Significance of Vascular Injury as a Factor in the Pathogenesis of Pancreatitis *

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WHEN REVIEWING the pathological changes observed in various forms of clinical pancreatitis, it is apparent that local vascular injury within the pancreas plays an important role in the type of lesion produced. In the milder forms of pancreatitis, varying amounts of edema fluid are lost into the interstitium of the gland and surrounding tissues. Hyperemia is the predominant vascular change; however, the integrity of the vascular system remains intact. In acute hemorrhagic or necrotizing pancreatitis, there is an extravasation of bloody fluid which may be of sufficient magnitude to produce serosanguinous ascites. Within the local vascular system there is a diffuse injury to small vessels characterized by arteritis, phlebitis, destruction of arteries and veins, and thrombosis of smaller vessels (Fig. 1).

This "vascular factor" in pancreatitis has received considerable attention as an important etiological mechanism responsible for pancreatic necrosis. Smyth injected droplets of mercury into the pancreatic arteries and produced focal areas of pancreatic necrosis.[8] Subsequently, Popper and Necheles [7] demonstrated that acute pancreatic edema, which followed duct ligation and exocrine stimulation, could be converted to hemorrhagic pancreatitis by temporary occlusion of the pancreatic blood supply. Thal observed that bile produced stagnation of blood flow when injected into the parenchyma of the pancreas.[9] More recently, Thal has used local hypersensitivity reactions to produce a severe pancreatitis which, in all probability, results from diffuse vascular damage.[10, 11]

Less attention has been directed to another manifestation of vascular injury, namely the release of either edematous or bloody fluid into surrounding tissues in response to pancreatic inflammation. We believe that the combination of these extravasated components of blood with enzymes, present in the interstitium of the pancreas during an attack of pancreatitis, may result in digestion products which play an important role in determining the extent and character of the final lesion.

Nemir and associates [5, 6] have shown that a mixture of whole blood, incubated with pancreatic enzymes, will cause severe necrotizing pancreatitis when introduced into the pancreas of the dog. These authors have concluded that this lesion results from the action of some toxic substance produced when blood is digested by pancreatic enzymes. The exact nature of this toxic product has not been defined, although hemolysis of the red blood cell by bacteria was considered a necessary part of the reaction.

We have produced a similar type of necrotizing pancreatitis by injecting an incubated mixture of autologous whole blood and sterile lyophilized trypsin into the pancreas of the dog.[2] This resulted in a highly reproducible lesion which caused death in 90 per cent of the animals tested, and closely resembled the pathological

* Submitted for publication December 13, 1960.

This work supported in part by Grant A-2188 (C-3) from the National Institutes of Health, U. S. Public Health Service.

changes found in the human form of the disease. In addition to wide-spread patchy areas of parenchymal necrosis, there was severe inter- and intralobular edema, leukocytic infiltration, fat necrosis and a characteristic vascular lesion consisting of arteritis, phlebitis and thrombosis of small arteries and veins (Fig. 2). Cultures of the incubated mixture were sterile in most instances.

The present study was originally undertaken to determine the specific fraction of whole blood which was responsible for this form of experimental pancreatitis. Accordingly, autologous canine whole blood was separated into washed red-cell, serum or plasma fractions, and one of these was incubated with sterile lyophilized trypsin prior to injection into the duct system of the dog pancreas. Surprisingly, none of these combinations produced a necrotizing pancreatitis comparable to that which followed injection of the whole blood-trypsin incubate. Instead, we found that the serum and plasma incubates produced a severe,

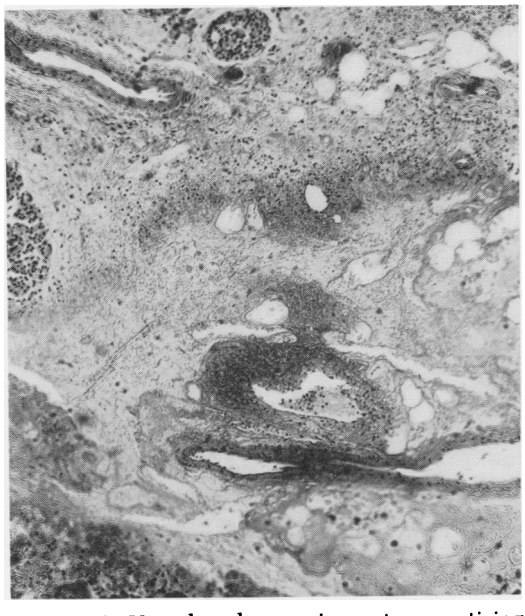

FIG. 1. Vascular changes in acute necrotizing pancreatitis in man. Arteritis and phlebitis involving vascular structures which are surrounded by areas of fat necrosis, severe edema and necrotic pancreatic parenchymal tissue (from × 100).

