Experimental Pancreatitis: *

A Possible Etiology of Postoperative Pancreatitis

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Introduction

Acute hemorrhagic pancreatitis is a well recognized complication of gastroduodenal surgery, but the mechanism of its causation has not been established. Surgical trauma to the pancreas has been considered by many to be the most important factor in its production, but others have suggested that spread of infection or reflux of duodenal contents into the pancreatic ducts may be the causative agents.

The present investigation was designed to test the hypothesis that this form of pancreatitis is associated with reflux of duodenal contents into the pancreas.

Historical Survey

Heffernon and Cassiet[5] reviewed 100 cases of acute hemorrhagic pancreatitis at the Lahey Clinic and found that 14 had had previous gastric surgery; acute pancreatitis immediately followed the operation in ten of these cases. Burton, Eckman and Haxo[2] reported five fatal cases of acute postoperative pancreatitis in 348 gastrectomies and stated that trauma to the head of the pancreas, and to the biliary or pancreatic ducts was the most important causative factor.

This traditional concept which relates pancreatitis to surgical trauma has been vigorously challenged by Warren.[12] He recorded seven cases of severe pancreatitis following partial or total gastrectomy, in some of which, no undue manipulation of the pancreas occurred. In contrast, five cases in which the main pancreatic duct was divided accidentally, and thereafter implanted in the mobilized duodenum, did not develop pancreatitis.

Many years previously Perman[7] described four cases of pancreatitis, one following gastroenterostomy and the other three following Billroth II partial gastrectomy. The afferent loop was markedly dilated in three cases, and he attributed the pancreatitis to stagnating bile-mixed contents being forced up the pancreatic duct and activating pancreatic secretions. More recently, Byrne and Boyd[3] reported two patients with acute mechanical small bowel obstruction and serum amylase values in the pancreatitis range. From experiments on dogs in which high intestinal obstruction was produced, they concluded that the elevated serum amylase levels were due to increased intraduodenal pressure causing pancreatic duct obstruction.

Support for Perman's view has come from Wallensten[11] who analyzed a large series of Billroth types I and II gastric resections. The striking features were 12 deaths from acute postoperative pancreatitis in 1,769 Billroth II resections and no deaths from pancreatitis in a series of 605 Billroth I resections. When the more extensive mobilization of duodenum in the latter operation is considered it is plain that pancreatitis following Billroth II resections is unlikely to be due simply to trauma of the pancreas, its ducts, or its

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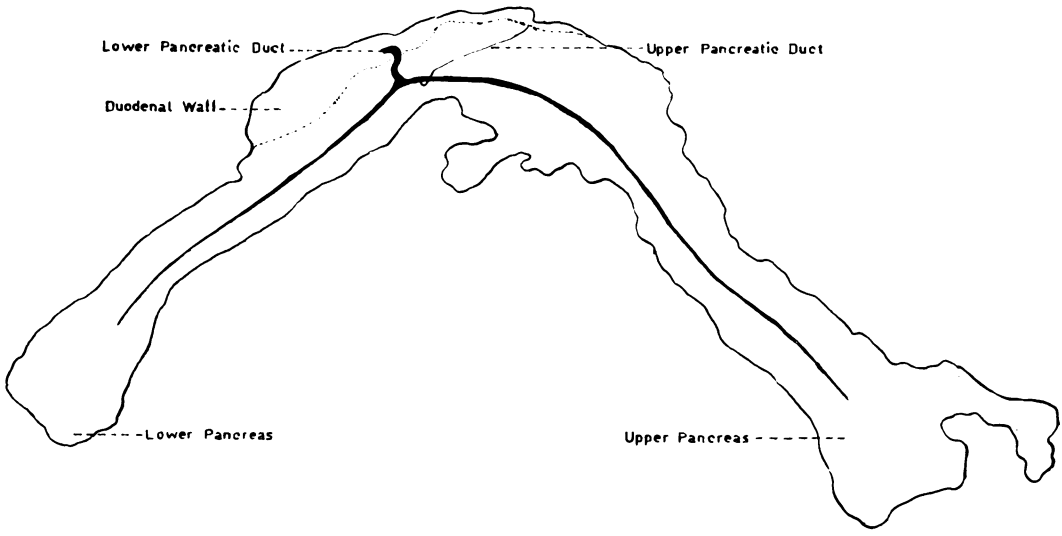

