MORPHOLOGY AND PATHOMORPHOLOGY

MORPHOLOGIC CHANGES IN THE PANCREAS IN HEMORRHAGIC SHOCK AND IN THE POSTRESUSCITATION PERIOD

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Recent investigations have ascribed an important role to the pancreas in the pathogenesis of shock [1, 3]. It is very sensitive to ischemia, and in shock the blood flow in it is sharply reduced [5, 6, 8, 9]. Pancreatic ischemia increases the serum cathepsin D activity by 27 times [9]. In experimental hemorrhagic shock a tenfold increase in trypsin activity has been found in the arterial blood of dogs, whereas in depancreatized animals, levels of trypsin activity were unchanged. The fact that the proteolytic activity of the blood is increased in shock and other extremal situations can be taken as established, but problems to do with the identification of the enzymes appearing in the blood and their source still remain matters for debate. Some workers [7] consider that the sources of proteases are the lysosomal enzymes released on death of the cells, whereas others [2, 6] associate the increased proteolytic activity with enzymes secreted by the pancreas.

The aim of this investigation was a morphological study of the pancreatic acinar tissue after acute massive blood loss and at different stages of the postresuscitation period.

EXPERIMENTAL METHOD

Experiments were carried out on adult mongrel dogs (20) of both sexes weighing 10-12 kg. Experiments on the animals were done under superficial pentobarbital anesthesia with trimeperidine premedication, and with the use of local procaine anesthesia during dissection and catheterization of the carotid and femoral arteries. There were two series of experiments. In series I (five dogs) a terminal state (clinical death lasting 3 min) was simulated, by a single massive bleeding from the femoral artery. In series II (15 dogs), after blood loss and the terminal state, the animals were resuscitated by the use of a combination of measures (intraarterial injection of the animal's own blood, artificial ventilation of the lungs, indirect cardiac massage and, if necessary, defibrillation). For the morphological investigation biopsy specimens of the pancreas were taken from anesthetized animals in the terminal state and 1, 24, and 48 h after resuscitation. Material for histological investigation was fixed in neutral formalin and embedded in paraffin wax; sections were stained with hematoxylin and eosin and by Mallory's method. Pancreatic tissue for electron-microscopic investigation was fixed in 2.5% glutaraldehyde solution, postfixed in 1% OsO_4 solution, and embedded in Araldite. The sections were stained with methylene blue and azure, and ultrathin sections with lead nitrate, and examined in the ÉVM-100B electron microscope. The serum enzyme profile of the animals was determined at the same time, with determination of α-amylase activity by the method in [5] and of trypsin activity by Erlanger's method in the modification in [4].

EXPERIMENTAL RESULTS

After acute blood loss, the pancreas preserved its lobular structure histologically. Arteries in spasm and dilated, congested veins could be seen in the edematous connective tissue of the interlobular spaces. Secretory ducts of all calibers, free from secretion, were clearly outlined. The cytoplasm of the acinar cells (AC) was characterized by increased basophilia and by narrowing of the zone of eosinophilia. Secretory granules in the cytoplasm of AC in sections stained by Mallory's method and in semithin sections could not be identified (Fig. 1a).

