MORPHOLOGY AND PATHOMORPHOLOGY

Structural and Functional Morphology of Acute Phase Reaction in the Liver in Experimental Hypercholesterolemia

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Synthesis of acute phase transmitters, migration and degranulation of pseudoeosinophils in hepatocytes, and damage to hepatocytes are observed during the first few weeks of atherogenic diet. It is hypothesized that the acute phase reaction determines disturbances in the synthesis and catabolism of lipoproteins in the liver.

Key Words: *hypercholesterolemia; acute phase proteins; pseudoeosinophils; damage to hepatocytes*

Recent concept considers atherosclerotic lesions in arteries as a focus of immune inflammation [2,3]. It was shown that arterial atherosclerotic plaques and blood of atherosclerotic patients contains autoimmune complexes that include apolipoprotein B-containing lipoproteins as the antigen [8,14]. We have previously demonstrated generation of antibodies against homologous apolipoprotein B-containing lipoproteins in rabbits maintained on atherogenic diet for 2 weeks [1]. These autoimmune reactions can be induced by unusual apolipoprotein B-containing lipoproteins, which differ from native lipoproteins in the antigenic composition.

The liver not only synthesizes and catabolizes lipoproteins, but also produces acute-phase proteins [5,9], in particular, C-reactive protein (CRP) and serum amyloid P (SAP) [4,10]. CRP and SAP bind to low, very low, and high density lipoproteins (LDL, VLDL, and HDL) [13,15] and induce modification and scavenging of LDL and VLDL by macrophages [12]. Acute-phase proteins may modulate metabolism of lipoproteins, but their role in atherogenesis remains poorly studied.

This study is focused on the role of acute phase reaction and structural and functional rearrangements in the liver of rabbits with experimental hypercholesterolemia.

MATERIALS AND METHODS

Experiments were carried out on 11 male rabbits weighing 2.8-3 kg. Experimental atherosclerosis was modeled by the method of N. N. Anichkov. To this end the animals received through a tube 200 mg/kg cholesterol (5 times per week in 5 ml sunflower oil).

Serum concentrations of CRP, SAP, and lipids were measured on days 4, 11, 20, and 25 after the start of experiment. Commercial antiserum against human CRP (Institute of Vaccines and Sera, St. Petersburg) and rabbit antiserum against human SAP (Institute of Experimental Medicine, Russian Academy of Medical Sciences) were used. SAP was isolated from serum of

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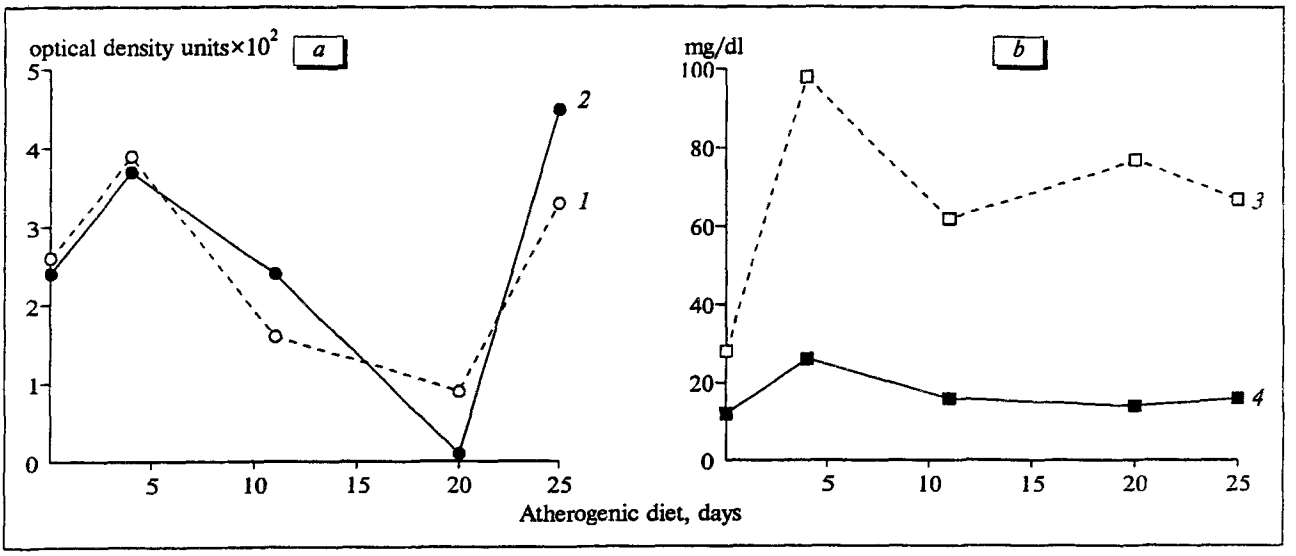