FIG. 1. Tracing from roentgenogram of dog pancreas in which the pancreatic ducts were outlined by injection of Hypaque.

blood supply. Wallensten suggested the following sequence of events:

1. The Billroth II operation transforms the duodenum and first part of the jejunum into a blind sac in which fluid collects and may become infected.

2. Reflux of stagnant duodenal contents up the pancreatic ducts, or the spread of infection (direct, lymphatic or blood stream) may subsequently give rise to pancreatitis.

The idea that pancreatitis might be caused by reflux of duodenal contents up the pancreatic duct has been largely rejected by British and American workers. Experiments such as those of Archibald,[1] in which the duodenum was obstructed above and below the papilla, have shown that it is impossible to force duodenal contents back into the common bile duct or pancreatic duct; these experiments, however, were done on postmortem material in which the dynamic processes of contraction and relaxation of bowel and sphincter muscle were absent.

It was decided to investigate this problem using the technic of Pfeffer, Stasior and Hinton,[8] in which a blind duodenal loop is created in the dog. This procedure regularly results in hemorrhagic pancreatitis and the conditions are in many ways comparable to an obstructed afferent loop following Billroth II partial gastrectomy.

Paulino-Netto and Dreiling,[6] have recently investigated this form of pancreatitis using the Pfeffer technic. They concluded: "the primary mechanisms in the production of this variety of pancreatitis, are incomplete pancreatic duct obstruction and perhaps backflow of the duodenal loop fluid into the pancreatic ductal system."

Our experiments, which were almost complete when we saw this report, were designed somewhat differently and we believe that they show clearly: 1) That this form of pancreatitis is due to reflux of duodenal contents into the pancreatic duct; and 2) That pancreatic duct obstruction helps prevent the onset of pancreatitis.

Method

Sixteen mongrel dogs weighing between 12.5 and 16 Kg. were given a pre-anesthetic dose of morphine sulfate 32 mg. and atropine sulfate 0.6 mg. and anesthesia was

induced with pentothal. The trachea was intubated with a standard Magill's catheter and artificial respiration instituted using an AGA pulsator. Anesthesia was continued with a gas mixture of oxygen (2.0 l./min.) and nitrous oxide (1.0 l./min.). Blood was taken for serum amylase and lipase determinations.

Using a standard surgical technic a midline incision was made into the peritoneum. The duodenum was mobilized by tying and cutting a few small blood vessels passing from the pyloric region. The common bile duct was identified, transfixed and tied. The procedure then varied.

A. In eight dogs, blind duodenal loops were made according to the description by Pfeffer *et al.*[8] The main blood supply to both pancreas and duodenum was not disturbed. An end-to-end anastomosis between the pylorus and jejunum was performed.

B. In another eight dogs, the above procedure was combined with ligation of the pancreatic ducts. The ducts were sought by gentle blunt dissection between duodenum and pancreas.

The upper pancreatic duct is small, often difficult to find and enters the duodenal wall at right angles at least 1.0 cm. below the point where the bile duct pierces the duodenal wall. Because of the oblique path of the bile duct, these two ducts usually open into the duodenal lumen, side-by-side in a well-marked papilla. The larger lower pancreatic duct is the main pancreatic duct in the dog. It enters the duodenum approximately 3.0 cm. below the upper duct, usually from the last island of attachment of pancreas to duodenum. It is comparatively easy to find. Injection of radiopaque material into the ducts shows that the main lower pancreatic duct drains most of the pancreas (Fig. 1).

