Enzyme Replacement Therapy in the Pancreatic Duct Ligated Swine (36779)

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Oral enzyme therapy has been reported to be of some benefit in the treatment of various types of exocrine pancreatic insufficiency (1). However, the results have not always been favorable (2) and the levels of nutrient absorption by these patients generally remain below normal in spite of the use of large and frequent doses of pancreatin (3).

Assessment of the problems associated with digestive enzyme replacement therapy has been hampered by the lack of an animal model in which pancreatogenous steatorrhea can be consistently maintained for extended periods of time. The pancreatic duct ligated dog has been used, but the degree of steatorrhea tends to be variable and may correct itself spontaneously during the course of study (4, 5). Although the creation of pancreatic insufficiency in the rat is possible by ductal ligation, the operative procedure is difficult because there are several pancreatic ducts which enter a common bile duct. The swine, however, with its single pancreatic duct has been a useful animal for study of pancreatic insufficiency (6). The present studies demonstrate that the pancreatic duct ligated pig will exhibit frank steatorrhea of extended duration and minimal variability which can be allevated by the oral administration of pancreatin.

Materials and Methods. Twelve-week-old (8–10 kg) male and female West African guinea swine which were farrowed and raised at the Warren-Teed Research Center were used for these studies. Double ligation and section of the pancreatic duct was performed under halothane anesthesia after exposing the duct through a ventral midline incision. An accessory duct, which is present in about 10% of these animals, was located, ligated and sectioned. After a 3-day recovery period, the animals were confined to narrow metabolism cages which prevented coprophagy by not allowing the swine to turn around.

Nutrient absorption was determined by the Cr_2O_3 indicator method (7) and expressed in the tables as the percentage of ingested protein and fat not recovered in the feces. It was found that 3 days were required for the level of Cr_2O_3 in the feces to reach equilibrium. Therefore, fecal collections were routinely made on the fifth and sixth days after beginning a treatment. Although total fecal collections are not required in this type of digestibility study, an attempt was made to collect all feces on the collection days.

Diets and feces were homogenized with an equal volume of water prior to analysis. Fatty acids in both the diet and feces were determined according to the Van de Kamer method (8). Nitrogen was determined using a Hewlett-Packard 185 CHN analyzer and was used as an index of protein content of diet and feces. Cr_2O_3 was analyzed according to the method of Clarkson (9).

The experiment was carried out over an 8-wk period. The first and last weeks were control periods during which no pancreatin was added to the diet. During the 6-wk treatment period, pancreatin 4X NF (Ilozyme, Warren-Teed Pharmaceuticals, Inc.) was added to the diet at levels of 0.1, 0.5 and 1.0% by weight. The diet was a commercial swine diet containing 1 part Master Mix Sow and Pig Concentrate (Central Soya, Fort Wayne, IN), 2.7 parts wheat and 1.3 parts oats which was adjusted to contain 20% fat by weight by the addition of olive oil.

The swine were given access to feed for two 1-hr periods daily. Feed consumption was

TABLE I. Absorption of Fat by Pancreatic Duct Ligated Swine[a] Before, During and After Dietary Supplementation with Pancreatin 4X NF.

Pancreatin % of diet[b]	Postsurgery	Treatment (week): 1	4	6	Posttreatment
0.1	34.9 ± 12.2[c]	72.1 ± 3.5[d]	79.0 ± 2.7	75.4 ± 3.7	43.2 ± 8.2
0.5	29.7 ± 9.8	85.3 ± 2.5	79.9 ± 2.3	73.2 ± 3.2[e]	33.4 ± 2.8
1.0	19.1 ± 6.8	90.4 ± 3.6	89.0 ± 0.4[d]	83.5 ± 2.3[e]	40.5 ± 8.1

[a] Fat absorption in sham-operated swine on this diet was 90–95%.
[b] Pancreatin 4X NF (Ilozyme) in a diet containing 20% fat.
[c] Percentage of ingested fat not recovered in feces. Mean of 4 animals ± standard error.
[d] Different from other treatments during same week ($p < .05$).
[e] Different from respective treatment during the first treatment week ($p < .05$).

not routinely monitored but averaged approximately 300–400 g/feeding except during the first postoperative week when the feed intake was highly variable.

Just before the morning feeding on the first day of each study week, blood was drawn from the anterior vena cava for glucose determination. At the end of the study, following an overnight fast, each swine was administered an oral dose of glucose (5 g/kg). Blood glucose was determined 1 hr later, the time which in preliminary studies was shown to produce peak levels of blood glucose in PDL swine receiving this glucose load. Blood glucose levels in control swine were found to remain relatively constant during a 4-hr period following the same dose of glucose.

