Clasmatosis and Proliferation of Hepatocytes at the Initial Stages of Experimental Hypercholesterolemia in Rabbits

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Profound dystrophic changes, which involved granulocytes and were associated with acute inflammatory reaction, were observed in rabbit liver at the initial stage of experimental hypercholesterolemia. It was suggested that "acute phase" is important for the formation of a modified lipoprotein with autoantigenic properties.

Key Words: *hypercholesterolemia; C-reactive protein; leukocyte decationization; clasmatosis*

It was hypothesized that modified apoprotein B-containing lipoprotein (apoB-LP) with autoantigenic activity [1,7] is an important factor of atherosclerosis [5]. Proceeding from this hypothesis, one can regard atherogenesis as a local immune inflammation [2]. The vascular wall is a target organ in which the modifications occurring in the organism are realized. The liver is the major organ that synthesizes and catabolizes various LP which were isolated from aortic intima [1]. Hepatocytes are the major cell type producing the acute inflammation proteins C-reactive protein (CRP) and serum amyloid P (SAP). These proteins play an important role in the immunoregulation and activation of granular and agranular leukocytes [4]. A relationship mediated by the phospholipid groups has been established between the acute-phase proteins and low- and very low-density LP [6]. As we are aware, morphological manifestations of structural and functional modifications occurring in hepatocytes during the development of hypercholesterolemia have not been investigated except fatty dystrophy of the liver.

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Our objective was to analyze the morphofunctional changes in the liver at the initial stages of experimental hypercholesterolemia when modified apoB-LP emerge in peripheral blood and the concentration of CRP and SAP increase.

MATERIALS AND METHODS

Experiments were performed on 28 male rabbits weighing 2.8-3.0 kg. Hypercholesterolemia was induced by feeding the animals with cholesterol dissolved in sunflower oil (200 mg cholesterol/kg body weight) through a gastric tube 5 times a week. Serum lipids were measured in a Technicon AA-II analyzer before and on days 2, 4, 7, and 14 of hypercholesterolemia. The CRP and SAP contents were determined by light absorbance changes after the precipitation reaction.

Blood smears as well as liver prints and sections were stained for cationic proteins by the method of V. E. Pigarevskii. Liver sections were stained for lipids with oil red O and with hematoxylin and eosin. Immunohistochemical investigations were performed with the use of anti-CRP and anti-SAP polyclonal antibodies. Proliferative activity of hepatocytes was determined after a single intravenous injection of the synthetic analog of thy-

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midine 5-bromo-2'deoxyuridine (BrdUrd, 60 mg in 5 ml normal saline) with its subsequent immunohistochemical identification. The preparation was injected 1 h before sacrifice. Paraffin sections were stained with monoclonal antibodies to halogenized deoxyuridine IU-4. Proliferative activity was determined by counting stained nuclei in 50 fields of view at magnification 650. Liver specimens were processes for analysis in a Hitachi H-300 electron microscope.

RESULTS

After 2 days of CL feeding, blood concentration of total CL increased 3-fold. There were no changes in blood concentrations of SAP and CRP.