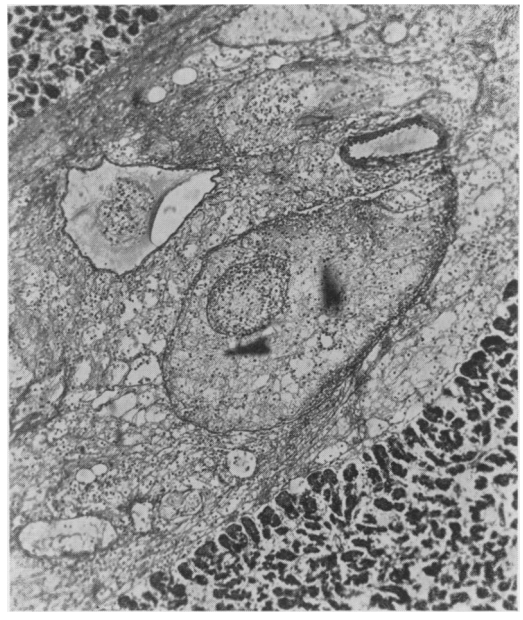

FIG. 2. Vascular changes in acute necrotizing pancreatitis produced by an injection of autologous whole blood and trypsin into the pancreas of a dog. Changes are similar to that observed in Figure 1. Severe edema, destruction of walls of vessels, thrombosis of veins, leukocytic infiltration and parenchymal necrosis (from × 100).

but sublethal, acute edematous pancreatitis which later progressed to a stage of marked chronic interstitial pancreatitis. Similar, but less pronounced changes were observed with the washed red-cell trypsin incubate. We have attempted to correlate these findings with current concepts related to the pathogenesis of pancreatitis, and have proposed what we believe to be a logical explanation for various forms of pancreatic inflammatory disease.

Method

A total of 113 healthy mongreal dogs weighing from 8 to 20 kg. were anesthetized with pentobarbital and, utilizing sterile technics, the pancreas was mobilized into an upper abdominal midline incision. The lesser pancreatic duct was ligated and the main duct cannulated through a small duodenotomy incision (Fig. 3). A variety of agents were in-

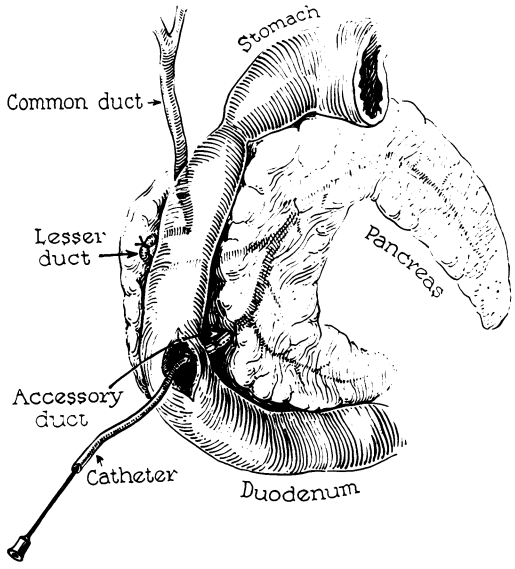

FIG. 3. Illustration of operative method used to inject agents into pancreatic duct system. Pressures were controlled with a side-arm manometer at between 110 and 140 mm. Hg. This level of pressure was sufficient to cause a diffuse interstitial extravasation of the injected material.

troduced into the main duct under pressures varying from 110 to 140 mm. Hg, the latter being sufficient to cause duct rupture and extravasation of the injected material into the interstitial tissues of pancreas. The pancreatic duct was ligated as the cannula was withdrawn, and the duodenotomy and abdominal incisions were closed.

Two series of animals served as controls for the remainder of the study. In one group, simple pancreatic duct ligation was performed, and in the second, saline was injected into the obstructed pancreas under controlled pressures. The remainder of the study consisted of an evaluation of the effect of trypsin and whole blood or blood fractions when injected, either separately or combined, with or without incubation, into the main pancreatic duct of the dog. Blood specimens were obtained from the femoral vein of the animal to be injected, utilizing sterile precautions. When blood or one of its fractions was mixed with trypsin, a volume of 10 ml.[3], consisting of 5 ml.[3] of autologous whole blood, serum, plasma or washed red blood cells and 5 ml.[3] of sterile lyophilized trypsin (Tryptar *), containing 50,000 tryptic units, were injected. Incubated specimens were placed in a water bath at 37.5° C. for 24 to 28 hours before injection.

Following operation, each animal was carefully observed for clinical signs of weakness, vomiting, shock, coma and convulsions. Survivors were given food and water as desired, and the time between operation and alimentation was noted. Each animal which died after operation was autopsied as soon as possible, and specimens were obtained from the head, body and tail of the pancreas. Survivors were sacrificed at weekly intervals up to one month.

Results

Ten experiments were used to identify the active fractions of the blood-trypsin combination (Table 1).

