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Pancreatic Duct Occlusion in the Rat: Report and Assessment of a New Technique (42378)

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Abstract. Temporary reduction of the exocrine pancreatic secretion may be desirable in various experimental models. In the rat this can be achieved by obstructing the connection between the pancreas and the duodenum. A new, simple technique of pancreatic duct occlusion using metal hemostatic clips is described. The reduction of secretion produced by the procedure was assessed by measuring duodenal protein, amylase, and trypsin during stimulation with cholecystokinin. Stimulated duodenal amylase activity 1 and 4 weeks following duct occlusion was reduced by approximately 80% compared with sham-operated controls, whereas proteolytic activity was reduced by 96 and 60%, respectively. The magnitude and duration of pancreatic insufficiency achieved by this technique is equivalent to that achieved with more complicated methods.

Temporary insufficiency of the exocrine pancreas in the rat may be produced by surgically obstructing the connection between the pancreas and the duodenum. Such models are useful in experimental studies concerning the influence of pancreatic secretions on various factors (1–4).

In the rat, the pancreatic gland is a diffuse organ with numerous excretory ducts opening into the common bile duct along its entire length. Therefore, the recommended technique (5) consists of proximal and distal ligature of the common bile duct and restoration of biliointestinal continuity by anastomosing the common bile duct to the duodenum or jejunum. In the present paper, a new simple method of pancreatic duct occlusion using metal hemostatic clips is described, and the reduction of pancreatic secretion achieved is estimated. This technique leaves the common bile duct *in situ* and obviates the need for a biliointestinal anastomosis.

Methods. *Surgical procedure.* The duodenum is exposed through an upper midline incision and separated from the transverse colon by blunt dissection. The duodenal loop is then pulled caudally by an assistant, thus exposing and slightly stretching the common bile duct and the duodenal portion of the pancreatic gland. Starting distally at the choledochoduodenal junction, hemostatic clips (Hemoclip "Weck," Ligaclip "Ethicon") are applied on both sides of the common bile duct along its entire length (Fig. 1). The staples are applied approximately 1 mm from the common bile duct, and care is taken to clamp all connections to the pancreatic gland. In order to achieve this, the superior pancreaticoduodenal artery sometimes has to be sacrificed. The abdominal incision is closed with nonabsorbable suture material.

The procedure takes about 10–15 min (skin to skin).

Secretion studies. Conventional outbred female Wistar rats (Mol:WIST, Mollegaards Breeding Center Ltd, L1.Skensved, Denmark) weighing 180–200 g, aged 60–90 days, were used for the experiments. The animals were kept in groups of four to five in plastic cages closed with metal wire netting, the bottom being covered by sawdust. Ambient temperature was 22 ± 1°C, and the relative humidity was 55 ± 5%. The rats had free access to tap water in drinking bottles and to standard pellets (Evos R3, Evos AB, Sodertalje, Sweden).

A total of 24 rats were utilized. Pancreatic duct occlusion was performed as described in 18 rats. Six animals were sham operated and used as controls. The sham operation consisted of laparotomy, separation of the transverse colon from the duodenum, and exposure and manipulation of the pancreatic gland.

In 12 of the 18 pancreatic duct occluded rats, stimulated exocrine pancreatic secretion

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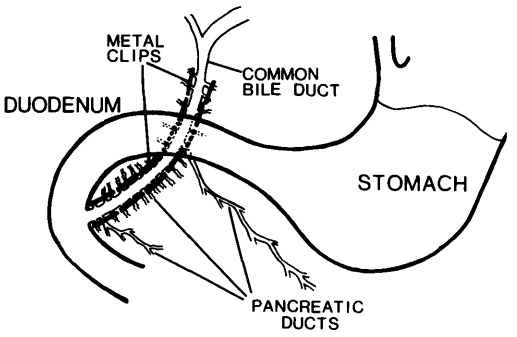

FIG. 1. Surgical procedure: Metal hemostatic clips applied on both sides of the common bile duct in order to occlude all connections from the pancreatic gland.

was examined after 1 week ($n = 6$) and 4 weeks ($n = 6$). The 6 controls were examined 1 week following sham operation.

In nonfasting animals, laparotomy was performed under neuroleptic-opioid anesthesia (Hypnorm Vet., "Mekos," 0.1 ml/100 g body wt). The duodenum was incised 1.5–2 cm proximal and distal to the choledochoduodenal junction, two polyethylene catheters were inserted, and the duodenum was occluded around the catheters with ligatures.

