Experimental Pancreatitis: *

Use of a New Antiproteolytic Substance, Trasylol

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Introduction

Acute hemorrhagic pancreatitis which closely resembles the human variety can be produced in dogs by the creation of a blind duodenal loop.[17] It has been shown that the vascular changes occurring in the pancreas under these circumstances are secondary to, and dependant upon, the reflux of duodenal contents into the pancreatic duct.[15] We believe that this reflux allows active proteolytic enzymes to diffuse across the duct wall and act on the blood vessels in the interstitial tissue. This mechanism probably is responsible for the hemorrhagic pancreatitis which may follow Billroth II gastric resections, and duodenal reflux may be an important etiological factor in other forms of acute hemorrhagic pancreatitis.[14, 15]

Various workers have stressed the importance of local vascular injury in the etiology of pancreatitis,[2, 22, 23] and Rich and Duff [21] have shown that commercial trypsin will produce the characteristic vascular lesions seen in acute pancreatitis. These facts suggest that pancreatic proteolytic enzymes are involved in the pathogenesis of acute hemorrhagic pancreatitis and it follows that proteolytic enzyme inhibitors ought to be of value both in the prevention and treatment of pancreatitis.

A new proteolytic enzyme inhibitor Trasylol ** is now available. According to the suppliers, it is a trypsin-kallikrein inactivator obtained from the parotid glands of cattle and the active substance is a polypeptide with a molecular weight about 11,500. Trasylol can be injected in high doses intravenously or intraperitoneally without risk, and it is not inactivated by human blood. It has been used successfully in many cases of human pancreatitis, often with rapid relief of pain.[6]

It is difficult to evaluate the efficacy of a new drug in a human disease in which the majority of patients will recover with adequate conservative treatment. It was decided therefore to test the effectiveness of Trasylol in preventing acute hemorrhagic pancreatitis in dogs subjected to the blind duodenal loop procedure.

Methods

Blind duodenal loops were made in 15 dogs by the technic of Pfeffer, Stasior, and Hinton,[17] and continuity of the gastro-intestinal tract was restored by gastrojejunostomy. The pancreatic ducts were left intact but bile was excluded from the loop by ligation and section of the common bile duct. Surviving dogs were sacrificed after 23 to 24 hours. Sections of pancreas and ducts were made and samples of serum and peritoneal fluid analyzed for amylase and lipase levels.[15] Two groups of dogs were compared.

A. The control group (seven dogs) was not given Trasylol. They were also the control group for a previous experiment [15] and therefore did not receive an intravenous infusion of plain saline,

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** Obtained from Farbenfabriken Bayer AG. Germany.

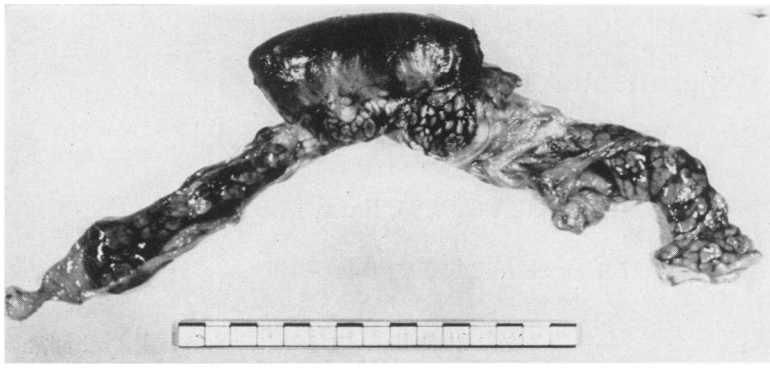

FIG. 1. Blind duodenal loop and attached hemorrhagic pancreas. Untreated dog.

which would have made them more strictly comparable with the second group. However, saline does not protect dogs against this experimental pancreatitis.[8]

B. In the second group (eight dogs) an intravenous infusion via a fine polythene catheter into the femoral vein, was started at the end of the blind loop operation. The infusate consisted of normal saline plus Trasylol (10 ml. of Trasylol/L. of saline, which equals 10,000 Kallikrein inactivator units) plus heparin (1,000 units/L. of saline).

The drip was set to run at approximately 0.5 ml./minute.

Results

A. In the control group not given Trasylol all seven dogs developed hemorrhagic pancreatitis which was severe in four and mild to moderate in three. Three dogs died less than 23 to 24 hours after operation. The typical picture of the distended loop with hemorrhagic pancreas is seen in Figure 1.

