Perfusion of the Dog Pancreas with Bile Without Production of Pancreatitis *

THOMAS T. WHITE, M.D.,** D. F. MAGEE, M.D., PH.D.†

From the Departments of Surgery and Pharmacology, School of Medicine, University of Washington, Seattle, Washington

DOES REFLUX of bile into the pancreatic duct trigger the onset of acute pancreatitis? This has been suggested by many people since Opie [9] demonstrated, in 1901, this could occur in a patient with a stone lodged in the ampulla of Vater, and Archibald, in 1919,[1] found that simple spasm of the ampulla could produce this situation. More recently, Doubilet has suggested that section of the sphincter of Oddi relieves pancreatitis and that this is because reflux is eliminated.[5] On the other hand, he points out, the majority (96 per cent) of individuals have a physiologic, if not an anatomic connection of the two ducts in the vicinity of the ampulla of Vater.[6] We have wondered why pancreatitis was not much more prevalent if reflux of bile was etiologic and reflux occurs so frequently.

Early experiments demonstrated that bile alone, injected under considerable pressure into the pancreatic ducts of dogs, would produce pancreatitis. Later experiments, such as those of Wangensteen [12] (1931) in cats, and de Almeida [3] (1955) in dogs, with connection of the pancreatic and bile ducts, showed no consistent untoward changes in the pancreas. These experiments, of necessity, were short-lived, owing to the absence of an exit point into the alimentary canal for the bile and pancreatic juice. In later experiments in dogs (Cross *et al.*,[2] 1955), all the liver bile was directed through the pancreas by connecting the gallbladder to one of the pancreatic ducts, thus allowing bile to pass through the pancreas and out the other duct. The gallbladder was thus nonfunctional. Whitrock *et al.*[13] redirected the entire bile flow through the goat pancreas, leaving the gallbladder functional. In the goat, however, the pancreatic duct normally opens into the side of the common bile duct about 3 cm. from the duodenum and often contains bile.[10] Although none of these perfusion experiments produced pancreatitis, the objection has been raised that gallbladder bile is necessary to produce the disease,[4] and that a carnivorous animal is needed. Powers [11] implanted the main pancreatic duct into the common bile duct of the dog, and produced acute pancreatitis, when morphine was given to cause sphincter spasm. Further, pancreatic juice incubated with gallbladder bile and introduced into the pancreatic duct under "physiologic pressure" produced necrotizing pancreatitis.[7] The inference is, pancreatic juice, under certain circumstances, will run up the common bile duct into the gallbladder, remain there for a period, and then run back into the pancreas to produce acute inflammatory processes. The following experiment is intended to produce a situation in which pancreatic juice passes to and fro between gallbladder and pancreatic duct, and the entire bile flow of both gallbladder and liver may pass through the pancreatic ductile system into the duodenum.

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** Clinical Assistant Professor of Surgery.

† Associate Professor of Pharmacology.

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Technic

1. In five dogs, the main pancreatic duct was ligated with a .61-mm. polyethylene catheter. The dogs were put back in their cages. At reoperation, three to five days later, the ligature was cut. The main duct was cannulated with a previously prepared 1.14-mm. polyethylene catheter, over each end of which a cuff of similar material was heat-annealed. The accessory duct remained undisturbed. Ligatures were placed around the duct and cannula. The opposite end was threaded into the cut end of the common bile duct and tied in place. In three instances, the catheter was left completely within the belly and in three instances, it was brought out to the surface of the abdomen and then back again. The dogs were observed for signs of prostration, bile or pancreatic juice in the externally placed cannulae, and color of stool. Blood was drawn, at intervals, for plasma, amylase and bilirubin determinations. Sections of the pancreas and liver were taken at autopsy. Amylase was determined, using Nelson's[8] method to determine the milligrams of maltose liberated from starch substrate in 30 minutes, at 37° C.

2. In another three dogs, the main pancreatic duct was tied as before. In these dogs, the pancreas was cross cut, three to five cm. from the tail, and a cannula was inserted into the pancreatic duct at one end and the common bile duct on the other. In the fourth and fifth dogs, the ducts were connected primarily. Three dogs had external placement of the cannulae, while two had the cannulae completely within the abdomen. These dogs were observed as in the first group.

3. Another three dogs were prepared as in group two with the exception that they were fed one Gm. desoxycholic acid, daily.

