PATHOMORPHOLOGY OF EXPERIMENTAL PANCREATIC NECROSIS TREATED BY A SINGLE ENDOLYMPHATIC INJECTION OF 5-FLUOROURACIL

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Many steps in the pathogenesis of pancreatic necrosis have now been reliably established by clinicians, experimental workers, and pathologists [8, 9, 14], and this has led to the complete abandonment of the surgical approach to uncomplicated forms of pancreatitis by clinicians, and to a radical revision of the tactics of conservative treatment of these patients [1, 4, 6]. In particular, the arsenal of antienzyme preparations (trasylol, gordox, contrykal) has been shown to act only on pancreatic enzymes in the blood and to be unable to arrest the autocatalytic autodigestion process taking place in the pancreas [3]. An intensive search has been conducted in recent years for preparations which would act directly on the acinar cells (AC) of the pancreas to suppress their enzyme-synthesizing activity temporarily [7, 11, 12, 13], and to determine the most effective ways of their administration. This group of preparations includes cytostatics and regulators of protein synthesis [2, 5, 10].

The aim of this investigation was to determine the morphogenesis of the positive effect of 5-fluorouracil (5-FU) in the treatment of pancreatic necrosis, administered by a relatively new (endolymphatic) route.

EXPERIMENTAL METHOD

Experimental pancreatic necrosis (EPN) was produced in 14 mongrel dogs of different weights under general anesthesia, by injection of infected autologous bile under pressure into the pancreatic duct in a dose of 0.5 ml/kg body weight, after functional loading of the pancreas with secretin (1 clinical unit/kg body weight, from Boots, England). Five of these animals served as the control. Animals of the experimental group were treated 2 h after production of EPN by injection of a single dose of 5 mg/kg of a 5% solution of 5-FU via a catheter introduced into a lymphatic vessel of the hind limb. Biopsy specimens were taken from the head, body, and tail of the pancreas of all animals 2 h after production of EPN (before the beginning of treatment), and 6, 18, and 24 h and 3 and 7 days after the beginning of treatment. The biopsy material was fixed in formalin solution buffered according to Lillie, and embedded in paraffin wax. Histological sections were stained with hematoxylin and eosin, for RNA by Brachet's method, and by Mallory's method. Pancreatic tissue for electron-microscopic study was fixed by Palade's method and embedded in Araldite. Ultrathin sections were cut on the LKB 8800 III ultrotome after preliminary examination of semithin sections stained with methylene blue and azure. Electron micrographs were obtained on the EVM-100B electron microscope. Enzyme profiles were determined in blood serum and lymph obtained from the thoracic duct from animals of both groups: amylase activity was determined by Caraway's method, lipase by Natelson's method, total BAEE-esterase activity after Werle and Trautschold, and antitryptic activity after Nartikova and Paskhina.

EXPERIMENTAL RESULTS

A distinct pattern of diffuse-focal centrolobular hemorrhagic necrosis was discovered macroscopically in the pancreas 2 h after production of EPN. Histologically, the necrosis in AC was colliquative in character. Necrobiosis and necrosis of AC were observed in areas of the pancreas bordering on the necrotic focus. The microcirculation in the pancrease was greatly disturbed: aggregation of platelets and erythrocytes in the capillaries and venules, erythro-

