Changes in Cerebral Vessels during Experimental Hyperlipidemia

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Both specific (lipid deposition in endotheliocytes, myocytes, and pericytes) and non-specific (intense formation of apical processes and vacuoles in endotheliocytes and vacuolization of myocytes) changes in cerebral vessels of different diameter were revealed in rabbits and rats after 4 months of experimental hyperlipidemia. These changes suggest reduced endothelium athrombogeneity and intensified transendothelial transport. Biochemical and morphological manifestations of hyperlipidemia in rabbits were more pronounced than in rats.

Key Words: *experimental hyperlipidemia; cerebral vessels*

Disturbed lipid homeostasis is now considered to be the key patho- and morphogenetic factor of atherosclerosis. Elevated content and enhanced transport of blood lipoproteins through the vascular wall affect both large arteries and microcirculatory vessels (MCV) of different organs [3,5]. Diffuse microcirculatory disturbances in the myocardium, liver, lungs, kidney, and other organs, the earliest manifestations of experimental hyperlipidemia (HLP), are considered to be the microangiopathy of lipid origin preceding pathological changes in large arteries (lipid strips and spots, fibrous plaques, and others) [2,4,5,7-9]. Morphological changes in large cerebral vessels and MVB at the early stages of HLP have not been studied. Some peculiarities of cerebral angioarchitectonics and hemodynamics (multilevel system of blood supply), morphogenetic specificity of cerebral endothelium, and neurochemical heterogeneity of brain structures can determine some peculiarities in the development of cerebral atherosclerosis in comparison with other organs and systems [5,6]. This study was designed to investigate early manifestation of HLP in cerebral vessels at cellular and subcellular levels.

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MATERIALS AND METHODS

Experiments were carried out on 20 Chinchilla rabbits (2.5-3.0 kg) and 16 outbred male albino rats (220-250 g). Control animals (8 rabbits and 8 rats) were kept on a standard diet. Experimental HLP was induced by atherogenic diet: the rabbits (n=12) received cholesterol (0.3 g/kg) and the rats (n=8) received oil suspension (1 ml/100g) containing 10% cholesterol and 1% cholic acid. Blood samples taken after 4-month diet followed by 16-18 h food deprivation were analyzed to determine serum concentration of total cholesterol (CH) by the method of Ilca [1], high-density lipoprotein cholesterol (HDLCH) after heparin-manganese precipitation, low-density lipoprotein cholesterol (LDLCH) [13], very low-density lipoprotein cholesterol (VLDLCH) [11], and triglycerides [10]. The coefficient of atherogenicity was calculated [6]. A morphological study was performed with light and electron microscopies after the brain was fixed with 10% formaldehyde and glutaraldehyde-osmium, respectively. All levels of the cerebrovascular system were investigated: major head arteries, basal and superficial cerebral vessels, intracerebral vessels and MCV. For light microscopy, histological sections were stained with hematoxylin and eosin, by Van-Gieson's method, with fuxelin for elastic fibers, by Weigert for fibrin, with

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sharlach, and by Nissl method. Semithin sections were stained with methylene blue, ultrathin sections were stained with uranyl acetate and lead citrate and examined under a JEM-100B electron microscope.

RESULTS

Animals kept on the atherogenic diet for 4 months showed considerable disturbances of lipid homeostasis: the content of triglycerides significantly increased, total CH increased 28-fold in rabbits and 1.7-fold in rats, the content of CH increased in atherogenic lipoprotein fractions and decreased in HDL, the index of atherogenicity increased 4.4-fold in rabbits and 4.8-fold in rats (Table 1).

Light microscopy revealed similar changes in the brains of rabbits and rats kept on the atherogenic diet: hypovolemia, MCV stenosis, and enlargement of perivascular areas around capillaries, arterioles, and venules. A dramatic enlargement of endothelial cytoplasm occluding capillary lumens was noted in the MCV of the cerebral cortex, cerebellum, and white matter (Fig. 1, *a*). A narrow luminal space in venules was filled with erythrocytes arranged in coins columns. Some capillaries, venules, and small veins showed signs of plasmatization. Other capillaries, venules and small arteries contained only erythrocytes with signs of conglutination. Hypertrophy and proliferation of pericapillary astrocytes (Fig. 1, *b*) were observed in the cerebral cortex. Some capillaries contained hyalin microthrombi. Pericapillary and, especially, perivenular areas were enlarged. Chorioid plexus in cerebral ventricles contained edematous villi with vacuolized cytoplasm and lipophage clusters arranged primarily perivenularly (Fig. 1, *c*). Vacuolized myocytes were observed in the media of superficial brain arteries (Fig. 1, *e*), which endotheliocytes in this arteria showed signs of enhanced permeability. No changes were found in the internal carotid arteries. The study of internal organs revealed variously pronounced fatty degeneration in the liver, kidney, and myocardium, and extensive lipid deposits in coronary arteries. It should be noted that morphological changes in the brain were less pronounced than in internal organs and that rabbits exhibited more pronounced morphological and biochemical manifestations of dislipidemia.

