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Ultrastructure of Acinar Cell Injuries in Experimental Acute Pancreatitis Created by Common Bile Duct Ligation

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Morphogenesis of acute pancreatitis induced by ligation of the common bile duct and the ultrastructure of autolytic transformations of acinar cells were studied. Autolytic changes in acinar cells started from the basal zones and then involved the apical zones. Violation of the zymogen granules integrity, interactions of their contents with the adjacent ultrastructures, destruction (melting) of ultrastructures, and formation of huge autophagosomes play an important role in the development of autolysis. Disordered secretion of zymogen granules (foci of their accumulation in the apical zone), hyperplasia and hypertrophy of centroacinar cells and ductal epitheliocytes aimed at restoration of the pancreatic secretion discharge pathways were seen in the retained acini.

Key Words: *acute pancreatitis; common bile duct ligatation; acinar cells; ultrastructure*

Impairment of the extrahepatic bile ducts is one of the main etiological factors of acute pancreatitis (AP) development [1,2]. Pancreatitis of biliary etiology is diagnosed in 45% cases [9]. Common anatomy and functions of the pancreatobiliary system are responsible for frequent development of AP in cholelithiasis, choledocholithiasis, stenosis, spasm, edema, or inflammation of the major duodenal papilla and choledochal dyskinesias of different etiology. Experimental and clinical findings suggest that the duodenal contents reflux into the pancreatic duct is one of the most important early mechanisms of AP pathogenesis.

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An important part in studies of the mechanisms of damage to the pancreatic exocrine cell populations is played by morphological analysis essential for evaluation of the sequence and severity of the structural and functional changes [3,4]. Ultrastructural modifications of the acinar cells in the development of acute and chronic pancreatitis remain least studied, which precludes comparison of the patterns and sequence of structural changes and biomolecular findings characterizing changes in activities of enzymes and other bioactive substances involved in AP pathogenesis.

We studied the ultrastructure of acinar cell damage in AP induced by the common bile duct ligation.

MATERIALS AND METHODS

Acute pancreatitis was simulated in Wistar rats (n=30) by ligation of the common bile duct (reproducing duc-

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tal hypertension). The animals were narcotized and laparotomy was carried out under sterile conditions. The duodenum was brought into the operation wound and the choledochus was ligated with a silk thread at the site of its flow in the duodenum, after which 3-h observation was carried out at the wound open. The wound was then tightly sutured and the animal was placed into the cage under common conditions. Material for light and electron microscopic studies was collected 24 h after the intervention. The animals were sacrificed by ether overdosage.

Specimens of the pancreatic head and tail were fixed in 10% neutral formalin, dehydrated in ascending alcohols, and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin and Perls reaction was carried out. Tissue specimens for semithin and ultrathin sections were fixed in 4% paraformaldehyde, postfixed in osmium tetroxide, and embedded in epon and araldite mixture. Semithin sections were stained with azur II. Paraffin and semithin sections were examined under a Leica DM 4000B universal microscope. Microphotographs were made using Leica DFC 320 digital photocamera and Leica Qwin V3.0 software. Ultrathin sections were sliced on an ULTRACUT EM UC7 ultratome (Leica), examined in a JEM-1400 electron microscope (Jeol) at accelerating voltage of 80 kV, and photographed with a Veleta digital cam using iTEM software (Olympus).

RESULTS

Pronounced edema of various compartments of the pancreas was seen 24 h after common bile duct ligation. As a result, interlobular interstitial spaces and the subcapsular zone were dilated. Fibrin depositions were found in dilated connective tissue interlayers. Plasmorrhages, hemorrhages, inflammatory cellular infiltration were seen. The arteries and veins were unevenly plethoric, some small vessels contained the plasma. An important feature of pathomorphological changes in the pancreas during this period was mosaic pattern of the acinar structures damage: foci of acinus necrosis and discomplexation alternated with zones with normal structure. The architectonics of the majority of acini was retained in the pancreatic head, the necrotic foci were small. Small and large foci of acinar cell necrosis were seen in the pancreatic tail in many animals; abscesses were forming in some places.