Fig. 1. Plasma content of acute-phase proteins (*a*) and cholesterol in rabbits feeding an atherogenic diet. *1*) C-reactive protein; 2) serum amyloid P; 3) total cholesterol; *4*) high density lipoprotein cholesterol.

healthy donors as described previously [16]: lipoproteins were removed from serum by ultracentrifugation, and the serum was mixed with Sepharose 4B in the presence of Ca^{2+}. After SAP had been specifically bound to agarose polysaccharides, the mixture was washed, transferred to a column, and SAP was eluted in the presence of EDTA. The protein was additionally purified by gel filtration on AcA34 Ultragel. The protein was stored at -18°C before use. Rabbits were immunized into pads with complete Freund's adjuvant and 2 weeks later into lymph nodes without adjuvant; the resultant antiserum was adsorbed with human erythrocytes. The serum was monospecific and had a titer of 1:8 in the Ouchterlony double diffusion test. Since human and rabbits SAP and CRP are similar, the corresponding antiserum can be cross used [6].

SAP and CRP in rabbit serum were measured by optical density in the precipitation reaction. To this end, 50 μl serum and 50 μl anti-CRP or anti-SAP (1:15 dilution) were transferred to a 96-well plate and incubated for 1.5-2 h at 37°C. Then 100 μl polyethylene glycol (PEG-600) was added to a final concentration of 3.5%, and the samples were incubated for 18-20 h at 4°C. Control samples contained 50 μl test serum, 50 μl phosphate-buffered physiological saline, and 100 μl borate buffer (pH 8.6, solvent for PEG-6000). The samples were measured in a Spectra multichannel spectrophotometer at 620 nm. The concentrations of CRP and SAP were calculated by the difference between control and experimental sample, and the data were expressed in optical density units.

