ULTRASTRUCTURAL CHANGES IN PANCREATIC ACINAR CELLS INDUCED BY COLD

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In the course of its vital activity every cell is exposed to the influence of various external environmental factors, to which it responds with reversible and irreversible changes. Irreversible changes in the cell are preceded, as we know, by a phase of paranecrosis [1, 3, 4, 9].

Electron-microscopic studies have shown that the phase of paranecrosis is characterized by injury to membranous structures and vital intracellular organoids, so that the cell loses its ability to localize vital dyes in a granular form [7].

The action of low temperatures on organs and tissues, which lies at the basis of a new branch of science, namely cryobiology, has been widely studied in recent years. Methods of cryosurgery, based on cold destruction of tissues which require to be removed, have become widely adopted in medicine. Many workers have shown by their research that cell membranes are the first structures to be damaged by low temperatures [2, 5, 10-13]. Their mechanical strength and their permeability for electrolytes and metabolites are changed under these circumstances [6]. However, the action of low temperatures on different organs and tissues has not yet been adequately studied.

The object of this investigation was to study the time course of ultrastructural changes in the acinar cells (AC) of the pancreas during cooling of the gland with ethyl chloride.

EXPERIMENTAL METHOD

The method of freezing an area of the pancreas with ethyl chloride, suggested as a model of experimental pancreatitis by Simavoryan [8], was used. Experiments were carried out on 30 male rats weighing 180-200 g. Laparotomy was performed under ether anesthesia, the splenic part of the pancreas was mobilized into the wound, and for 1 min both surfaces were cooled with a jet of ethyl chloride until the appearance of a deposit of hoarfrost, which corresponded to a temperature of −30°C. After rapid thawing the gland was replaced in the abdomen and the wound in the abdominal wall was closed without drainage. The animals were decapitated 5, 30, and 60 min and 3, 6, and 24 h after the operation. Pieces of pancreatic tissue for electron-microscopic investigation were fixed in 1% osmic acid solution by Palade's method, then in absolute acetone, and embedded in Araldite. Semithin sections were cut in each case and stained with a mixture of azure II and methyl blue. Sections obtained by microtomy of trimmed blocks were stained with uranyl acetate and lead nitrate by Reynolds' method and examined under the ÉMV-100L electron microscope.

EXPERIMENTAL RESULTS

The structure of the pancreatic acini was preserved on histological examination after 5 min in the zone of exposure to cold and periacinar edema, marked congestion, and stasis of blood in the capillaries were seen. The boundaries of AC and the centroacinar ducts were indistinctly outlined. The cytoplasm of many AC appeared vacuolated and weakly basophilic in its basal portions, evidence of a decrease in the RNA content. No secretory zymogen granules

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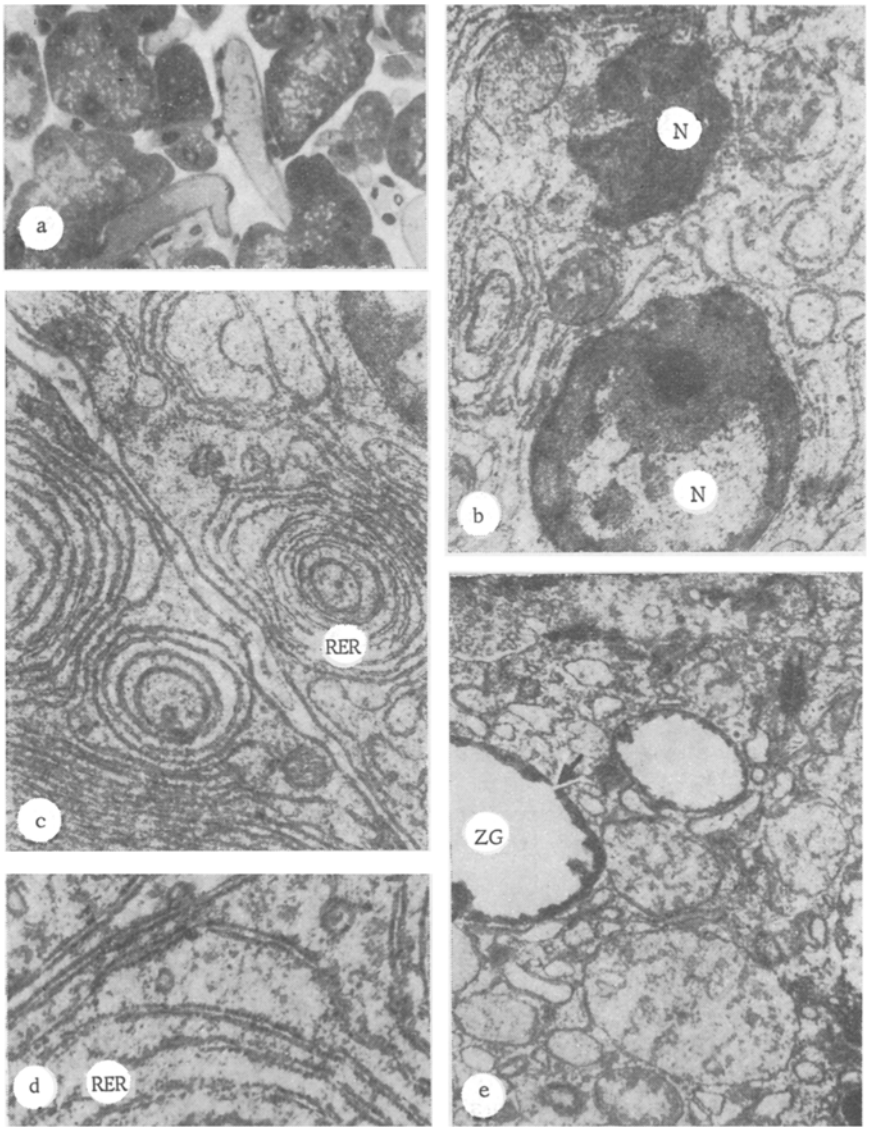

