# Ultrastructural Analysis of Liver Biopsy Specimens in Diabetes Mellitus Associated with Chronic Opisthorchiasis

**G. I. Nepomnyashchikh, O. A. Pavlenko, S. V. Aidagulova, and D. L. Nepomnyashchikh**

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Degenerative and compensatory adaptive changes in hepatocytes and other cell populations were detected in liver biopsy specimens from patients with diabetes mellitus concomitant with chronic opisthorchis invasion. The degenerative changes included alteration of hepatocyte nuclei, infiltration of the nucleoplasm with glycogen, and formation of cricoid nuclei. The cholestatic syndrome manifested in increased intracellular cholestasis. Perihepatocellular fibrosis characteristic of combined disease leads to "autonomization" of hepatocytes, "activation" of all poles of the plasmalemma, and formation of numerous microvilli not only at the biliary and sinusoidal poles, but also on lateral surfaces. These changes are directed at improvement of hepatocyte functional activity and intensification of metabolic processes.

**Key Words:** *diabetes mellitus; chronic opisthorchiasis; liver biopsy; electron microscopy*

Diabetes mellitus (DM) ranks third among the immediate causes of death after cardiovascular diseases and cancer [3,4]. Gastrointestinal diseases are common visceral manifestations of DM, in particular, diseases of the liver, which plays an important role in the regulation of glycemia and insulinemia [1]. Involvement of the hepatobiliary system leads to fatty hepatosis and gallbladder atonia [10]. However, pathogenesis of liver involvement in DM remains unclear.

The main manifestations of DM as a systemic disease are hyperglycemia and vascular disorders induced by high blood glucose [12,16] and leading to severe ischemia of various organs. Insulin-dependent DM is associated with structural and functional changes in the liver, in particular, hepatomegaly caused by hypertrophy, hyperplasia, and apoptosis imbalance [15].

The problems of mixed pathology "diabetes mellitus-chronic opisthorchiasis" are little studied in the Ob'—Irtysh basin, a highly endemic region for opisthorchiasis. This makes antidiabetic therapy difficult and therefore studies in this field are of primary importance.

We investigated the ultrastructure of the liver in patients with DM associated with chronic opisthorchiasis (CO).

## MATERIALS AND METHODS

We examined 77 patients (age 17-52) with types I and II DM of moderate severity. The history of DM varied from several months to 15 years, mainly less than 10 years. Patients with type I DM received insulin therapy, patients with type II DM received sugar-lowering sulfonylurea drugs; 75% patients presented with late complications: diabetic retinopathy, diabetic nephropathy, arterial hypertension, and sensorimotor neuropathy.

Chronic opisthorchiasis was verified by duodenomicroscopy, coproovoscopy (repeated 3 times), and serological tests. The intensity of opisthorchis invasion was evaluated using a chemical sedimentation

Institute of Regional Pathology and Pathomorphology, Siberian Division, Russian Academy of Medical Sciences, Novosibirsk; Siberian State Medical University, Ministry of Health of the Russian Federation, Tomsk. ***Address for correspondence:*** pathol@cyber.ma.nsc.ru. Nepomnyashchikh G. I.

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method; it was low in all cases. The duration of parasitic invasion was more than 10 years in 88% examinees; the majority of patients received antihelminth therapy with biltricide (Bayer AG) or poputril (dry extract of aspen bark).

Needle biopsy of the liver was carried out in diabetics with concomitant CO with hepatomegalia. Disposable kits for transcutaneous liver biopsy (Braun) were used. The samples were divided into 2 parts. Specimens for light microscopy were fixed in 10% neutral formalin. Paraffin sections were stained with hematoxylin-eosin in combination with Perls reaction, by Van Gieson method with poststaining of elastic fibers by resorcin-fichsin and Shiff reagent.

Specimens for electron microscopy were fixed in cold (4°C) 4% paraformaldehyde, postfixed in 1% $OsO_4$ were then treated as described previously [6]. Semithin sections were stained with Azur II and Schiff reagent. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope.