Electron microscopy of extracranial parts of the internal carotid arteries, anterior, middle, and posterior cerebral arteries and their branches, as well as fragments of vertebral and basillar arteries and their extra- and intracerebral branches revealed lipid droplets and vacuoles in endotheliocytes (Fig. 2, *a*), myocytes (Fig. 2, *b*), subendothelial layers, pericytes (Fig. 2, *c*), astrocytes (Fig. 2, *d*), and the lumens of different intracerebral vessels. It should be noted that these changes were present only in some brain regions. The increased number of apical processes (pseudopodia) protruding into the vascular lumen (Fig. 3, *a*), small vesicles, and large vacuoles (Fig. 3, *b, c*) often occupying more than 50% cell volume were the most common changes observed in all examined brain vessels. Loosening and vacuolization of the interstitial substance, electron-dense depositions, and the formation of myelin bodies were observed in the subendothelial layer. Vascular myocytes contained vacuoles of different size (Fig. 3, *d*) with electron-lucid material, membranous structures, and electron-dense granules. Disruption of endotheliocytes exposing the subendothelial layer was observed less frequently (Fig. 3, *e*). Thus, after 4 months of atherogenic diet cerebral arteries of different diameter showed initial manifestations of atherosclerosis such as lipid deposition in different structures of the arterial wall and functional changes in the endothelium. According to previous classification, the former are specific for HLP, while the latter are nonspecific [4,5,8,9]. Lipid depositions in the vascular lumen, endotheliocytes, myocytes, subendothelial layer, pericytes, and perivascular astrocytes were discovered in cerebral arteries of a different diameter. Large cerebral arteries accumulated lipids primarily in the region of branching. In the brain, lipid deposition in structural components of the vascular wall were less common

TABLE 1. Serum Content of Cholesterol and Triglycerides in Rabbits and Rats with Experimental Hyperlipidemia (*M±m*)

Index		Rabbits		Rats	
		control (*n*=8)	AD (*n*=12)	control (*n*=8)	AD (*n*=8)
Cholesterol, mg/dl	total	31.0±2.4	878.0±126.5*	87.7±7.7	155.0±12.9*
	HDL	15.4±1.3	164.8±24.7*	52.5±3.1	37.9±3.1**
	VLDL	13.5±1.7	121.9±24.2**	16.9±0.6	29.3±2.3*
	LDL	3.3±0.9	591.0±97.3*	18.3±1.0	87.8±7.9*
Triglycerides, mg/dl		67.5±8.7	609.5±121.2**	84.9±3.2	146.5±12.9*
Coefficient of atherogenicity, arb. units		1.03±0.14	4.53±0.84**	0.67±0.002	3.2±0.25*

Note. AGD, atherogenic diet. *$p<0.001$, **$p<0.01$ in comparison with the corresponding control.

