Experimental Production of Pancreatitis by Infusion of Mixtures of Bile and Pancreatic Juice into the Pancreatic Duct *

RENE JOYEUSE, M.D.,** GEORGE A. HALLENBECK, M.D.,***
WILLIAM T. E. MCCAUGHEY, M.D.†

From the Mayo Clinic and Mayo Foundation, Rochester, Minnesota

ELLIOTT, Williams and Zollinger[1] have proposed the theory that acute pancreatitis occurs if pancreatic juice enters the obstructed common bile duct and reacts there with bile to form a toxic mixture that can flow into the pancreatic ductal system under low pressure and damage the pancreas. In support of this theory, Elliott and his associates presented evidence that 1) although, initially, pressure in the obstructed pancreatic duct of the dog exceeds that in the obstructed common bile duct, the two pressures approach one another after 24 hours of obstruction, so that movement of fluid from bile duct to pancreatic duct is at least conceivable if a common channel exists; and 2) although bile or unincubated mixtures of bile and pancreatic juice infused at 40 cm. of water pressure enter the pancreatic ducts of dogs only in small quantities and produce minimal pancreatitis, bile incubated with pancreatic juice or trypsin is accepted readily by the pancreas at this pressure and produces fatal hemorrhagic pancreatitis.

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** Fellow in Surgery, Mayo Foundation.

*** Section of Surgery.

† Research Associate.

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The purpose of the present study was to repeat some of the observations of Elliott and co-workers concerned with the efficacy of the intraductal infusion of bile, pancreatic juice and mixtures thereof in producing pancreatitis in dogs.

Methods and Procedure

General Plan of the Experiment. In tests on 67 dogs, bile, pancreatic juice or mixtures of these substances were infused into the pancreatic ducts for 30 minutes at a pressure of 40 cm. of water. Surviving animals were anesthetized and killed 72 hours later; all animals were examined by gross dissection and inspection and by study of microscopic sections of the pancreas to determine the extent of pancreatitis.

In some experiments bile was infused alone; in others bile was mixed with equal volumes of either pancreatic juice or isotonic solution of sodium chloride and given with or without a 24- to 36-hour period of incubation at 37° C. In still other tests, pancreatic juice was administered without bile, sometimes with and sometimes without a period of incubation.

Technic of Infusion into Pancreatic Duct. Dogs were anesthetized with pentobarbital sodium administered intravenously; aseptic technic was used throughout the operative procedure. In each case the abdomen was opened through an upper mid-

line incision, the minor pancreatic duct was divided between ligatures of fine silk and the major pancreatic duct was isolated at its junction with the duodenum. The duodenum was opened; a short metal cannula was introduced into the major pancreatic duct transduodenally and was tied in place. After the infusion was completed, the ligature and cannula were removed and the pancreatic duct was left patent. The opening in the duodenum was sutured closed, the abdominal incision was closed, and the dog was returned to its cage for observation.

When pressures for infusion into the pancreatic duct are low, it is difficult to be certain whether or not the limited flow of fluid into the duct is the result of obstruction at the tip of the cannula, if the usual type of cannula is used. For this reason a cannula was made with a tip that has three lateral openings in addition to one at the end, so that neither lateral nor terminal pressure applied singly could obstruct it (Fig. 1).

Pressure during infusion was controlled by a simple, sterile glass manometer connected to the barrel of a 10-ml. syringe and to a polyethylene tube leading to the cannula in the pancreatic duct (Fig. 2). The zero mark on the manometer was placed at the level of the papilla of the pancreatic duct, and the level of fluid in the reservoir was repeatedly adjusted to maintain the level in the manometer at 40 cm. during the 30-minute period of infusion. The volume of fluid that entered the pancreas was calculated by noting the volume of fluid in the reservoir at the beginning and end of the experiment and adding to it the volume, if any, added during the experiment.

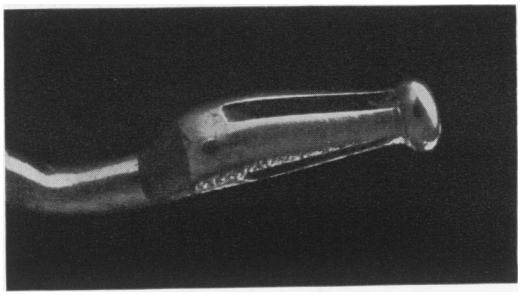

FIG. 1. Special cannula tip used for the infusion of fluids into the pancreatic duct (× 5½).