1. *Simple ligation of both pancreatic ducts* was followed by a rapid recovery in each of ten animals. Examination of the pancreas at autopsy revealed mild edematous changes during the first few days, characterized microscopically by widening of the interlobular spaces (Fig. 4). Later the parenchymal tissues became atrophic; however, there was little or no fibrosis or chronic inflammatory reaction.

2. *Simple duct ligation combined with an injection of 10 cu. ml. of sterile saline into the main pancreatic duct* resulted in an acute necrotizing pancreatitis in one of ten dogs studied. In the others, the recovery was uneventful. All animals were able to stand and eat within 48 hours of the operation. The degree of pancreatic edema was comparable to that observed with duct ligation alone, and after one

* Supplied by Armour Pharmaceutical Company, Kankakee, Illinois.

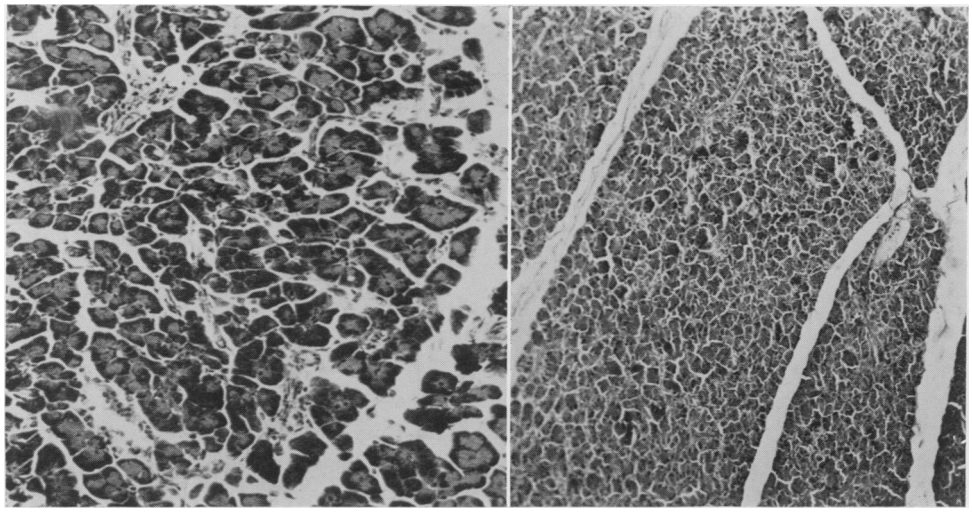

FIG. 4. (Left). Microscopic appearance of the canine pancreas 5 days following simple duct ligation. There is a diffuse inter- and intralobular edema, but no evidence of parenchymal injury. The vessels in the pancreas were hyperemic but no loss of vascular integrity was observed (from × 150). FIG. 5. (Right). Appearance of pancreas 24 days after 10 cc. of sterile saline were injected into the main pancreatic duct. The parenchymal architecture is preserved. There is no evidence of fibrosis or chronic inflammation (from × 100).

month there was only moderate atrophy without fibrosis or chronic inflammatory cell infiltration (Fig. 5).

3. *Sterile lyophilized trypsin* was injected into the main pancreatic duct of 12 animals and none died of acute pancreatitis. Each

TABLE 1

Injection	Animals	Predominant Pancreatic Lesion	Mortality from Pancreatitis	Comments on Usual Clinical Course
Pancreatic duct ligation only	10	Mild pancreatic edema	0	Rapid recovery
Saline	10	Mild pancreatic edema	1	Rapid recovery
Trypsin	12	Mild pancreatic edema and fibrosis	0	Rapid recovery
Fresh whole blood	7	Mild pancreatic edema	1	Rapid recovery
Fresh whole blood-Trypsin	15	Mild pancreatic edema and fibrosis	1	Rapid recovery
Incubated whole blood-Trypsin	20	Severe necrotizing pancreatitis	18	Rapid downhill course
Incubated serum-Trypsin	10	Severe interstitial pancreatitis	2	Slow recovery; initially ill
Incubated plasma-Trypsin	10	Moderate interstitial pancreatitis	1	Slow recovery; initially ill
Incubated washed red cells-Trypsin	10	Mild interstitial pancreatitis	3	Slow recovery; initially ill
Incubated serum-saline	4	Mild pancreatic edema	0	Rapid recovery