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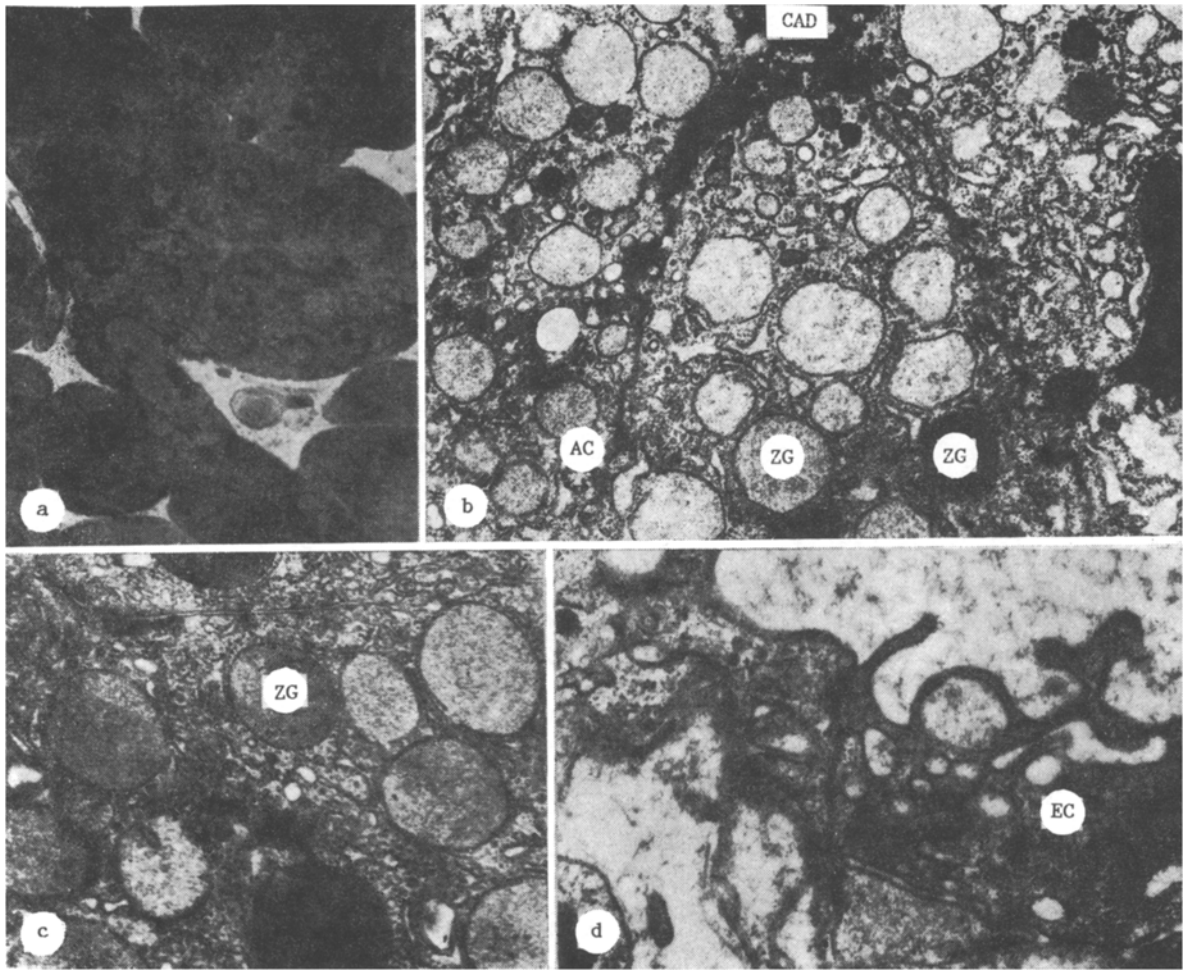

Fig. 1. Changes in pancreas associated with acute blood loss. a) Accumulation of secretion in lumen of centroacinar ducts (CAD), absence of ZG in cytoplasm of AC. Semithin section. Methylene blue and azure. 900×; b) accumulation of immature zymogen granules (IZG), of low electron density, in cytoplasm of AC. 14,000×; c) Interrupted outlines of membranes of ZG with formation of micropores (arrow). 38,000×; d) Macro- and micropinocytotic vesicles in cytoplasm of a papillary endotheliocyte (EC). 32,000×.

Ultrastructural study of the apical zones of the cytoplasm of AC revealed numerous immature zymogen granules (ZG) of low electron density. Numerous micropores were found in the membranes of the granules (Fig. 1b, c). The apical plasmalemma of AC had numerous microvilli. The ZG did not make contact with membranes of the central acinar ducts, and no signs of extrusion of secretion were present. The location, shape, and structure of the mitochondria were unchanged. The narrow lumen of the interacinar capillaries was filled with plasma, The number of micro- and macropinocytotic vesicles in the cytoplasm of the endotheliocytes was increased (Fig. 1d).

At the height of hemorrhagic shock an increase in the α-amylase and trypsin titers was observed in the blood serum of the animals (Table 1), evidence of incretion of the secreted enzymes into the blood stream.

Acute blood loss is thus accompanied by changes in the blood supply to the pancreas, manifested as anemia in the arterial system and capillary bed and congestion of vessels of the venous collector. Structural changes in endotheliocytes of the capillaries and venules indicate increased vascular permeability, mainly for plasm.

A study of the pancreocytes of the pancreas revealed reduced electron density of ZG, evidence of reduction of the protein fractions and of their composition. The discovery of defects in the membranes of ZG and the formation of micropores may be evidence of "leakage" of the protein component from the granules.

