REPAIR PROCESSES IN THE PANCREAS AFTER ETHYL CHLORIDE COOLING

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Regeneration of the pancreas after degenerative and necrobiotic changes in the organ has not been adequately studied. Most workers have studied reparative regeneration of the pancreas after partial resection. However, these investigations have led to contradictory results: some workers consider that reparative regeneration of the acinar parenchyma is possible through the formation of new acini by differentiation of proliferating ducts [3, 5, 9, 10], whereas others have claimed that compensation of the lost parts of the acinar parenchyma is effected through hypertrophy of residual acini, i.e., by intracellular regeneration [2, 4, 6-8, 11-16]. Some workers [17, 18] have denied that partial or complete recovery of the lost part of the pancreas is possible.

Differences in view on the regenerative powers of the pancreas can be explained by inequality of the experimental conditions, for when the regenerative capacity of the pancreas is assessed it is essential to bear in mind a number of factors and, in particular, the species of experimental animal, the character of injury, and so on. Electron-microscopic investigations have shown that the manifestations of regeneration are not confined to an increase in the number of cells; they are much more varied and include some complex and little studied intracellular repair processes [14].

The object of this investigation was to study the dynamics of regeneration of the pancreas in experimental pancreatitis.

EXPERIMENTAL METHOD

The method of freezing an area of the pancreas with ethyl chloride, suggested as a model of experimental pancreatitis by P. S. Simavoryan in 1973, was used. Under ether anesthesia laparotomy was performed on rats, the splenic segment of the pancreas was mobilized into the wound, and for 1 min both surfaces were cooled with a jet of ethyl chloride until a covering of frost appeared, equivalent to a temperature of −30°C. After rapid thawing the pancreas was returned into the abdomen and the wound in the abdominal wall was closed without drainage. The animals were decapitated 5, 30, and 60 min, 3, 6, and 24 h, and 3, 7, 14, 21, and 30 days after the operation. Pieces of pancreas for electron-microscopic study were fixed in 1% osmic acid solution by Palade's method, post-fixed in absolute acetone, and embedded in Araldite. In each case semithin sections were cut and stained with a mixture of azure II and methyl blue. Ultrathin sections cut from trimmed blocks were stained with uranyl acetate and lead nitrate by Reynolds' number and examined in the ÉMV-100L electron microscope.

EXPERIMENTAL RESULTS

Widespread destruction of cell membranes was observed in the zone of direct action of cold, terminating in the course of 12-24 h in the formation of a focus of necrosis [1].

Areas of pancreas bordering on the epicenter of the lesion, being less severely injured, acted as the source for repair processes, which proceeded in three directions: 1) intracellular regeneration of partially damaged acinar cells; 2) proliferation of the

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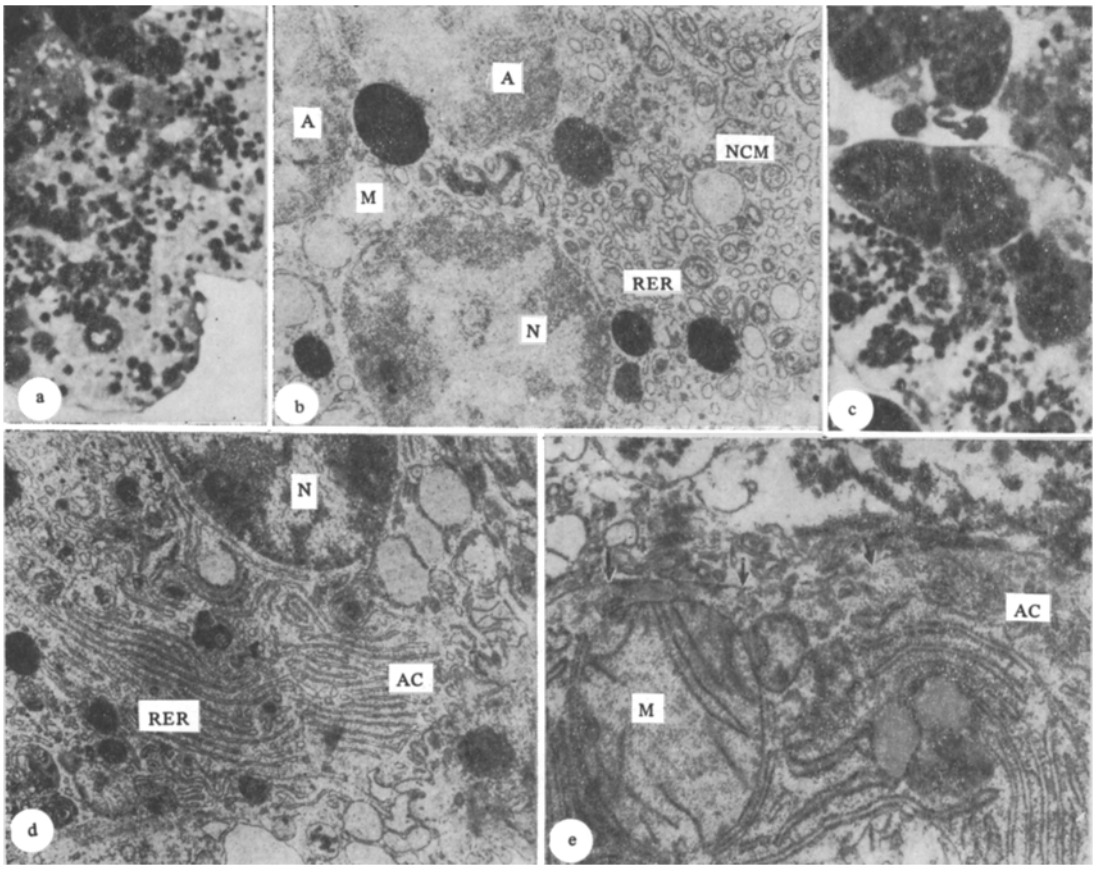