Intraduodenal enzyme levels were determined on 3 separate occasions in 2 normal and 2 PDL pigs previously prepared with a jejunal fistula approximately 50 cm from the pylorus. The animals were fasted for 18 hr prior to being given 100 g of ground Purina Laboratory Chow with or without 4 g of pancreatin. At selected time intervals, samples were collected through the fistula with an infant feeding tube. The pH was determined and the coarser food particles in the samples were then removed by slow speed centrifugation. The samples were stored frozen until assayed.

Trypsin and chymotrypsin were assayed titrimetrically (10) using BAEE and ATEE as substrates. The amount of NaOH required to maintain a pH of 7.6 for 1 min was used to quantitate enzyme activity. One microequivalent of NaOH per minute was designated as 1 unit. Crystalline trypsin and chymotrypsin (Grade A, Calbiochem) were used to establish the range over which the reaction rates were proportional to the enzyme concentration.

Lipase was assayed according to a modification (11) of the method described by Sammons, Frazer and Thompson (12). The liberated fatty acids were extracted into benzene and titrated with methanolic NaOH. One unit of lipase activity is the amount which releases 1 μEq fatty acid/min.

Analysis of variance and t tests were used to test the statistical significance of the nutrient absorption data without transformation.

Results. Pancreatic duct ligation and section in the swine resulted in marked impairment in the uptake of dietary fat (Table I). The fat absorption during the first postoperative (pretreatment) week for 11 of the animals used in the study ranged from 2 to 45% of the ingested fat. In 1 animal which received the 0.1% pancreatin treatment, fat absorption was 68% during both the pretreatment and posttreatment weeks. The mean fat absorption in 4 unoperated control swine and 3 sham-operated control swine receiving the same diet was 92% (range 90–95%). Previous studies in our laboratory indicated that the PDL swine, although unable to gain weight, could survive for several months when the basal diet without added fat was fed *ad libitum*. However, these animals survived for only a few weeks when maintained

TABLE II. Absorption of Protein by Pancreatic Duct Ligated Swine[a] Before, During and After Dietary Supplementation with Pancreatin 4X NF.

Pancreatin % of diet[b]	Postsurgery	Treatment (week): 1	4	6	Posttreatment
0.1	9.3 ± 20.1[c,d]	41.5 ± 11.2	42.1 ± 5.6	46.0 ± 6.7	39.4 ± 7.7
0.5	−5.3 ± 12.7[d]	37.3 ± 6.7	55.0 ± 3.9	36.8 ± 6.2	36.5 ± 3.8
1.0	4.2 ± 8.3[d]	51.8 ± 1.8	58.6 ± 2.1[e]	50.8 ± 3.2	44.9 ± 4.5

[a] Protein absorption in sham-operated swine on this diet was 75–80%.
[b] Pancreatin 4X NF (Ilozyme) in a diet containing 20% fat.
[c] Percentage of ingested protein not recovered in feces. Mean of 4 animals ± standard error.
[d] Different from treatment and posttreatment ($p < .05$).
[e] Different from 0.1% pancreatin group during the same week ($p < .05$).

on a high fat diet without enzyme supplementation. It was also very difficult to collect meaningful data from such animals because of a severe diarrhea. The present studies, therefore, do not contain a placebo group of animals, *i.e.*, PDL animals receiving no pancreatin.

The inclusion of pancreatin in the diet of the PDL swine at any of the 3 dosage levels resulted in a rapid and highly significant ($p < .01$) increase in fat absorption. There was also a concomitant reduction in experimental variation which is shown by the smaller standard errors during treatment weeks. The analysis of variance showed a significant dose-related response for the first ($F = 12.7$) and fourth treatment weeks ($F = 6.8$) but not for the sixth treatment week ($F = 2.9$). During the first treatment week, the animals receiving 0.5 and 1.0% pancreatin diets absorbed significantly more fat ($p < .05$) than did those receiving the 0.1% pancreatin diet. By the fourth treatment week, the PDL swine fed the 1.0% pancreatin diets absorbed significantly more fat ($p < .05$) than either of the other 2 treatment groups. There was a trend for the swine on the 0.5 and 1.0% pancreatin diets to absorb less fat with time, and the absorption during the final treatment week was significantly less ($p < .05$) than it was during the first week of treatment. Normal levels of 90–95% fat absorption exhibited by sham-operated and unoperated controls, was never attainable except occasionally during the first and fourth weeks with 1.0% pancreatin. When the animals were returned to the pancreatin-free diets, steatorrhea resumed within 2 days and was not significantly different than that during the postsurgery (pretreatment) week.