The animals were allowed to survive for 18 to 22 hours and were then killed by exsanguination performed under deep pentothal anesthesia. Blood was taken for postoperative serum amylase and lipase estimations. Three dogs died shortly be-

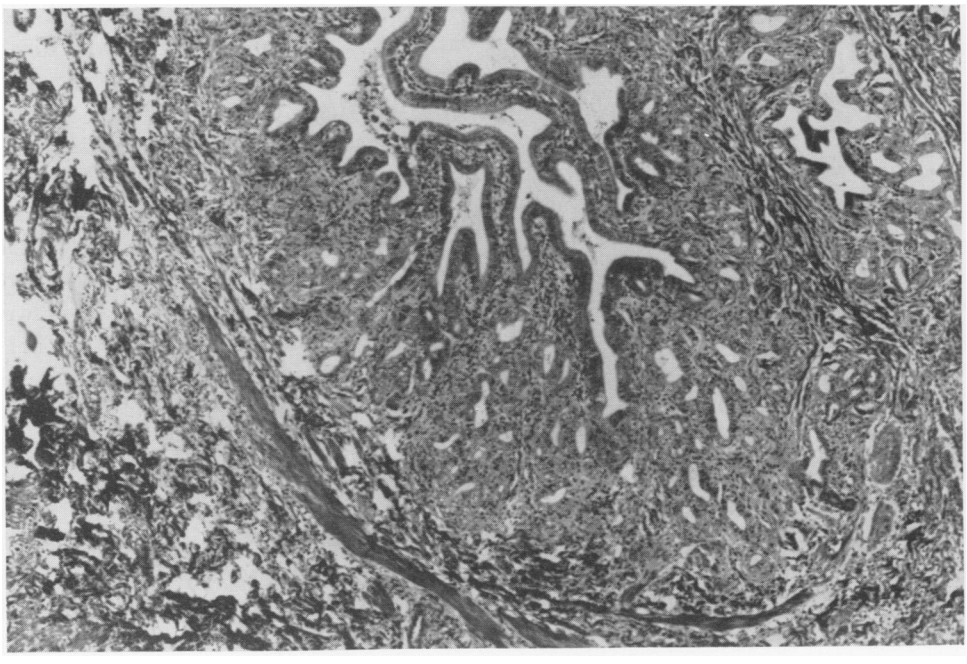

FIG. 2. Unobstructed main (lower) pancreatic duct in the dog.

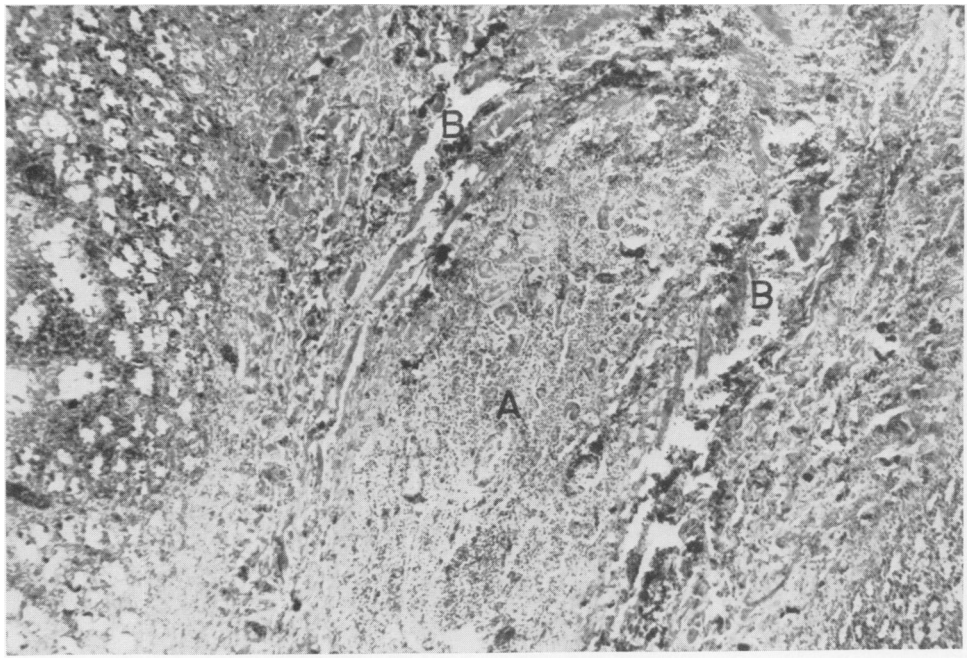

FIG. 3. Area of pancreatic duct (A) showing loss of the normal duct outline, with obstruction by cellular debris, and surrounded by the partly fragmented muscle and connective tissue sheath (B).