4. In order to determine the effect of bile on proteolytic zymogens, equal volumes of gallbladder bile and pancreatic juice with 100 units of penicillin and 100 mg. of streptomycin, were incubated for 24 hours in one experiment, and for 20 minutes in another, after which proteolytic activity was determined by the method of Anson and Mirsky. The results were compared with enterokinase-activated juice and with incubated pancreatic juice controls, to which bile was added at the time of determination.

Results

Group 1: All five dogs were anorexic for from four to five days, after which time their appetites gradually improved in proportion to relief of their biliary obstruction. One dog (A) was re-operated on, on the tenth day, at which time the gallbladder was immensely dilated with evidences of marked biliary stasis and bile passed into the pancreas, which was yellow tinged. Biopsies of the pancreas, taken at this time, were normal. The liver showed extensive biliary obstruction. This dog died several days later of bile peritonitis without pancreatic change of note. The next dogs (B and C) died of biliary obstruction, one after three days and the other after 22 days. Dogs D and E died on the third and 20th days after pulling out their catheters. There was no gross or microscopic evidence of pancreatitis at autopsy (Fig. 1). Amylases were done on dogs C, D and E as

TABLE 1. *Blood Amylase as Gm. Maltose per 100 cc. Plasma*

	C	D	E	F	G	H	I
Days 1–3	2.46			1.64	1.37	.99	.94
3–6	1.65						
Weeks 2		1.39	2.08	1.21	1.67	1.31	.73
3	3 12		1 84		1 36		
4			2 39		1.06		1.55
5				1.04	1.67		
6							
7				.82			
8					1.20		
9							
10					.79		

Control 1.10 (average of 5)

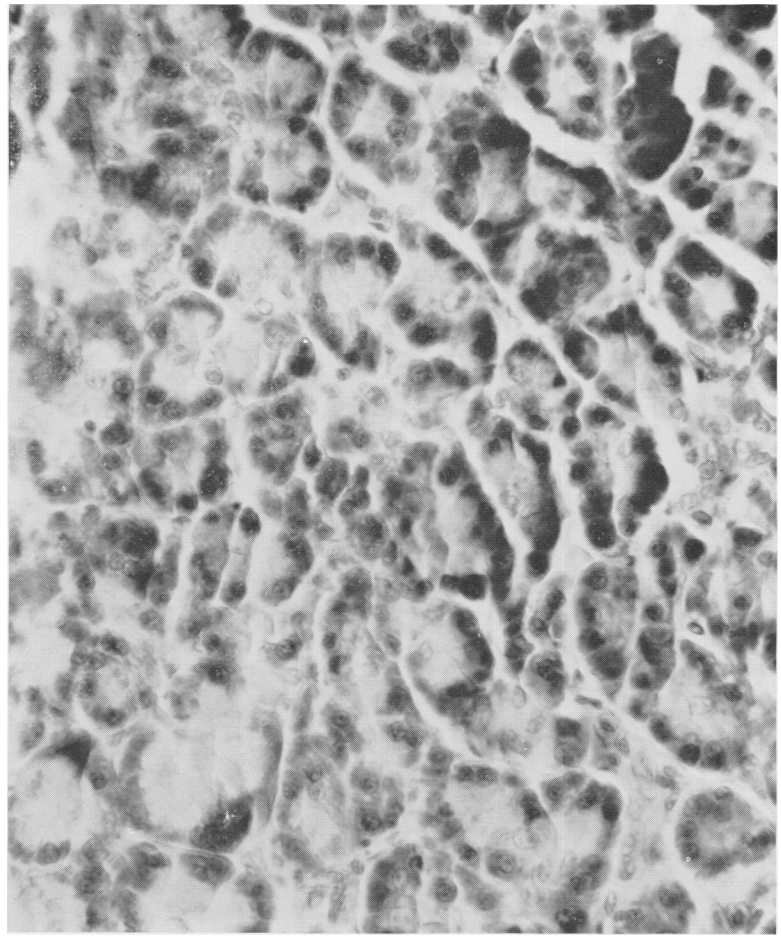

FIG. 1. Autopsy section of the pancreas in dog C shows no autodigestion after three weeks perfusion with bile.

indicated in Table 1. Moderate elevation of amylase was seen in all these dogs.