Protein absorption was also sharply reduced following pancreatic duct ligation (Table II). During the first postoperative week, the values for 11 of the animals ranged from −46 to 25%. The negative values were only observed during the first postoperative week when some of the PDL swine ate very little and all had some diarrhea. Under these conditions, the Cr_2O_3/nitrogen ratio in the feces was higher than that in the feed. No attempt was made to quantitate endogenous fecal nitrogen loss during this period. In the animal which had a 68% fat absorption, protein absorption was almost 50%. However, this was an exception since the correlation between fat absorption and protein absorption within animals was not statistically significant during either control or treatment periods.

Pancreatin at each of the 3 dose levels resulted in a significant increase in protein absorption ($p < .05$). However, in contrast to what had been observed for the absorption of fat, there was only a slight and nonsignificant decrease in protein absorption upon cessation of pancreatin therapy. The posttreatment protein absortion was significantly higher than it was during the postsurgery (pretreatment) week. During the 6 treatment weeks, protein absorption tended to be somewhat better in the swine receiving 1% pancreatin in the diet, but a significant dose-

TABLE III. Enzyme Activity in Jejunum of Normal and Pancreatic Duct Ligated Swine.

Enzyme[b]	Pancreas	Postprandial[a] (hr): 0.5	1	1.5	2	3	4
Lipase	Normal	—[c]	7.8 (2.5–13.8)	—[c]	5.6 (2.9–13.8)	—[c]	7.8 (5.8–10.5)
	PDL	4.6 (1.7–6.6)	3.4 (1.0– 5.7)	1.7 (0.3–5.1)	2.0 (1.7– 2.3)	0.8 (0.3–1.5)	1.2 (0.2– 3.8)
Trypsin	Normal	25 (10–45)	25 (21–28)	21 (6–35)	18 (12–28)	21 (16–24)	38 (26–55)
	PDL	4 (3–6)	4 (2–7)	3 (1–4)	2 (0–4)	2 (1–2)	1 (0–1)
Chymotrypsin	Normal	59 (21–106)	62 (38–82)	38 (10–78)	37 (23–62)	41 (31–59)	73 (54–106)
	PDL	14 (10–25)	10 (8–11)	10 (8–12)	6 (2–10)	7 (6–9)	4 (1–8)

[a] Normal swine received 100 g ground Purina Lab Chow and PDL swine received the same meal with 4 g pancreatin 4X NF added. Activity was nil in PDL swine without pancreatin.

[b] Units of activity per milliliter of jejunal contents. Refer to Materials and Methods for definition of units. The data for the means were collected from 2 normal and 2 PDL swine on 3 separate occasions. The range for the 6 individual values at each time is shown in parentheses below the mean.

[c] Not determined.

related response was only observed during the fourth week ($F = 4.4$) when the 0.1 and 1.0% pancreatin groups differed significantly ($p < .05$).

In spite of the improvement in absorption afforded by the pancreatin treatment, there was still gross evidence of severe muscle atrophy in all PDL swine. The animals receiving 0.1% pancreatin were emaciated near the end of the study. Those receiving the high levels of pancreatin did show body weight gains of up to 4 kg, but these increases were less than the 7–8 kg gains shown by control animals fed this same diet for 6 wk.

Throughout the 8 wk of study, the fasting blood glucose levels tended to be higher in the PDL swine (range 90–130 mg/100 ml) than in control swine (80–100 mg/100 ml). When the oral load of glucose (5 g/kg) was administered, blood glucose levels 1 hr later increased to 190 ± 18 mg/100 ml in PDL swine but remained unchanged in controls.

Gross examination of the pancreas of the PDL swine at necropsy revealed an atrophic and fibrous gland with no functional excretory ducts. Only a few recognizable acini and islets were present within the gland on histological examination.

Lipase, trypsin, and chymotrypsin activities were determined on jejunal aspirates taken at periods up to 4 hr after a 100 g test meal. These results are shown in Table III. The samples were obtained from 2 PDL swine both with and without pancreatin 4X NF and from 2 control swine without pancreatin. Enzyme levels in the PDL swine intestine following the pancreatin-free meals were essentially zero and are, therefore, not included in Table III. The addition of 4 g of pancreatin to the meal elevated each of the enzyme concentrations for the first or second postmeal hours. Lipase levels during the first hr after dosing were within the range observed in normal swine. Trypsin and chymotrypsin were moderately and transiently increased to only about 20–30% of normal. There was practically no detectable trypsin activity and only slight chymotrypsin activity by the fourth postprandial hour in the PDL swine jejunum. The pH of the jejunal aspirates from both PDL and control swine ranged from 6 to 7 and bore no relationship to the presence or absence of pancreatin in the test meal.