fore this preliminary period of 18 to 22 hours had elapsed.

Postmortem, the state of the pancreas, duodenal loop, anastomosis site and degree of peritoneal effusion, were noted. After photography, the pancreatic ducts were carefully dissected to see whether they had been ligated.

Duodenal wall containing the region of both pancreatic duct papillae, was fixed and sections cut at intervals of 150μ from within out, in a direction at right angles to the direction of the duct and approximately parallel to the mucosal surface. These were stained with hematoxylin and eosin, and also by the method of Picro Gomori. Sections from the upper, middle and lower pancreas, and duodenal wall, were stained with hematoxylin and eosin.

Amylase in serum and peritoneal fluid was measured by the method of Wohlgemuth. For lipase determinations the Sigma modification of Tietz, Borden and Stepleton[10] was used.

Results

Group A: Of the eight dogs in which a blind duodenal loop alone was formed, six were found to have unobstructed pancreatic ducts confirmed by histological examination of sections of the ducts (Fig. 2). In two dogs the main lower pancreatic duct was blocked in its superficial papillary portion just before it opened into the duodenal lumen. The blockage was due to a combination of infarction of the papilla with swelling and collapse of the walls of the ducts, congestion, cellular infiltration and accumulation of debris (Fig. 3).

Group B: Of the eight dogs in which a blind duodenal loop operation was combined with ligation of the pancreatic ducts, one was found postmortem in which neither duct had been tied, the ties instead being round blood vessels. In the other seven dogs, the main lower pancreatic duct was properly ligated, but the upper pancreatic duct was not ligated in five of these seven dogs.

The animals were therefore regrouped according to whether the main lower pancreatic duct was patent (seven dogs) or blocked (nine dogs). Striking differences in the degree of pancreatitis were found in these two groups, despite similar vascular and pathological changes in the blind loop.

Group 1. Unobstructed Main Pancreatic Duct and Blind Duodenal Loop (Seven Dogs). Severe hemorrhagic pancreatitis was present in four dogs (Fig. 4) and three of them were dead within 18 to 20 hours of operation. Mild to moderate pancreatitis was present in the other three dogs, consisting of interstitial hemorrhages and cellular infiltration, with patchy areas of necrosis of pancreatic acini (Fig. 5). The clinical state of the surviving dogs was poor; they were obviously sick and apathetic.

In one dog (with severe pancreatitis) the lower end of the duodenal loop had 'blown' through a necrotic patch in the wall. In the remaining animals the duodenal loop was distended and plum colored (Fig. 6). In one dog, the blind loop was much longer than in any of the others and this animal had the mildest degree of pancreatitis.

Sections of the wall of the duodenal loop showed areas with loss of mucosal epithelium and a varying degree of necrosis of the underlying lamina propria. Other mucosal areas were relatively normal except for marked vascular congestion. Hemorrhages and vascular congestion were most marked in the deeper muscle layer.