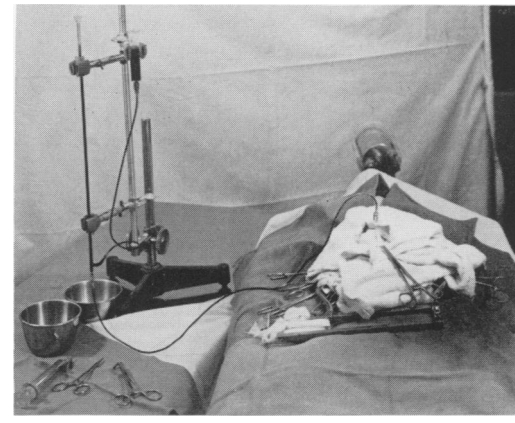

FIG. 2. System used to infuse fluid into the pancreatic duct at controlled pressure. See text for explanation.

Source of Bile. Bile was aspirated aseptically with needle and syringe from the gallbladders of dogs at the time of laparotomy. In many experiments the bile infused into the pancreatic ducts was autologous; in those experiments in which bile was first incubated for 24 to 36 hours with either isotonic solution of sodium chloride or pancreatic juice, the bile was homologous.

Source of Pancreatic Juice. Juice from donor dogs was collected from external pancreatic fistulas made by the insertion of one No. 20 polyvinyl tube through a transverse incision in the major pancreatic duct toward the pancreas, and another through the same incision in the duct through the papilla and a few millimeters into the duodenum. Both catheters were tied to the duct and to each other, and small pieces of Ivalon sponge were sutured around the catheters at the points of junction with the pancreatic duct and with the parietal peritoneum. The two tubes could be connected when collections of juice were not being

made. By means of this system donors could be used to provide pancreatic juice for 2½ weeks in all cases. The duodenal catheter had to be irrigated at intervals to keep it open.

Pancreatic juice was collected in sterile containers, chilled at once by ice water and preserved for later use by freezing. The flow of juice was stimulated by the feeding of horse meat and by the subcutaneous administration of a cholinergic drug (Urecholine) every 20 minutes after feeding. In most experiments an extract of duodenal mucosa, prepared according to the technic of Waldschmidt-Leitz,[8] was added to the juice immediately before the juice was used in order to activate trypsinogen to trypsin.

Each sample of pancreatic juice was cultured immediately before use. Only two samples were found to be contaminated by bacteria; data from these two experiments were discarded.

In a few experiments, plasma amylase activity was determined by the method of Norby, as modified by Lagerlöf,[4] at intervals after infusion of the test substance.

At the end of each experiment the degree of pancreatitis was assessed grossly at postmortem examination, and the pancreas with part of the duodenum attached was removed and fixed in a 10 per cent solution of formalin. Microscopic sections stained by hematoxylin and eosin were cut from the head of the pancreas near the attachment of the major duct to the duodenum, from the uncinate process and from the tail of the gland.

The degree of pancreatitis was graded from 1+ to 4+ as follows:

1. *Minimal pancreatitis* was graded 1+. This grade denotes pancreatitis in which no significant thickening of the gland had occurred and in which both the gross and histologic changes were inconspicuous and focal.

2. *Mild pancreatitis* was graded 2+. This grade applied to localized or mild generalized enlargement of the gland with some induration or edema and with mild subcapsular and interstitial inflammation. No more than an occasional focus of fat necrosis occurred in animals with pancreatitis classified in this group.

3. *Moderate pancreatitis* was graded 3+. This grade was said to be present when there was a substantial degree of enlargement and induration of much of the pancreas, with extensive subcapsular and interstitial edema and inflammation in one or more areas of the gland. Adhesions of the gland to adjacent viscera and a considerable degree of fat necrosis were common, as was the occurrence of small amounts of serous or serosanguineous fluid in the abdominal cavity. There were never more than a few tiny foci of hemorrhagic necrosis.

4. *Severe pancreatitis* was graded 4+. This grade signified the presence of marked generalized enlargement and induration of the pancreas, with conspicuous acute inflammation and edema of the subcapsular and interstitial tissues, and often extensive fatty and hemorrhagic necrosis. The accumulation of up to several hundred milliliters of serosanguineous peritoneal exudate was common, and usually there were numerous adhesions of the gland to the omentum and adjacent intraperitoneal structures. Seven of the 24 animals in which pancreatitis was graded 4+ died before 72 hours had passed after the infusion was completed.

