SOME PROBLEMS IN THE PATHOGENESIS OF ACUTE PANCREATITIS

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The development of edematous or hemorrhagic pancreatitis in albino rats as the result of injection of 1 ml of a 0.001 N solution of hydrochloric acid into the pancreatic duct of the animals does not lead to an increase in the quantity of active trypsin or in the total proteolytic activity in a pancreatic homogenate. No disturbance of the zymogen system was observed under these conditions. Intracanalicular injection of 1 ml of crystalline trypsin, dissolved immediately before injection in 1 ml of 0.85% NaCl solution, into the pancreas does not cause the development of acute pancreatitis.

Several factors are concerned in the pathogenesis of acute pancreatitis, but the key to the complex pathogenetic mechanism is digestion of the gland by its own activated proteolytic enzymes, and above all by trypsin. This theory, which became generally accepted has recently been disrupted by a number of investigators [1-6]. A study of the role of tryptic proteolysis in the pathogenesis of acute pancreatitis and pancreonecrosis is particularly important at the present time because of the wide introduction of inhibitors of proteolytic enzymes (Trasylol, Uniprol, etc.) into clinical practice for the treatment of acute pancreatitis.

Some insight into the pathogenesis of acute pancreatitis can be obtained by investigation of the intrapancreatic activity of trypsin and the total proteolytic activity in experimental pancreatitis and to compare it with the character and dynamics of the morphological changes.

EXPERIMENTAL METHOD

Experiments were carried out on 162 albino rats weighing 180-250 g. To produce acute pancreatitis in the animals, the most popular model was used: pancreatitis was produced by injecting 1 ml of crystalline trypsin, dissolved in 1 ml 0.001 N hydrochloric acid, into the common bile duct (in rats it is also the pancreatic duct). Control animals received an injection of 1 ml of a 0.001 N solution of hydrochloric acid and 1 mg trypsin dissolved in 0.85% sodium chloride solution. The groups of animals and times of the experiments are given in Table 1.

The experiments were carried out by Heinkel's method [6]: the abdomen was opened under ether anesthesia, and a fine needle was passed blindly through the wall of the duodenum into the common bile duct. The duct was clamped at the hilus of the liver and at its entry into the duodenum, after which the solution was injected into it. The wound was sutured in layers. After the end of the experiment the pancreas was excised from the animal under ether anesthesia, part of the organ was subjected to histological and histochemical examination, and the rest was homogenized (the homogenate was prepared by Creutzfeldt's method [4]). The activity of trypsin, content of trypsinogen, and total proteolytic activity were determined in the homogenate.

Activity of trypsin was determined by Erlanger's method in Shaternikov's (1965) modification, and proteolytic activity was determined by Anson's method in Sabsai's modification.

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TABLE 1. Distribution of Animals by Groups and Times of Experiments

Group	Substance injected	Duration of experiment					
		1-3 min	15 min	1 h	6 h	24 h	48 h
1	1 ml trypsin + 1 ml 0.001 N HCl	12	15	19	10	26	8
2	1 ml 0.001 N HCl	–	–	12	–	13	–
3	1 ml 0.85% NaCl	–	–	8	–	5	–
4	1 mg trypsin + 1 ml 0.85% NaCl	–	–	5	3	12	3
5 (healthy)		11	–	–	–	–	–

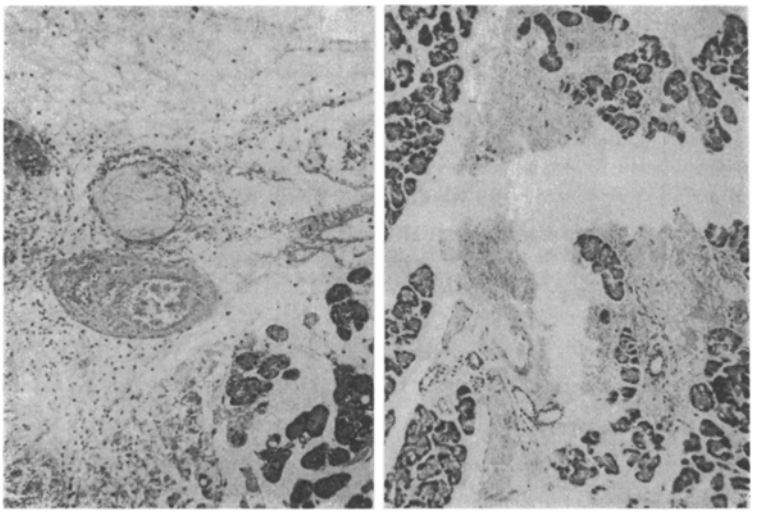

Fig. 1. Marked inter- and intralobular edema and hemorrhages. Dilatation of blood vessels. Hematoxylin-eosin, 100×.