The pancreatic ducts were obviously patent and in some cases dilated. They frequently contained debris, erythrocytes and polymorphs. In two dogs, 6.0 to 7.0 ml. of a barium sulfate suspension were introduced into the duodenal loop before closure: when the pancreatic ducts of these two dogs were examined histologically, particles of barium could be seen inside the main duct (Fig. 9). There was insufficient intraductal barium for radiological demonstration. In all of the animals there

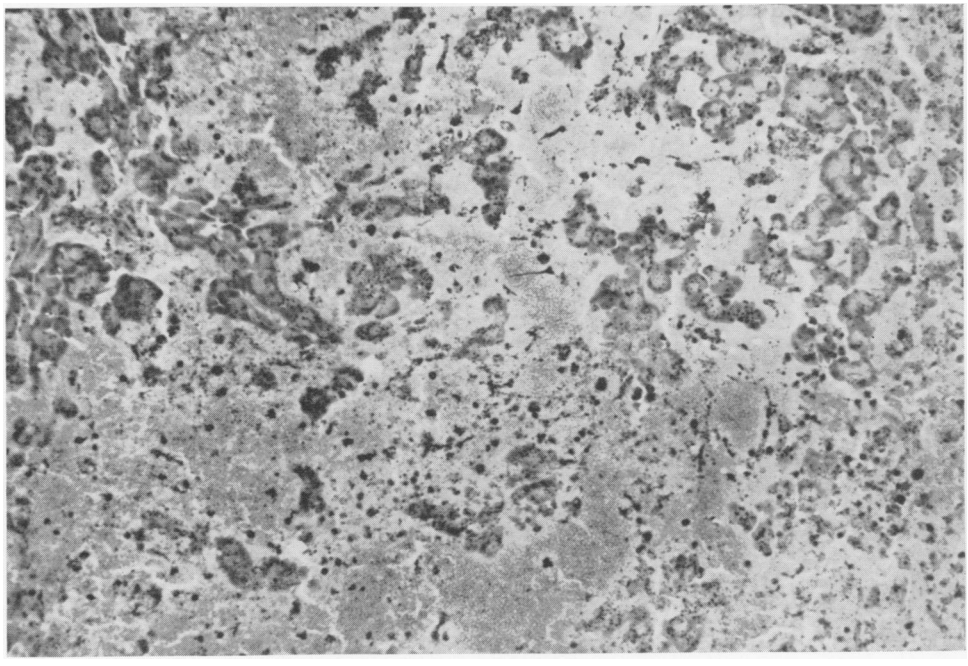

FIG. 4. Severe hemorrhagic pancreatitis with small islands of acinar cells surrounded by blood, necrotic tissue and clumps of pigment.

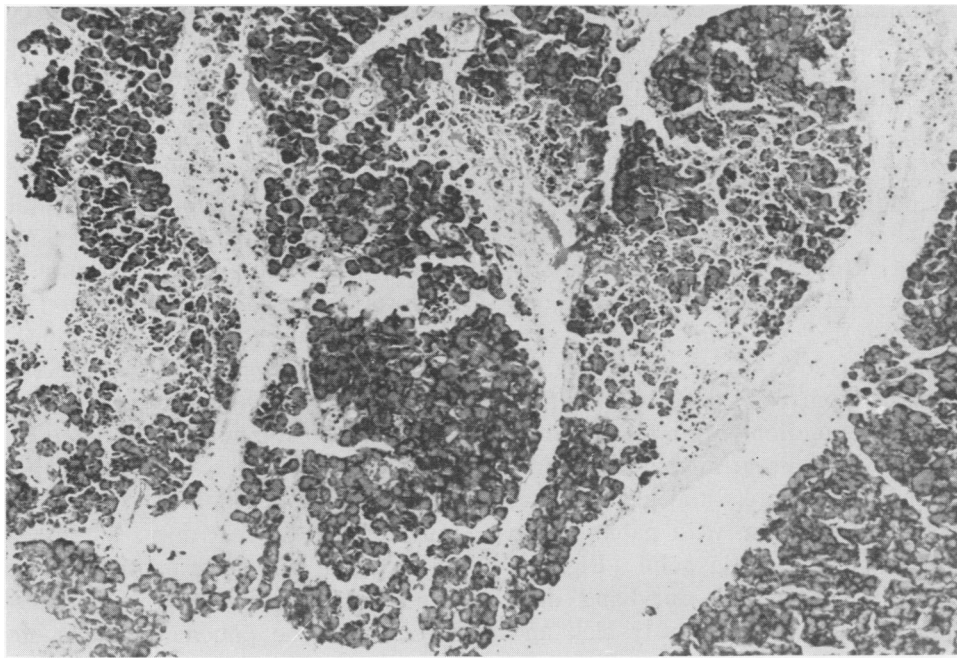

FIG. 5. Mild pancreatitis showing interstitial hemorrhages and patchy areas of necrosis of acini.