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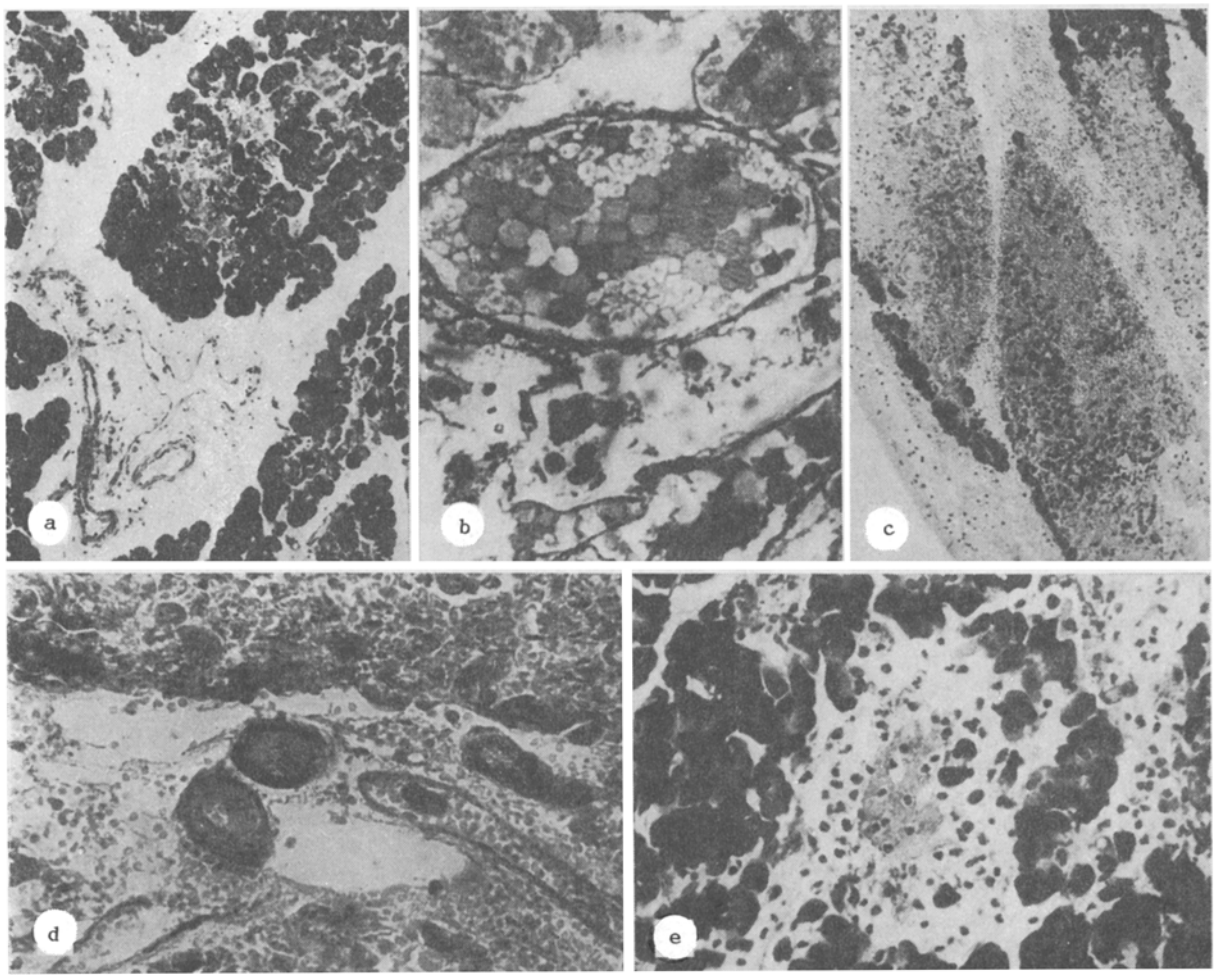

Fig. 1. Histological changes in pancreas before (a-d) and after (e) treatment of EPN. a) Centrilobular foci of necrosis, edema of interlobular spaces. Hematoxylin and eosin, 100 ×; b) aggregated erythrocytes and platelets in lumen of intralobular venule. Semithin section. Methylene blue and azure, 900 ×; c) hemorrhagic necrosis of pancreatic lobules. Hematoxylin and eosin, 100 ×; d) thrombi in lumen fibrinoid necrosis of wall of arteriole. Mallory's stain, 400 ×; e) focus of intralobular necrosis with inflammatory infiltration and clear boundary between it and undamaged acinar parenthyma. Hematoxylin and eosin, 400 ×.

stasis, swelling of endotheliocytes and pericytes in the vascular walls (Fig. 1a, b). The lymphatics were morphologically unchanged while they preserved their drainage function.

In the control group the disease progressed: large foci of hemorrhagic pancreatic necrosis developed, or the lesion was subtotal, and led to death of the animals within 24-30 h. Gross rheologic disturbances were found histologically: hemolysis of erythrocytes against the background of erythrostasis, thrombi composed of fibrin and erythrocytes, with fibrinoid necrosis of the blood vessel walls and with lymphothrombosis (Fig. 1c, d).

