Alteration and Intracellular Regeneration of Hepatocytes under the Effect of Hepatitis C Virus

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In chronic virus hepatitis C, two types of structural changes dominate in hepatocytes: multivesicular lipid infiltration and cell involution degeneration characterized by reduction of all cytoplasmic organelles and light-optic phenomenon of cytoplasma depletion with preserved structure of the nuclear compartment. These changes can be considered as adaptive cell response to cytopathic influence of hepatitis C virus.

Key Words: *hepatitis C virus; hepatocytes; degeneration; regeneration; pathomorphology*

Hepatitis C virus (HCV) genome contains single-strand RNA encoding structural and nonstructural viral proteins. HCV infection, one of the most common cause of morbidity and mortality in the world [8], induces a wide spectrum of liver disorders from silent carrier state to fatal hepatic insufficiency. HCV infection is characterized by a higher rate of transformation to chronic disease in comparison with other hepatotropic viruses. HCV infection is also associated with the development of hepatocellular carcinoma [13].

The role of viral cytopathic effects remains poorly understood, since experimental models, in particular transgene expression of HCV structural proteins, revealed no histological changes related to the expression of these proteins in animals [12].

The aim of the present study was to analyze cell and intracellular reactions of the liver in chronic hepatitis C verified by clinical, biochemical, and immunoserological tests.

MATERIALS AND METHODS

Autopsy materials (46 samples) from 37 patients with chronic hepatitis C obtained through transcutaneous biopsy were analyzed. Each specimen was divided into 2 parts: the larger part was used for preparing paraffin sections, while the lesser samples were used for preparing semithin and ultrathin sections.

For light microscopy, the specimens were fixed in 10% neutral formalin. Paraffin sections were stained with hematoxylin and eosin followed by Perls' reaction, according to van Gieson method combined with visualization of elastic fibers with Weigert resorcin-fuchsin, and PAS reaction was carried out.

Electron microscopy specimens were fixed in 4% paraformaldehyde and processed routinely [2]. 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.

Stereological analysis of liver specimens was based on counting the test system points within the test structure [7,11]. Tissue structure was assessed on semithin sections. Structural density of different parenchymal and stromal components (hepatocytes, endotheliocytes, sinusoids, stroma, and cell infiltrates) was measured using an ocular test system consisting of short lines (n=36, P=72, L=650 μ) and derivative stereological parameters (stroma to parenchyma and vessels to parenchyma ratios) were calculated. The differences were significant at $p<0.05$ [1].

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RESULTS

Light microscopy of liver samples from patients with hepatitis C revealed structural alterations in both the stromal and parenchymal compartments.

In the parenchymal compartment degenerative changes were most abundant. Hepatocytes often exhibited signs of lipid degeneration of varying degree: from multivesicular lipid inclusions seen only on semithin sections (Fig. 1, *a*) to huge lipid drops occupying the cytoplasm and inducing deformation and dislocation of the nucleus (Fig. 1, *b*). Multivesicular lipid droplets diffusely distributed in the cytoplasm or concentrated near the plasmalemma sometimes in the form of pearl necklace were often findings. These multivesicular inclusions probably reflect emergency reparative reaction of the cell in response to cytopathic action of HCV. The second most abundant pathological phenomenon in parenchymal cells was cell involutive degeneration. Under light microscope these hepatocytes were characterized by cytoplasm depletion, sharply decreased glycogen content, and hypochromatic nucleus (Fig. 1, *c*). They formed large aggregates sometimes occupying several lobules or the majority of the lobule fragments in the sample.

More than half samples contained parenchymal cells with signs of acidophilic degeneration, extremely eosinophilic homogenous cytoplasm, and pyknotic nuclei. These hepatocytes were primarily localized in the periportal area, but in some samples expansion of acidophilic degeneration resulted in disintegration of cells and hepatic trabeculae and irregular dilatation of the sinusoidal system.

In the majority of samples, portal tracts were dilated and deformed due to cell infiltration and sclerosis, the boundaries between the parenchymal and stromal elements were usually preserved. The infiltrate consisted of lymphocytes, macrophages, and fibroblasts. The proportion between high and less mature fibroblasts and other cell elements depended on the predominance of fibrosis or infiltration in the portal tract. Hyperplasia of lymphoid tissue in the portal stroma in the form of round follicle-like bodies was observed in half of samples (Fig. 1, *d*); some specimens contained large lymphoid follicles with germinal centers, a morphological marker of HCV infection; intrasinusoidal and interhepatocellular lymphocyte chains and intralobular lymphoid infiltrates appeared due to lymphocyte diapedesis.

