr>
<tr>
<td>Choline-Cl</td>
<td>0.15</td>
</tr>
<tr>
<td>Cellulose powder d</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Manufacturers: * Oriental Kobo Ltd., Tokyo, Japan; b Mitsubishi Shoji Ltd., Tokyo, Japan; c Daito Ltd., Tokyo, Japan; d Toyo Roshi Ltd., Tokyo, Japan.

Fig. 1. Diagram of the procedure for pancreatic duct ligature. Ligatured points are indicated by arrows. Bile was introduced into the upper duodenum through a polyethylene tube (PE50).

Experiment-1. Nitrogen intake, the dry weight of faeces, faecal nitrogen and fat and urinary nitrogen were determined, and from the values obtained, the nitrogen balance and apparent digestibility in various periods were calculated. Periods one, two and three indicate days 3–5, 7–9 and 12–14 after PDL, respectively.

Experiment-2. Rats were maintained on feeding schedule 1 and killed by a blow on the head at 10:00 h on day 3, 7 or 14. The entire small intestine was removed and the intestinal contents were collected by washing with cold saline. Their total volume was adjusted to 25 ml with cold saline. The resulting solution was centrifuged and the supernatant was used for determination of trypic, chymotryptic and caseinolytic activities. A mucosal homogenate, prepared as described under Analyses, was used for determining caseinolytic activity. The gastric mucosa and gastric contents, and intestinal mucosa and intestinal contents were treated as described for experiments 2 unless otherwise mentioned.

Experiment-3. Peptic activities in the mucosa and contents of the stomach at 10:00 h on day 14 of rats trained to eat from 06:00 to 10:00 h for 2 weeks (feeding schedule 2) were determined.

Experiment-4. The acid protease activity of the intestinal contents of PDL and control rats maintained on feeding schedule 1 was determined at 10:00 h on day 14.

Experiment-5. Animals were maintained on feeding schedule 1. The entire small intestine was cut into two segments of equal length, and the intestinal contents
were collected by washing each segment with cold saline. The optimal pH of proteolytic activity in the contents of each segment was then measured with hemoglobin as substrate (21).

Experiment-6. Animals were maintained on feeding schedule 1. The stomach and first segment of the small intestine from the pylorus to the aperture of the common bile duct were removed, and the rest of the small intestine was cut into 4 segments of equal length. The tryp tic and caseinolytic activities of the contents of these segments were measured. The acidic protease activities and pH values of the contents of the stomach and each segment were also determined. The volumes of the contents and washing fluid of the stomach and intestine were adjusted to 12.5 ml in this experiment.

Experiment-7. Animals were maintained on feeding schedule 1. Rats were killed at 10:00 h. The entire small intestine was cut into two segments of equal length, and gastric and intestinal contents were collected by washing each segment with cold saline. Acidic protease activities were determined with bovine serum albumin as substrate.

Experiment-8. For determination of the transit time of administered diet through the GI tract, rats were trained to eat from 18:00 to 14:00 h (feeding schedule 3). At 18:00 h on days 3, 7 and 14, they were given a diet containing a red dye (50 μg/g diet; New coccine: Phloxine = 3:17) and the time when the red color appeared in the faeces was recorded.

Analyses

Enzyme activities. The stomach and intestinal segments were everted and the mucosa was scraped off with a slide glass and homogenized in 10 volumes of cold distilled water in a Teflon homogenizer. Tryptic and chymotryptic activities in the intestinal contents were measured spectrophotometrically with N₂-p-toluene-sulfonyl-L-arginine methyl ester as substrate at 247 nm and N-benzoyl-L-tyrosine ethyl ester as substrate at 256 nm, respectively, by the method of Rick (22). One unit of activity of each enzyme was defined as the amount that caused an initial change in optical density of 1.000 per min under the assay conditions. Peptic activity in the mucosa and contents of the stomach and caseinolytic activity in the contents and mucosa of the intestine were also assayed by the method of Rick (22). In some experiments, acidic protease activity was measured with hemoglobin as substrate at pH 2.8 instead of pH 1.8 in the method of Rick and with bovine serum albumin as substrate by the method of Press et al. (23).