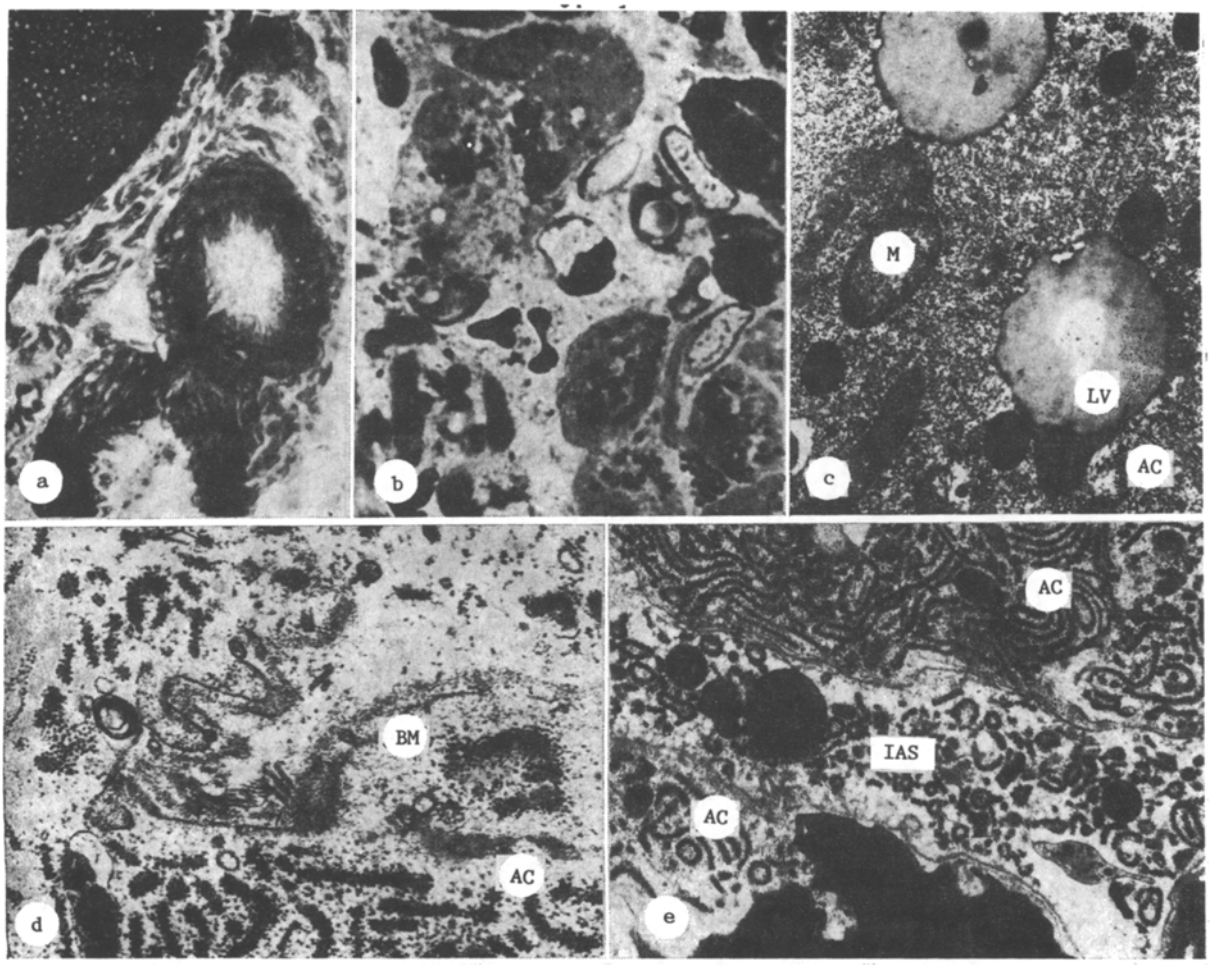

Fig. 2. Changes in pancreas in postresuscitation period of hemorrhagic shock: a) anemia of artery and venous erythrostasis. Hematoxylin and eosin. 400×; b) outlines of basement membrane of AC indistinct, fragments of destroyed AC, erythrocytes, and fibrin in interacinar space (IAS). Methylene blue and azure. 900×; c) lipid vacuole (LV) in contact with mitochondria (M) in cytoplasm of AC. 800×; d) Fragmentation and rupture (arrow) of basement membrane (BM) of AC. 18,000×; e) Organelles of destroyed AC in IAS. 18,000×.