Pancreatic secretion was maximally stimulated by continuous intravenous infusion of cholecystokinin (CCK "Kabi Diagnostica") in a dose of 80 IDU/kg/hr (6). The stimulation period was 60 min, during which the contents of the isolated duodenal loop were removed at 10-min intervals by perfusion with 0.2 ml physiological saline.

The concentration of amylase, protein (micro-Kjeldahl technique), and proteolytic activity as described by Berstad (7) was determined in the perfusate, and output per hour was calculated. At the end of the stimulation period, blood was drawn from the inferior vena cava for determination of glucose concentration, as well as serum levels of bilirubin and alkaline phosphatase (conventional clinical chemical methods).

The two-tailed Mann–Whitney test (8) was used for comparisons between groups.

Results. Three rats died during the first week after pancreatic duct occlusion due to severe pancreatitis. The remaining animals appeared to be in good health throughout the experimental period. The 12 rats used for secretion studies were randomly selected from the 15 survivors.

One week after the operation there was no significant difference in body weight between pancreatic duct occluded animals and controls. The mean body weight of pancreatic duct occluded animals examined at 4 weeks was about 5% less than predicted from the normal growth curve; however, the difference was not statistically significant.

One week following pancreatic duct occlusion, the macroscopic appearance on laparotomy was clearly suggestive of pronounced pancreatitis. Most animals exhibited gross peritonitis, ascites, and chalky, white deposits in and near the pancreatic gland.

Animals examined 4 weeks after the duct occluding operation also showed areas of fat necrosis, but less pronounced than at 1 week. Ascites and peritonitis were absent, and the pancreas appeared atrophic. Histological examination showed marked acinar atrophy, but preserved excretory ducts and islets of Langerhans (Fig. 2).

Output per hour of trypsin and amylase, and the amylase/protein ratio are shown in Table I.

Compared with sham-operated controls, the reduction of proteolytic activity, amylase output, and amylase/protein ratio were all highly significant both 1 and 4 weeks after pancreatic duct occlusion ($P < 0.005$ for all three parameters).

Proteolytic activity was reduced by 96% compared with controls 1 week after duct occlusion, but rose to about 40% of the control level at 4 weeks following the operation ($P < 0.05$). Amylase output was reduced to about 20 and 16% after 1 and 4 weeks, respectively (difference not significant). The median protein output was reduced by approximately 60% ($P < 0.05$), whereas the amylase/protein ratio was reduced to about 35% of that of the sham-operated animals.

There were no significant differences in blood glucose, serum bilirubin, and serum alkaline phosphatase levels in duct occluded rats as compared with the controls (Table II).

Discussion. Pancreatic exocrine insufficiency in the rat may be produced by pharmacological agents (9–11), dietary manipulations (12), and by different surgical procedures (13–17).