Enzyme activity was expressed as the amount of tyrosine (μmol) produced per min under the assay conditions. Protein was measured by the method of Lowry et al. (24) with bovine serum albumin as a standard. The nitrogen contents of the food, faeces and urine were determined by the modification (25) of the method of Kjeldahl, and faecal fat was extracted with diethyl ether, evaporated to dryness and weighed. The pH values of samples were measured with a pH meter (F7II, Hitachi-Horiba, Horiba Seisakusho, Kyoto, Japan).
Reagents. Enzyme substrates were obtained commercially. N\textsubscript{\alpha,p}-toluenesulfonyl-L-arginine methyl ester and N-benzoyl-L-tyrosine ethyl ester were supplied by Nakarai Chemical Co., Kyoto, Japan. Bovine hemoglobin and albumin were obtained from Wako Pure Chemical Industries, Osaka, Japan. Hammarsten casein was obtained from E. Merck A. G. Other reagents were commercial products of the highest purity available.

Statistical analysis of the results was done by the Student's t-test.

RESULTS

The changes in body weight of PDL and control rats are shown in Fig 2. The body weight of PDL rats decreased for several days after the operation, but began to increase on day 7, and was restored to that at the time of the operation on day 14. Figure 3 shows the changes in food intake of PDL and control rats. The PDL group consumed less food than the control group for several days, but from about 1 week after the operation, the food intake, on the basis of body weight was almost equal to that of the control group. These changes in food intake of the PDL group were reflected in changes in their body weight, as could be expected. Figure 4 shows the nitrogen intake, dry weight of faeces and faecal nitrogen excretion of the two groups in periods 1, 2 and 3, respectively. In period 1, the nitrogen intake of the PDL group decreased to about 50% of that of the control, without any difference in dry weight.
Fig. 3. Changes in food intake of control and PDL rats. Rats were starved for 1 day before and after the operation and then given food *ad libitum* (feeding schedule 1). Open and closed circles and bars represent values for control and PDL rats, respectively; values are means ± SD for 35 rats.

Fig. 4. Changes in nitrogen intake, amount of faeces and faecal nitrogen of control and PDL rats. Rats were maintained on feeding schedule 1. Periods one, two and three indicate days 3–5, 7–9, 12–14, respectively, after the start of the experiment. Open and solid bars represent values for control and PDL rats, respectively; values are means ± SD for 8 rats. Significantly different from the control value at levels of 1% (*) and 0.1% (**).
Fig. 5. Urinary nitrogen, nitrogen balance and apparent digestibility of food in control and PDL rats. Rats were maintained on feeding schedule 1. Periods one, two and three indicate days 3–5, 7–9 and 12–14, respectively, after the start of the experiment. Open bars and circles and closed bars and circles represent values for control and PDL rats, respectively; values are means ± SD for 8 rats. Significantly different from control value at levels of 1% (*) and 0.1% (**).

Table 2. Proteolytic activities of intestinal contents and mucosa in control and PDL rats.

<table>
<thead>
<tr>
<th>Intestinal mucosa</th>
<th>Intestinal contents</th>
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<tbody>
<tr>
<td></td>
<td>Caseinolytic activity (μmol tyrosine/mg protein/min)</td>
</tr>
<tr>
<td>Control (40)</td>
<td>0.054±0.008</td>
</tr>
<tr>
<td>PDL rats on day 3 (10)</td>
<td>0.044±0.004*</td>
</tr>
<tr>
<td>PDL rats in week 2 (60)</td>
<td>0.043±0.001*</td>
</tr>
</tbody>
</table>

Rats were maintained on feeding schedule 1. Values in parentheses indicate numbers of rats. Values are means ± SD. Significantly different from control value at levels of 0.1% (*).
Table 3. Proteolytic activities of gastric contents and gastric mucosa in control and PDL rats (hemoglobin as substrate).

<table>
<thead>
<tr>
<th></th>
<th>Gastric mucosa (µmol tyrosine/mg protein/min)</th>
<th>Gastric contents (µmol tyrosine/total contents/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal-fed (4 h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(6)</td>
<td>1.39 ± 0.30</td>
</tr>
<tr>
<td>PDL rats in week 2</td>
<td>(5)</td>
<td>1.24 ± 0.09</td>
</tr>
</tbody>
</table>

Values in parentheses indicate numbers of rats. Rats were fed by feeding schedule 2. Values are means ± SD.

Fig. 6. Changes in food intake, dry weight of faeces and faecal fat of rats after pancreatic duct ligature. Rats were maintained on feeding schedule 1. Open circles indicate values for 3 days before pancreatic duct ligature, and closed circles those for periods one, two and three after the operation; values are means ± SD for 11 rats. Significantly different from control value at levels of 1% (*) and 0.1% (**).