Group 2: The first three dogs had bile in their catheters after the first day and in the stool after the third day. The dogs were jaundiced at first, but were largely free of icterus by the third week. Amylase determinations on dog I were 0.94 Gm. maltose, per 100 cc. plasma, the third day; 1.04 Gm. the eighth day; 0.73 Gm. the fourteenth day; and 1.55 Gm. the twenty-ninth day. On dog G, they were 1.37 Gm. the second day; 1.67 Gm. the eleventh day; 1.36 Gm. the nineteenth day; and 1.06 Gm. the twenty-second day and 79 Gm. after 10 weeks. All three dogs pulled their catheters out by the end of a month, yet there was no gross or microscopic evidence of pancreatic disease at autopsy. The last two dogs had catheters completely within the abdomen. Stools were normal in color, on the third and fourth days, respectively. These dogs were well on the seventy-seventh and sixty-second days, respectively. X-rays of biliary and pancreatic ductal systems by injection of dye are illustrated in Figures 2 and 3. The biliary radicals are enormously dilated while the pancreatic ducts are relatively normal. Sections of the pancreas of these dogs, taken at sacrifice, are normal (Fig. 4).

Group 3: The dogs survived 3, 7 and 21 days, respectively, without evidence of pancreatitis. Death was owing to biliary ob-

TABLE 2. *Activity of Pancreatic Juice After Incubation With and Without Bile as Mg. Tyrosine Released from Hemoglobin in 10 Minutes*

	Without Activator	With Activator
No incubation	36.6	151.5
24 hours incubation		
Pancreatic juice only	1.5	0.3
Pancreatic juice and bile	6.25	18.2
20 minutes incubation		
Pancreatic juice and bile	6.25	52.5

struction in each instance, bole flow having been greatly increased by the addition of desoxycholic acid to the diet.

Group 4: Table 2 shows the results of the protease activation studies. Activity after incubation with bile is small compared with that produced by an enterokinase source and very little greater than occurring in the control juice tubes.

Summary and Conclusions

The entire biliary flow has been diverted through the pancreas in 11 dogs. In the six dogs with bile passing into the main pancreatic duct and out the accessory duct, there was persistent jaundice and elevation of amylase, probably due to the inability of the pancreatic ductal system to handle such a large flow. There was no instance of acute pancreatitis, in any of the five dogs, in which the bile passed into the tail and out through the two pancreatic openings to the duodenum. Nor was there an elevation of amylase in any of these. Jaundice in these dogs disappeared after three to four weeks. Addition of desoxycholic acid

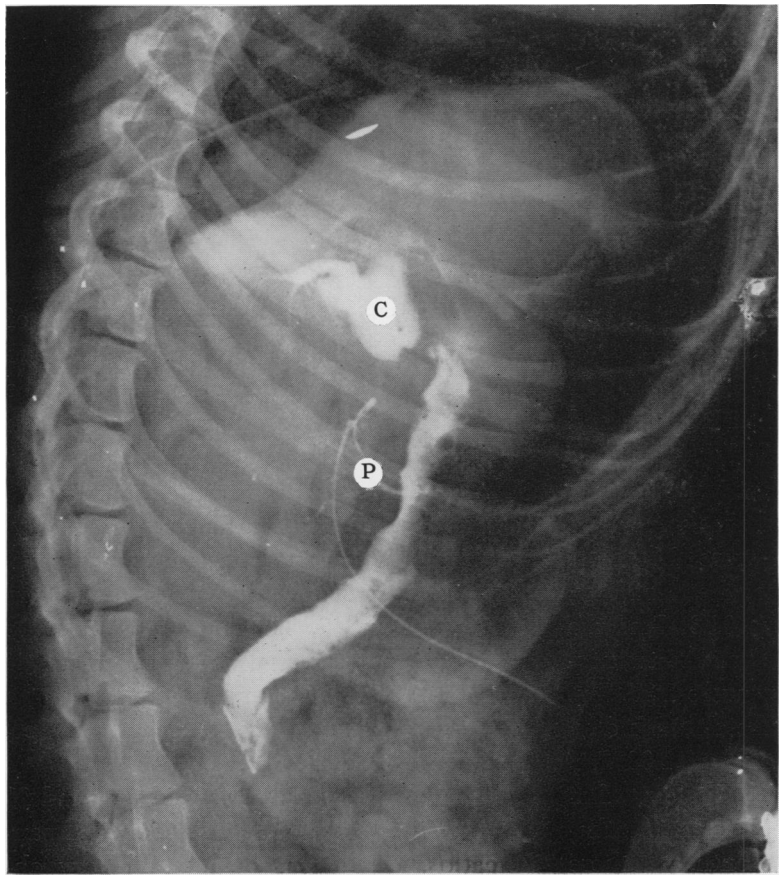

FIG. 2. Cholangiogram (C), pancreatogram (P), and dye in duodenum after injection of radiopaque dye shows great dilatation of biliary radicals.