Results

The results are summarized in Table 1. Undiluted fresh bile infused into the pancreatic ductal system under the conditions of the experiments was accepted in very small quantities, the average being 1.6 ml. in 14 dogs. When only about 1.0 ml. of bile entered the pancreas, only that part

TABLE 1. *Receptivity of Pancreas to Infusions of Bile, Pancreatic Juice, and Mixtures Thereof, and Severity of Resulting Pancreatitis*

Infusion Fluid, Nature	Dog, No.	Fluid Entering Pancreas, ml.		Pancreatitis, Severity*			
				1+ or 2+		3+ or 4+	
		Mean	Range	No.	%	No.	%
Fresh undiluted bile	14	1.6	1.0– 3.0	14	100	0	0
Bile diluted 1:1 with isotonic sodium chloride solution	14	3.8	1.0–12.0	12	86	2	14
Pancreatic juice and enterokinase, incubated 24–36 hr. at 37° C.	8	10.4	0.2–26.0	7	87	1	13
Bile and pancreatic juice 1:1 and enterokinase, incubated 24–36 hr. at 37° C.	24	15.7	0.4–42.0	14	58	10	42
Same, without enterokinase	7	1.5	0.2– 7.0	5	71	2	29

* See text for explanation of the grading of severity of pancreatitis.

of the gland near the major pancreatic duct became edematous and yellow-green during the infusion. Entry of as little as 2.0 or 3.0 ml. sufficed to make the entire pancreas appear swollen and yellow-green. Despite these immediate changes, the dogs appeared to be normal postoperatively. Seventy-two hours later the degree of pancreatitis was minimal (1+) in 13 animals and mild (2+) in one.

The pancreas accepted a larger amount of an equal mixture of bile and isotonic solution of sodium chloride, the average in 14 dogs being 3.8 ml. The glands became more edematous but less deeply yellow-green than those infused with undiluted bile. The resulting pancreatitis was a little more severe. One dog died with severe pancreatitis and in another the process was graded 3+. In the remaining 12 cases (86%) the pancreatitis was graded minimal (seven cases) or mild (five cases).

When the infusing fluid was pancreatic juice with enterokinase added, the pancreases of eight dogs accepted an average of 10.4 ml. during the 30 minutes of infusion. Despite the fact that more pancreatic juice entered the glands than was the case when diluted bile was used, the resulting pancreatitis was similar. In seven of the eight cases pancreatitis was graded 1+ or 2+; in the remaining case it was graded 3+.

Incubated mixtures of equal volumes of pancreatic juice and bile with enterokinase added were accepted in even larger quantities by the pancreas, the average in 24 dogs being 15.7 ml. When a very large volume of infusate entered a gland, the mixture would exude from the surface of the pancreas into the abdominal cavity. The resulting pancreatitis was severe in a greater percentage of animals than in the other groups studied; it was graded 3+ or 4+ in 10 animals (42%) and 1+ or 2+ in 14 animals (58%). Seven dogs (29%) died within 72 hours and three more were in shock when sacrificed.

Omission of enterokinase from the infusing fluid in seven animals appeared to cause both the acceptance of the fluid by the pancreases and the severity of the pancreatitis to diminish.

Comment

In agreement with the observations of Elliott, Williams and Zollinger, our observations show that fresh bile infused into the pancreatic duct for 30 minutes at a pressure never exceeding 40 cm. of water is accepted only in small quantities by the pancreas. The resulting pancreatitis in all cases was minimal or mild; subcapsular and interstitial changes, when present, were patchy only.

Also in agreement with the observations of Elliott and associates is the observation that when bile was diluted with equal parts of isotonic solution of sodium chloride under the same experimental conditions, the pancreas admitted increased quantities of the mixture but the resulting pancreatitis as a rule still was not severe. Bile from the gallbladder mixed with isotonic solution of sodium chloride is less viscous than bile alone. It is possible that this lessened viscosity is important in the acceptance of increased volumes of this mixture by the pancreatic ductal system.

Homologous incubated pancreatic juice with enterokinase added was accepted in even larger quantities than fresh or diluted bile. However, the acceptance of larger quantities of infusion material did not result in a more severe degree of pancreatitis than that observed when diluted bile was infused. These data supplement those of Elliott and associates, who did not make this test.

The observation that a mixture of an equal volume of bile and pancreatic juice (in this study, with enterokinase added to ensure activation), incubated 24 to 36 hours at 37° C., is more readily accepted than either bile or pancreatic juice alone, was confirmed. In 24 dogs an average of 15.7 ml. of the mixture was accepted under the conditions of the experiments. This quantity is about 10 times that of bile given alone, but is only 1½ times that of pancreatic juice given alone. It was also confirmed that the resulting pancreatitis is significantly more severe when this mixture of bile and pancreatic juice and enterokinase is used than when either of its constituents is administered alone (42% of moderate or severe pancreatitis against 0 per cent when bile was given and 13% when pancreatic juice alone was given). However, Elliott and his group reported the occurrence of lethal pancreatitis in 100 per cent of their cases; in the present study pancreatitis, after the infusion of bile and pancreatic juice, was lethal in only 29 per cent of cases and was classified as moderate or severe in 42 per cent. This difference can be explained by a consideration of Elliott's method of selecting cases.

