Ultrastructural Changes in the Pancreas and Their Role in the Pathogenesis of Myocardial Injury in Endotoxin Shock

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The pancreas in endotoxin shock is characterized by the hypoperfusion, prolonged cycle of acinar cell secretion, and activation of their lysosomal system. The rearrangements in the exocrine part of the gland involve the production of a myocardial depressant factor exerting a negative inotropic effect on the heart. Myocardial dysfunction is aggravated by myofibrillar contractures, myocytolysis, glycogenolysis, and circulatory disorders.

Key Words: *pancreatic capillaries and acinar cells; myocardium; endotoxin shock; ultrastructure*

The development of endotoxin shock (ES) depends on the cardiac contractile [2] and endocrine [6] functions, which are determined by the effects of endotoxin [4]. While considering the mechanisms of myocardial injury in ES, we have analyzed the role of the myocardial depressant factor (MDF) [1], a cardiotoxic peptide released by acinar cells of the pancreas under conditions of the organ hypoperfusion [14].

The relationship between destructive processes in the pancreas and heart is manifested as the pancreatocardial syndrome, which was confirmed by experimental, clinical, and pathological data [7,12]. In order to elucidate the role of the exocrine part of the pancreas in the mechanism of myocardial damage we examined the ultrastructure of the pancreas and myocardium over the course of ES.

MATERIALS AND METHODS

Experiments were carried out on 39 mongrel dogs weighing up to 10 kg, 26 Chinchilla rabbits weighing 3 kg, and 39 male rats weighing 200-250 g. The animals were maintained under standard vivarium conditions with free access to water. Dogs and rabbits were intravenously injected with the *Escherichia coli* endotoxin (5 mg/kg). Rats were injected with the endotoxin into the tail vein (2 mg/100 g). The animals were sacrificed by a lethal dose of Nembutal after 30 min (initial period of ES), 5 h (intermediate period of ES), and 3 days (late endotoxemia). Control animals (6 rabbits, 9 dogs, and 9 rats) were injected with an equal volume of sterile NaCl solution. Since the specific activity of pancreatic acinar cells and capillaries depends on food, secretory phase, etc. [9], all experiments were carried out under conditions of basic (fasting) secretion (the animals were neither fed nor given water the evening before). Fragments of the myocardium from both ventricles and material collected mainly from the body and head of the pancreas were fixed in 2.5% glutaraldehyde on phosphate buffer, then in 1% osmium tetroxide on the same buffer, and after dehydration embedded in Epon. Morphological changes were initially studied using semithin (1-2 μ) sections stained with Toluidine Blue. The sections were cut in an LKB-8800 ultramicrotome, contrasted with uranyl acetate and lead citrate, and examined under a JEM-100S electron microscope.

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RESULTS

Intravenous injection of sterile NaCl (control) induced no light optic or ultrastructural disorders in the myocardium or pancreas; the content of zymogen granules in the acinar cells was moderate, with predominant localization in the apical portion. The microcirculatory bed was intact, which agrees with the findings in rats and cats [9,11].

During the initial period of ES (30 min) the most pronounced changes were detected in pancreatic capillaries. Intravascular disorders manifested themselves in the plethora hemorrhages (stasis, erythrocyte aggregation, and sludging). Endotheliocytes contained numerous micropinocytous vesicles, evenly distribution in the cytoplasm and opening from the side of the luminal surface or toward the basal layer. The endotheliocyte plasma membrane was characterized by increased lability; numerous microvilli were formed, generally they were subjected to clasmatosis (Fig. 1, *a*). Increased vascular permeability resulted in infiltration of the stroma with plasma and migration of individual blood cells, often eosinophils, into the interstitium. Mast cells with slight degranulation were released (Fig. 1, *b*). Unlike the liver, where degranulated mast cells were detected during the initial period of ES [8], in the pancreas no total degranulation was observed in any case during the entire period of observation.

Microcirculatory abnormalities promoted early disorders in the oxidation of the exocrine parenchyma of the pancreas, and hypoxia affected primarily the acinar cells. The most obvious reaction was due to reduced count of zymogen granules (Fig. 1, *c*). The majority of exocrine pancreocytes were devoid of granules during the initial period of ES, though some of them had occasional condensation vacuoles and few immature zymogen granules. In parallel with this, a substance of moderate electron density appeared in the lumina of acini and insertion compartments of central and interacinar ducts.