Fig. 1. Histological (a, c) and electron-microscopic signs of regeneration of pancreatic acinar cells (AC) in zone of degeneration and necrosis. a) Pancreatic acini appear as nucleo-cytoplasmic mass (NCM) are indistinguishable cell boundaries. Semithin section, methyl blue and azure, 900 ×; b) fragment of Fig. 1a. NCM consisting of numerous nuclei (N) with clumpy chromatin, distinct nuclear membranes, mature zymogen granules (ZG), mitochondria (M), and vesicular or narrow cisternae of rough endoplasmic reticulum (RER), 18,000 ×; c) formation of a separate AC in an acinus among NCM. Semithin section, 900 ×; d) concentration of organelles in cytoplasm of AC around nucleus in absence of plasma membrane, 20,000 ×; e) partially formed plasma membrane (arrow) of AC on boundary with sequestered necrotic masses, 36,000 ×.

epithelium of residual ducts; 3) activation of fibroblasts with the formation of a connective-tissue capsule.

The initial stages of restoration of the acinar parenchyma, which followed the lines of intracellular regeneration, could be observed as early as 6 h after the beginning of the experiment in the zone of degenerative and necrotic changes in the acini. Under the light microscope many acini in this zone had the appearance of a mass of nuclei and cytoplasm with indistinguishable cell boundaries (Fig. 1a). Under the electron microscope these nucleo-cytoplasmic masses appeared to be formed by fragments of cytoplasm oriented around the nucleus. As a rule the nuclear pores of these nuclei were wide open, and relatively intact mitochondria and zymogen granules (ZG), distributed among the densely packed cisternae of the rough endoplasmic reticulum (RER), were arranged in direct contact with them (Fig. 1b). The nuclei of the acinar cells were evidently the most resistant to the action of cold. Many of them 3-6 h after ethyl chloride injury were undergoing amitotic division, but without cytotomy, with the formation of bi-, tri-, and tetranuclear complexes.

The cytoplasmic fragments described above were bounded by cell membranes only in certain parts, and elsewhere the role of cell membrane was evidently played temporarily by the somewhat condensed membranes of the RER, surrounding the irreversibly damaged fragments of cytoplasm. By the end of 24 h, a newly formed outer cell membrane, sequestering the necrotic masses, could be seen (Fig. 1c-e).