Acinar cells in little changed acini often contained numerous (more than in the control) zymogen granules (Fig. 1, *a*). This fact could reflect disorders in their secretion to the ductal system because of mechanical obstruction of the duodenal duct. In semithin sections, secretory granules often occupied a great volume of the cells, not just their apical part. In some cases, secretory granules were released into the insertion duct. Flat centroacinar cells in the insertion compartments of the exocrine glands were often seen in the lumens of the pancreatic terminal compartments and between acinar cells. A special feature of this period of the study was moderate hyperplasia of the centroacinar cells and excretory duct epitheliocytes, which formed elongated structures, probably additional insertion and interacinar ducts (Fig. 1, *b*).

Some acini were characterized by pronounced structural and functional heterogeneity of acinar cells: they contained "dark" and "light" cells (Fig. 1, *c*). Cells of both types could contain numerous zymogen granules or be completely devoid of them. Light cells included also cells with "lamellar" structure containing solitary secretion granules (Fig. 1, *c*), while dark cells often had lipid incorporation in the basal zone. Small more compact cells without granules were seen among acinar cells with secretory granules; these smaller cells could be referred to cambial elements; binuclear acinar cells were often found. The appearance of these cells indicated that the regeneratory reactions in the pancreatic exocrine pool were retained. Studies of semithin sections showed that necrobiotic changes in the acinar cells in this AP model always started from the basal zone, in which optically clear foci of partial necroses appeared, as a result of which the cells looked "corroded" (Fig. 1, *d*). Pancreatic stellate cells in with lipid incorporation (Fig. 1, *b*, *c*) were often seen in the connective tissue interlayers between the acini.

According to light microscopy, Langerhans' islets underwent less severe changes after the common bile duct ligation than the pancreatic exocrine compartments. Insulocytes forming the islets were smaller than acinar cells, with predominantly homogeneous cytoplasm; granularity was seen mainly in the insulocytes located at the islet periphery. The capillaries in the islets were as a rule dilated and plethoric.

Other authors found similar pathomorphological changes in the pancreas after the common bile duct ligation (ductal hypertension simulation) in small rodents [10,12,13]. The pathomorphological changes reflected the development of the so-called mixed fatty pancreonecrosis [5], characterized by the formation of foci of coagulation necrosis of the acinar cells and demarcation (perifocal) inflammatory cellular infiltration with plethoric vessels. It was assumed that the lipolytic enzymes (lipase and phospholipase A), promoting the destruction of acinar cell membranes [2], play the key role in the pathogenesis of fatty pancreonecrosis (and its mixed forms).

Appearance of sites of suppurative melting of acinar structures as early as 24 h after common bile duct ligation indicated not only activation of proteolytic reactions, but also secondary infection. Parapancre-