## RESULTS

Light microscopy of liver biopsy specimens showed changes in all compartments of the liver, however, general structure of the organ was preserved. Hepatocytes were characterized by moderate polymorphism due to irregular manifestations of cell involution degeneration (partial or total devastation of the cytoplasmatic matrix) [9], intracellular cholestasis syndrome, and lipid infiltration. Lipid infiltration foci were located priparily pericentrally: lipid inclusions (from small- to large-vesicular) sometimes occu-pied the whole cytoplasm. The most pronounced fatty degeneration was observed in obese pati-ents. These metabolic disorders reflected the interrelated changes in the protein-lipid-carbohydrate metabolism [13].

Portal tracts were unevenly dilated mainly due to sclerosis; portal fibrosis was paralleled by pericentral and perihepatocellular fibrosis accompanied by Ito cell hyperplasia and focal capillarization of the sinusoids. Cell infiltration of portal tracts was weakly expressed and these infiltrates consisted of fibroblasts, macrophages, eosinophils, and individual lymphocytes; sinusoids contained numerous eosinophils.

Electron microscopy showed that most hepatocytes were characterized by a high content of cytoplasmic glycogen presented by small granules and rosettes (α-glycogen). Glycogen was diffusely spread in the cytoplasm, primarily perinuclearly and between membrane organelles; sometimes glycogen formed large fields, which later were focally sequestrated.

It should be noted that glycogen inclusions in some hepatocytes were located in the nuclei as centronuclear accumulations, which were seen under light microscope as ring-shaped nuclei (Fig. 1, *a*). In these nuclei, finely dispersed heterochromatin was evenly distributed along the nucleolemma, with a nucleolus often seen against this background (Fig. 1, *b*); large nucleoli make the nuclei look like a ring. The major components of the nuclei (heterochromatin, nucleolus, nucleolemma) remained apparently intact, in contrast to the ring-shaped nuclei with different ultrastructure, characteristic for the hepatitis B virus infection (a ring-shaped composition of rough osmiophilic heterochromatin lumps with nucleolemma destruction) [8, 14]. Nuclei without ring-like transformation were round, with even contour, contained decondensed chromatin and one nucleolus. Some nuclei were characterized by segregation of the granular and fibrillar components, collapse, and occasionally were ring-shaped.

As a rule, carbohydrate infiltration of hepatocyte nuclei was not associated with essential changes in the cytoplasm; there was no clear-cut correlation between glycogen content in the caryoplasma and cytoplasm. The majority of parenchymatous cells had polymorphous elongated, club-shaped mitochondria, located perinuclearly and diffusely near solitary membranes of the granular cytoplasmatic reticulum; these mitochondria had moderately dense homogenous matrix and small, thin, chaotically oriented cristae. In some hepatocytes the mitochondria were grouped at the sinusoidal pole near long cytolemma protrusions.

The protein-synthesizing compartment of the majority of hepatocytes was reduced and represented by only cisterns or a group of short profiles of the granular cytoplasmatic reticulum without predominant location; this was paralleled by hyperplasia of the smooth cytoplasmatic reticulum (Fig. 2, *a*), with elements of the Golgi complex seen in some cells. Large lipid incorporations were characterized by different osmiophilia; lipid infiltration of the cytoplasm was more intense and polymorphic in obese patients. In addition, solitary small and medium-sized lipid incorporations were seen in hepatocyte nuclei, probably as a result of the nucleolemma invagination. Hyperplasia of Ito cells, whose cytoplasm was filled with large lipid vacuoles, was seen in these patients and in those with DM of long duration.

In diabetics with CO, the parenchymatous cells of the liver acquired characteristics indicating involvement of the biliary tract and development of intracellular cholestasis. Many hepatocytes contained bile components (granules with osmiophilic flocculate or dense contents) obviously moving towards dilated bile capillaries (Fig. 2, *b*); heterogeneous lipofuschine granules were seen.