Early changes in cardiomyocytes were as follows: low glycogen content, intracellular myocytolysis, and contractures involving several sarcomeres. In other cases vacuolized sarcoplasm looked like "Swiss cheese" [17]; insertion disk membranes were dissociated, which may lead to changes in mechanical strength and impair the metabolic cooperation of cardiomyocytes (Fig. 1, *c*). Microcirculatory disorders (rheological shifts and formation of "coin bars" by erythrocytes) and migrating extravasates promoted hypoxia.

During the intermediate period of ES (5 h) the bulk of acinar cells did not contain zymogen granules and was in the secretion phase. Considerable portions of acinar tissue contained no granules. During this period of ES massive secretion was synchronous with the production of numerous secondary autophagal lysosomes (autophagolysosomes, Fig. 2, *a*). Ultrastructurally phagocytized organelles and incorporations were generally seen as electron-dense material (membranes, granules, small corpuscles, and myelin-like figures at different stages of digestion). Microcirculatory shifts at the intravascular, vascular, and stromal levels progressed. Local foci of the endotheliocyte plasma membrane destruction and separation of these cells were observed (Fig. 2, *b*). Labilization of the histohematic barrier augmented vascular permeability. Cellular content of extravasates changed: after 5 h polymorphonuclear leukocytes were often detected in the stroma in comparison with the previous period.

Ultrastructural changes in the pancreas represent the so-called asymptomatic or preclinical period of the disease [10] and develop during the first 0.5-5 h, anticipating of clinical manifestation.

The lesions in the myocardium progressed during the intermediate period of ES. Desquamated endotheliocytes were seen in the capillary lumen, there were sites with separation of endothelial cells, all this creating conditions for extravasation of blood cells. Activation of intracellular proteolysis caused partial lysis of the sarcoplasm and led to irreversible changes in cardiomyocytes. Myocardial contractility was impared due to interstitial edema, myocardial cell hydration, and contractures (Fig. 2, *c*). Myofibril contractures depended on activation of the sympathoadrenal system and subsequent development of hypercatecholaminemia persisting during the first two periods of ES development. Catecholamine granules almost completely disappear from the adrenomedullary cells, leaving optically blank profiles [3].

During late endotoxemia, hemodynamic and rheological shifts were still observed in the pancreas; twisted basal membrane and lumen dilation indicated the development of vasospasm in some arterioles. Necrotic acinar cells have been first detected after 3 days (Fig. 3, *a*). There were adaptive changes due to improvement of microcirculation and, as a result, recovery of normal function of pancreatic acinar system. Acinar cells with two nuclei were more numerous than in the control; there were virtually no secondary lysosomes in the cytoplasm. The phase of secretion, which predominated during the previous periods of ES, was replaced in the majority of cells by production, maturation, and accumulation of zymogen granules. The phenomenon of local loss of ribosomes in the rough endoplasmic reticulum and their transformation into smooth "ring-like" structures looking like cisternae or chains consisting of

small vesicles should be noted (Fig. 3, *b*). Previously, a similar phenomenon was observed after long-term protein-free nutrition [9], but the physiological significance of this transformation after exposure to endotoxin is not clear.

The signs of intracellular regeneration were observed in the myocardium after 3 days, namely, increased size and hyperplasia of the mitochondria and the appearance of glycogen granules (Fig. 3, *c*). The presence of hemodynamic and hemorheological