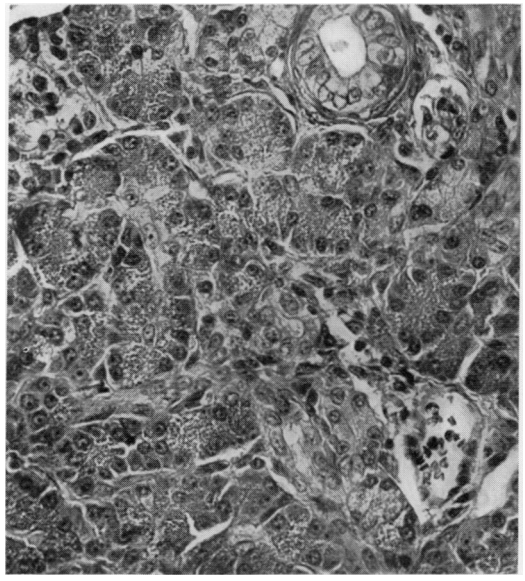

FIG. 6. Microscopic appearance of the canine pancreas one week following an injection of 10 cu. ml. of sterile lyophilized trypsin into main pancreatic duct. The acinar structure is preserved; however, a moderate fibroblastic reaction is seen both surrounding acini and in the interlobular spaces. Ductal dilatation secondary to obstruction of the pancreatic ducts at the time of injection (× 190).

dog responded rapidly following operation and was able to stand and eat by the second postoperative day. At autopsy, the pancreas was uniformly pale and indurated, but lacked evidence of fat necrosis or hemorrhage. Microscopic sections revealed diffuse interacinar and interlobular fibroplasia (Fig. 6). These changes resulted in a mild degree of interlobular fibrosis after a period of one month.

4. *Fresh autologous whole blood* introduced into the main pancreatic duct caused death from acute pancreatitis in one of seven animals. Survivors recovered quickly, and at autopsy the pancreas was pale and indurated, but there was no evidence of recent hemorrhage or fat necrosis. The microscopic changes included edema, interlobular fibroplasia and leukocytic infiltration; however, within one month little evidence of pancreatic inflammatory disease remained (Fig. 7).

5. *Fresh autologous whole blood and sterile lyophilized trypsin* produced a fatal pancreatitis in one of 15 dogs injected. The remaining animals made an uneventful recovery and at autopsy the pancreas was grossly and microscopically similar to that observed when trypsin was injected alone (Fig. 8).

6. *Incubated autologous blood and sterile lyophilized trypsin* caused a fatal necrotizing pancreatitis in 18 of 20 animals in-

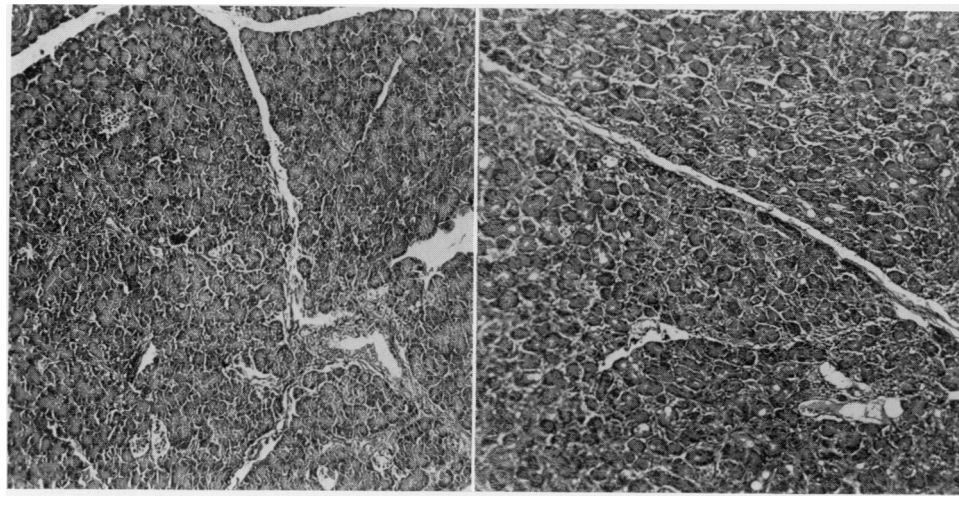

FIG. 7. Microscopic findings 21 days after an injection of fresh autologous blood into main pancreatic duct. Except for evidence of obstruction in the small ramifications of the pancreatic duct system and mild increase in fibrous tissue in the interstitial spaces, the pancreas appears normal (from × 100). FIG. 8. Appearance of pancreas 30 days after injection of a mixture of fresh autologous blood and trypsin. Mild infiltration of fibrous tissue but no evidence of chronic inflammation. Parenchymal architecture intact (from × 100).

jected. Fourteen died during the first 36 hours following operation with progressive weakness, prostration, shock, coma and convulsions preceding death. None of the dogs was able to eat for at least four days and the recovery of the two survivors was doubtful for the first two weeks. Autopsy revealed a diffusely swollen, boggy pancreas studded with foci of fat necrosis and infiltrated with hemorrhagic fluid. All of the animals had a serosanguinous accumulation of fluid in the peritoneal cavity. Microscopic sections showed severe edema, leukocytic infiltration, diffuse hemorrhage, spotty parenchymal necrosis, arteritis, phlebitis and thrombosis of small vessels (Fig. 9).