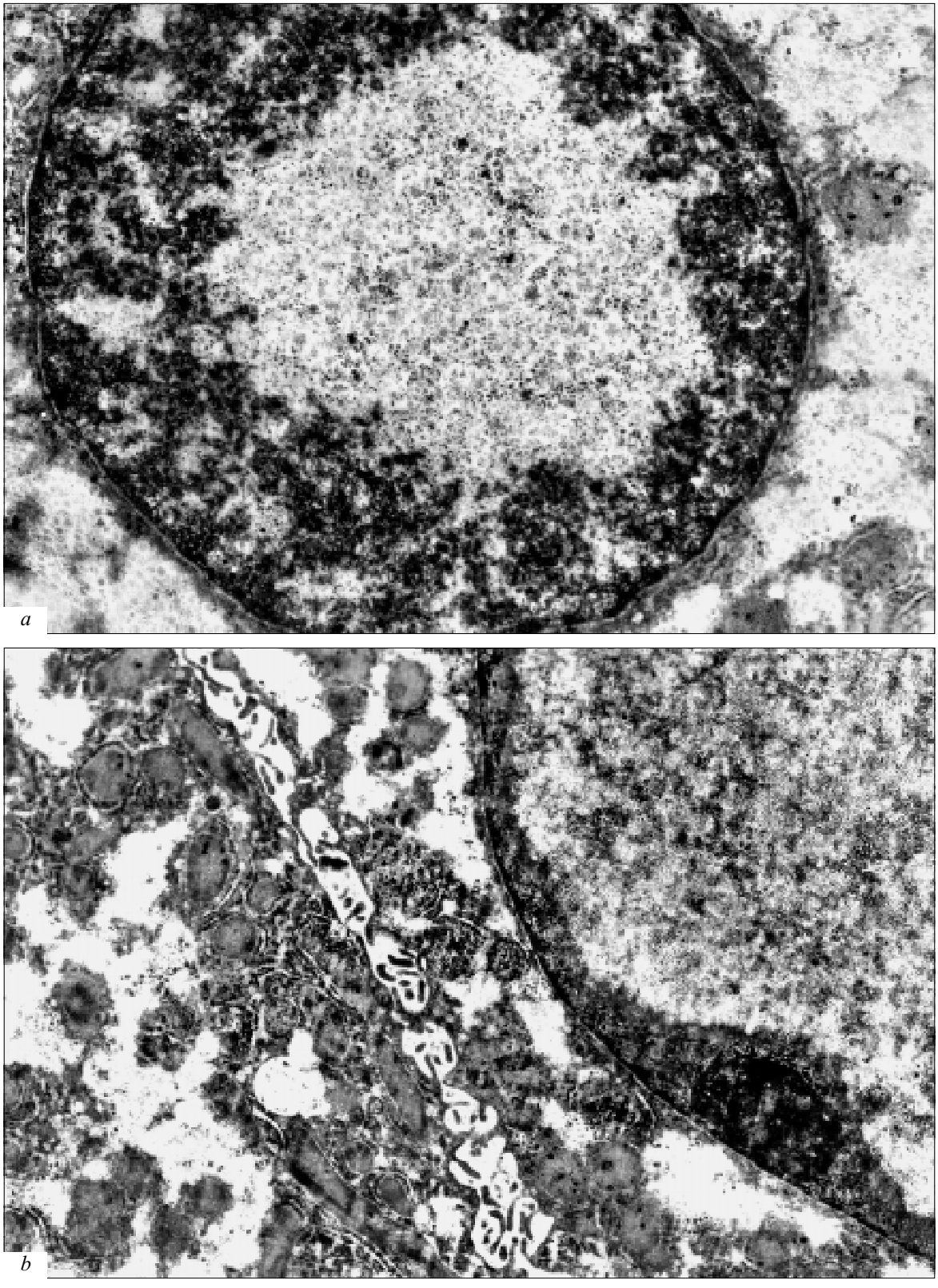


**Fig. 1.** Ultrastructure of ring-shaped nuclei in hepatocytes in patients with diabetes mellitus and chronic opisthorchiasis. *a*) centronuclear grains and rosettes of glycogen, diffuse accumulations of fine heterochromatin, ×15,000; *b*) marginal location of the nucleolus, high content of glycogen in the nucleoplasma, intact nucleolemma, ×12,000.