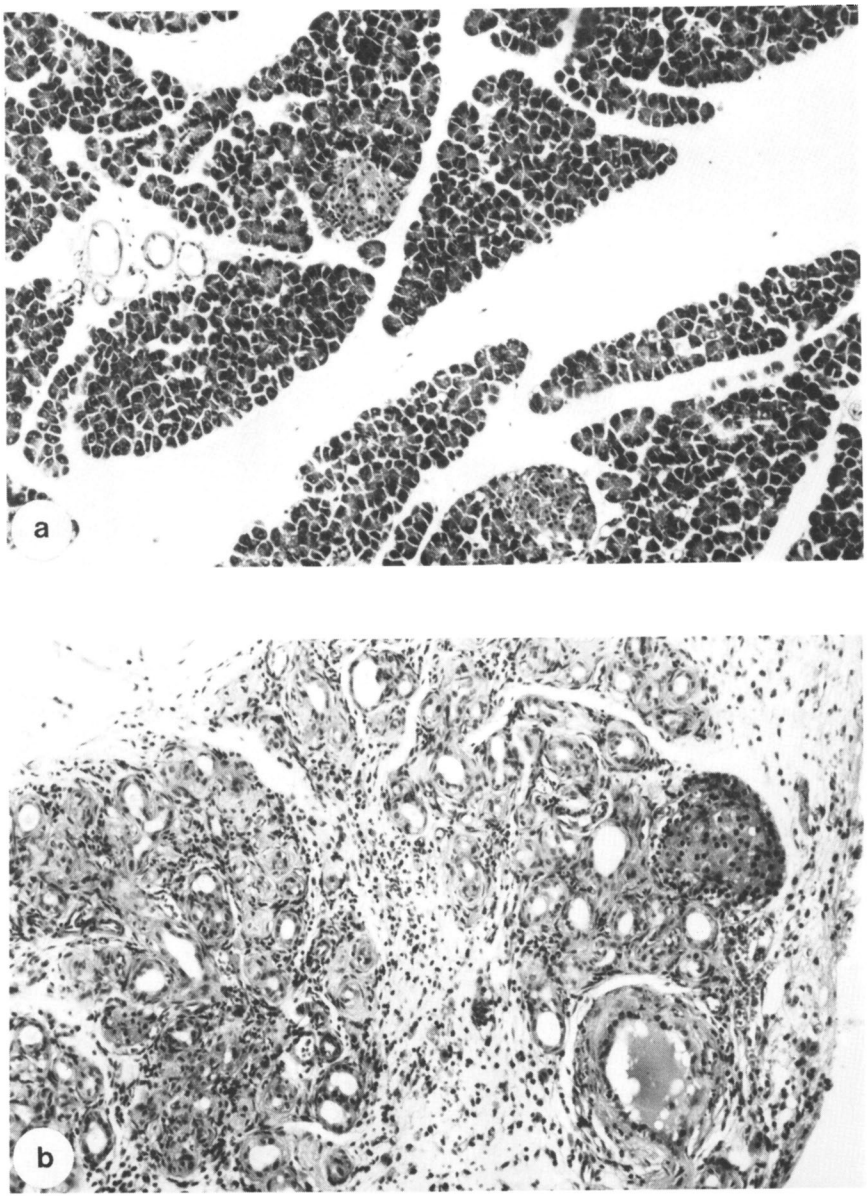

FIG. 2. (a) Normal pancreatic gland in control animal. (b) Marked acinar atrophy, preserved excretory ducts and islets of Langerhans 4 weeks after pancreatic duct occlusion.

Feeding rats a protein-free diet containing ethionine leads to extensive pancreatic exocrine lesions with inflammatory changes and a resulting secretory insufficiency lasting 1–2 weeks following cessation of the diet (12). However, the mortality associated with this treatment is relatively high, e.g., 42% in the study of Michel *et al.* (12).

In many experimental models, surgical methods of inducing exocrine insufficiency are more convenient and possibly also preferable to pharmacologic and dietary methods because undesirable effects on other organ systems are minimized.

Due to the particular anatomy of the rat it is necessary to perform a rather complicated

TABLE I. OUTPUT OF TRYPSIN AND AMYLASE, AND AMYLASE/PROTEIN RATIO IN CONTROLS AND PANCREATIC DUCT OCCLUDED ANIMALS AT 1 AND 4 WEEKS

	Trypsin (μg/hr)			Amylase (1000 Units/hr)			Amylase/protein ratio (Units/mg)		
	Median	Range	Reduction	Median	Range	Reduction	Median	Range	Reduction
Controls (n = 6)	139	86–184	—	843	395–1895	—	258	184–421	—
1 week (n = 6)	5	0–13	96.2%	168	17–178	80.1%	86	14–176	66.7%
4 weeks (n = 6)	55	33–98	60.5%	134	86–330	84.1%	91	43–174	64.5%

total or subtotal pancreatectomy (14, 16, 17) in order to make the animals completely and permanently devoid of pancreatic enzymes. However, a temporary partial exocrine insufficiency often suffices for experimental purposes, and this may be achieved by duct occlusion alone (2, 4, 15).

Most authors agree that in the rat all excretory pancreatic ducts drain into the common bile duct; the number of pancreatic ducts has been estimated as high as 40 (5). Therefore, the technique described by Clowes and MacPherson (13) has usually been preferred to individual ligature of the ducts. Their procedure consists of ligating the common bile duct at its junction with the duodenum and proximal to the point where it becomes surrounded by pancreatic tissue, and creating a biliointestinal anastomosis by use of a thin polyethylene catheter. In addition to proximal and distal ligature of the common bile duct, injection of tissue glue or similar substances into the common duct and pancreatic ducts may be utilized (15). With this modification, the cystic dilation of the common bile duct which follows ligation alone (2) is avoided. However, it is still necessary to perform a bile drainage procedure.