Macroscopically the pancreas 6 h after the beginning of treatment differed only a little from the untreated control. Histologically, however, changes were found in both the character and the scale of pancreatic necrosis: small zones of coagulation necrosis, affecting 5-15 acini, and with a leukocytic inflammatory response to them, predominated; foci of intralobular interstitial hemorrhages were reduced, the blood flow in the pancreas was restored, and signs of necrobiosis and disturbances of the complex structure of the acini had disappeared in zones bordering on the affected areas, evidence that necrosis of the acinar tissue had ceased to progress (Fig. 1e, f). A high degree of accumulation of zymogen granules (ZG), observed in AC in the absence of secretion in the ducts, was evidence of delay of secretion of enzymes into the duct system.

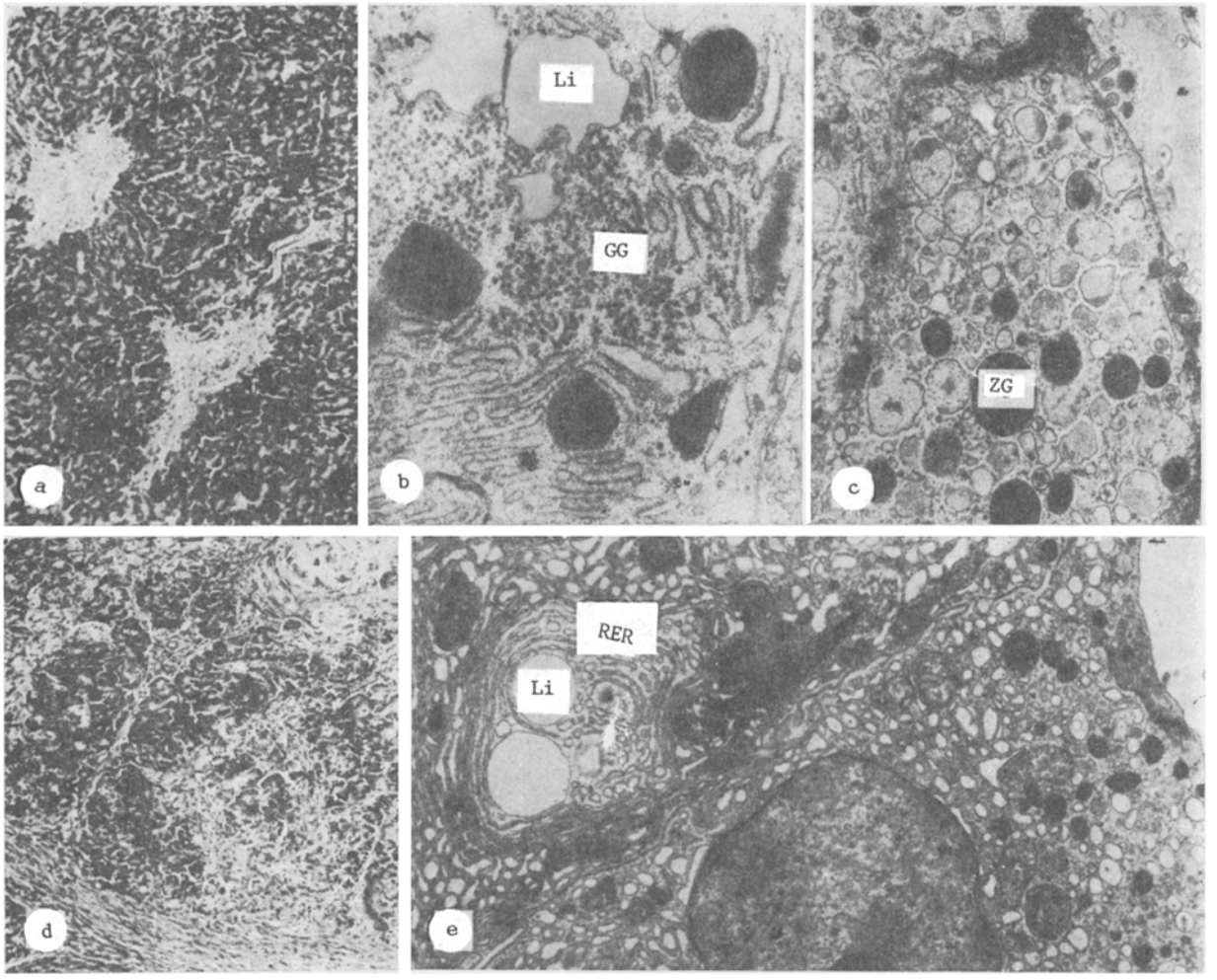

Fig. 2. Histological (a, d) and electron-microscopic (b, c, e) changes in pancreas after treatment of EPN with 5-FU. a) Intralobular foci of necrosis clearly demarcated from acinar parenchyma. Hematoxylin and eosin, 100 ×; b) glycogen granules (GG) and lipid inclusions (LI) in cytoplasm of AC, 28,000 ×; c) immature zymogen granules (ZG) in cytoplasm of AC, 18,000 ×; d) structural pattern of acini indistinct due to formation of epithelial tubular structures. Hematoxoylin and eosin, 100 ×; e) small ZG visible in cytoplasm of AC, with concentric figures of rough endoplasmic reticulum (RER), 18,000 ×.

Edema of the pancreas disappeared 24 h after the beginning of treatment. Interstitial foci of steatonecrosis, still observable histologically, were demarcated by a leukocyte barrier. Lymphatic plethora was observed, with protein-enriched lymph in the lumen. In most of the large lobules of the pancreas lesions were present and were well demarcated from the intact parenchyma (Fig. 2a). Electron-microscopically the necrotic AC consisted of cell debris with destruction of polymorphonuclear leukocytes and macrophages in them. Fragments of