At all times of the postresuscitation period, arterial and capillary anemia persisted histologically in the pancreas, while the cells of the vessel walls were swollen. Vessels of the venous collector were dilated and congested at these times, with evidence of aggregation of platelets and erythrocytes and stasis of the latter. Signs of increased vascular exudation included perivascular plasmorrhagia, deposition of fibrin, and focal perivascular hemorrhages (Fig. 2a).

Signs of desynchronization of synthesis and accumulation of enzymes in AC within the lobule were found 1 h after resuscitation of the animals. Whereas in centrolobular zones signs of accumulation of mature secretory granules and of extrusion of secretion into the lumen of the duct were observed, in the peripheral zones of the lobules no ZG were discovered. Ultrastructural study of AC in the centrolobular zones revealed signs of fragmentation and microvesculation of the rough endoplasmic reticulum, which preserved the form of elongated, flattened cisterns only in "dark" pancreocytes. Numerous lipid vacuoles, in close contact with mitochondria, could be seen in the cytoplasm of AC (Fig. 2c). Differences in structure of the acini within the pancreatic lobule became more marked 24 and 48 h after resuscitation of the animals. Cell membranes of AC in the acini of the peripheral zones of the lobules were indistinct, the basement membrane in some cells showed evidence of destruction, and fragments of cytoplasm of the destroyed pancreocytes filled the interacinar spaces. Among the cell debris, permeated with fibrin, erythrocytes and labrocytes were found (Fig. 2b).

TABLE 1. Serum α-Amylase and Trypsin Levels in Dogs during Acute Blood Loss and after aesuscitation

Character and period of experiment	Number of animals	Amylase, U, M ± m	Trypsin, IU, M ± m
Intact animals	5	25,4±5,8	0,2±0,02
Acute blood loss	5	152±9,6	2,1±0,1
After resuscitation of animals:			
1 h	5	187±14	4,9±0,4
24 h	5	210±15,5	5,5±0,5
48h	5	230±18,8	6,7±0,5

Legend. $p < 0.001$ compared with intact animals in all series.

Under the electron microscope the pancreocytes of the peripheral zones of the lobules were characterized by diffusion of ZG in all parts of the cytoplasm. The basal plasmalemma of AC underwent fragmentation and separation into layers, or even rupture in some areas, assuming the shape of a spiral. The contents of the cytoplasm of AC escaped through defects in the cell membranes into the interacinar and perivascular space (Fig. 2d, e).

Biochemical study of the serum α-amylase and trypsin titers of animals in the postresuscitation period indicated a progressive increase in their concentrations compared with those in the period of acute blood loss (Table 1).

Resuscitation of the animals and restoration of the lost blood thus did not restore the circulation in the pancreas. In the early part of the postresuscitation period, degenerative changes in the smooth and rough endoplasmic reticulum, mitochondria, and protein-lipid complexes of the cytoplasm dominated the morphologic picture of the pancreocytes, with unmasking of lipids. In the late postresuscitation period intracellular metabolic disturbances gave way to destructive. It can be concluded from the morphological investigation that the source of the enzymes in the blood in hemorrhagic shock and in the postresuscitation period is the proteolytic enzymes of the pancreas, penetrating into the blood stream through "leaks" from the secretory granules after destruction of the membrane structures of the pancreocytes.

LITERATURE CITED

1. El. V. Vashetko, Traumatic Shock [in Russian], Leningrad (1982), pp. 22-26.
2. E. N. Meshalkin, V. S. Sergievskii, A. V. Suvernev, and G. K. Gleim, Trypsinemia and Reactions of the Organism to Injury [in Russian], Novosibirsk (1982).
3. A. V. Suvernev and A. S. Kogan, Abstracts of Proceedings of the Second All-Union Congress of Pathophysiologists [in Russian], Vol. 2. Tashkent (1976), p. 427.
4. V. A. Shaternikov and L. A. Savchuk, Biochemical Methods of Investigation in Clinical Medicine [in Russian], Moscow (1963), pp. 189-191.
5. W. Caraway, Am. J. Clin. Path., **32**, 97 (1969).
6. R. Jones, J. H. Garcia, and W. Mergner, Arch. Patk., **99**, No. 12, 634 (1975).
7. A. Lefer and T. Glenn, Steroids and Shock, Baltimore (1974), pp. 234-251.
8. M. Sato, K. Yamamoto, and N. Ozawa, J. Surg. Res., **23**, No. 1, 19 (1977).
9. J. Spath, R. Gorezynsky, and A. Lefer, Am. J. Physiol., **226**, No. 2, 443 (1974).