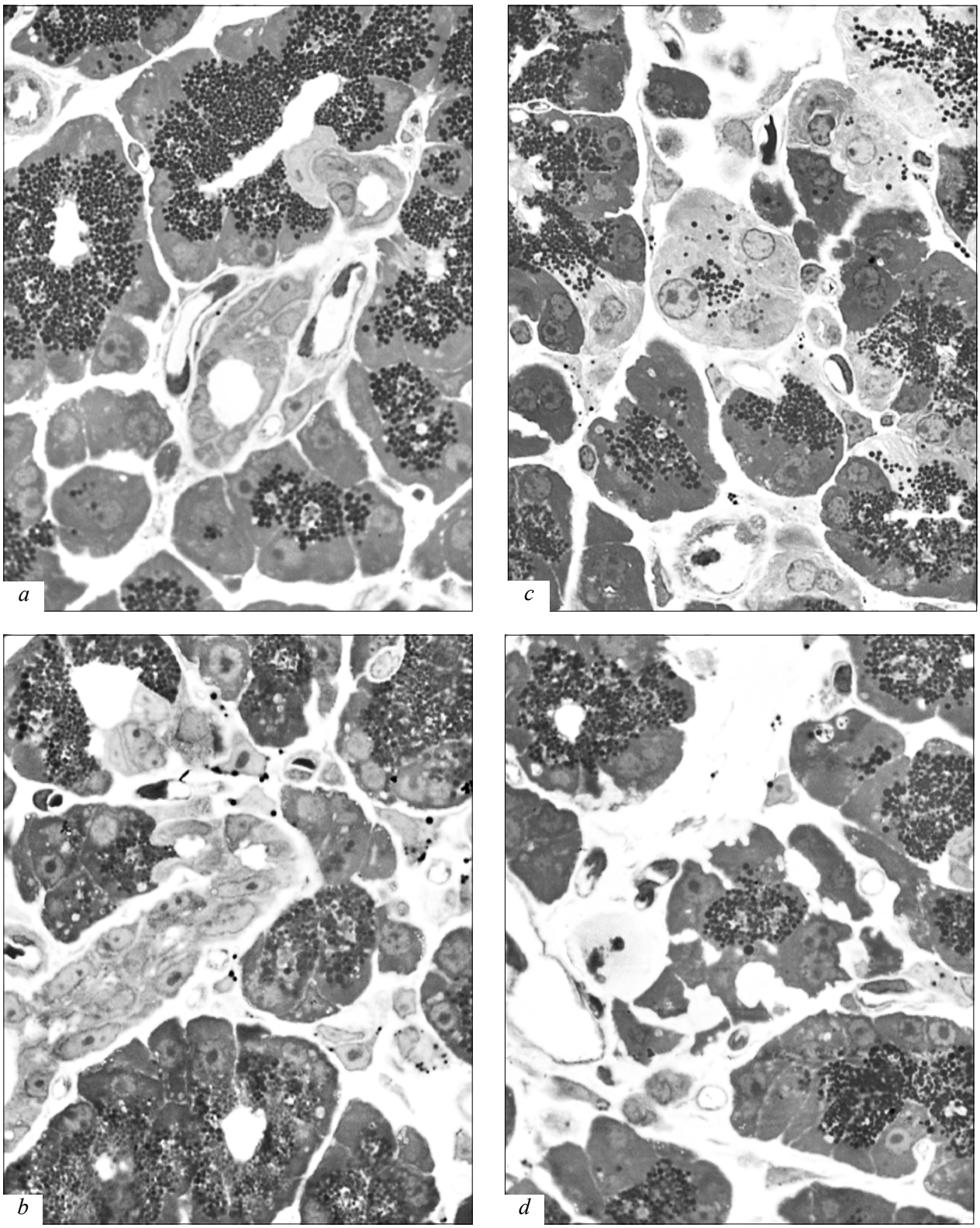

Fig. 1. Morphological changes in the exocrine part of the pancreas in Wistar rats 24 h after the common bile duct ligation. Semithin sections. Azur II staining, ×1000. *a*) significant accumulation of zymogen granules in the acinar cell apical zones; *b*) centroacinar cell hyperplasia and formation of insertion ducts, increase in the count of stellate cells in the periacinar interstitium; *c*) pronounced heterogeneity of acinar cells, appearance of "lamellar" cells; *d*) dissemination of necrobiotic changes in acinar cells from the basal zones.