EXPERIMENTAL RESULTS

At laparotomy on the animals of the first group, marked edema was observed after 15 min. After 1 h the intensity of the edema was increased as hemorrhages appeared. Histological examination of the pancreas after 15 min revealed interlobular edema, and after 1 h, intralobular edema with disturbance of the structural pattern of the lobules and acini, hemorrhages, and slight infiltration with leukocytes. Later, at laparotomy on all the rats, "stearin" patches could be seen on the omentum and parietal peritoneum. Loops of intestine were adherent with each other and with the parietal peritoneum, and the pancreas of all the animals was edematous with large, diffuse hemorrhages. Microscopic examination revealed marked dilatation of the blood vessels, and protein-rich edema fluid permeated the cellular and interstitial tissues, separating the lobules and acini. In all cases marked leukocytic infiltration, both diffuse and focal, was observed (Fig. 1). A morphological picture of acute edematous and hemorrhagic pancreatitis was also observed in the animals of group 2, completely indistinguishable from that of pancreatitis in the rats of the experimental group. The ability of 0.001 N hydrochloric acid to produce changes characteristic of acute pancreatitis does not, however, rule out the possible action of injected trypsin in the animals of group 1, especially because in most investigations in which this particular model of acute pancreatitis has been used, the changes which have developed have been regarded as due to trypsin. To shed light on the precise role of trypsin in the development of the morphological changes thus obtained, 1 mg of crystalline trypsin, dissolved immediately before injection in 0.85% sodium chloride solution, was injected into a group of animals (group 4). Preliminary examination in vitro showed that the activity of trypsin in physiological saline is indistinguishable from its activity in 0.001 N hydrochloric acid. Macroscopic and histological investigations of material from the pancreas in the animals of this group revealed no marked changes. In some animals, in the early stages, slight edema of the pancreas was observed, but it later disappeared, (Fig. 2). Injection of crystalline trypsin, dissolved in 0.85% sodium chloride, thus does not cause the development of acute pancreatitis.

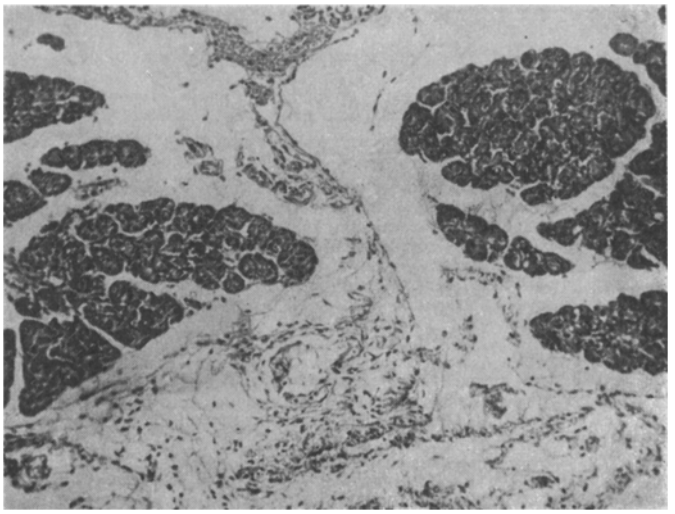

Fig. 2. Mild edema of pancreas after injection of trypsin in physiological saline. Hematoxylin-eosin, 100×.

This suggests that in the experimental model used, trypsin is not the primary cause of development of the acute pancreatitis. Irrespective of the agent primarily responsible for acute pancreatitis, whether this be the 0.001 N HCl or any other substance, the problem of the fate of trypsin injected into the pancreas and the role of tryptic autolysis in the development of the changes observed in the experimental animals in this series remains unsolved.

Investigation of the activity of trypsin in the pancreatic homogenate from the animals showed that 2-3 min after injection of trypsin solution into the pancreatic duct, active trypsin either could not be found or was present only in traces. Later, against the background of a morphological picture of severe hemorrhagic pancreatitis, neither tryptic activity, nor proteolytic activity in general, could be found in the pancreatic homogenate.

To assess the state of the zymogen system of the pancreas, the trypsinogen content was determined in the homogenate. The results of these tests showed that the trypsinogen content in all groups of animals was the same as in the intact animals. At later stages of the experiment in some animals the trypsinogen content was actually higher than in homogenate from healthy rats. This rules out the possibility of a disturbance of the zymogen system as the reason for the absence of tryptic activity.

Histochemical tests for RNA and SH-groups in the material, to investigate the state of protein synthesis and biological activity of protein, confirmed the results of the biochemical tests.

LITERATURE CITED

1. M. Anderson and F. van Hagen, Surg. Gynec. Obstet., 107, 693 (1958).
2. I. T. Beck, E. J. Pinter, J. Solymar, et al., Gastroenterology, 43, 60 (1962).
3. I. T. Beck, D. S. Kahn, J. Solmar, et al., Gastroenterology, 46, 531 (1964).
4. W. Creutzfeldt and H. Schmidt, Klin. Wschr., 43, 15 (1965).
5. W. Doerr, P. Diezel, and K. Grözinger, Klin. Wschr., 43, 125 (1965).
6. K. Heinkel, Klin. Wschr., 31, 815 (1953).
7. J. Hurean, P. Vayre, H. Audhoui, et al., Arch. Mal. Appar. Dig., 54, 323 (1965).