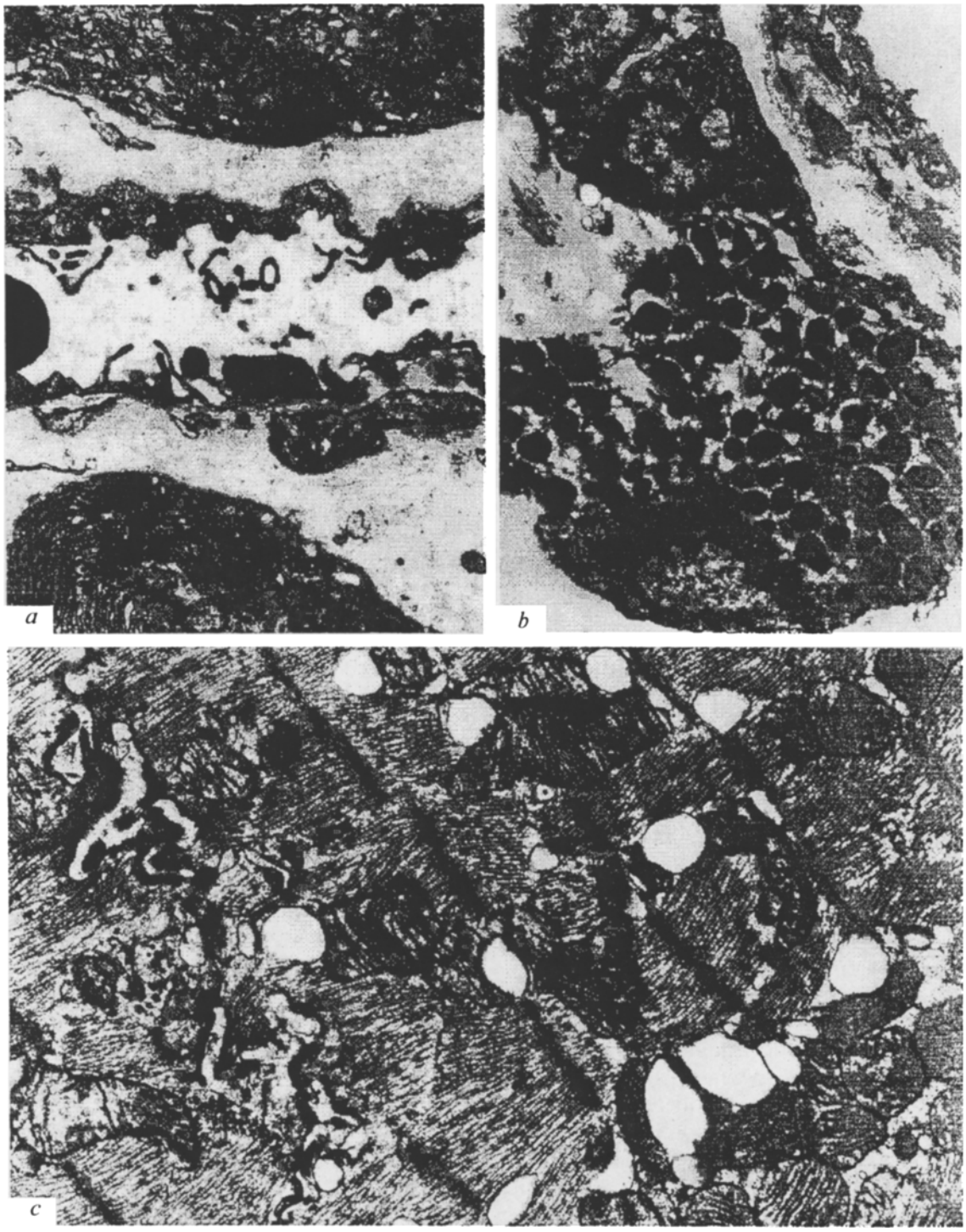

Fig. 1. Ultrastructural changes in the pancreas and myocardium during the early period of endotoxin shock. *a*) numerous micropinocytous vesicles in endothelial cells, clasmatosis of microvilli, ×5600; *b*) mast cell with occasional granules of low electron density, ×4200; *c*) sarcoplasma vacuolation, separation of the insertion disk membranes, ×7000.

disorders in the capillaries of surviving animals is rather an exclusion than a regularity.

In shock of different origin, MDF accumulates in the plasma and reaches the toxic level after 2-7 h [16]. A decrease in arterial pressure to 45 mm Hg decreases the blood flow in the pancreas by 70% after 30 min and by 80-85% after 2 h [15]. In ES circulation decreases by 50% only, which is sufficient for MDF level to start growing in the plasma and for the activity of cathepsine D to increase 12-fold [15].