Discussion. It has been reported (13) that pancreatectomized dogs may absorb as much as 75% or more of ingested fat. Unpublished observations in our laboratory demonstrated that in 8 PDL dogs, in which communication between the pancreas and duodenum was prevented by wrapping the duodenum with omentum (14), absorption of fat ranged from 7 to 84% while protein absorption ranged from 7 to 49%. In 3 of these dogs, fat absorption was greater than 75% and protein absorption was above 40%. Pancreatic duct ligation in the swine, however, resulted in azotorrhea and steatorrhea which tended to be relatively less variable. Furthermore, in every case, the fat absorption during the posttreatment period decreased significantly from what it had been during the treatment period, which indicates that spontaneous recovery did not occur during the study. The reasons for this difference between the dog and swine are not clear. However, the marked impairment in fat absorption encountered in the PDL swine would argue against any significant contribution of intestinal lipase to the intraluminal digestion of fats in this species (15).

During the early treatment periods it was possible to achieve nearly normal levels of fat absorption by the addition of 0.5 or 1.0% pancreatin to the diet. However, protein absorption remained well below the range of 75–80% found in control swine fed this diet. The failure to observe a significant decrease in protein absorption when pancreatin was eliminated from the diet suggests that the response noted during the first treatment week may have been due, in part, to the more pronounced effect which the pancreatin had on fat absorption and the concomitant alleviation of diarrhea.

In view of the enzyme activities in the jejunum of the PDL swine following an oral dose of pancreatin, it is not surprising that steatorrhea was more responsive to pancreatin therapy than was azotorrhea. The mean lipase levels were within the normal range for the first hour, whereas trypsin and chymotrypsin levels remained well below those found in normal swine.

The pancreatin 4X NF used in this study had specific activities of 4.5, 12.4, and 2.5 units/mg of lipase, chymotrypsin and trypsin, respectively. One-half hour after the oral dose of pancreatin, the enzyme activity in the intestine of the PDL swine was 4.6, 14, and 4 units/ml of intestinal juice. These results indicate that lipase was destroyed or inactivated to a greater extent than either of the proteases, and are in agreement with an earlier report concerning the relative stabilities of these enzymes (1).

The present studies also show that there is a considerable difference between the amounts of enzyme normally present in the swine small intestine and the amounts provided by orally administered pancreatin, a situation not unlike that which exists during pancreatic replacement therapy in man (16). The 4 g dose of pancreatin used in the present studies for determination of intestinal enzyme levels is similar to the amount of pancreatin ingested during one meal by PDL swine on the 1% pancreatin diet. Since this amount of pancreatin restored fat absorption to nearly normal levels in the PDL swine, the quantity of lipase secreted by the swine pancreas, as in humans (17), must be greatly in excess of that normally required.

The development of diabetes in the PDL swine is consistent with what has been generally found in patients with chronic pancreatitis (18). It is not known whether the diabetes is a direct result of exocrine pancreatic insufficiency or to the disorganization of islet tissue which was seen histologically. Structural disorganization of the islets of Langerhans has been implicated in diabetes secondary to cystic fibrosis (19).

The studies reported herein clearly demonstrate the importance of the exocrine pancreatic secretion to the digestive processes in the swine. Exclusion of pancreatic juice from the intestine of this animal produces a severe, yet relatively uniform impairment of nutrient absorption which can be partially reversed with oral enzyme supplementation. These findings also suggest that the swine, which in recent years has become an important laboratory animal in gastrointestinal re-

search, may serve as a useful model for studying enzyme supplements used in the treatment of exocrine pancreatin insufficiency.

Summary. Pancreatic duct ligation (PDL) in swine reduced absorption of dietary fat and protein to less than 50%. Supplementation of the diet with pancreatin 4X NF at 0.1, 0.5 and 1.0% resulted in a significant improvement in fat absorption which approached normal on the highest level of pancreatin. Protein absorption was also improved but remained well below that observed in normal swine.

Intestinal levels of trypsin, chymotrypsin, and lipase were elevated in the PDL pig for up to 2 hr following oral administration of 4 g pancreatin 4X NF. However, only in the case of lipase did the levels approach those found in the normal swine intestine.

These studies also point out that the PDL may have applicability as a model for investigating pancreatin enzyme replacement therapy.

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