Sometimes infiltrates penetrate through the boundary lamina into the liver parenchyma and induced degenerative changes in periportal hepatocytes and degradation of the boundary lamina, so that portal tracts looked corroded. Necrobiotic foci in the parenchyma were observed only in high HCV activity, while intralobular postnectrotic granulomas consisting of macrophages and lymphocytes were more common findings.

Mild necrosis was revealed in the majority of specimens. Fibrous changes involved primarily portal stroma. However, in half of cases focal or diffuse perisinusoidal sclerosis associated with poorer prognosis was found; sometimes sclerosis of the central vein with perivascular fibrosis was noted. Thus, sclerosis in HCV infection was characterized by low intensity, but wide spreading in the liver tissue.

Ultrastructural analysis revealed marked heterogeneity of parenchymal cells; some cells had regular ultrastructure, but most of them underwent degenerative alterations. Of particular interest were hepatocytes characterized by cytoplasm depletion under light microscope. Electron microscopy showed that this depletion was associated with reduction of all cytoplasmic organelles (Fig. 2, *a*). It should be noted that cytological analysis of different specimens or different cells in the same specimen allowed us to follow the dynamics of degenerative changes from partial reduction of cytoplasmic organelles to total cytoplasm depletion. This degeneration was accompanied by the formation of multiple residual bodies diffusely scattered over the cytoplasm (Fig. 2, *b*), sometimes migrating to the vascular pole of hepatocyte and Disse space. Moreover, these cells had large clustered lamellar structures formed by osmiophilic membranes (Fig. 2, *c*).

The nuclear compartment was preserved in most hepatocytes with depleted cytoplasm. Many of them contained perinuclear islets of cell regeneration (Fig. 2, *d*) including elements of granular endoplasmic reticulum, free ribosomes, polysomes, mitochondria, and glycogen granules. Segregation of the granular and fibrillar components in nucleoli and ring-shaped structures were only occasional findings.

Electron microscopy of the endoplasmic reticulum and cytoplasm revealed round membrane-bound viral particles with a diameter of about 50 nm with moderate electron density.

Endotheliocytes in sinusoids were characterized by high electron density and poorly defined ultrastructure. Solitary Kupffer cells with signs of high functional activity (developed endoplasmic reticulum and abundant polymorphic lysosomes) were noted.

Stereological analysis was based on the comparison of liver specimens from patients with and without virus replication verified by PRC-analysis of the serum. Volume density of parenchymal liver cells, sinusoids, and depleted hepatocytes, as well as vessels to parenchyma volume/volume ratio were similar in the two patient group. However, in patients with HCV replication a significantly ($p<0.05$) higher volume density of mononuclear infiltration and stromal compartment was noted. The latter determined a considerable