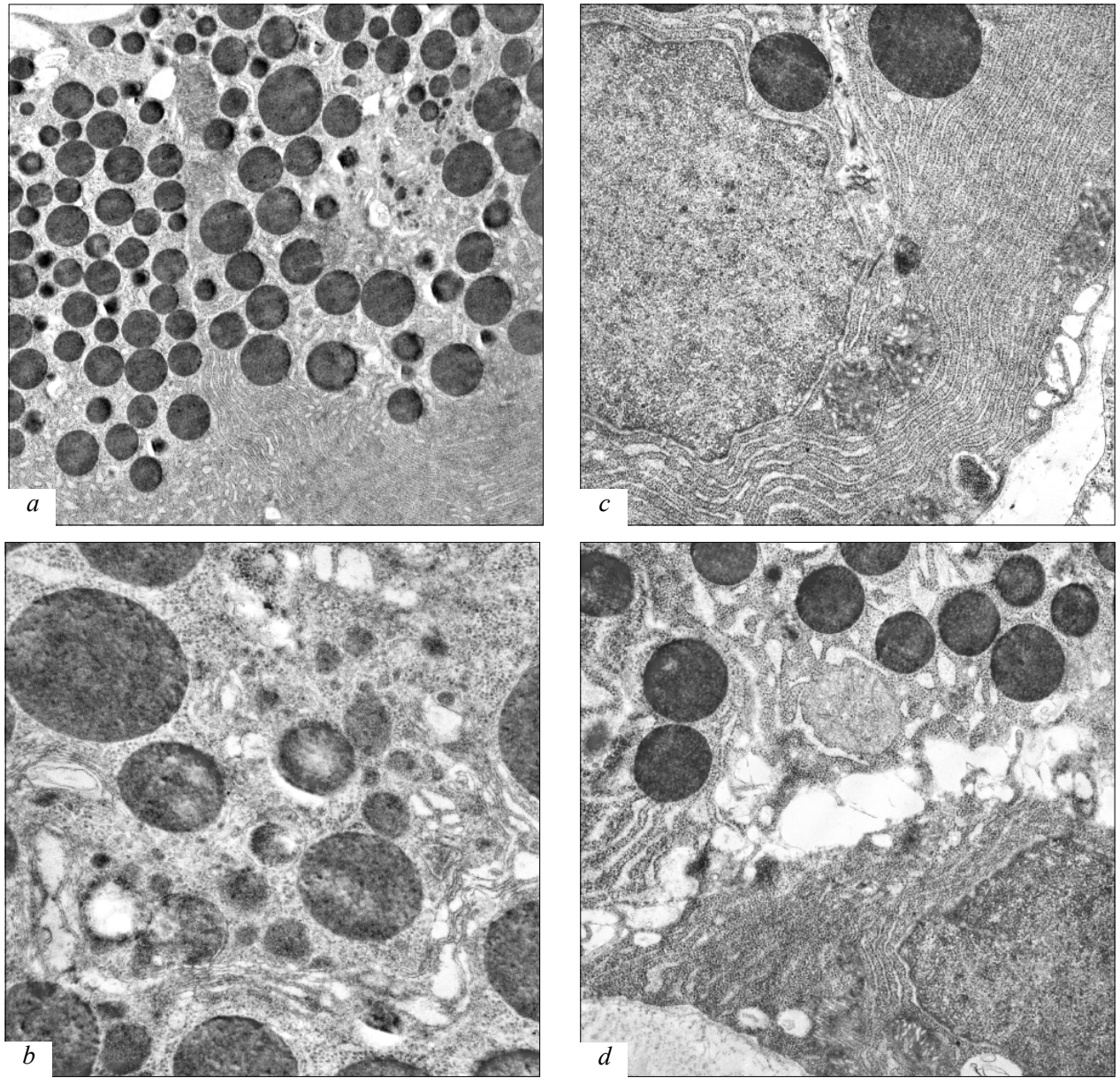

Fig. 2. Ultrastructure of acinar cells in Wistar rats 24 h after the common bile duct ligation. *a*) numerous zymogen granules in apical zones, ×12,000; *b*) formation of zymogen granules near hypertrophic Golgi complex, ×40,000; *c*) solitary mitochondria, zymogen granules, and osmiophilic residual bodies in acinar cell basal layer, ×25,000; *d*) cambial (progenitor) cell in the acinus, significant dilatation of cell-to-cell space, ×25,000.

atic tissues and organs were involved in the process. Pancreatogenic intoxication, developing as a result of release of pancreatic enzymes, proinflammatory cytokines, and autolysis products into the blood and lymph, caused the development of cardiac, pulmonary, hepatic, and renal failure and even comatose states, which could lead to a lethal outcome in AP [2,5].

Ultrastructural analysis showed compact packing of cisterns of the granular cytoplasmic reticulum (sometimes unevenly dilated) in the acinar cells with numerous zymogen granules (Fig. 2, *a*). Hypertrophy and hyperplasia of structural elements of Golgi complex were always seen in these cells; Golgi complex was surrounded by forming zymogen granules of different size (Fig. 2, *b*) and was always located in the apical zone. The basal zones of acinar cells had no or just solitary zymogen granules. The basal zone contained cell nuclei, compactly packed cisterns of the granular cytoplasmic reticulum, solitary mitochondria always surrounded by cytoplasmic reticulum profiles (Fig. 2, *c*). Floccular substance was always present in the granular cytoplasmic reticulum profiles. The plasmalemma of acinar cell at the basal pole somewhere formed long processes contacting with each other and

forming cellular structures. Similar structures were seen on the lateral surfaces. Some acini had low electron dense cells with just few mitochondria in the cytoplasm, compactly packed cisterns of the granular cytoplasmic reticulum, and solitary lipid incorporations (Fig. 2, *d*). We referred these cells to cambial (progenitor) ones.