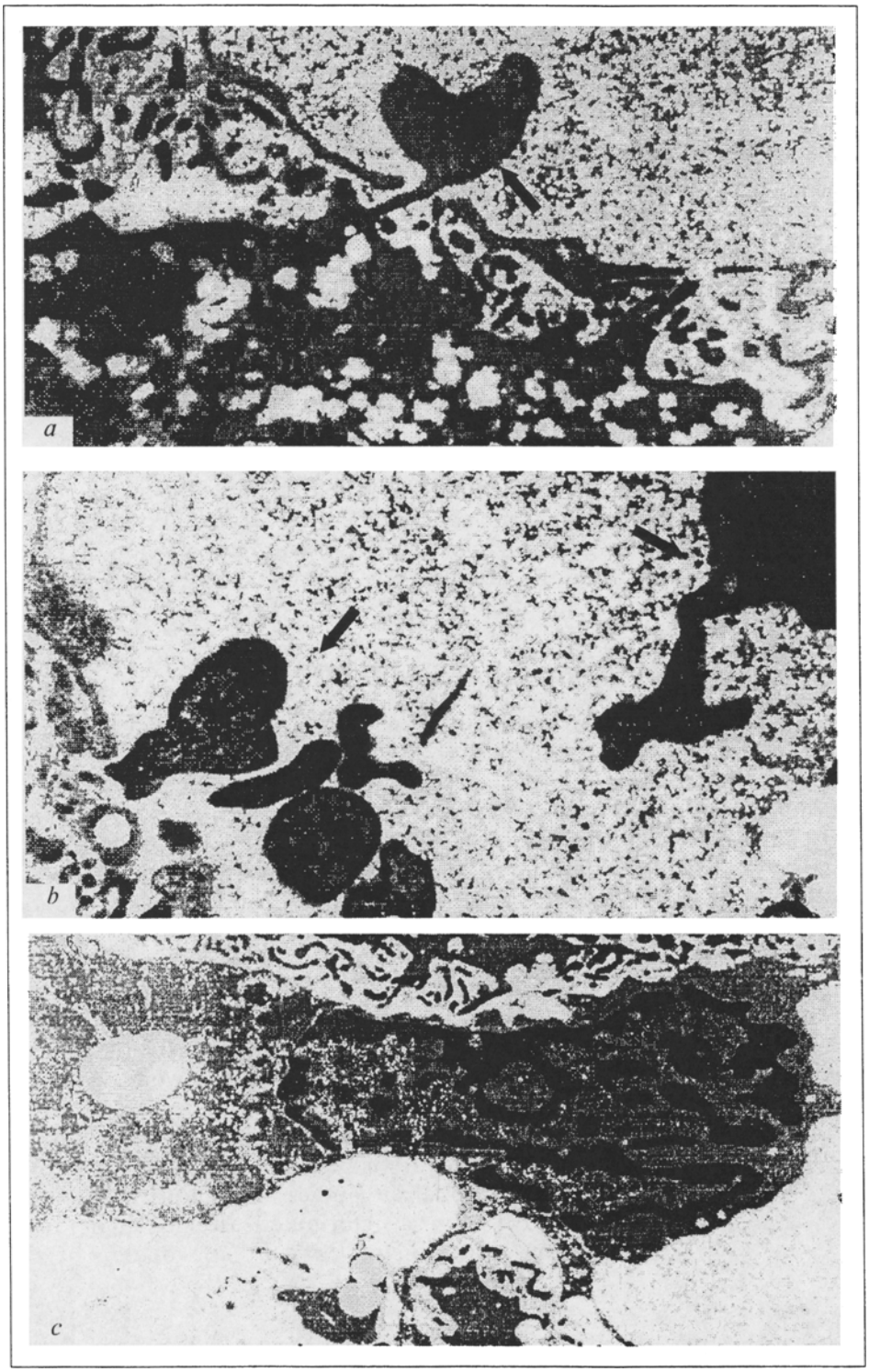

Fig. 1. Ultrastructural changes in rabbits liver on day 3 of cholesterol feeding. Transmission electron microscopy. *a)* microclasmatosis of villi and clasmatosis of hepatocyte (arrowhead), ×8000; *b)* hepatocyte fragments in the sinusoid, clasmatosis (arrowhead) ×12,000; *c)* granulocyte contacting with damaged hepatocyte in the clasmatosis zone, ×6500.

The dystrophic changes in hepatocytes coincided with formation of large and small cytoplasmic vacuoles both in the proximal and distal parts of the acinus. There was no fatty dystrophy; occasional lipid vacuoles were seen in some cells.

These changes were studied in more detail by electron microscopy. The thickening of the hepatocyte plasma membrane was accompanied by changes in the amount, length, and shape of microvilli and their edema and by increase or decrease in the number of pynocytotic vesicles in the space of Disse. Local damage to the plasma membrane and its disintegration were associated with microclasmatosis. Swollen microvilli with organelles separated from the hepatocyte were located in the space of Disse (Fig. 1, *a*).