B. In the group given Trasylol (eight dogs), some difficulties were encountered with the intravenous infusion which continued to drip satisfactorily all night in only four dogs. None of these four showed any evidence of hemorrhagic pancreatitis, the pancreas being quite white in appearance (Fig. 2); in two of them the bulk of the pancreas was also normal microscopically (Fig. 3); in the other two there were numerous neutrophil leukocytes in the interstitial tissue but no hemorrhages or necrosis of pancreatic acini.

In the other four dogs the Trasylol infusion had stopped running when the dogs were seen next morning and in three the infusion was recommenced. The blind loop had ruptured through a necrotic patch in three of the dogs, two of which showed mild hemorrhagic inflammation confined to the surface of the pancreas near the perforation (Fig. 4). The third dog in this group did not have hemorrhagic pancreatitis.

The fourth dog had an intact loop and hemorrhagic pancreatitis was absent. However the interstitial tissue was heavily infiltrated with neutrophil

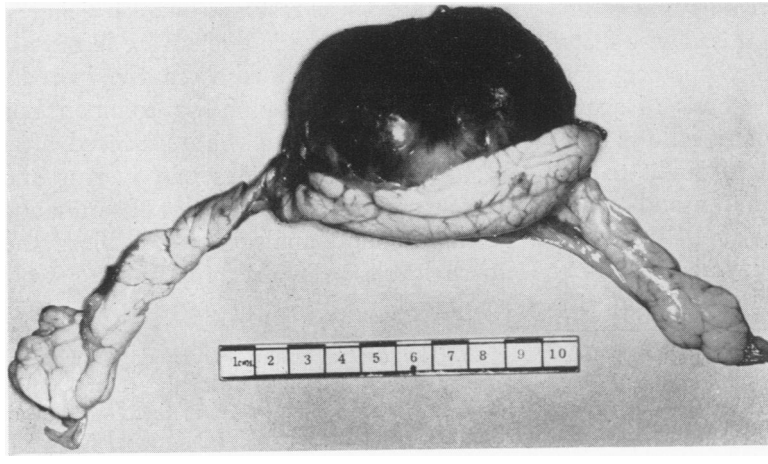

FIG. 2. Blind duodenal loop and normal pancreas. Dog treated with Trasylol.