...of the faeces from the control group. However, in this period, the faecal nitrogen excretion of PDL rats was twice as great as that of the control rats, indicating that digestibility of protein in the PDL group in this period might be significantly decreased. The nitrogen intake of the PDL group increased in period 2 and approached the control level in period 3. Faecal nitrogen excretion by PDL rats decreased in period 2 and reached nearly the control level in period 3. Urinary nitrogen excretion by PDL rats was somewhat lower than that by control rats in

Fig. 7. Change in apparent digestibility of fat after pancreatic duct ligature. Rats were maintained on feeding schedule 1. The open circle indicates the value 3 days before pancreatic duct ligature and closed circles indicate values in periods one, two and three after the operation; values are means ± SD for 11 rats. Significantly different from control value at levels of 1% (*) and 0.1% (**).

Table 4. Acidic protease activities of intestinal contents in control and PDL rats (hemoglobin as substrate).

<table>
<thead>
<tr>
<th>Protease at pH 1.8 (µmol tyrosine/total contents/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10)</td>
</tr>
<tr>
<td>PDL rats in week 2 (10)</td>
</tr>
</tbody>
</table>

Rats were maintained on feeding schedule 1. Values in parentheses indicate numbers of rats. Values are means ± SD. Significantly different from control value at levels of 0.1% (*).

period 1 but not in periods 2 and 3 (Fig. 5A). Nitrogen balance was negative in the PDL group in period 1, improved in period 2 and was restored to nearly the control level in period 3 (Fig. 5B). The apparent digestibility of dietary protein was calculated as the difference between nitrogen intake and faecal nitrogen excretion. In the control group the digestibility was found to be over 90% in all periods, but in the PDL group it was 65, 86 and 91% in periods 1, 2 and 3, respectively (Fig. 5C). The trypsinic, chymotryptic and caseinolytic activities in the intestinal contents were measured to examine whether pancreatic protease leaked out into the small intestine with time in spite of PDL. These enzyme activities in the intestinal contents of the PDL group were extremely low on days 3 and 14, showing that there was no leakage (Table 2). The mucosal caseinolytic activity of the PDL group was less than that of
Fig. 8. Effect of pH on protease activities of gastric and intestinal contents of control and PDL rats (hemoglobin as substrate). Rats were maintained on feeding schedule 1. The buffers were HCl/KCl buffer (pH 1.21–2.32), glycine/HCl buffer (pH 2.2–2.8) and citrate buffer (pH 2.71–6.72). Enzyme source: ■, gastric contents; *, proximal intestinal contents; +, distal intestinal contents; values are means ± SD for 8 rats.

the control group, indicating that mucosal proteolytic enzymes did not contribute to the improvement of digestibility (Table 2). Since hypergastrinemia (16,26) has been observed in PDL dogs, peptic activities in the gastric mucosa and stomach contents were measured in the PDL and control groups, but no difference was found in the values between the two groups (Table 3). Changes in digestion and absorption of dietary fat were similar to changes in those of dietary protein in the PDL group (Figs. 6, 7). When the pancreatic duct is ligatured and pancreatic juice is not secreted into the duodenum, acidic chyme should not be able to be neutralized appreciably in the small intestine, and peptic digestion should continue therein. To test this possibility we measured acidic proteolytic activity in the intestinal contents with hemoglobin as substrate by the method used to measure peptic activity. The activity was extremely high in the PDL group and very low in the control group (Table 4). The optimal pHs of the acidic proteolytic activities in the gastric and intestinal contents were both shown to be 2.8 (Fig. 8). However, acidic proteolytic activity in the intestinal contents was thought to be derived from pepsin and/or cathepsins in the desquamated epithelial cells. As shown in Fig. 9, unlike cathepsins, the intestinal contents catalyzed hydrolysis of hemoglobin and bovine serum albumin in the same manner as pepsin. These results indicate that the activity in the intestinal contents is due to pepsin.

As shown in Fig. 10, in the PDL group, the intraluminal pH of the upper part
Fig. 9. Acidic protease activities of gastric and intestinal contents in control and PDL rats (bovine serum albumin as substrate). Rats were maintained on feeding schedule 1. Control and PDL rats were killed at 10:00 h. The entire small intestine was cut into two segments of equal length. Open and solid bars represent values for control and PDL rats, respectively; values are means ± SD for 10 rats. Significantly different from the control value at levels of 1% (*) and 0.1% (**) of the small intestine was below 4.0 (segments 2 to 4), whereas in the control group, the pH of the distal segments, from the aperture of the common bile duct, was about 6 because of neutralization with bicarbonate in the pancreatic juice.