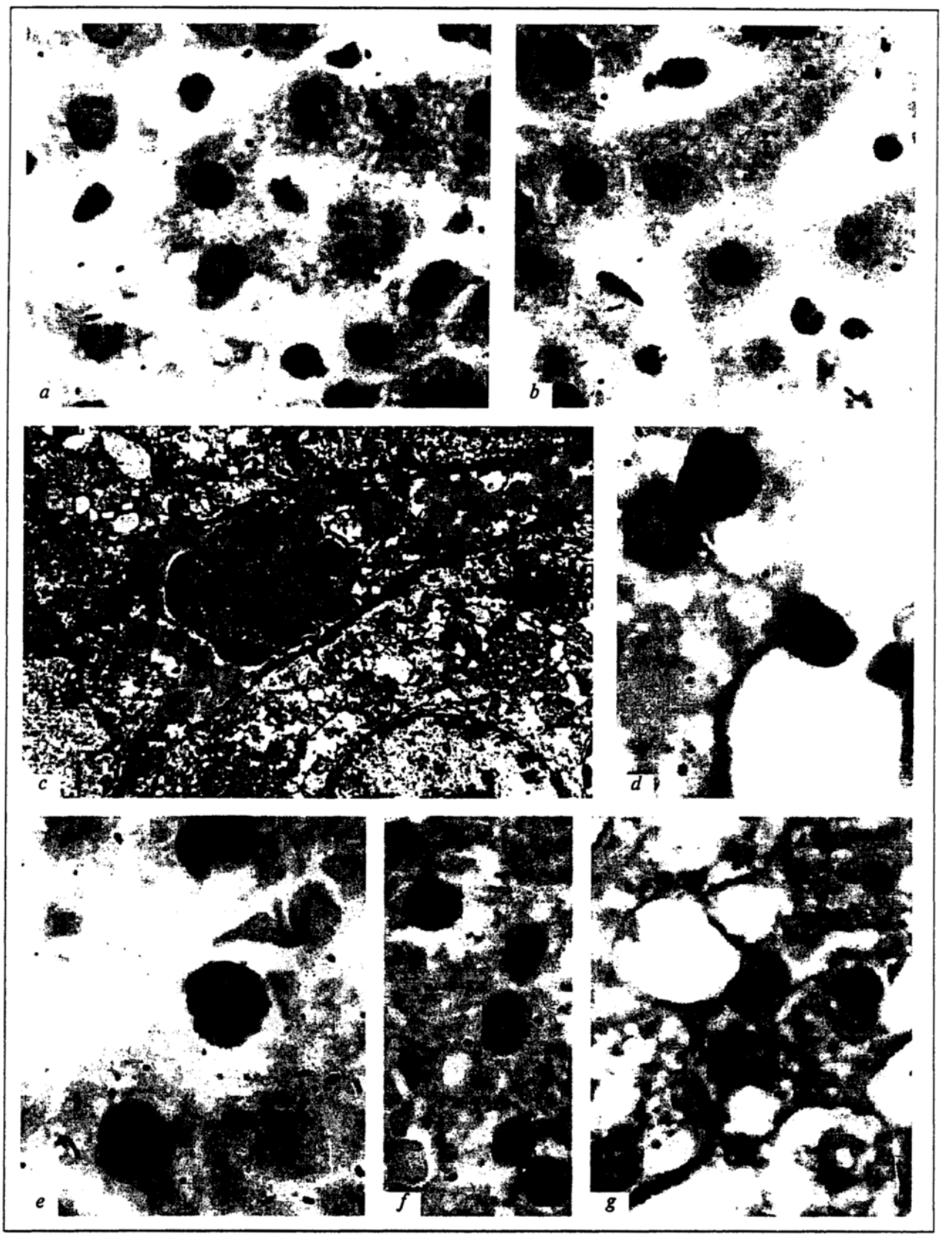
Blood lipids (total cholesterol, VLDL and HDL cholesterol, and triglycerides) were measured in a Technicon AA-II analyzer. The animals were sacrificed on days 11, 20, and 25 and 8 and 12 weeks after the start of atherogenic diet. Histological and electron microscopic examination of the liver was carried out. Liver sections were stained with Sudan IV (visualization of lipids), hematoxylin and eosin, and 0.1% fast green in 50% ethanol (pH 8.15) and examined under a Hitachi-H300 electron microscope.

RESULTS

The first rise of serum cholesterol was noted on day 4 of atherogenic diet: total cholesterol increased 4-5-fold. It coincided with maximum serum concentration of SAP and CRP (Fig. 1).

Since serum lipid content during the first few weeks varied from rabbit to rabbit, we present typical dynamics of acute-phase proteins and lipid parameters (development of hypercholesterolemia) (Fig. 1).

It was found that the higher cholesterol content on day 4 of the experiment the higher serum concentration of CRP and SAP, i.e., there was a strong correlation between the intensity of the acute phase reaction and hypercholesterolemia at the initial stages of experimental atherosclerosis. This correlation was observed throughout subsequent 2 weeks of the experiment. A decrease in serum CRP and SAP was accompanied by a drop in plasma lipids. This phenomenon is due to the fact that CRP is characterized by high affinity fo phospholylcholine and binds 90% apoprotein B [13,15]. Induction of CRP synthesis in the liver is accompanied by a decrease in plasma apoprotein A-I [7] and, consequently, in HDL content (Fig. 1, *b*).


a
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Two weeks after the onset of acute phase reaction in the liver induced by hypercholesterolemia, we observed accumulation of pseudoeosinophils (in rabbits presented primarily by neutrophilic granulocytes and to a lesser extent by monocytes) in the sinuses (Fig. 2, *a, b*) and transformation of Kupffer cells into foam cells (Fig. 2, *c*). No signs of fatty degeneration in hepatocytes was noted at this stage.