Clasmatosis of hepatocyte fragments, which enter the sinusoids (Fig. 1, *a*, *b*) and separate from the cell (Fig. 1, *b*), can be seen on the microphotographs.

After 2 days of experimental hypercholesterolemia, so-called "aggressive emperiopolesis" developed: granulocytes (in rabbits they are referred

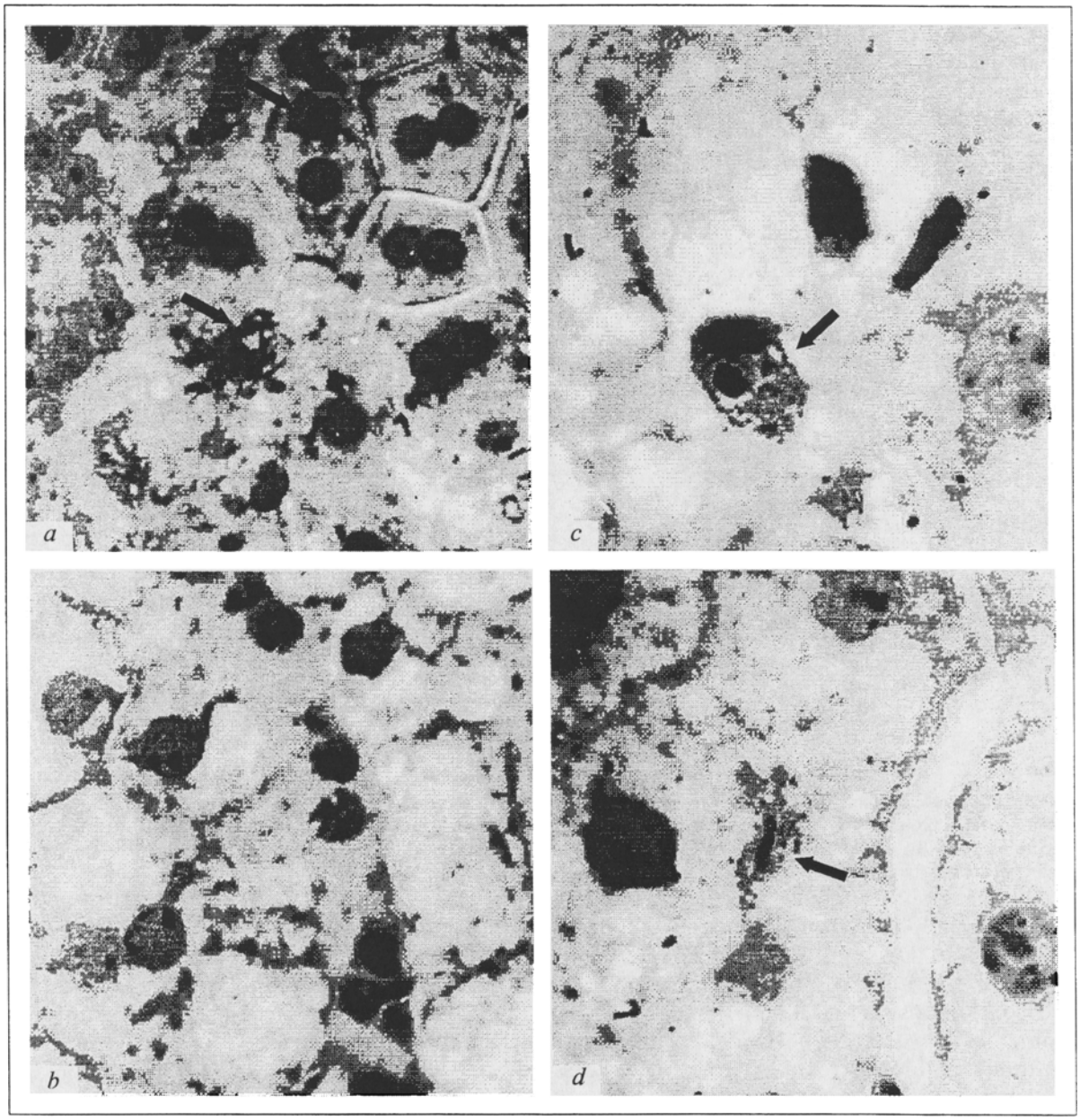

Fig. 2. Morphological changes in the liver at the initial stages of experimental hypercholesterolemia. *a)* pronounced production of C-reactive protein in hepatocytes after 2 days of experimental hypercholesterolemia (arrowhead), immunohistochemical staining and counterstaining with hematoxylin and eosin, ×1200; *b)* accumulation of pseudoeosinophils among damaged hepatocytes on the 8th day of the study, staining with hematoxylin and eosin, ×1200; *c, d)* presumable enter of granulocytes into hepatocytes and release of cationic proteins (arrowhead), day 3 of the study, staining with hematoxylin and eosin, ×1000.