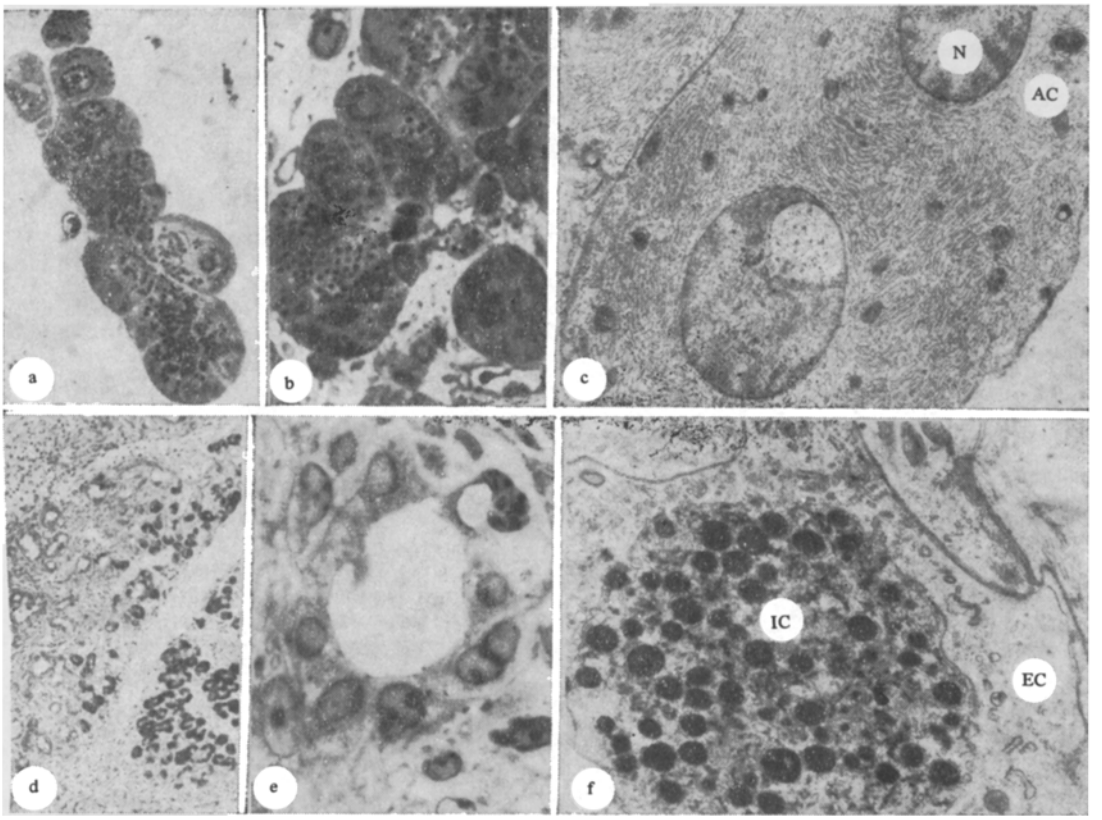

Fig. 2. Morphological changes in pancreatic acinar tissue during regeneration. a) Group of AC forming acinus with centroacinar duct. Semithin section. Methyl blue and azure, 900 ×; b) acini or groups of AC with developing intercalary ducts. Semithin section, 900 ×; c) binuclear AC incorporated into pancreatic acinus, 10,000 ×; d) multiple epithelial bands in boundary zone of pancreas after cooling. Photomicrograph, Romanovsky–Giemsa stain, 120 ×; e) epithelial tubules composed of undifferentiated cells. Semithin section, 900 ×; f) fragment of wall of epithelial tubule with an undifferentiated islet cell (IC) among duct epithelial cells (EC), 20,000 ×.

The subsequent fate of the nucleo-cytoplasmic complexes, having gone through a series of "surgical amputations" and having been shaped into cells, differed, mainly depending on the state of the nucleus. Some of them, formed around genetically deficient nuclei, underwent cytolysis, others exhibited all the features of specific differentiation with resumption of ZG production. Amputated cells, both inside the intact acinar membranes and also outside them, exhibited the property of adhesion with the formation of the renewed acinar complexes, forming a primary centroacinar duct by means of their apical regions (Fig. 2a, c). The fate of these renewed acini was determined by the degree of preservation of previous connections with the duct system or ability to restore connections with ducts proliferating toward them. Only in these cases was some chance of survival and restoration of normal secretory function present (Fig. 2b).

During the 3-7 days after cooling many epithelial bands of undifferentiated cells, in which many typical mitotic figures could be seen, appeared in the boundary zone. Later these bands differentiated in the direction of tubules, and separate islet cells could also be found among them (Fig. 2d-f).

The areas of tubular proliferation described above must be distinguished from the numerous tubules that were derivatives of atrophic acini which had lost their connection with the ducts.

By 6-12 h after cooling, activation of fibroblasts and of capillary endothelium, forming together with polymorphonuclear leukocytes and phagocytes, a granulation barrier, was observed in the boundary zone.

After 7-14 days nearly all the boundary zone was replaced by mature connective tissue, forming a capsule around the focus of necrosis. In the course of collagenization of the capsule nearly all the acinar-duct derivatives reflecting the attempts at complete regeneration of the excretory parenchyma described above, underwent atrophy and scar formation.

The degenerative-necrotic changes thus never terminated in complete recovery of the acinar parenchyma by a process of neogenesis: the dying parenchyma, just as in the myocardium, central nervous system, kidneys, and other organs, was replaced by a scar. The functions of the lost part were compensated by hypertrophy of the residual acinar parenchyma, i.e., by enhancement of the structural and functional potential of each acinar cell by intracellular regeneration of its organoids.

Evidence in support of this view is given by the unusual tenacity of the acinar cells after sequestration of a large part of their cytoplasm, and also the absence of mitoses and of cytotomy after anitosis. The formation of multinuclear cells also promotes the more rapid recovery of their structural potential.

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