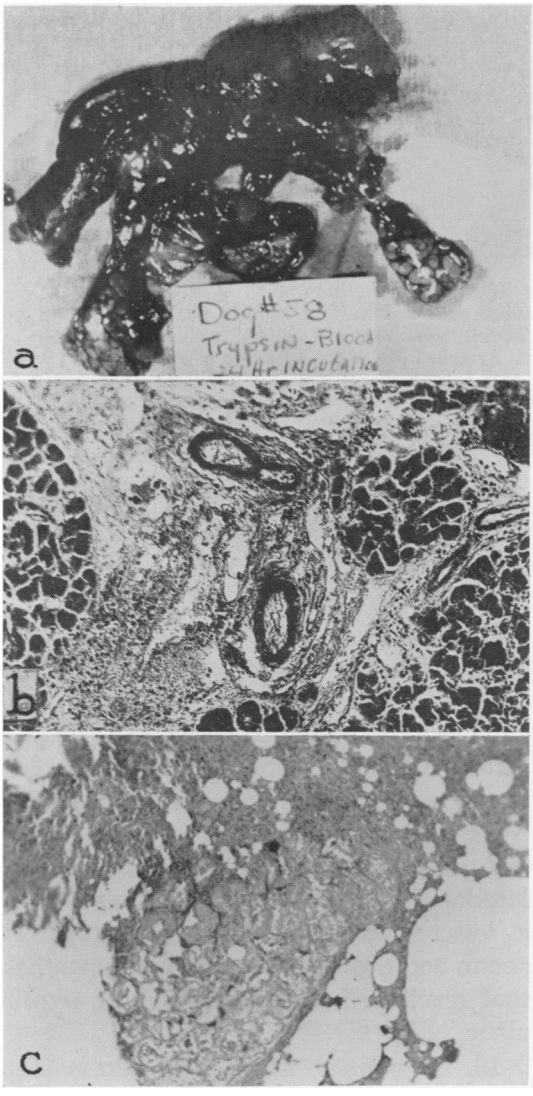

FIG. 9. a) Gross appearance of pancreas removed from a dog which died 24 hours after an injection of autologous whole blood and sterile lyophilized trypsin. The pancreas is edematous, hemorrhagic and necrotic. b) Typical microscopic findings, including acinar necrosis, edema, arteritis of small vessels and leukocytic infiltration (× 100). c) Fat necrosis.

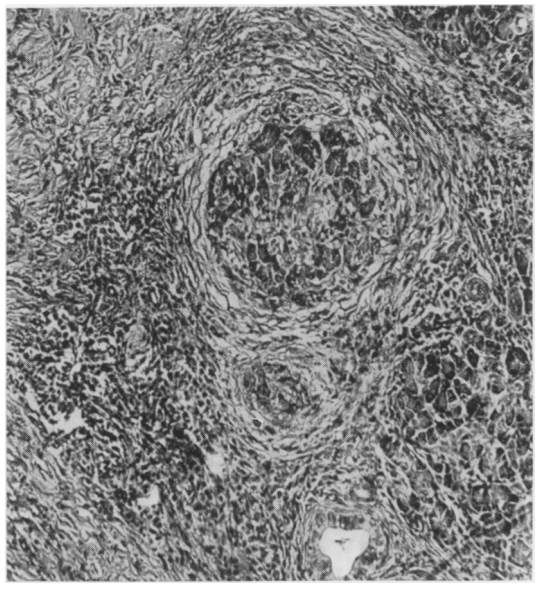

FIG. 10. Microscopic findings in a pancreas of a dog sacrificed one month following an injection of autologous serum and sterile lyophilized trypsin. Large areas of the gland are replaced by dense fibrous tissue which is infiltrated with chronic inflammatory cells. Only islands of parenchymal tissue remain (× 150).

7. *Incubated autologous serum and sterile lyophilized trypsin* produced a fatal acute hemorrhage pancreatitis in two of ten dogs. The other eight animals recovered; however, the clinical course was one of slow improvement. Weakness and vomiting were prominent for many days and a return to normal alimentation required at least one week. Autopsy revealed marked induration of the pancreas and peripancreatic tissues. Microscopic sections showed a progressively severe fibroplasia involving the interlobular areas. Dense fibrous tissue septa separated islands of atrophic acinar tissue, and foci of chronic inflammatory cells were prominent throughout the gland (Fig. 10).