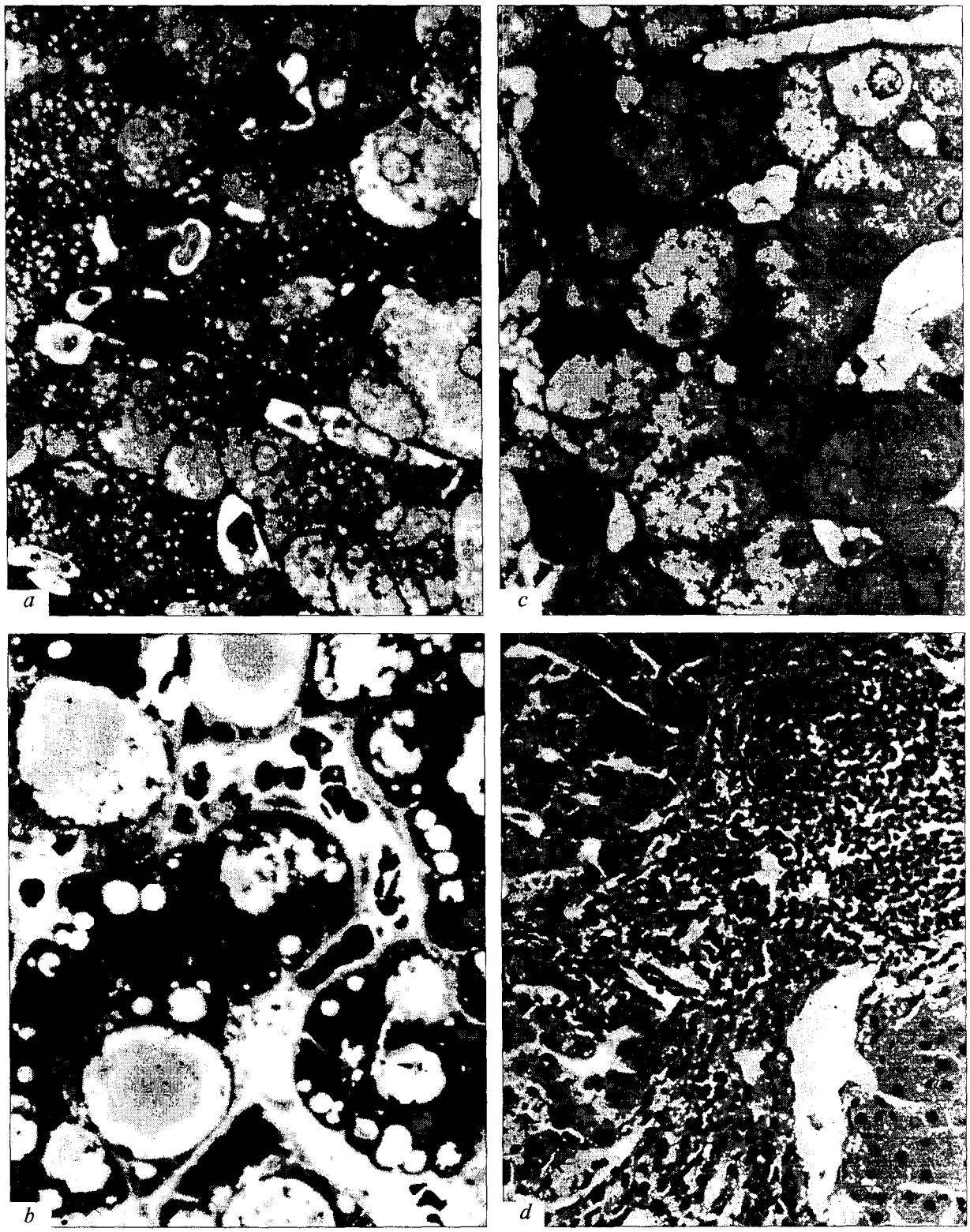

Fig. 1. Light microscopy of liver specimens in chronic hepatitis C. *a*) structural organization of hepatocytes: cells with light depleted cytoplasm and multivesicular lipid infiltration, ×250; *b*) polymorphic lipid infiltration of hepatocytes, ×200; *c*) most hepatocytes with depleted cytoplasm, ×250; *d*) lymphoid follicle in portal tract, hematoxylin and eosin staining, ×100. *a-c*) semithin section, staining with Schiff reagent and azur II.