intracellular necrosis were clearly demarcated from undamaged parts of AC. Changes characteristic of disturbed energy metabolism were found in AC: The mitochondria were in a state of increased functional activity, but demarcation of the cytoplasmic lipids, accumulation of glycogen granules, and contact between them and the mitochondria indicated replacement of oxidative phosphorylation by anaerobic glycolysis (Fig. 2b).

Macroscopically single foci of steatonecrosis could still be detected beneath the renal capsule and also in the greater omentum 2-3 days after the beginning of treatment. The pancreas was condensed, with a regular lobular structural pattern and with petechial hemorrhages in the centers of individual lobules. Histologically the general architectonics of the pancreatic lobules appeared regular. Only in a few lobules of the pancreas were small foci of centrilobular necrosis present, affecting about 20-30 acini. Small-scale zones of injury had been completely cleared of necrotic debris. Necrobiosis disappeared in the boundary zones. Prolifera-

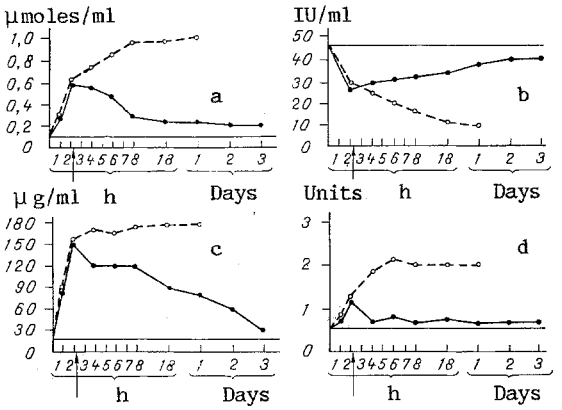

Fig. 3. Changes in total BAEE-esterase (a) and antitryptic (b) activity, α-amylase (c), and lipase (d) in blood serum during development of EPN (broken line) and after treatment with 5-FU (continuous line). Abscissa, time in hours and days; ordinate, enzyme activity in accepted units of measurement. Arrow indicates injection of 5-FU. Horizontal line — the normal state.

tive regeneration of the duct cells was observed. Outside the zones of injury electron-microscopic signs of depression of synthesis, maturation, and accumulation of secreted enzymes could be observed in the acini, due to the cytostatic effect of 5-FU on synthetic activity of AC: Accumulation of immature ZG was observed in the cytoplasm of AC, the elements of the lamellar network were collapsed, and the cisterns of the rough endoplasmic reticulum had undergone fragmentation and vacuolation, and their nuclear chromatin was reduced in density (Fig. 2c).