Structural and functional heterogeneity of acinar cells revealed by light microscopy was mainly caused by pronounced changes in the architectonics of the granular cytoplasmic reticulum (Fig. 3, *a*). The granular cytoplasmic reticulum cisterns in some cells were significantly unevenly dilated; at the light microscopy level, these cells looked "lamellar". As a rule, these cells contained few zymogen granules. Destructive changes in the mitochondria (mainly lythic changes in the matrix and crist destruction) were seen in the basal zones of acinar cells with unevenly dilated cisterns of the granular cytoplasmic reticulum (Fig. 3, *b*). Osmiophilic polymorphic residual bodies were often found in the basal zone near destroyed mitochondria; these bodies were almost exhausted zymogen granules

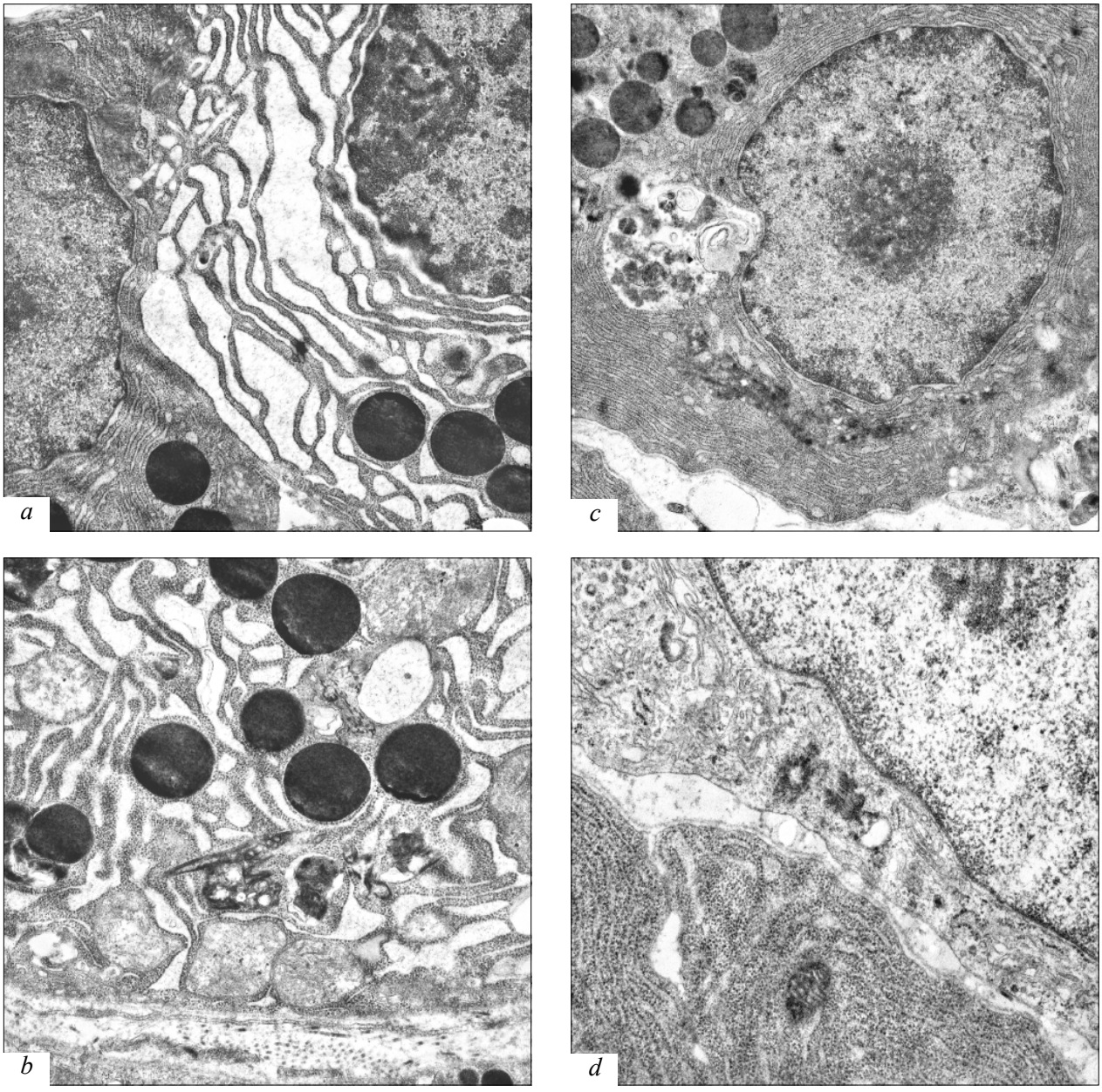

Fig. 3. Ultrastructure of acinar cells in Wistar rats 24 h after the common bile duct ligation. *a*) pronounced dilatation of the granular cytoplasmic reticulum cisterns, ×20,000; *b*) destructive changes in the mitochondria, formation of osmiophilic residual bodies, and uneven dilatation of the granular cytoplasmic reticulum cisterns in the basal zone, ×25,000; *c*) formation of giant autophagosomes, their fusion with zymogen granules, partial autolysis of the basal zone, ×15,000; *d*) centrioles in a centroacinar cell, ×30,000.

which sometimes fused to form elongated foci of melting ultrastructures.

These ultrastructural changes presumably represented processes of untimely activation of the pancreatic enzymes, contained in the zymogen granules. These enzymes promoted destruction of membranes in the granules, cytoplasmic reticulum, and mitochondria with their subsequent destruction and autolysis. Autolytic processes in the acinar cells induced by common bile duct ligation initially appeared in the basal zones and then progressed to the apical zones. These processes included the formation of extensive zones of the cytoplasmic reticulum and mitochondrial destruction (long osmiophilic conglomerations) in the subnuclear zone and the formation of large autophagic vacuoles gradually fusing with zymogen granules in the supranuclear zone (Fig. 3, *c*).

Autolytic processes did not develop in all acini simultaneously. However, if light microscopy showed significant necrobiotic changes in the basal zones of some acinar cells, electron microscopy showed more or less pronounced ultrastructural manifestations of autodigestion and autophagia in all cells of this acinus. These autolytic and autophagic changes involved predominantly the acinar cells.