After 4 weeks, we observed adhesion of pseudoeosinophils to the hepatocyte surface primarily at the sites of disappeared Kupffer cells (Fig. 2, *d*) and penetration into hepatocytes, which was accompanied by partial (Fig. 2, *e*) or complete (Fig. 2, *f, g*) degranulation. This reaction of pseudoeosinophils is probably due to the presence of two types of receptors to CRP and SAP (IgGR and FcγRII) on granulocytes [11] and the possibility of direct contact with hepatocytes actively producing acute-phase proteins.

This stage was characterized by profound degenerative alterations in pseudoeosinophil-adjacent hepatocytes, which first appeared after 2 week, i.e., when the acute phase reaction attained the maximum (Fig. 2, *c*). The most prominent features were focal necroses of hepatocytes (Fig. 3, *a*), mitochondria swelling, and enlargement of endoplasmic reticulum and the Disse space containing numerous vacuoles and vesicles. In parallel, solitary membrane-surrounded lipid vacuoles appeared in hepatocytes (Fig. 3, *a*).

After 8 week of atherogenic diet, fatty degeneration of the liver associated with accumulation of lipid vacuoles of various size in hepatocytes became a predominant phenomenon and after 12 weeks led to the development of cirrhosis accompanied by coagulation and colliquative necrosis of hepatocytes, degradation of nuclei and cytoplasmic organelles, and growth of fibrous tissue along portal tracts. Hepatocytes looked like empty honeycombs with fibrous tissue between them (Fig. 3, *b*). Flattened vascular sinuses contained primarily lymphocytes.

Thus, our experiments demonstrated an interrelationship between the initial stage of experimental hyperlipidemia and acute phase reaction in the liver. These processes are characterized by two types of hepatocyte damage, the damage induced by acute-phase proteins developed earlier than fatty degradation. The latter consideration allowed us to hypothesize that acute phase reaction is an important prerequisite for disturbances in lipoprotein synthesis and catabolism and leads to the formation of modified lipoproteins and the development of hypercholesterolemia.