Macroscopically the pancreas 7 days after the beginning of treatment was dense and a little atrophic, and composed of large and small lobules with solitary small foci of subcapsular steatonecrosis. Histologically, foci of fatty and parenchymatous necrosis of the pancreas were absent, and in zones where the acinar tissue was previously damaged, foci of replacement fibrosis had developed. In the centers and at the periphery of the lobules the structural pattern of the acini was indistinct because of their replacement by tubular epithelial structures, with cystic dilatation of their lumen (Fig. 2d). Electron-microscopically, epithelial complexes formed both true and accessory ducts, the rough endoplasmic reticulum formed concentric figures, and in the zone of the lamellar network lysosomes and myelin figures appeared (Fig. 2, f). All these changes were evidence of depression of the protein-synthesizing and energy-generating potential of AC, due both to incomplete reparative regeneration and to the disorganized state of the functional unit (the acinus), connected with the system of ducts. The disturbed secretory activity of the pancreatic cells, without release of the secreted enzymes into the lumen of the ducts and digestive tract, led to atrophy of AC and to their dedifferentiation into duct cells.

The study of the activity of the pancreatic enzymes (PE) and vasoactive peptides in the animals' blood serum during treatment of EPN showed that α-amylase activity (Fig. 3c) increased immediately after production of EPN, and by the beginning of treatment it was 5-7 times higher than initially. Under the influence of treatment α-amylase activity declined slowly, and did not approach subnormal values until the 3rd-7th day. Lipase activity (Fig. 3d), on the other hand, which was almost twice as high as initially by the beginning of treatment, fell under the influence of treatment to normal 1 h after administration of 5-FU, and did not rise again. Total BAEE-esterase activity (Fig. 3a), which showed a threefold increase toward the beginning of treatment, was unchanged 2 h after administration of 5-FU, and later it fell slowly to subnormal values, to return after 3-7 days toward the initial normal value. Antitryptic activity (Fig. 3b), which fell by one-third 2 h after the beginning of the experiment, remained unchanged for the first 3 h after treatment, but thereafter began to rise slowly, and remained for 3 days at close to its level at the beginning of treatment, returning to the initial value only on the 7th day.

Thus a single endolymphatic injection of 5-FU in the early stages of EPN had a positive effect with complete cure of the animals, whereas in the control group all the dogs died during 24-30 h after production of EPN. The pathomorphology of RPN, treated with 5-FU, is as fol-

lows: 1) In the early stages (6 h) the scale of destruction of the pancreas is reduced, zones of injury are demarcated, hemorrhages, inflammation, and vascular changes disappear, and the secretory activity of the pancreas is depressed; 2) after 1-3 days the zones of necrosis become smaller, demarcated, and later cleared of debris, and show evidence of initial repair; 3) after 7 days complete healing of the zones of injury takes place with signs of substitutive sclerosis of the pancreas and of incomplete regeneration; 4) the time course of the parameters of pancreatic activity corresponds completely to the morphologic data.

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MORPHOLOGIC STUDY OF THE EFFECT OF VAGOTOMY ON THE MUCOSAL MICROFLORA OF THE STOMACH AND DUODENUM

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In the modern view, besides the bacterial flora of the lumen, the luminal or L-flora in the small intestine of higher animals and man, there is also a flora which is directly connected with structures of the mucous membrane, the mucosal or M-flora [4-6]. It has been shown that the conditions of life of laboratory animals, and also various pathological processes involving the human digestive organs affect the qualitative and quantitative characteristics of both the L-flora and the M-flora. In particular, in duodenal ulcer, the quantity of both the L- and M-flora is increased [5, 6].

A widespread method of surgical treatment of duodenal and gastric ulcer with a hypersecretory syndrome at the present time is vagotomy [2, 3]. The reduction of the acidity of the gastric contents resulting from vagotomy is bound to affect the state of the microflora. However, no morphological investigations into the effect of vagotomy on the state of the M-flora and its interaction with the epithelial cells of the gastric and duodenal mucosa have yet been undertaken. The aim of the investigation described below was to study these problems.

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