Although Elliott and his associates reported the occurrence of lethal pancreatitis in all 11 dogs which underwent infusion of an incubated mixture of pancreatic juice and bile into the pancreatic ducts, they added that not all specimens of pancreatic juice were capable of effecting this change in bile. They concluded that bacterial contamination, when present, caused the glands to accept less of the mixture than was the case when the mixtures were sterile, and in this manner interfered with development of this type of pancreatitis. They also wrote that the ability of pancreatic juice to alter bile and render the mixture lethal depended upon the content of trypsin in the juice. To quote them, "If the donor pancreas was stimulated by secretin, which produces a thin watery juice, low in enzymes, the resulting juices would not produce the essential change in bile. . . . The animals reported here include every one which received a mixture containing pancreatic juice with a mini-

mum potency, as measured in our laboratory, of 1,000 trypsin (tryptar) units per cc."

By such selection of cases, Elliott and his group obtained their high incidence of lethal pancreatitis. It is likewise possible that variations in the tryptic activity of pancreatic juice used in our experiments were important in determining the lesser incidence of severe pancreatitis which we observed.

Although the mean volume of mixed bile and pancreatic juice accepted by the pancreas was greater than the mean accepted volume of the other fluids which produced less severe pancreatitis, no consistent correlation between the volume of fluid accepted and the severity of pancreatitis was seen when individual cases were reviewed. For example, the pancreas of one dog accepted only 2.0 ml. of bile and pancreatic juice and lethal pancreatitis developed, whereas the pancreas of two others accepted respectively 15 and 16 ml. of the mixture and only mild pancreatitis developed. In experiments in which large volumes of the mixtures were accepted, fluid began to enter the ductal system immediately and continued to flow in at approximately the same rate during the 30 minutes of infusion. When the larger amounts were used, fluid would be seen to weep from all parts of the surface of the gland. The nature of the toxic factor that results from the interaction of bile and pancreatic juice or bile and trypsin is not known. The question may be raised whether the toxic factor is similar to that obtained by Nemir and Drabkin[6] when trypsin and blood were incubated together.

The infusion pressure of 40 cm. of water was chosen to duplicate the pressure used by Elliott, Williams and Zollinger. These authors chose it because, after they had measured pressures in obstructed pancreatic ducts of dogs and observed maximums higher than 40 cm. of water, they concluded that this pressure was within the physiologic range. Many workers[2, 9] have observed occasional pressures of 40 cm. of water in the obstructed pancreatic duct; often, in the dog, pressures of this magnitude are transitory and values of 20 to 30 cm. of water are more usual.[3]

In the absence of obstruction, pancreatic and biliary intraductal pressures are quite different. Parry and associates[7] found the mean value for the pancreatic duct to be 9.1 cm. of water (range 6.8 to 13.1) and Menguy and his colleagues,[5] using the same method, reported a mean of 14.0 cm. of water for the pancreatic duct and 7.0 for the common bile duct. The least that can be said is that 40 cm. of water is at the extreme upper end of "physiologic pressure" for the pancreatic ductal system. It would be interesting to repeat some of these experiments with the use of lower infusion pressures.

Conclusion

Results of this study confirm the general conclusion of Elliott, Williams and Zollinger that incubation of canine bile with pancreatic juice produces a mixture which enters the pancreatic ductal system at a pressure of 40 cm. of water more readily than does bile alone, and which is capable of producing severe and even fatal pancreatitis.

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Annular Pancreas in Infants

P. Wittebol; A. Tjon Sien Kie, and J. Lunding, Amsterdam, Netherlands
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In this paper two cases of annular pancreas in infants, in whom the condition had caused complete duodenal occlusion, are presented; symptomatology, aetiology and treatment are discussed. In both cases the condition manifested itself when breastfeeding was started on the second day after birth by nonexplosive vomiting. The first case, a boy, showed mongoloid stigmata. Both were admitted in a very severe condition of dehydration and stupor.

In the first case contrast medium showed occlusion of the duodenum, in the second the diagnosis was made on the U-shaped air figure in the upper abdomen. In the first case the underlying cause of the stenosis was not recognized. First, bands crossing the distended duodenum were severed. Postoperatively vomiting continued; a second laparotomy became necessary and anastomosis between the distended duodenum and the proximal jejunal loop was made. Here the general condition deteriorated slowly postoperatively and the infant died 18 days later. Autopsy showed the ring of pancreatic tissue in inflamed condition. No leakage of the anastomosis was noted.

In the second case the ring of pancreatic tissue was recognized at operation. Duodenojejunostomy was performed: postoperative course was largely uneventful. Duodenojejunostomy is considered the safest treatment in cases of stenosing annular pancreas and to be preferred above cleavage of the ring, as pancreatic fistulation may result.

The aetiology of annular pancreas is found in developmental disturbance in the pancreasanlage. The various theories are given.