Fig. 1. Histological (a) and electron-microscopic (b-e) changes in pancreatic AC 5-30 min after exposure to cold. a) Vacuolation of cytoplasm of AC with absence of ZG, signs of stasis of erythrocytes in capillaries. Semithin section, methyl blue—azure II. 400 ×; b) coarse condensation of chromatin with rarefaction of karyoplasm in individual nuclei (N); c) color of cisterns of rough endoplasmic reticulum (RER), fragmentation, degranulation, and incorporation of ribosomes into membranes with formation of figures of "palisade" type (d); e) submembranous condensation of secretory material of ZG with formation of micropores in membranes (arrow); magnification: b, c, e) 22,000 ×, d) 48,000 ×.

(ZG) could be seen. The nuclei in the cells were oriented basally or centrally, they showed marked polymorphism, and they stained intensely with methyl blue (Fig. 1a).

Electron-microscopic examination revealed fragmentation and degranulation of the outer nuclear membranes while the inner membranes were preserved. The nuclear chromatin was submembranous in distribution with central rarefaction of the karyoplasm or it was concentrated into a single compact mass (karyopycnosis). The nucleoli were indistinctly outlined. Increased osmiophilia of the basement membrane of the acini and weakening of intercellular connections in the acinus were observed (Fig. 1b). No microvilli were seen. The greatest changes affected intracellular membranous structures. The rough endoplasmic reticulum (RER) in basal portions of the cytoplasm had the appearance of compact concentric figures