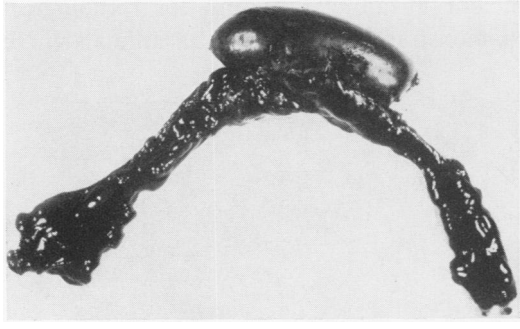

FIG. 6. Blind duodenal loop, attached to pancreas which is markedly hemorrhagic throughout.

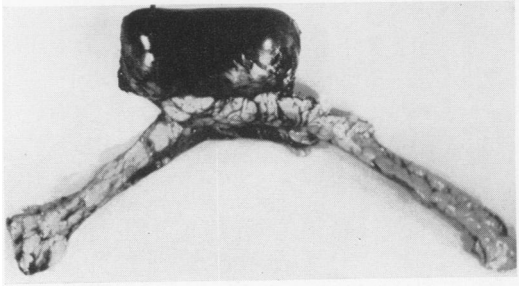

FIG. 7. Blind duodenal loop and pancreas in dog with obstructed pancreatic ducts. The blind loop is distended and hemorrhagic, but the pancreas is normal.

was a considerable collection of blood stained fluid in the peritoneal cavity.

Group 2. Obstructed Main Pancreatic Duct and Blind Duodenal Loop (Nine Dogs). None of these dogs died from pancreatitis. In all cases the bulk of the pancreas was macroscopically and microscopically normal (Fig. 7, 8). In seven of the dogs there was a mild degree of pancreatitis limited to a small part of the middle of the pancreas in the region where the upper smaller pancreatic duct entered the duodenum. The upper duct apparently was patent in these cases. In the two dogs in which both pancreatic ducts were ligated, the pancreas was normal throughout.

The blind loops in this group of dogs were similar macroscopically and microscopically, to those in the first group, providing a sharp contrast in color to the relatively normal pancreas (Fig. 7). There was a similar collection of blood-stained peritoneal fluid, but clinically the dogs were in much better condition, were more alert and responsive.