8. *Incubated autologous plasma and sterile lyophilized trypsin* caused fatal

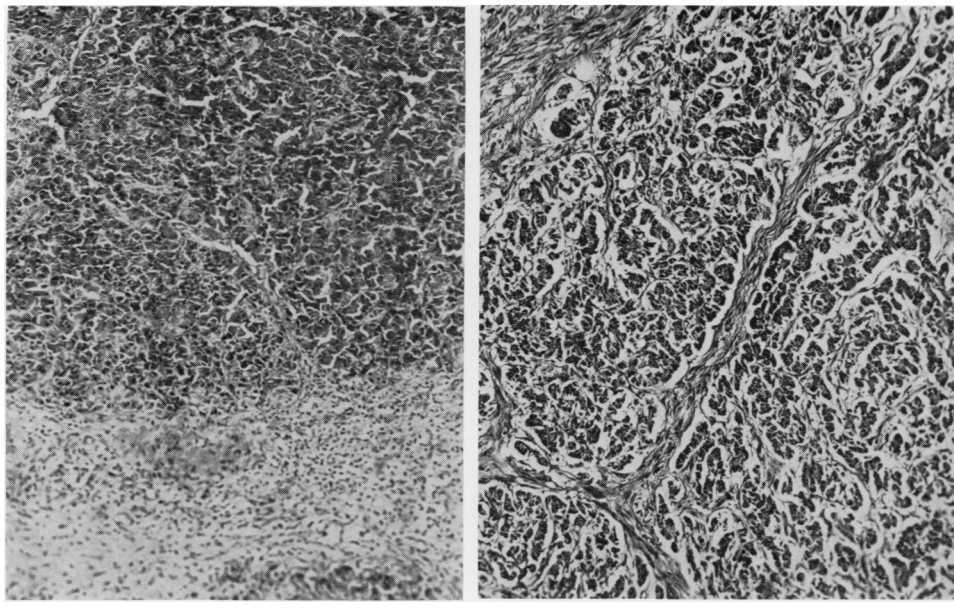

FIG. 11. Microscopic findings one month after injection of an incubated mixture of autologous plasma and trypsin. Severe fibroblastic reaction in lower half of section with preservation of parenchymal architecture in upper part. Chronic inflammatory cells prominent in area replaced by fibrous tissue (from × 100). FIG. 12. Appearance of pancreas one month after an injection of autologous washed red blood cells and trypsin incubated for 24 hours. Diffuse parenchymal atrophy and intralobular fibrosis. Little evidence of chronic inflammatory reaction. Lesion more severe than that observed with trypsin alone after similar time interval (from × 100).

pancreatitis in one of ten animals. Another dog died from postoperative hemorrhage. Survivors recovered slowly and at autopsy the pancreas was indurated. Microscopic findings were comparable to those observed with incubated serum and trypsin, but less severe (Fig. 11).

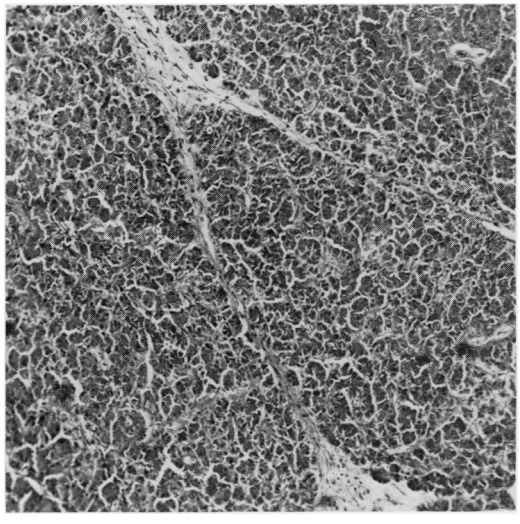

FIG. 13. Appearance of pancreas one month after an injection of autologous serum and saline incubated for 24 hours (× 100). Changes similar to those produced by an injection of saline alone (Fig. 5). Compare with Figure 10 where trypsin was incubated with serum.

9. *Incubated autologous washed red blood cells and sterile lyophilized trypsin* resulted in fatal pancreatitis in three of 15 animals. The others survived and, despite listlessness and anorexia during the first four to seven days, recovered completely. At autopsy, the pancreas was indurated and pale, and microscopic sections revealed interlobular and interacinar fibroplasia of a lesser degree than that produced by serum and trypsin, but more extensive than that resulting from an injection of trypsin alone (Fig. 12).

10. *Incubated autologous serum and sterile saline* injected into the main pancreatic duct was followed by rapid recovery in each of four animals tested. Gross and microscopic findings were identical to those observed when saline alone was injected (Fig. 13).

Discussion

These findings suggest that the type of fluid released from the vascular system into the interstitial tissues of the pancreas during an attack of acute pancreatitis may influence the character and extent of the lesion produced.