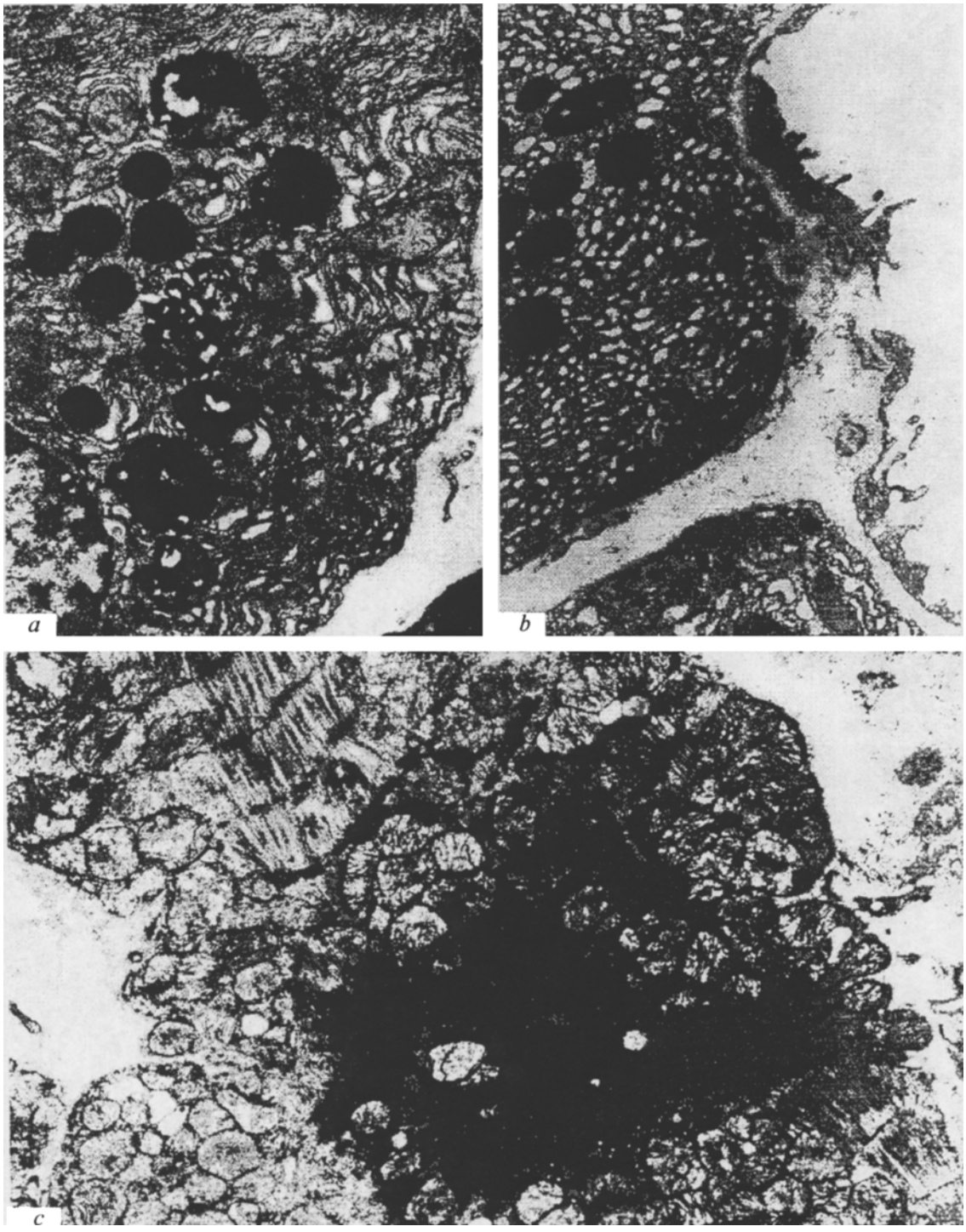

Fig. 2. Ultrastructural changes in the pancreas and myocardium during the intermediate period of endotoxin shock. *a*) formation of numerous secondary lysosomes in the acinar cell cytoplasm, ×5600; *b*) separation of endothelial cells and defect formation in the vascular wall, ×5600; *c*) myofibril contractures and injury to the majority of mitochondria, ×2800.

Hypoperfusion of the gland promotes the development of acidosis and hypoxia and subsequent destabilization of lysosomal membranes and activation of proteolytic enzymes (some cathepsines and peptidases), entering in high amounts the acinar cell cytoplasm. Extralysosomal (zymogenous) proteases