Finally, since recovery of the growth rate of the PDL group appeared to be difficult to explain entirely by a compensatory effect of pepsin in the small intestine, the transit time of the gut contents was determined by adding a red dye to the food in the diet. In the control group, the time for appearance of this red dye in the faeces was 9.2 ± 2.0 h. In the PDL group, it was similar to that of the control group on day 3 but was 19.8 ± 5.2 h on day 7 and 22.8 ± 3.8 h on day 14 (Fig. 11). Thus the transit time was longer in the PDL group than in the control group on days 7 and 14.

DISCUSSION

In this work, the authors found that the growth rate of PDL rats began to increase about 1 week after the operation, with improved food consumption (Figs. 2, 3). This phenomenon could not be explained by improved digestion resulting from leakage of pancreatic enzymes into the duodenum with time or by a compensatory increase in the activities of proteolytic enzymes in the intestinal
Fig. 10. pH values and proteolytic activities of gastric and intestinal contents of control and PDL rats (hemoglobin as substrate). Rats were maintained on feeding schedule 1. Control and PDL rats were killed on days 3, 7 and 14. The first segment of the small intestine was from the pylorus to the opening of the common bile duct. The rest of the small intestine was cut into 4 segments of equal length. Values in parentheses indicate numbers of rats. Values are means ± SD. Significantly different from control at 0.1% (*).

mucosa (Table 2). Since pancreatic enzyme activities in the blood are elevated in PDL animals (27,28), the very low, but not negligible, activities of trypsin and chymotrypsin in the intestinal contents of PDL rats can be explained by the finding of Rohr and Scheele that the fraction of $^{35}$S-methionine-labeled pancreatic enzymes injected into the blood circulation is secreted into the duodenum via the bile duct (29). Hypersecretion of gastric acid from the Heidenhain pouch in response to dietary intake in PDL dogs and hypergastrinemia or hypercholecystokininemia (6,30,31) in PDL animals have been reported. Hypersecretion of gastric acid and a very low pH of the duodenal contents (32,33) have also been observed in patients with pancreatic insufficiency. No data are available on pepsinogen excretion in...
PDL animals. However, the hypergastrinemia or hypercholecystokininemia observed in these animals suggests that pepsinogen secretion increases in parallel with the increase of acid secretion from the stomach. Unexpectedly, pepsin activity in the gastric contents and mucosa was not increased in PDL rats (Table 3). When the pancreatic duct is ligatured, acidic chyme is not neutralized appreciably in the small intestine by bicarbonate secreted from the pancreas. Consistent with this, we found that the intraluminal pH was maintained at below 4.0, especially in the upper part of the small intestine (Fig. 10). We also found that acidic proteolytic activity in the intestinal contents was higher in the PDL group than in the control group (Table 4). The acidic protease in the intestinal contents was active between pH 1 and 4, with an optimum at pH 2.8 (Fig. 8). Thus compensatory digestion by acidic proteolysis in the small intestine may contribute to the growth of PDL rats. Since proteolytic activities in the gastric and intestinal contents have the same optimal pH and the intestinal contents catalyzed hydrolysis of hemoglobin and bovine serum albumin in the same manner as pepsin in contrast to cathepsins (34,35), the activity in the intestinal contents seemed to be due to pepsin. In contrast, the activity in the intestinal contents of control rats increased with an increase in pH, suggesting that it was due to trypsin and chymotrypsin. The decrease in body weight of PDL rats in period 1 (Figs. 2, 4) was probably due to appetite loss after surgical damage, in spite of the high acidic proteolytic activity in the small intestine.
Digestion and absorption of dietary fat in the PDL group changed in parallel with those in the control group (Fig. 7). An acidic lipase that catalyzes the hydrolysis of long-chain triglyceride has been found in human stomach (36, 37). A similar enzyme might show compensatory activity in fat digestion in the intestine of PDL rats.

The transit time of dye through the GI tract was longer in the PDL group than in the control group on days 7 and 14, but not day 3, after the operation (Fig. 11). The reason for the prolongation in the transit time after PDL is uncertain.

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REFERENCES