In mild forms of pancreatitis, hyperemia is a prominent findings and the interstitium of the pancreas is filled with a serous type of edema fluid which contains variable numbers of leukocytes, depending upon the severity of the attack. Recurrent episodes of this nature result ultimately in the replacement of large areas of parenchyma with dense fibrous tissue interspersed with foci of chronic inflammatory cells. The latter lesion has been termed chronic interstitial pancreatitis. These changes can be accurately reproduced in the dog by an intrapancreatic injection of either autologous serum or plasma which has been previously incubated with the enzyme trypsin. The pancreas becomes filled with edema fluid, is boggy and studded with areas of fat necrosis. Later there is progression to a stage of severe brawny edema typical of chronic pancreatitis. Microscopically, the interstitial tissues are infiltrated with fibroblasts and ultimately islands of parenchyma are separated by a dense network of fibrous tissue containing foci of lymphocytes and plasma cells. Similar, but less extensive changes are observed when autologous washed red blood cells and trypsin are incubated and injected into the canine pancreas. A mild interstitial fibroplasia developed when trypsin alone was injected under similar conditions; however, this lesion failed to progress to the stage of diffuse chronic interstitial pancreatitis observed with either incubated serum and trypsin or plasma and trypsin. Likewise, we were unable to produce this chronic form of pancreatitis when serum was incubated with saline instead of trypsin. Hence, it would appear that pancreatic enzymes are capable of digesting the serum or plasma released from the vascular system during an attack of acute pancreatitis, and that a product is formed which incites severe fibroplasia. Such a mechanism would explain the extensive inflammatory changes and fibrosis which characterize chronic interstitial pancreatitis.

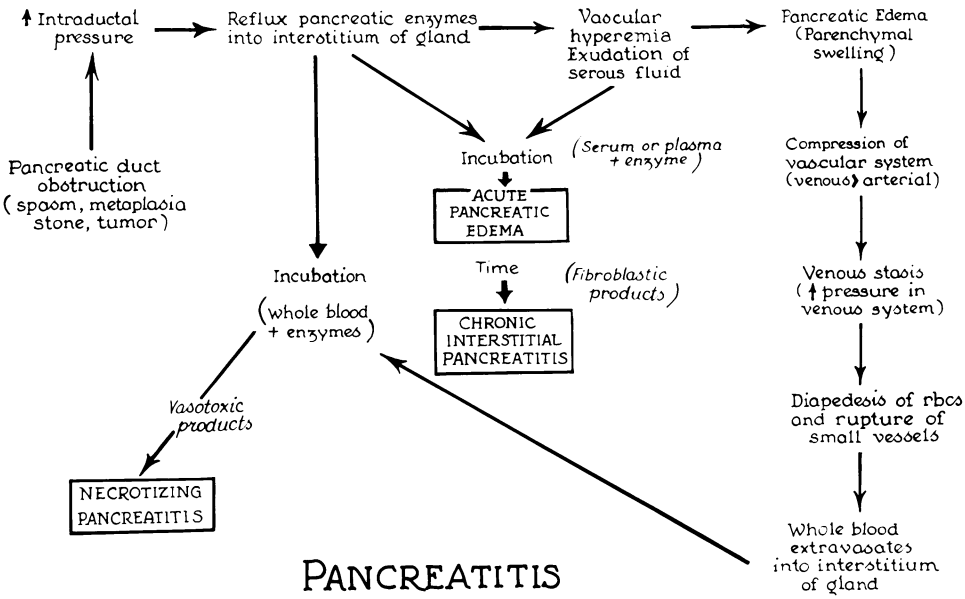

FIG. 14. Schematic representation of author's concept of the pathogenesis of pancreatitis.

In acute hemorrhagic pancreatitis, as the name implies, there is an extravasation of blood into the interstitium of the pancreas. The gland becomes edematous, covered with fat necrosis, and tense due to large amounts of serosanguinous fluid which are lost into surrounding tissues and into the peritoneal cavity.

Similar changes were observed in dogs when autologous whole blood and trypsin were incubated together and injected into the pancreas. In contrast to the chronic inflammatory lesion produced when autologous serum or plasma was incubated with trypsin, the whole blood-trypsin incubate produced a fulminating, highly fatal acute necrotizing pancreatitis. Enzymatic digestion of whole blood, released into the parenchyma of the pancreas consequent to disruption of the local vascular system integrity, results in a toxic product which increases materially the severity of the lesion produced. The predominant pathological change is a diffuse vascular injury. Similar findings were reported by Nemir and his associates [5, 6] who have isolated an abnormal hemin pigment from incubated dog blood and pancreatic juice. These authors believe that hemolysis of the blood-enzyme mixture is initiated by the action of bacteria present in pancreatic juice. In our studies, bacterial cultures of the incubated mixture were sterile in most instances, or contained only meager growths of nonpathogenic contaminants. Another interesting alteration was the failure of an incubated mixture consisting of either washed autologous red blood cells, serum or plasma and trypsin to produce an acute fulminating necrotizing pancreatitis comparable to that observed with incubated whole blood and trypsin. It would appear that the fluid fraction of whole blood, as well as pigment bearing red blood cells, is essential to produce this form of pancreatitis.

An apparent paradox in this study was the finding that neither fresh whole blood nor fresh whole blood combined with trypsin produced a lesion comparable in magnitude to that initiated by incubated whole blood and trypsin. One would expect that in either instance blood would incubate with pancreatic enzymes at the body temperature of the animal. It is possible that sufficient amounts of the enzyme are removed by the circulation of the intact canine pancreas to prevent the anticipated response. The severe vascular injury observed when *incubated* blood and trypsin are injected into the normal dog pancreas suggests that the toxic product elaborated by the enzymatic digestion of blood is responsible for the extensive vascular changes which characterize acute necrotizing pancreatitis.