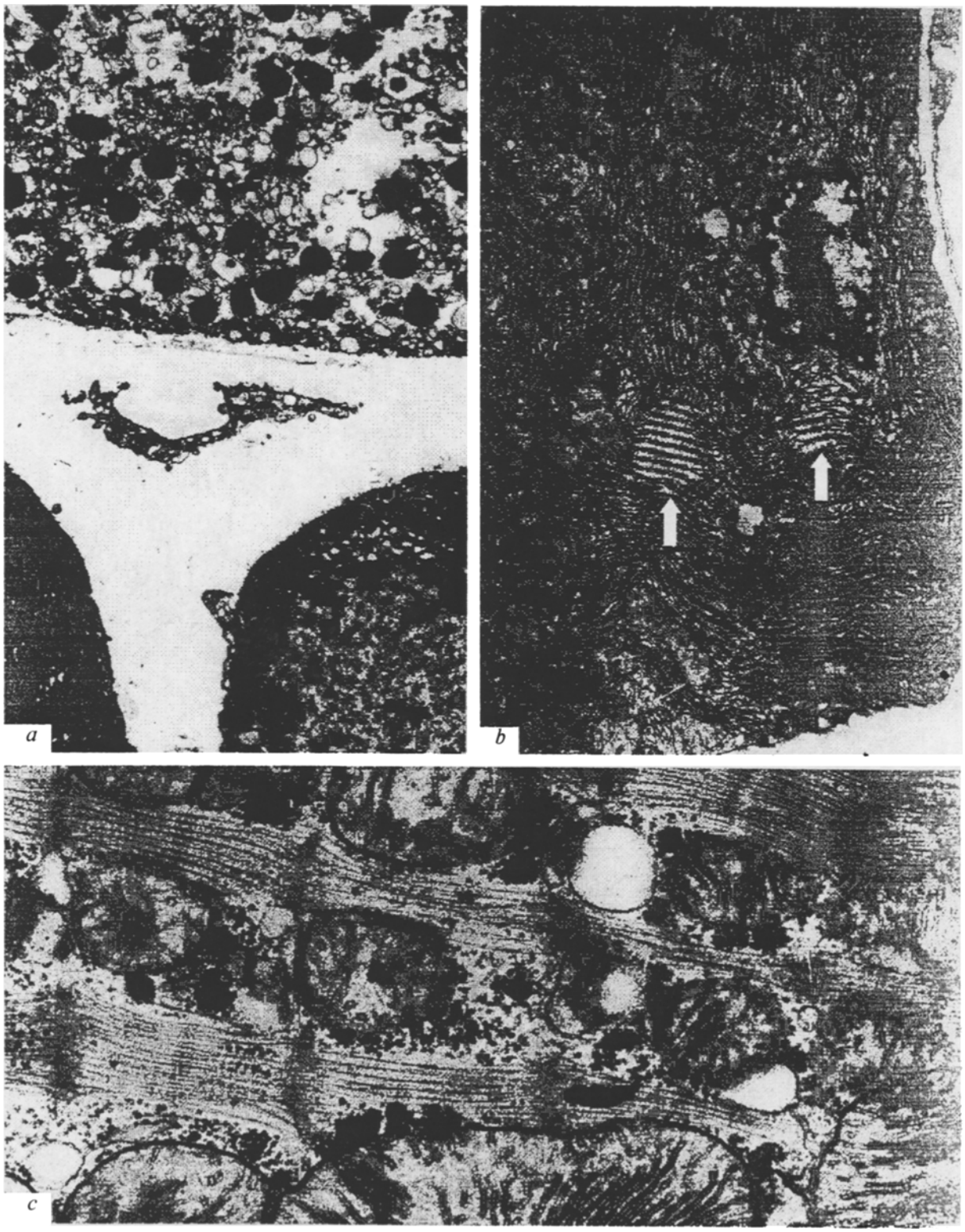

Fig. 3. Ultrastructural changes in the pancreas and myocardium during late endotoxemia. *a*) fragment of necrotic acinar cell (above), fragments of two intact exocrine pancreocytes (below), ×2800; *b*) "ring-like" structures (arrows) in the acinar cell cytoplasm, ×7000; *c*) glycogen granules contacting with external mitochondrial membrane, ×18,000.

also enter the cells [14]. Activation of zymogenous and lysosomal proteases promotes the release of many compartmentalized proteins that are then subjected to proteolysis; as a result, MDF and other peptide fragments are formed [14-16]. Presumably, MDF molecules enter the blood flow as free peptides or bind to large carrier proteins in the extracellular fluid, enter lymph vessels, and then into systemic circulation [16]. Cardiovascular effects of MDF have been observed in several types of shock (including ES) and are due to its negative inotropic effect on cardiomyocytes [13,16].

Thus, microcirculatory and rheological disorders develop during the initial period of ES and progress during the intermediate period in the exocrine portion of the pancreas in experimental endotoxemia. The secretory phase, coinciding with activation of cell lysosomes and formation of secondary lysosomes, predominates in the cytoplasm of acinar cells. Hypoperfusion of the pancreas, occurring during the entire intermediate period of ES and in some cases persisting during the first 3 days is paralleled by the production of MDF and its penetration into vessels. The relevant ultrastructural changes in the acinar cells and blood capillaries confirm such a course of events. Ultrastructural changes in the myocardium and microcirculatory bed together with the cardiodepressant effects of MDF aggravate cardiac dysfunction in ES.

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