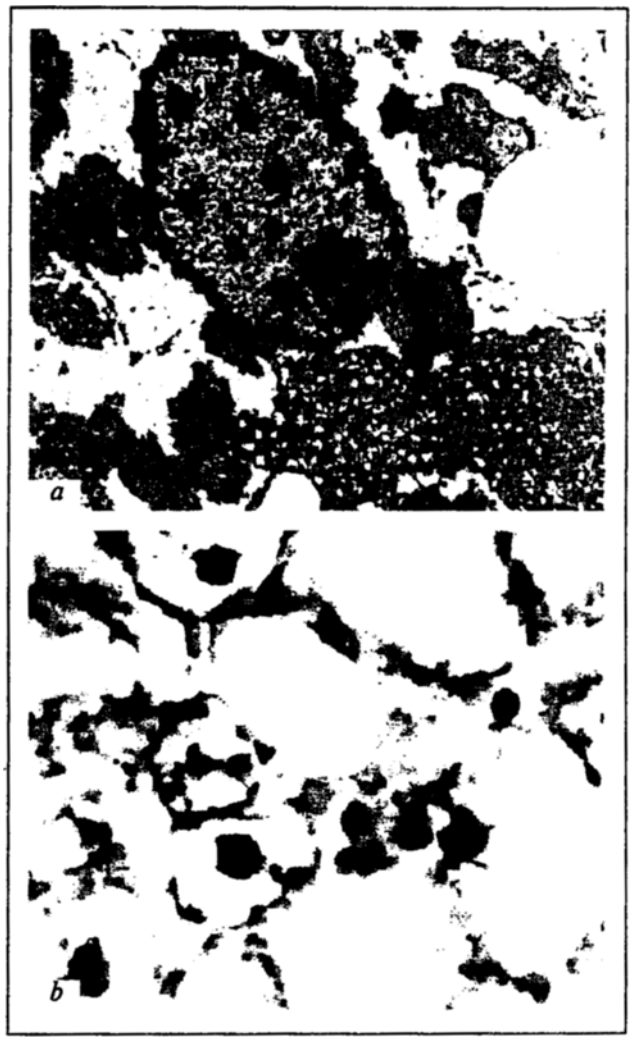

Fig. 3. Coagulation and colliquative hepatocyte necrosis after 12 weeks of hypercholesterolemia. *a*) focal hepatocyte necrosis, solitary lipid vacuoles, transmission electron microscopy, ×12,000. *b*) complete hepatocyte degradation, hematoxylin and eosin staining, ×1200.

Fig. 2. Morphological analysis of acute phase reaction in rabbit liver during the initial stages of experimental atherosclerosis. *a, b*) accumulation of pseudoeosinophils in the sinuses after 2 weeks of atherogenic diet, hematoxylin and eosin staining, ×1200. *c*) abundant lipid vacuoles in the cytoplasm of Kupffer cells, signs of edema and disorganization of hepatocyte cytoplasm, transmission electron microscopy, ×8000. *d-g*) 4 weeks of atherogenic diet, different stages of adhesion and migration of pseudoeosinophils to hepatocytes and their decationization, hematoxylin and eosin staining, ×1600.

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Effect of Calcium Antagonists on the Kidney Graft Injury Induced by Long-Term Cold Ischemia

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Ultrastructural changes in kidney graft induced by long-term cold ischemia and effect of calcium antagonists on these alterations are studied. It is shown that calcium antagonists prevent activation of lipid peroxidation and erythrocyte agglutination and adhesion in capillaries.

Key Words: *ischemia; calcium antagonists; malonic dialdehyde*

Long-term storage of kidney allograft required for choosing a recipient, preoperative procedures or transportation of the organ is a pressing medical problem. Acute tubular necrosis is often caused by cold ischemia longer than 24 h, while 72 h is the maximum time of graft storage [4]. However, long-term cold ischemia is associated with increased occurrence of acute tubular necrosis and graft dysfunction [12].

Calcium antagonists exerting vasodilating, mitochondrion-protective, and antitoxic (with respect to cyclosporine treatment) effects improve functional capacity of kidney grafts [3,8,11].

The present study explores ultrastructural changes in kidney graft during 96-h storage and the effect of calcium antagonists on these changes. To investigate peculiarities of oxidative stress induced by long-term cold ischemia in the kidney we compared electron microscopic picture and data on the intensity of lipid peroxidation in kidney parenchyma.

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MATERIALS AND METHODS

Experiments were carried out on New Zealand rabbits weighing 2.25-2.5 kg. The animals were intravenously narcotized with Phenobarbital (20 mg/kg) and Ketamine (25 mg/kg single injection+1 mg/kg/min infusion) and laparotomy and bilateral nephrectomy was performed. The animals were intravenously injected with 250 U/kg heparin 3 min before surgery and 50 ml/kg 0.9% NaCl was injected intraoperative.

In group 1 animals (n=12), renal arteries were cannuled, the kidneys were placed into physiological saline at the ice melting temperature and perfused with Euro-Collins solution at a perfusion pressure of 100 cm H_2O (heat ischemia did not exceed 2 min).

Group 2 animals (n=12) were intravenously injected with 0.35 mg/kg verapamil 30 min before nephrectomy, other procedures were as in group 1.

The kidneys were stored at 4°C.

In both groups the aorta was cannuled and 160 ml blood was drawn and mixed with citrate (4°C).

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