The centroacinar cells and ductal epitheliocytes underwent necrosis only in case of total destruction of the pancreatic acini. In the AP model used in our study we observed proliferation of centroacinar cells in intact acini. Centroacinar cells contained large nuclei; their cytoplasm contained numerous profiles of granular cytoplasmic reticulum, well-developed Golgi complex, small mitochondria, and often centrioles (Fig. 3, *d*). The plasmalemma facing the ductal lumen formed short microvilli. Hyperplasia and hypertrophy of the centroacinar cells after common bile duct ligation also noted by other authors [8], could be regarded as a compensatory adaptive reaction aimed at restoration of discharge of the secreted substances.

Initiation of early activation of the pancreatic enzymes and hence, the acinar cell autolysis/necrosis, could be caused by not only lipolytic enzymes. Molecular biological studies showed that acinar cell necrosis was caused by significant accumulation of Ca^{2+} in their cytosol [7,15]. Accumulation of Ca^{2+} in the cytosol and the presence of zymogen granules with cathepsin B in large cytoplasmic vacuoles of acinar cells promoted early stimulation of trypsin and other proteolytic enzymes and their release into the interstitium. This was paralleled by more intense synthesis of the proinflammatory cytokines (for example, TNF-α and IL-1β), stimulating macrophages and promoting attraction of leukocytes to the foci of acinar cell necrosis [6,11,14]. Early activation of proteolytic enzymes results in autolysis (autodigestion) of the acinar cells, realized to this or that measure in all AP forms.

Hence, common bile duct ligation initiated within 24 h the formation of foci of coagulation necrosis of acinar cells with the development of demarcation (perifocal) inflammatory cell infiltration. Autolytic changes in the acinar cells started from the basal zones and then involved the apical zones. The development of autolysis was associated with violation of zymogen granules integrity, interactions of their content with adjacent ultrastructures, their melting, and formation of huge autophagosomes. Disordered secretion of zymogen granules, centroacinar cells, and ductal epitheliocyte hyperplasia and hypertrophy aimed at restoration of the pancreatic secretion discharge unfolded in the retained acini.

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