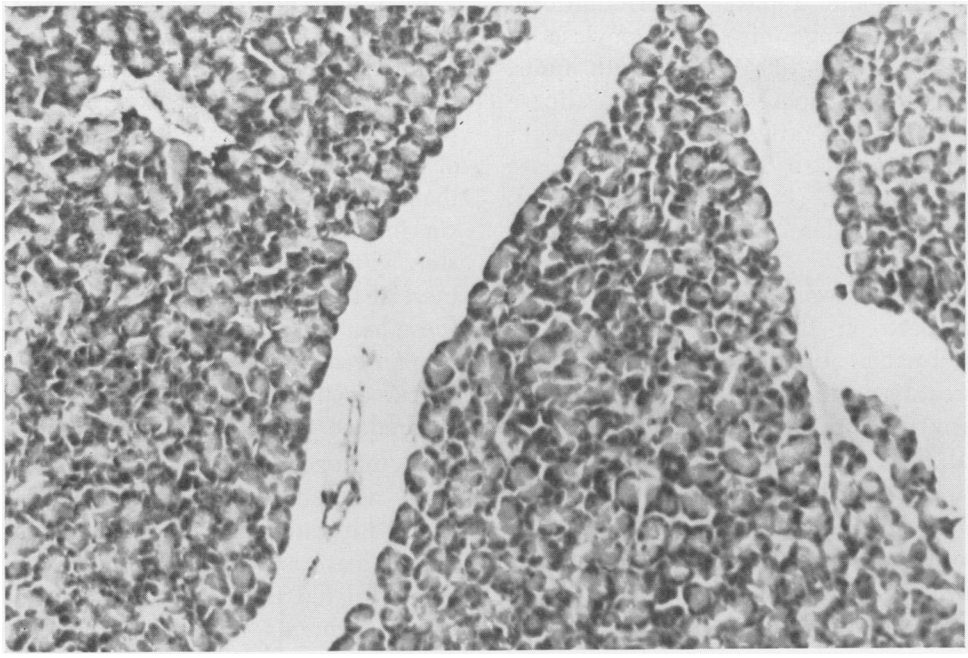

FIG. 8. Histologically normal pancreas from dog with a blind duodenal loop and obstructed main pancreatic duct.

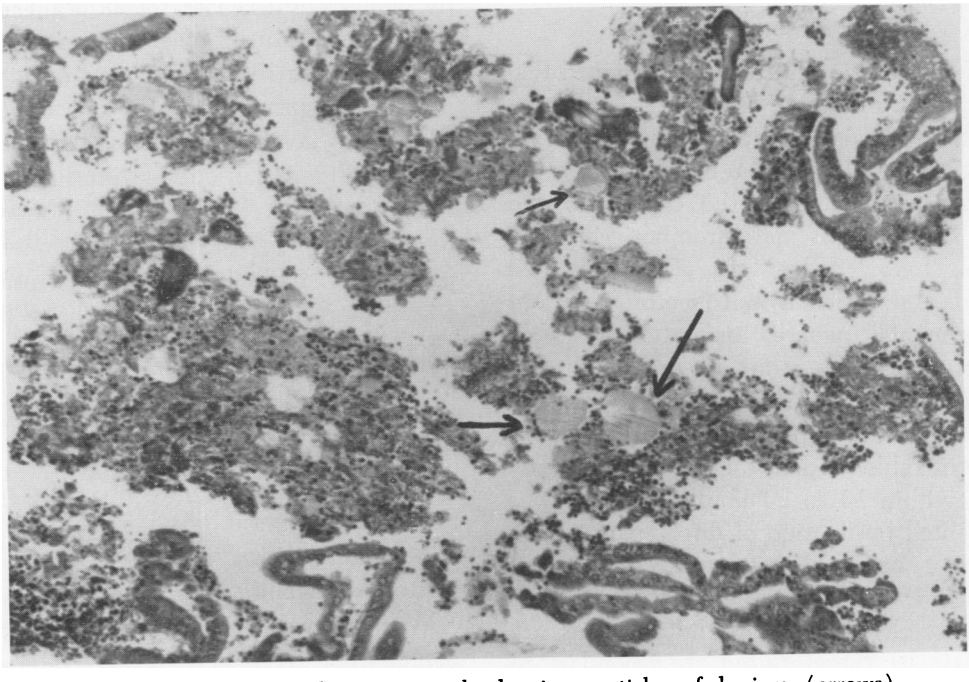

FIG. 9. High power photomicrograph showing particles of barium (arrows) within the pancreatic duct.

Amylase and Lipase Determinations. The normal range of serum amylase in 36 dogs was 33–100 Wohlgemuth units, and of serum lipase was 0.1–0.7 lipase units/ml. Postoperatively the ranges were: Group 1. Amylase 100–500 units; lipase 5.6–6.6 units/ml. (only 2 determinations). Group 2. Amylase 100–500 units; lipase 1.6–7.1.

Thus there were no significant differences between the two groups despite marked pancreatitis in one group and little pancreatitis in the other. A comparison of the enzyme estimations done on the peritoneal fluid however, showed differences which were probably significant. Group 1. Amylase (peritoneal fluid) 200–4,000 units; lipase (peritoneal fluid) 12.8–29.1 units/ml. Group 2. Amylase (peritoneal fluid) 100–1,000 units; lipase (peritoneal fluid) 2.7–10.9 units/ml.