We believe that many problems related to the pathogenesis of pancreatitis can be explained on the basis of these findings (Fig. 14). Pancreatic duct obstruction produces a reflux of pancreatic juice into the interstitial tissues of the pancreas and enzymes are removed by the local circulation.[3] The pancreatic circulation responds to the presence of enzymes by the development of hyperemia and the release of a serous exudate containing variable numbers of leukocytes. Lium and Maddock have demonstrated that the severity of this inflammatory response to pancreatic duct obstruction is related to the level of secretory activity of the pancreas.[4] Administration of secretin to animals subjected to pancreatic duct obstruction increases the magnitude of the pancreatic edema as well as the number of inflammatory cells released from the local vascular system. When the degree of pancreatic edema is severe, the local vascular supply to the duodenum and head of the pancreas, as well as intrapancreatic vascular ramifications, may be sufficiently compressed to result in venous stasis. Adams and Musselman [1] have demonstrated that venous stasis will cause a necrotizing form of pancreatitis which

must be similar to the "wet gangrene" observed in venous obstruction elsewhere. Venous obstruction may allow diapedesis of red blood cells or cause disruption of small vessels within the pancreas. The combination of venous stasis and intrapancreatic bleeding then would set the stage for blood to incubate with retained enzymes in the interstitial tissues of the pancreas. Enzymatic digestion of whole blood releases a toxic substance which produces widespread vascular injury and consequently acute necrotizing pancreatitis.

If the disease is arrested in a stage of pancreatic edema either spontaneously or as a result of exocrine secretory depression by an active medical regimen, then only a serious exudate is released into the interstitium of the gland. Incubation of this blood fraction with enzymes results in a digestion product which initiates interstitial fibrosis and chronic interstitial pancreatitis, particularly following repeated attacks of acute pancreatic edema.

Summary

Evidence is presented which suggests that fluid, extravasated from the local pancreatic vascular system in response to the inflammatory effects of acute pancreatitis, may incubate with pancreatic enzymes and form digestion products which influence the character and extent of the disease.

Autologous serum or plasma incubated with trypsin produces acute edematous pancreatitis and later a severe chronic interstitial pancreatitis when introduced into the pancreas of the dog. Autologous whole blood produces diffuse intrapancreatic vascular injury and consequently a fulminating necrotizing pancreatitis when incubated with trypsin and injected into the canine pancreas.

The relationship of these findings to the pathogenesis of human pancreatitis is discussed.

References

1. Adams, T. W. and M. M. Musselman: Pancreatic Venous Thrombosis as an Etiologic Factor in Acute Necrotizing Hemorrhagic Pancreatitis. Surg. Forum, Am. Coll. Surg., **4**:401, 1953.
2. Anderson, M. C., W. H. Mehn and H. L. Method: Treatment of Acute Hemorrhagic Pancreatitis with Adrenocorticosteroids. Clinical and Experimental Observations. A. M. A. Arch. Surg., **78**:802, 1959.
3. Howard, J. M., A. K. Smith and J. J. Peters: Acute Pancreatitis: Pathways of Enzymes into the Blood Stream. Surg., **26**:161, 1949.
4. Lium, R. and S. Maddock: Etiology of Acute Pancreatitis, an Experimental Study. Surg., **24**:593, 1948.
5. Nemir, P., Jr. and D. L. Drabkin: The Pathogenesis of Acute Necrotizing Hemorrhagic Pancreatitis. An Experimental Study. Surgery, **40**:171, 1956.
6. Nemir, P., Jr., S. J. Mucha, H. R. Hawthorne and D. L. Drabkin: A Further Inquiry into Enzymatic Digestion of Blood. Ann. Surg., **150**:469, 1959.
7. Popper, H. L., H. Necheles and K. C. Russell: Transition of Pancreatic Edema into Pancreatic Necrosis. Surg., Gynec. & Obst., **87**:79, 1948.
8. Smyth, C. J.: Etiology of Acute Pancreatitis with Special Reference to Vascular Factors. Arch. Path., **30**:651, 1940.
9. Thal, A.: Studies on Pancreatitis IV. The Pathogenesis of Bile Pancreatitis. Surg. Forum, Am. Coll. Surg., **5**:391, 1954.
10. Thal, A. and E. L. Brachney: Acute Hemorrhagic Pancreatic Necrosis Produced by the Local Schwatzman Reaction: Experimental Study on Pancreatitis. J. A. M. A., **155**:569, 1954.
11. Thal, A.: Studies on Pancreatitis. II. Acute Pancreatic Necrosis Produced Experimentally by the Arthus Sensitivity Reaction. Surgery, **37**:911, 1955.