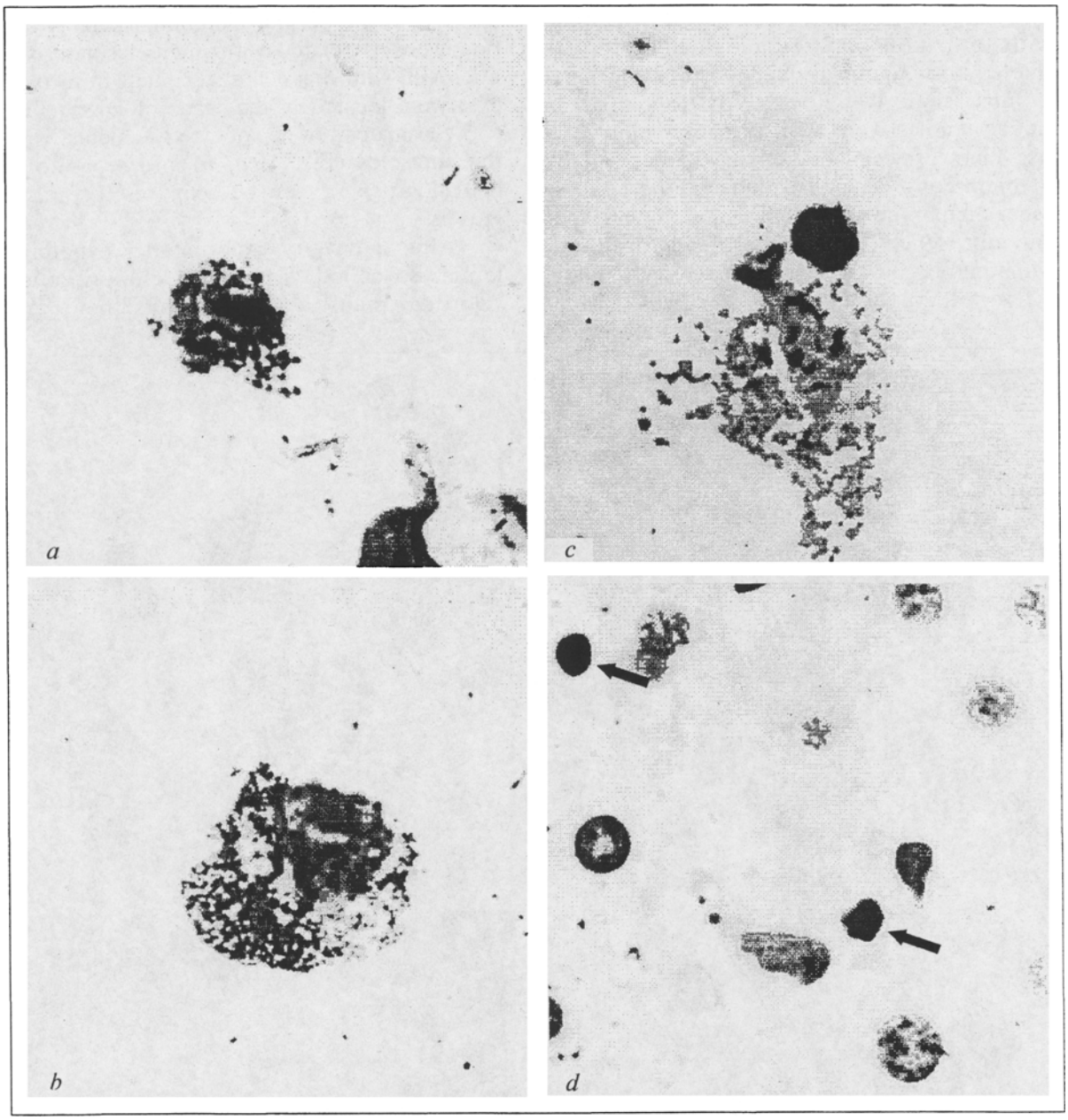

Fig. 3. Release of cationic proteins from granulocytes and proliferation of hepatocytes at the initial stages of experimental hypercholesterolemia. *a-c)* release of cationic proteins from pseudoeosinophils in the liver 2 *(a)* 4 *(b)* and 7 *(c)* days of hypercholesterolemia. Staining of liver prints for cationic proteins, ×1000; *d)* proliferation of individual hepatocytes after 2 weeks of experimental hypercholesterolemia (arrowhead); immunohistochemical staining for BrdUrd and counterstaining with hematoxylin, ×1000.

to as pseudoeosinophils) from peripheral blood entered the space of Disse and formed contacts with hepatocyte in the area of the plasma membrane damage or clasmatosis (Fig. 1, *c*).

Kuppfer cells were transformed into foam cells which entered the bloodstream. There were no dystrophic changes in endothelial cells.

On day 4, blood CRP concentration increased 1.5-2-fold, and the signs of colliquative necrosis of hepatocytes appeared. The mitochondria were swollen, their cristae disappeared, and the matrix became clarified. The rough endoplasmic reticulum was destroyed, glycogen granules disappeared, and light empty spaces similar to necrotized cytoplasm and containing occasional lipid vacuoles were observed in hepatocytes.

What causes such a rapid development of dystrophic changes in hepatocytes? We propose the following scheme for this phenomenon: exogenous CL 1) stimulates the production of apoB-100 and formation of very low-density lipoprotein and 2) increases CRP synthesis.

Immunohistochemical analysis has shown that on the third day of experimental hypercholesterolemia CRP production in hepatocytes is increased (Fig. 2, *a*) in comparison with the control (rabbits fed standard diet). Granulocytes have two CRP receptors (CRP-r and FcgRII) that mediate their interaction