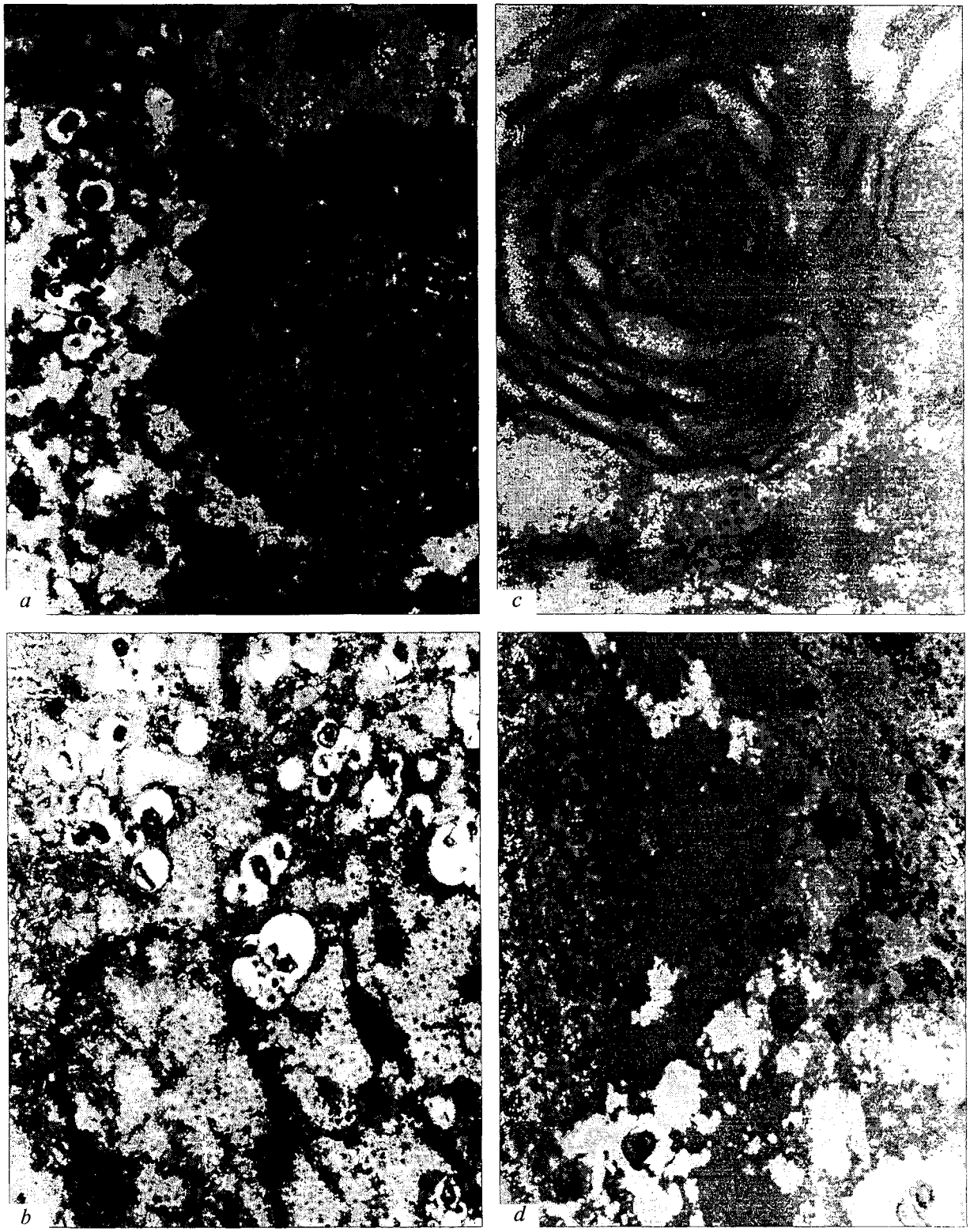

Fig. 2. Ultrastructure of hepatocytes in chronic hepatitis C. *a*) reduction of cytoplasmic organelles, residual bodies, foci of intracellular regeneration, and euchromatic nucleus, ×4000; *b*) reduction of cytoplasmic organelles, residual bodies, multiple polymorphic residual bodies, ×10,000; *c*) cytoplasm sequestration with osmiophilic membranes, ×50,000; *d*) fragments of hepatocyte and endotheliocyte: reduction of cytoplasmic organelles, perinuclear focus of regeneration. Residual bodies in the cytoplasm and Disse space, ×8000.

increase in the stroma/parenchyma ratio. Structural density of endotheliocytes tended to increase in HCV RNA-positive patients.

Morpho- and cytogenesis of virus-induced infectious processes largely depends on the ratio of altered and regenerating hepatocytes [5,6]. We found that apart from previously described structural changes in the liver [9,10], chronic hepatitis C manifests itself in a peculiar form of hepatocyte damage characterized by reduction of cytoplasmic organelles and visual picture of cell depletion.

This type of cell degeneration is underlain by impaired recovery of damaged organelles due to inhibition of protein synthesis (syndrome of regeneration and plastic insufficiency [3,4]), rather than by hepatocyte alteration and necrosis. This type of hepatocyte degeneration represents a phenotypical involution, but this state is reversible, because of the presence of preserved nuclear compartment and intracellular regeneration foci.

Hepatocyte depletion is probably associated with activation of protein kinase P1, which reduces the intensity of protein synthesis in the cell and, consequently, inhibits replication and assembly of virus particles [13]. These changes can be interpreted as a protective reaction of the cells under conditions of virus infection.

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