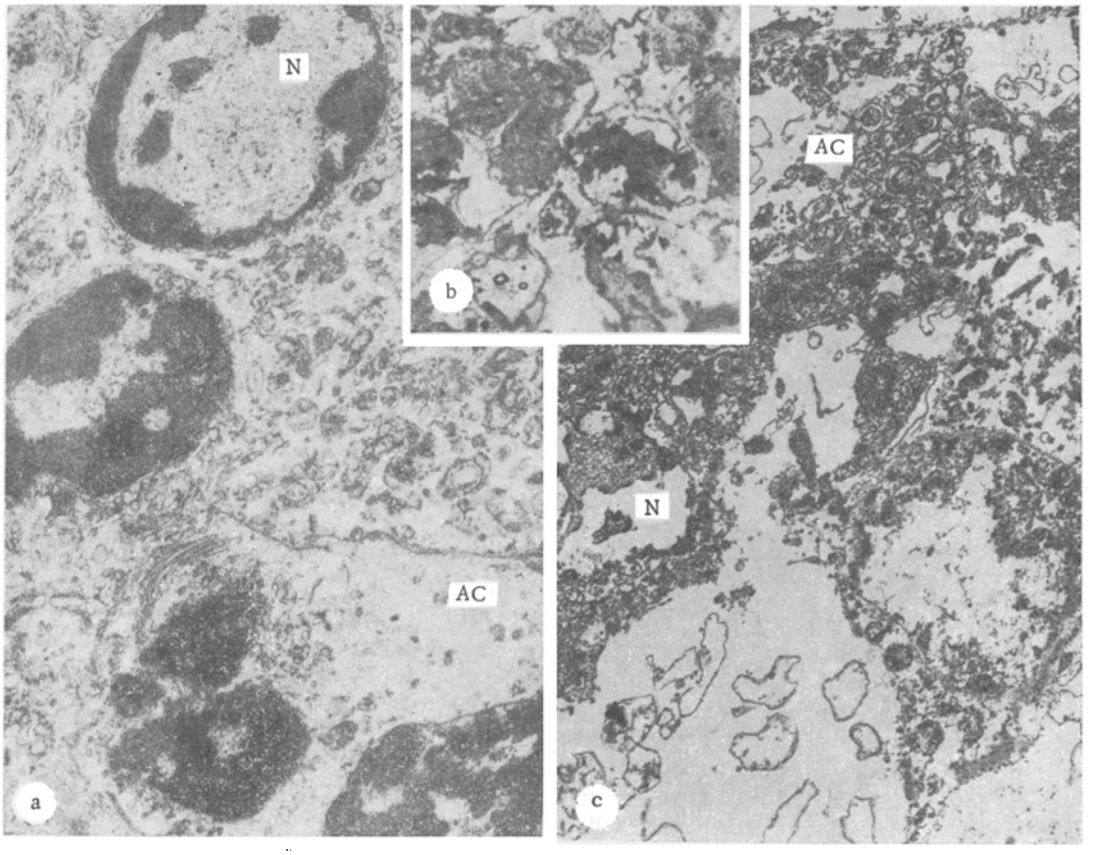

Fig. 2. Ultrastructural (a, c) and histological (b) changes in AC in zone of cooling with progressive destruction of membranous structures of cytoplasmic organelles, with evidence of pycnosis, karyorrhexis (a), karyolysis (c), and cytolysis. a, c) Electron micrographs. 22,000 ×; b) semithin section. Methyl blue and azure II. 400 ×.

formed from collapsed cisterns of the RER, whereas in the apical portions it appeared to be highly vacuolated. Diffuse fragmentation and degranulation of RER membranes and a decrease in size and deformation of RNP granules were noted. The ribosomes became drop-like or rod-like in shape, and being arranged in parallel rows perpendicularly to the length of the membranes, they formed figures of "palisade" type (Fig. 1c, d). Elements of the lamellar complex appeared vacuolated and they were difficult to find among vacuoles of the rough and smooth endoplasmic reticulum. The ZG had the appearance of vacuoles with submembranous condensation of secretory material. Fragmentation and micropore formation in their membranes were found, and were accompanied by leakage of enzymes into the hyaloplasm (Fig. 1e). The mitochondria mainly had their usual structure.

After 30-60 min progressive destruction of all tissue elements of the gland was observed in the zone of cooling. The pattern of the acini was outlined only by the loosely packed basal layer. The cytoplasm of AC consisted of an amorphous structureless mass, with fragments of chaotically arranged membranous structures. Focal disintegration of the basal portions of some AC with the outflow of cell debris into the interacinar spaces occurred as a result of these changes. Fragmentation of the nuclear membranes was observed. As a result of this, chromatin penetrated into the cytoplasm of the cells, leading to partial or complete destruction of the nuclei — karyorrhexis (Fig. 2a).

After 3 h the cytoplasm of AC consisted of debris of cytoplasmic membranes and vacuoles, among which single parallel smooth cisterns could be distinguished. Edematous and pycnotic nuclei with indistinctly outlined membranes apparently "floated" in this structureless mass.

After 6 h the AC were in a state of almost total destruction. Only in places were the outlines of the acini visible on account of the relatively preserved basal layers.

After 24 h expansion of the foci of cytolysis was observed and nuclear ghosts with remnants of chromatin masses were difficult to distinguish in the cell debris (Fig. 2b,c).

Considerable disturbances of the microcirculation (congestion, erythrocyte stasis, thrombosis, hemorrhage) were observed histologically in the zone of immediate exposure to cold, but more especially in the boundary zones. These changes were accompanied by marked edema of the interstitial tissues and diapedesis of polymorphs, the first signs of which were detectable as early as after 30 min, and became more evident later. Foci of fatty necrosis could be identified as early as after 30 min under the light microscope in the fatty areolar tissue.

At least three factors are thus involved in the development of the pathogenesis of irreversible destruction of AC: 1) destruction of cellular ultrastructures by the action of cold; 2) enzymic autolysis; 3) increasing circulatory hypoxia on account of disturbances of the microcirculation.

As the observations showed, widespread injuries to membranous structures of the cell were detected by the electron microscope 5 min after cooling, in the first place in the membranes of the RER and in ZG. Considerable deformation of ribosomes also was found. These changes were undoubtedly attributable to the destructive action of cold, and they were evidently responsible for the state of parabiosis, manifested histologically as diffuse staining of the cytoplasm with acid dyes. Later, destruction of the membranes spread to all intracellular organelles, progressing both in depth and in severity. In the course of necrobiosis the AC lost its ability to carry out autophagocytosis of its injured ultrastructures and it was in a state resembling "cold shock." Its subsequent fate depended on the degree of cold destruction of the membranous structures. In severe injuries it underwent total destruction after only 30 min, but in less severe injuries this process extended over 3-6 h or more. In this case additional harmful factors are involved: enzymic autolysis and circulatory hypoxia. The proteolytic effect of autologous enzymes of AC is evidently sharply reduced by the rapid release of ZG into the interstitial tissues and their inactivation by cold [6]. This effect is exhibited mainly in the interstitial spaces in the form of the rapid formation of foci of fatty necrosis (30 min) and injuries to the microcirculatory system.

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