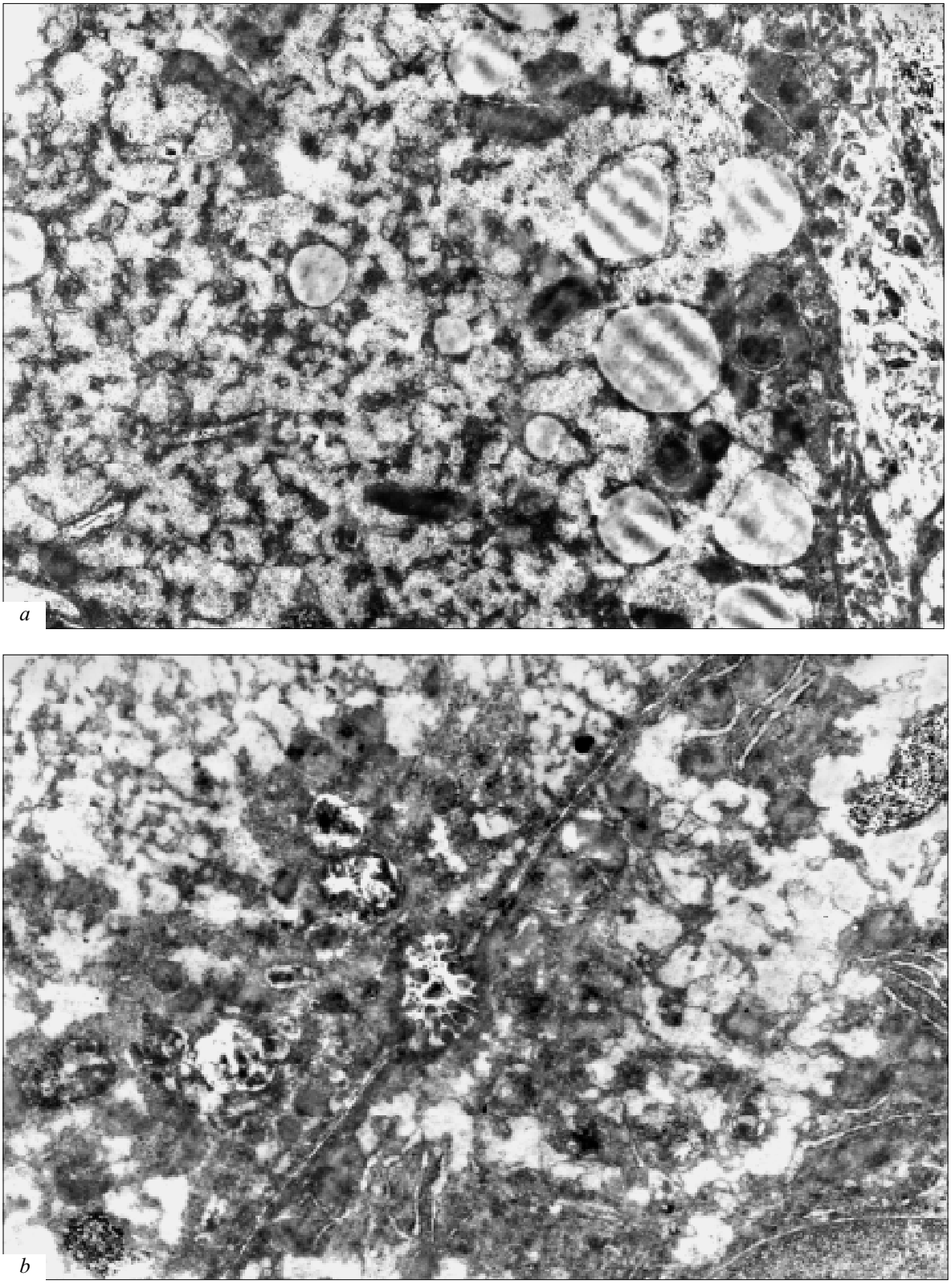


**Fig. 2.** Ultrastructure of hepatocyte cytoplasm in patients with diabetes mellitus and chronic opisthorchiasis. *a*) numerous glycogen inclusions, hyperplasia of the smooth cytoplasmatic reticulum, polymorphous lipid infiltration, ×12,000; *b*) biliary poles of two hepatocytes with biliary capillary between them and concentration of heterogenous components of the bile in this zone, ×15,000.

Notable collagenization of Disse spaces and interhepatocellular spaces with appearance of microvilli on the lateral surfaces of hepatocytes was observed in patients with longer history of combined disease; this indicated more intense metabolic processes, specifically, increased excretion through the lateral plasmalemma. An important phenomenon of ultrastructural reorganization of liver parenchymatous cells typical of combined disease was a more complex relief of the sinusoidal poledue to cytolemma hyperplasia and polymorphism of the microvilli, where numerous mitochondria concentrated. This also can be regarded as a compensatory cell reaction aimed at intensification of the transsinusoidal exchange. Analysis of serial electronograms showed that this phenomenon reflects collagen resorption, which was demonstrated for experimental cirrhosis of the liver [5].

Ductal epithelium of the portal tracts was characterized by marked heterogeneity due to nuclear polymorphism, high variability of cytoplasm ultrastructure and electron density, which was explained by destruction and proliferation of epitheliocytes. Proliferating and actively functioning epitheliocytes were presented by large cubic cells with numerous free ribosomes and polysomes, cytoplasmatic reticular tubules and mitochondria, well-developed Golgi structures, and numerous vesicles. Degenerative cells were flattened, had electron-dense cytoplasmic matrix, mitochondria with signs of destruction, and often dilated cell-cell spaces. The basal membrane of the ductal epithelium was thickened in all cases, something splitted, with numerous incorporations of collagen fibrils.