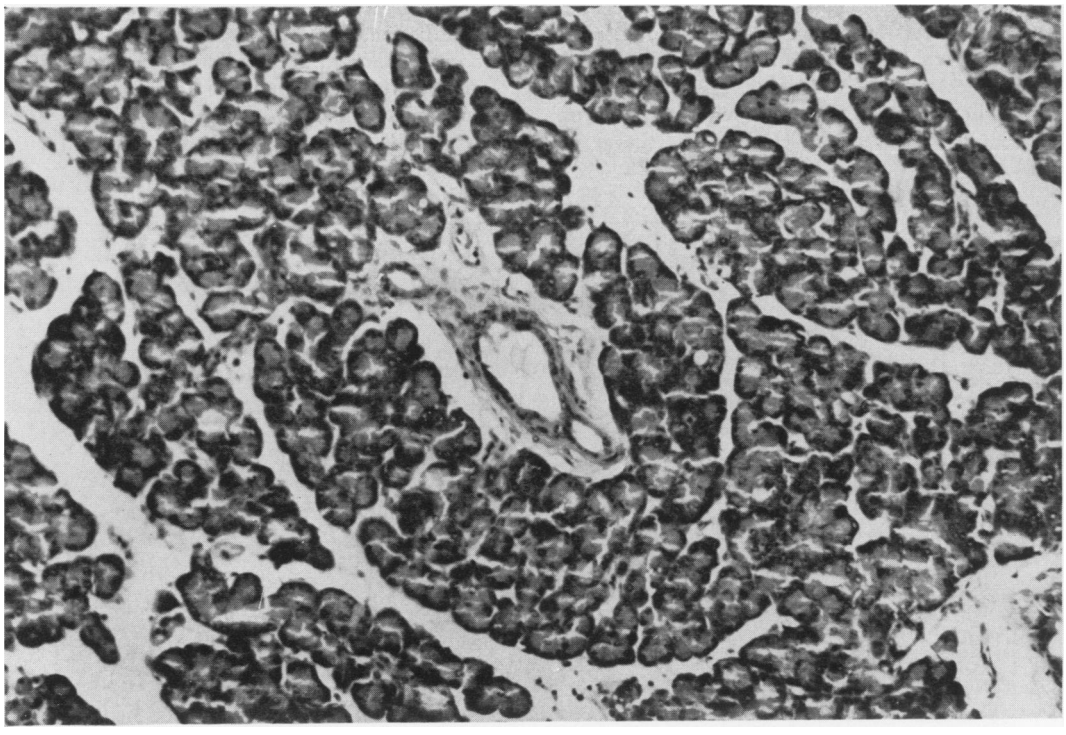

FIG. 3. Section of pancreas seen in Figure 2.

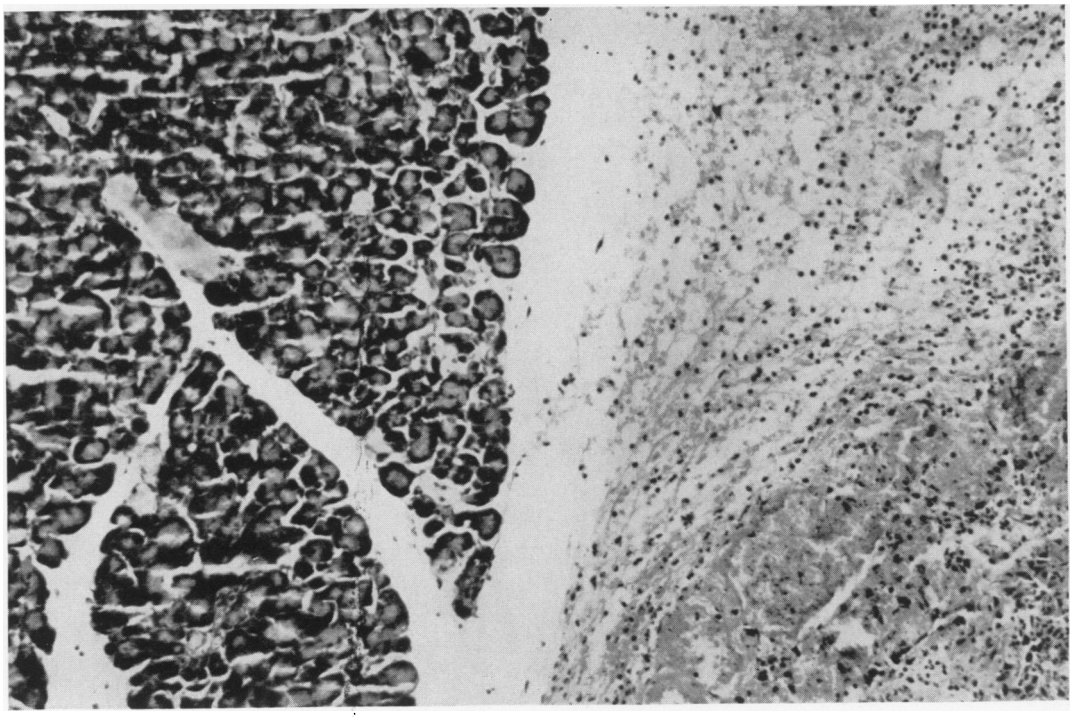

FIG. 4. Mild hemorrhagic inflammation confined to the surface of the pancreas. Pancreatic acini appear normal.

leukocytes and destruction of small areas of acinar tissue with abcess formation was found (Fig. 5).

Of the eight dogs given Trasylol none had severe or extensive hemorrhagic pancreatitis and their clinical condition was superior to that of dogs in the control group. Only one of the eight dogs died prematurely (20 hours postoperatively); its pancreas was normal and death was probably due to the fact that it received more than one liter of normal saline in 20 hours.