We have used pancreatic duct occlusion with metal clips to investigate the influence of pancreatic secretion on late radiation enteropathy (1), small intestinal mucosal enzyme content (manuscript in preparation), and intestinal damage following hyperthermia (manuscript in preparation). In these studies, the mortality due to the duct occluding procedure has been less than 10%.

Pancreatic duct occlusion with metal hemostatic clips is easy and quick to perform, does not result in dilation of the common bile duct, and the technique obviates the need for a biliointestinal anastomosis. The operation did not appear to cause biliary stasis, as there was no difference in the serum levels of bilirubin and alkaline phosphatase between pancreatic duct occluded animals and controls.

TABLE II. SERUM BILIRUBIN AND ALKALINE PHOSPHATASE AND BLOOD GLUCOSE CONCENTRATIONS (MEDIAN VALUE AND RANGE)

	Bilirubin (μmole/liter)	Alkaline phosphatase (Units/liter)	Glucose (mmole/liter)
Controls (n = 6)	2 (1–4)	111 (106–174)	7.4 (6.4–10.5)
1 week (n = 6)	3 (1–4)	147 (110–224)	7.3 (5.0–12.6)
4 weeks (n = 6)	2.5 (1–4)	151 (109–284)	7.5 (5.8–11.8)

In the present study the food intake of the animals was not monitored, but the postoperative weight gain was close to normal and the animals appeared healthy. Thus, the effects of the procedure on nutrition seem to be minor.

There was atrophy of the pancreatic acini 4 weeks after duct occlusion. However, the islets of Langerhans were preserved and the blood glucose level was normal. Although no glucose-tolerance tests were performed, our results suggest that the procedure did not cause any major endocrine abnormality. This is in accordance with observations following other methods of pancreatic duct occlusion in the rat (2, 3, 15).

Pancreatic trauma due to sham operation significantly affects secretion only during a few hours (5). Therefore, the use of a single group of controls for comparison with pancreatic duct occluded animals at 1 and 4 weeks was considered justified.

One week following the operation, the median duodenal amylase output was reduced by 80%, whereas the proteolytic activity was reduced by 96% compared with sham-operated control animals. Four weeks after the operation amylase output was unchanged, whereas the trypsin output was approximately 40% of that in the controls.

These figures are in agreement with those of Klein *et al.* (3) who measured pancreatic function with the pancreolauryl test following a modified Clowes and MacPherson procedure with intraductal prolamine gel injection.

It has been shown that anesthesia, the time elapsed since surgical preparation, and recirculation of bile and pancreatic secretions in the intestine influence both basal pancreatic secretion and the response to cholecystokinin stimulation (6, 18). Pancreatitis induced by intraductal sodium taurodeoxycholate and trypsin injection is also associated with a markedly reduced secretory volume and protein output (19). The factors mentioned above may to some extent influence the enzyme output measurements in the present study, and possibly also explain the discrepancy between amylase and trypsin output after pancreatic duct occlusion, due to a different influence on the various types of secretory cells. Following pancreatic duct occlusion, the relative reduction of protein output was less marked than that of amylase output. This could be due to proteins of biliary and intestinal origin, and may explain the reduced amylase/protein ratio found in pancreatic duct occluded animals.

The increase in duodenal proteolytic activity from 1 to 4 weeks after pancreatic duct occlusion may be the result of recanalization, either across the staples or by the direct route from pancreas to duodenum described by Uram *et al.* (4). In their study, complete recovery of digestive capacity was observed 6 weeks following pancreatic duct occlusion with a modified Clowes and MacPherson procedure. Furthermore, lampblack injected into the duodenum rapidly appeared in the pancreas, indicating reestablished continuity. Using a similar approach, Isaksson *et al.* (2) found that even after presumed complete duct ligation there was a substantial amount of enzyme activity of probable pancreatic origin in the intestinal contents.

There was no difference in amylase activity between samples obtained 1 and 4 weeks following duct occlusion. This finding may possibly be due to differences in compensatory adaptation between trypsin and amylase secretory mechanisms, or by relative differences in enzyme activity of intestinal origin (4, 20).

Pancreatic duct occlusion using metal clips is a rapid and simple procedure. The resulting impairment of the exocrine pancreatic secretion seems to be of the same magnitude and duration as that achieved with more complicated procedures.

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