Endothelial cells of sinusoids and portal veins were also heterogeneous and characterized by high functional activity. However some endotheliocytes had flattened cytoplasm with solitary pinocytotic vesicles, smoothened luminal and basal surfaces, and reduced fenestra. Signs of basal membrane formation (thin layer of amorphous substance along the basal surface of endotheliocytes) were sometimes seen in Disse spaces. Kupffer cells were numerous and polymorphic.

Hence, in patients with DM and CO, electron microscopy of liver biopsy specimens showed both degenerative and compensatory adaptive changes in hepatocytes and other cell populations. The degenerative changes included alteration of hepatocyte nuclei caused by disturbed carbohydrate metabolism and extensive glycogen infiltration of the nucleoplasm, which led to the formation of ring-shaped nuclei specific for DM. The cholestatic syndrome caused primarily by chronic opisthorchis invasion manifested by intracellular cholestasis of different severity.

Degenerative changes in hepatocytes were paralleled by changes in all cell populations of the liver: alteration of sinusoidal and ductal epitheliocytes, activation of Kupffer and Ito cells, and activation of collagen production. Pronounced perihepatocellular fibrosis led to "autonomization" of hepatocytes and "activation" of all poles of the plasmalemma, which formed numerous microvilli at the biliary and sinusoidal poles and on lateral surfaces. This modification of the embryonal phenotype was aimed at improvement of the hepatocyte functional activity and intensification of metabolic processes [2,7].

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