There were no significant differences between serum amylase and lipase estimations in the two groups of dogs. However, in the peritoneal fluid, the range of lipase determinations was 12.8 to 29.1

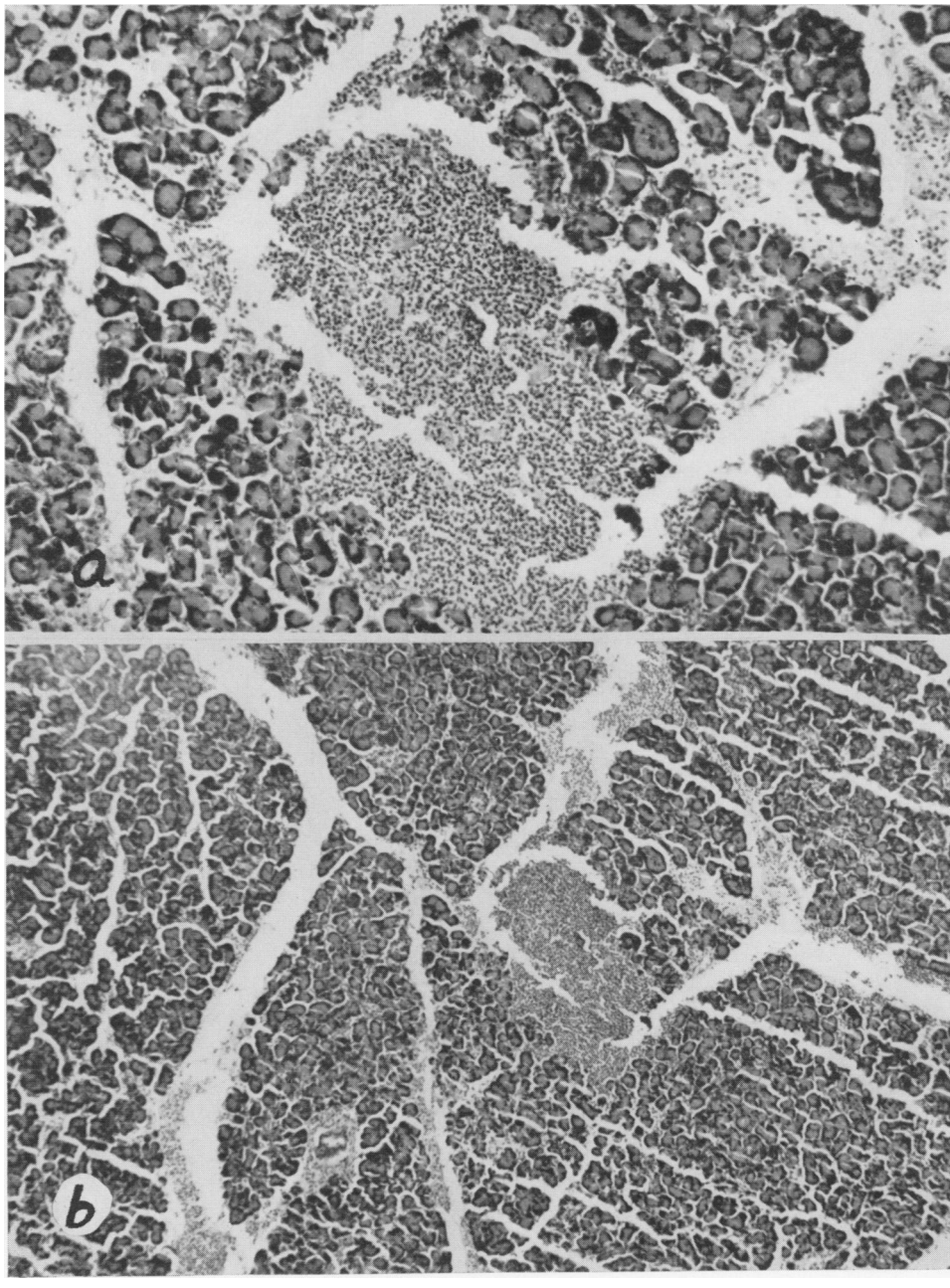

FIG. 5. (a) Infection with abscess formation within the pancreas. (b) Infiltration of interstitial tissue with neutrophil leukocytes. No hemorrhagic pancreatitis.

units/ml. in the control group with pancreatitis, and 2.0 to 14.0 in the Trasylol group.

Discussion

Many workers have shown that extensive activation of proteolytic enzymes occurs in acute hemorrhagic pancreatitis.[4, 5, 16, 24] Others have demonstrated that such proteolytic enzymes are capable of producing the typical vascular lesions seen in acute hemorrhagic pancreatitis;[13, 21] it would seem reasonable to conclude that in human pancreatitis with its characteristic pathology, the vascular lesions are in fact produced in this way.