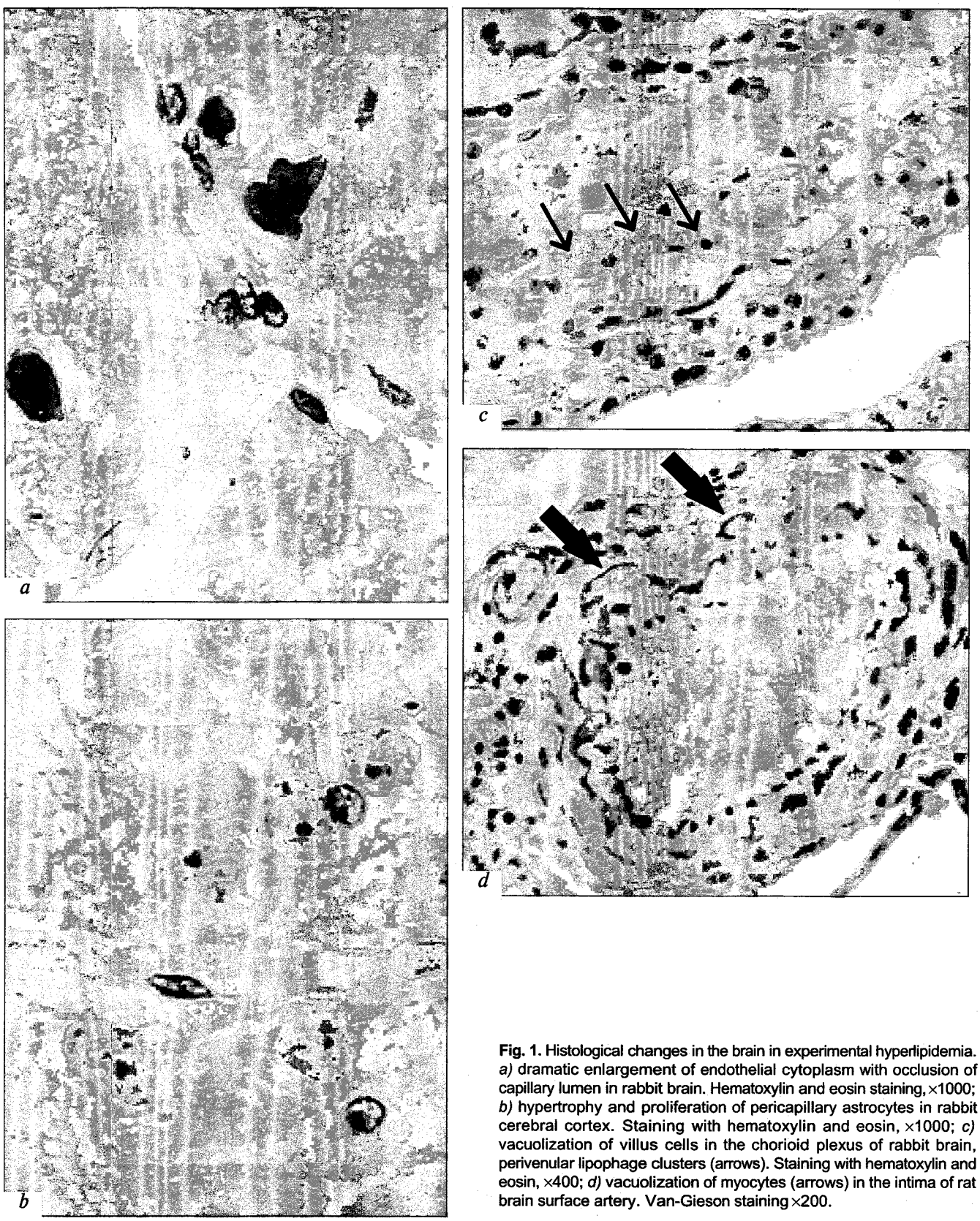

Fig. 1. Histological changes in the brain in experimental hyperlipidemia. *a)* dramatic enlargement of endothelial cytoplasm with occlusion of capillary lumen in rabbit brain. Hematoxylin and eosin staining, ×1000; *b)* hypertrophy and proliferation of pericapillary astrocytes in rabbit cerebral cortex. Staining with hematoxylin and eosin, ×1000; *c)* vacuolization of villus cells in the chorioid plexus of rabbit brain, perivenular lipophage clusters (arrows). Staining with hematoxylin and eosin, ×400; *d)* vacuolization of myocytes (arrows) in the intima of rat brain surface artery. Van-Gieson staining ×200.