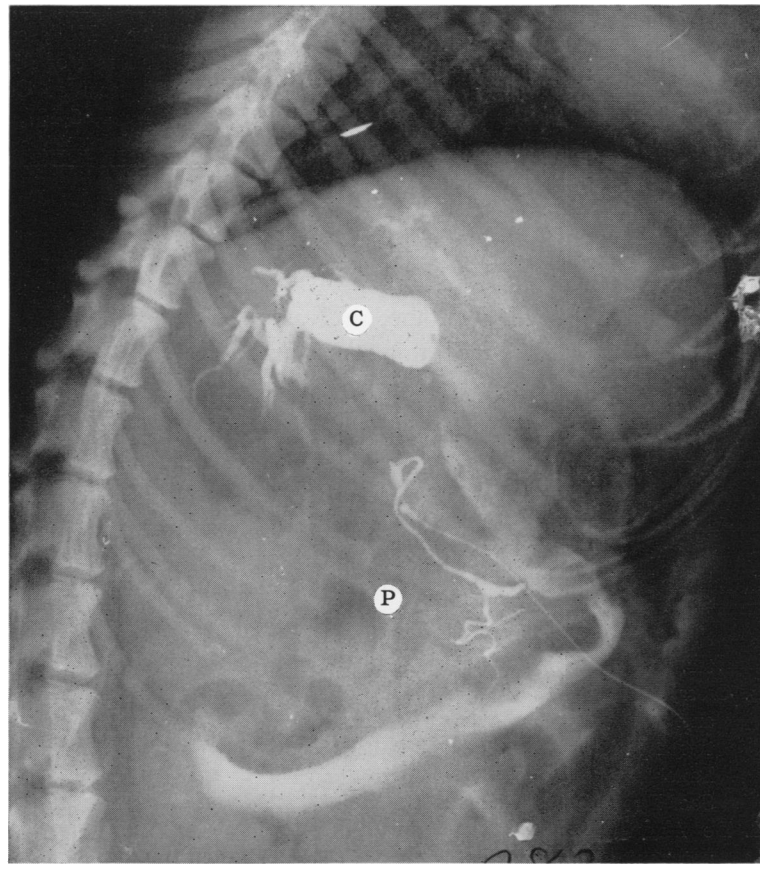

FIG. 3. Cholangiogram (C) Pancreatogram (P), and dye in duodenum again shows a patent system and enormous dilatation of biliary radicals.

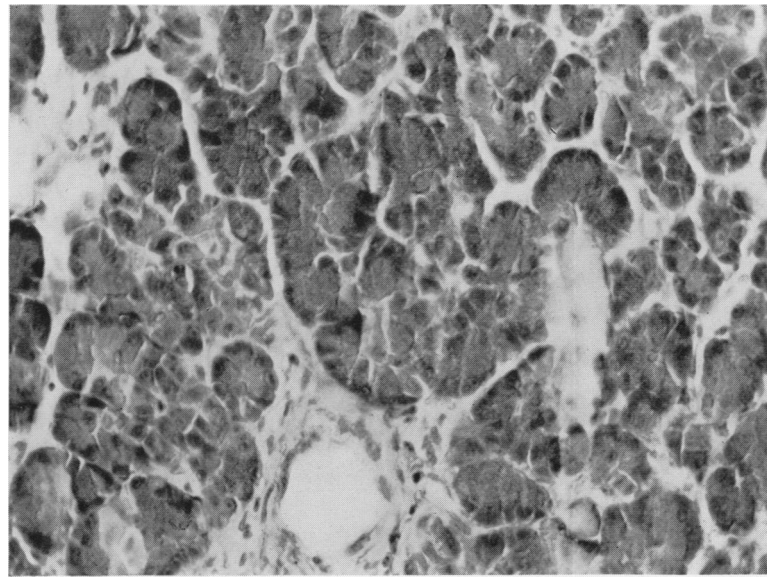

FIG. 4. Dog F. No evidence of pancreatitis is seen on sections of the pancreas after seven weeks perfusion with bile.

to the diet, to raise the dog bile-desoxycholic acid level towards human levels, did not alter the picture.

Previous studies in this and other laboratories have shown that bile is not a potent activator of pancreatic proteases. The fact that addition of enterokinase to a mixture of bile and pancreatic juice results in threefold increase in protease activity leads us to suspect that bile may protect proteases from auto-activation. This makes it unlikely that pancreatitis produced by intraductal injections of 24-hour incubated bile and pancreatic juice is due to the presence of active proteases.

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