These figures suggest that lipase estimations on the peritoneal fluid were a more accurate guide to the presence of pancreatitis than serum amylase or lipase.

Discussion

Pfeffer, Stasior and Hinton, believed that the primary changes in experimental pancreatitis associated with a blind duodenal loop, were vascular in nature. They described congestion in the capillaries and thin walled veins and thrombosis in some areas, followed by foci of extravasated blood and parenchymal necrosis. In our experiments pancreatitis occurred only when there was reflux of duodenal contents up the pancreatic duct. Barium introduced into the duodenum at operation was demonstrable within the main pancreatic duct within 10 to 20 hours. If reflux was prevented by ligation of ducts, pancreatitis did not occur despite marked vascular changes within the duodenal loop. The vascular changes within the pancreas are not primary therefore but are secondary to reflux of duodenal contents.

Rich and Duff[9] described the characteristic vascular changes which they found in all their cases of hemorrhagic pancreatitis whether human or experimental, as a peculiar and rapid necrosis of the walls of the pancreatic vessels. They believed that this was due to the liberation of trypsin into the inter-acinar tissue and they showed that subcutaneous injection of commercial trypsin in dogs exactly reproduced these hemorrhagic lesions. Similar injection of pancreatic juice in which the inactive precursor trypsinogen was present, failed to produce the hemorrhagic lesions.

Trypsinogen in pancreatic juice is normally activated in the duodenum by contact with duodenal secretions containing enterokinase and other substances. It may be postulated therefore, that reflux of duodenal contents under pressure into the pancreatic duct, allows activation of trypsinogen to trypsin within the pancreas and that escape of active trypsin (and other enzymes) into the inter-acinar tissue is responsible for the hemorrhagic pancreatitis which ensues. The secondary vascular phenomena induced by trypsin may largely determine the subsequent pathology. That is, the final process may be one of infarction from involvement of multiple small vessels, rather than one of autodigestion.

The similarity between this form of experimental pancreatitis and postoperative pancreatitis following Billroth II resection where there is inadequate drainage of the afferent loop, suggests that the latter form is also due to reflux of duodenal contents up the pancreatic duct. It underlines the necessity for urgent surgical relief of such obstruction should pancreatitis arise in the postoperative period, and is in agreement with similar views expressed by Paulino-Netto and Dreiling.[6]

With regard to the etiology of acute pancreatitis in general, several interesting points emerge from these experiments:

1. Bile is not necessary in the production of this form of pancreatitis, since it was excluded from the duodenum by ligation of the common bile duct. This is of great interest in view of the importance some workers attach to the participation of bile in activating pancreatic trypsinogen.[4]

2. *In vivo,* duodenal contents under pressure can be forced past a normal pancreatic duct papilla, despite experiments on postmortem material which seemed to show that it was impossible to force fluid from the duodenum back past the papilla into the common bile duct or pancreatic duct.[1]

3. The theoretical possibility arises that pancreatitis may be produced by much smaller pressure changes in the duodenum provided the duct papilla is damaged or incompetent. Reflux of duodenal contents into the pancreatic duct must therefore be reconsidered as a possible etiological factor in all cases of acute hemorrhagic pancreatitis.

Summary and Conclusions

1. Acute hemorrhagic pancreatitis may be produced in the dog by the creation of a blind duodenal loop where the conditions are comparable to an obstructed afferent loop after Billroth II resection.

2. The experimental pancreatitis is due primarily to reflux of duodenal contents up the pancreatic duct, and only secondarily to vascular changes within the pancreas. It may be prevented by ligation of the pancreatic ducts.

3. It is likely that pancreatitis following gastric surgery in man, is also due to reflux of duodenal contents up the pancreatic duct.

4. Lipase estimations on the peritoneal fluid were a more accurate guide to the presence of pancreatitis than serum amylase or lipase.

5. The relevance of these findings to other forms of acute pancreatitis is discussed.

Acknowledgment

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