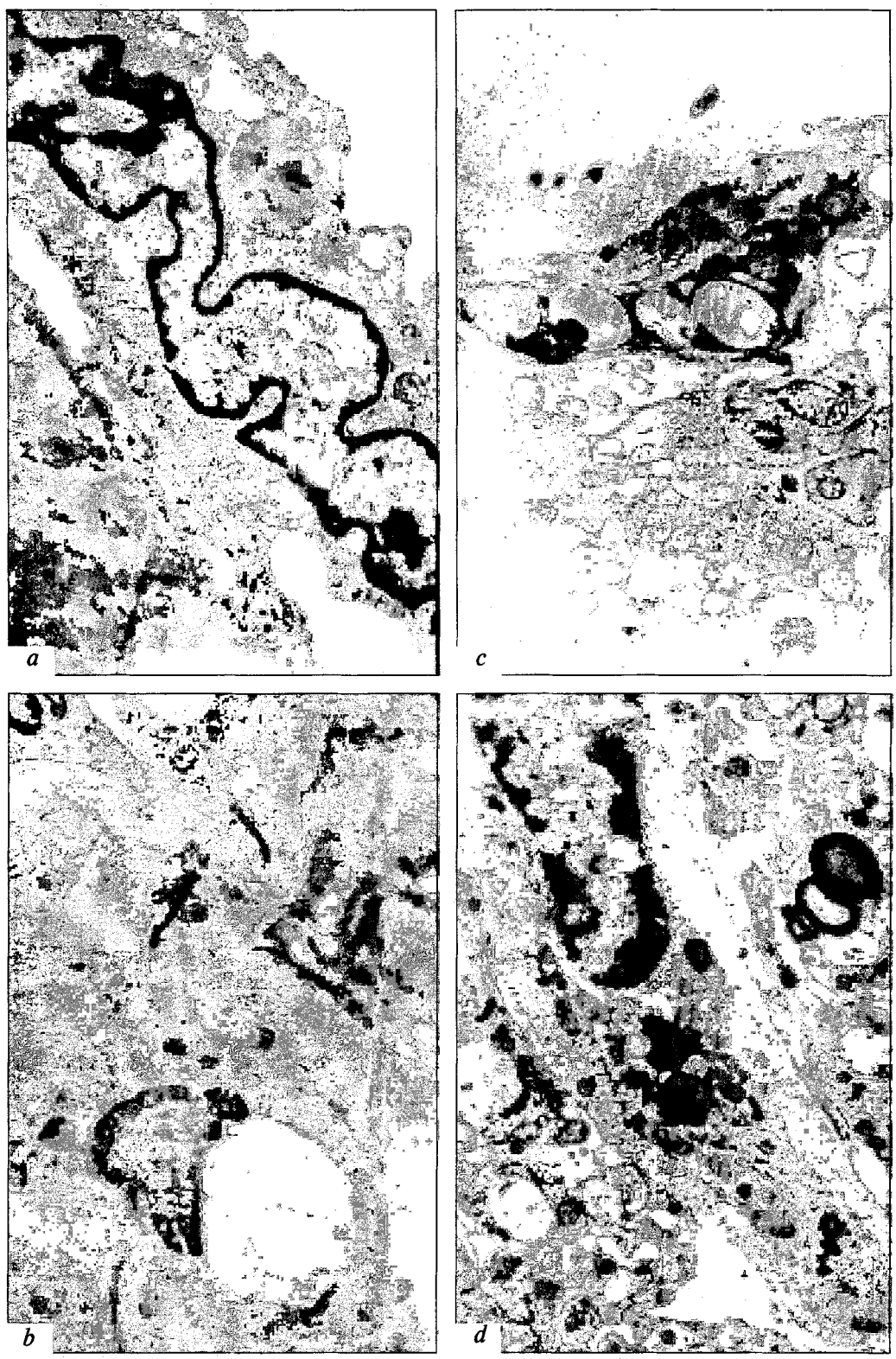

Fig. 2. Lipid inclusions in vascular cell structures and brain tissue (electron microscopy). *a)* large lipid droplet and multiple vacuoles in the apical parts of endotheliocyte in the brain surface artery, ×30,000; *b)* lipid droplets and large vacuole containing fine granular material of moderate electron density in arterial myocytes, ×18,750; *c)* multiple lipid droplets in pericyte of intracerebral vessel, ×33,750; *d)* deposition of lipids and lipid-lipofuscine complexes in astrocyte cytoplasm, ×15,000.