Hansson, Lundh and Stenram,[7] and Anderson [1] have brought forward evidence that trypsin alone does not cause pancreatitis, but the commercial trypsin preparation used by Rich and Duff to produce pancreatitis undoubtedly contained other proteolytic enzymes as well, such as elastase, and these may be of more importance than trypsin in digesting the wall of small blood vessels.[19]

Two recent reports have shown that trypsin inhibitors derived from egg white or soybean are effective in suppressing the development of experimental pancreatitis in dogs.[3, 20] In an earlier study, however, crystalline soybean trypsin inhibitor was found to be ineffective in the treatment of experimental pancreatitis, but the method used to produce pancreatitis involved forcible injection of bile into the pancreatic duct at a pressure sufficient to rupture the small ducts.[9] It is unlikely that this violent method of producing pancreatitis bears any relation to human pancreatitis and it does not provide a suitable model for studying the effects of enzyme inhibitors.

Our experiments with the Pfeffer loop procedure show that an acute hemorrhagic pancreatitis closely resembling the human variety can be produced by the action of pancreatic enzymes, and that it can be prevented by a proteolytic enzyme inhibitor. The effectiveness of Trasylol in suppressing this form of pancreatitis despite patency of the main pancreatic duct, has also demonstrated that the pancreatitis is not due simply to the transmission of increased pressure from the duodenum with rupture of the smaller ducts, nor is it due to infection.

The possible role of infection in the etiology of pancreatitis has been frequently discussed [12] and the general consensus of opinion is probably in agreement with Ivy's comment that there is little evidence to support infection as a cause of pancreatitis.[11] Our results strengthen this view because several dogs which failed to develop hemorrhagic pancreatitis, did show extensive neutrophil infiltration of the interstitial tissue and one progressed to the stage of formation of small abcesses. These were obvious infections and it is of interest that the histological and macroscopic picture could be clearly distinguished from that of hemorrhagic pancreatitis.

It is clear that Trasylol is a very effective proteolytic enzyme inhibitor and that our results provide a sound theoretical basis for its use in man. The two main indications for its use would be: 1) Therapeutic—in the treatment of established acute pancreatitis; and 2) Prophylactic—in the prevention of development of pancreatitis, under circumstances likely to produce pancreatitis, especially certain operative procedures in the gastroduodenal region.

The effectiveness of Trasylol in the treatment of acute pancreatitis must depend on the length of time which elapses between the onset of the condition and the development of irreversible changes in the pancreas. The sooner antiproteolytic therapy can be instituted, the better. As methods of treatment improve it becomes imperative therefore to find more rapid and sure means of diagnosis.

The diagnostic value of peritoneal paracentesis has been stressed by Hinton and his associates.[8, 18] They regard a hemorrhagic peritoneal fluid with a high amylase

concentration as pathognomonic of acute hemorrhagic pancreatitis. Howard and Jordan,[10] however, have described high amylase levels in the peritoneal fluid of patients with perforated peptic ulcers, and they consider that this test has the same limitations as serum enzyme determinations. Hinton [8] has concluded that serum lipase determinations are of no additional diagnostic help, but he does not discuss peritoneal fluid lipase levels.

We have found in the above experiments that lipase determinations on the peritoneal fluid are of much more diagnostic value than the peritoneal fluid amylase, the serum amylase or the serum lipase.[15] Further investigation is needed to show whether peritoneal fluid lipase levels are also high in perforated peptic ulcer. Meanwhile, the advent of effective proteolytic enzyme inhibitors means that all measures must be taken to make an early diagnosis in acute hemorrhagic pancreatitis and it would seem that these measures should include paracentesis with estimation of peritoneal fluid lipase.

Summary and Conclusions

1. The acute hemorrhagic pancreatitis which follows the creation of a blind duodenal loop in dogs, is due to the action of proteolytic enzymes.

2. A new proteolytic enzyme inhibitor, Trasylol, is very effective in preventing the development of this type of pancreatitis.

3. There is a sound theoretical basis for the therapeutic and prophylactic use of Trasylol in man, and therefore more rapid and sure methods of diagnosis of pancreatitis are necessary, so that early treatment may be instituted.

4. Peritoneal paracentesis with estimation of the peritoneal fluid lipase level is probably of greater diagnostic value in pancreatitis than the other enzyme tests.

5. Infection probably plays little part in the pathogenesis of hemorrhagic pancreatitis, and under our experimental conditions it gave a different macroscopic and microscopic picture to that seen in acute hemorrhagic pancreatitis.

Acknowledgment

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