with cells producing acute-phase proteins [8]. Considerable amounts of pseudoeosinophils were accumulated in the liver sinuses on the 8th day of experimental hypercholesterolemia (Fig. 2, *b*). Receptor binding of these cells is probably responsible for aggressive emperiopolesis and migration of leukocytes not only in the space of Disse (Fig. 1, *c*), but also (probably) in hepatocytes (Fig. 2, *c, d*). Cationic proteins were released from granulocytes, as evidenced by specific staining (Fig. 3, *a-c*). We think that the profound dystrophic changes in hepatocytes are associated with decationization of granulocytes.

It can be suggested that clasmatosis of hepatocytes and deposition of fat in Kuppfer cells induce proliferation of these cells. In normal liver, cells do not proliferate and are in the G0-phase of cell cycle [3]. Qualitative and quantitative analysis of cells that are in the S-phase revealed no pronounced proliferation of hepatocytes practically within the first weeks of hypercholesterolemia; the nuclei of occasional cells contained BrdUrd (Fig. 3, *d*). After 2 weeks of hypercholesterolemia, BrdUrd incorporation in Kuppfer cells was more pronounced. However, the amount of Kuppfer cells in the S-phase was very small. The possibility that hypercholesterolemia blocks the G2-phase and affects replication cannot be ruled out.

Thus, the acute-phase proteins are synthesized in rat liver in response to hypercholesterolemia, which leads to migration of granular leukocytes from the circulation, release of cationic proteins from them, and formation of contacts with hepatocytes with their subsequent damage. This reaction develops prior to fatty dystrophy of the liver and is probably an important factor in the formation of modified lipoprotein with autoantigenic activity.

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