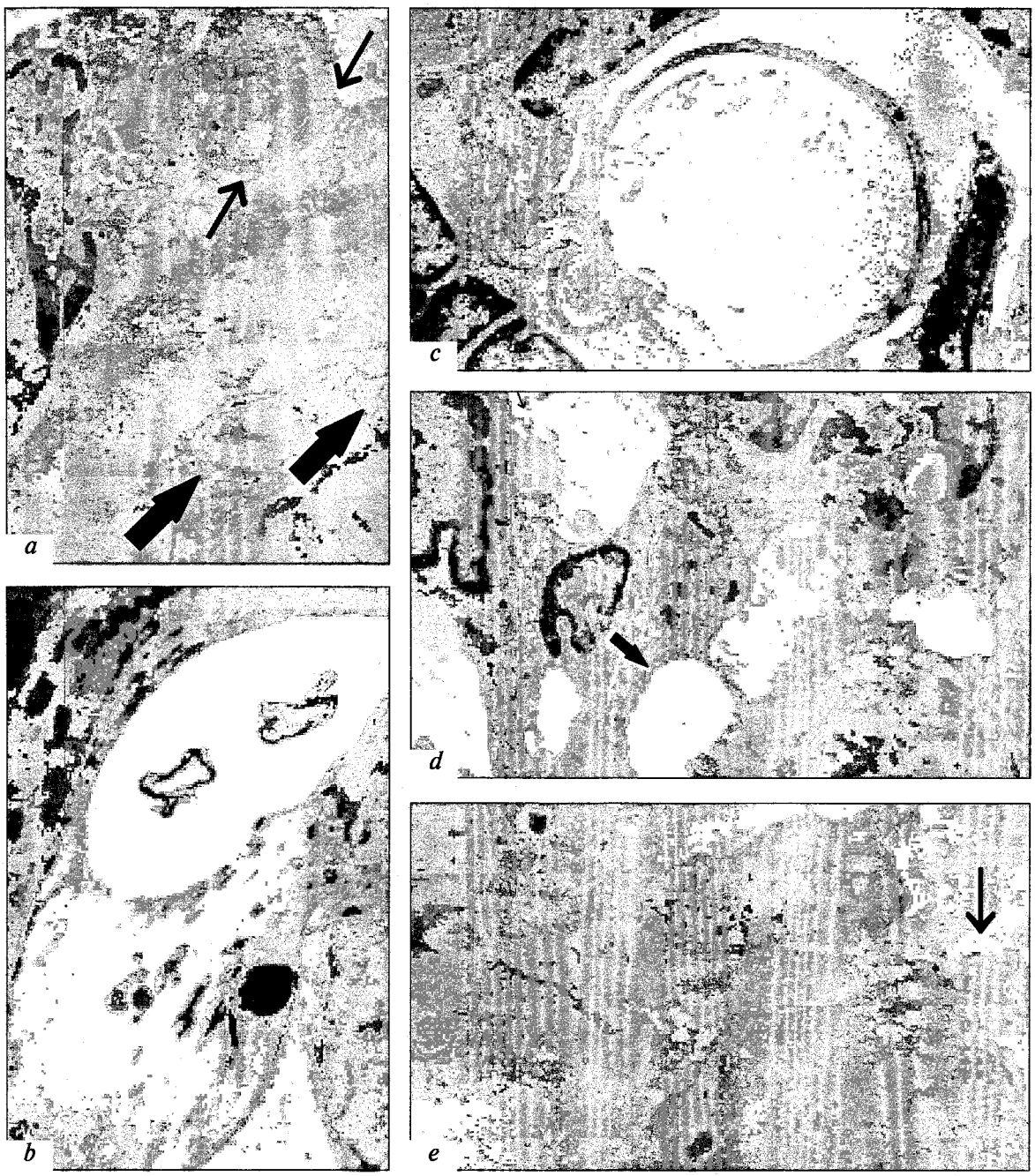

Fig. 3. Nonspecific changes in different cell structures of cerebral vessels (electron microscopy). *a)* apical processes of endotheliocytes with multiple branches, platelet adhesion to endothelial surface (small arrows), vacuoles among collagen fibers in the muscular layer of artery (big arrows), ×30,000; *b)* large vacuole with membranous structures in the cytoplasm of cerebral artery myocyte, ×30,000; *c)* large vacuole occupies almost the entire area of endotheliocyte and bordering with internal elastic membrane, ×30,000; *d)* multiple large vacuoles in the apical and basal parts of endotheliocytes bordering with vascular lumen (small arrow) and basal membrane (big arrow), ×25,000; *e)* endotheliocyte degeneration, exposed basal membrane (arrow), ×45,000.

and frequent than in the heart, liver, and other organs, while nonspecific changes in the endothelium, especially the formation of multiple apical processes protruding into the vascular lumen, were most typical. These changes affect negatively the major endothelial function – the formation of athrombogenic internal surface [14]. This dysfunction promotes fixation of different blood cells to the endothelial surface in both small and large arteries. Multiple small vesicles and large vacuoles in the apical parts and their presence in the central and basal parts of endotheliocytes attests to enhanced transport through the endothelium and

increased vascular permeability. Damage to the apical parts or the whole endotheliocytes, followed by disruption of cell membranes and the exposure of subendothelial structures observed in some cerebral vessels disturb the transmembrane transport, in particular lipid transport and promotes thrombosis. Structural and functional changes in the endothelium can lead to changes in other layers of the vascular wall [12].

Thus, HLP is characterized by accumulation of lipids in endotheliocytes, myocytes, and pericytes of cerebral arteries of different animal species and by nonspecific functional changes in endotheliocytes indicating increased transendothelial transport and impaired athrombogenic properties of the vascular wall. Destructive changes in endotheliocytes are less common. It can be assumed that HLP-induced functional and structural changes in cerebral vessels should be considered as precondition for the development of typical atherosclerotic changes. It is important to note that brain vessels undergo less pronounced changes than vessels of internal organs probably due to morphogenetic specificity of brain vascular endothelium.

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