STRUCTURAL CHANGES IN THE MICROCIRCULATORY BED AND PERIVASCULAR CONNECTIVE TISSUE OF THE MYOCARDIUM IN EXPERIMENTAL HYPERCHOLESTEREMIA

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KEY WORDS: microcirculatory bed; myocardium; hypercholesteremia; lipid metabolism.

It is now considered that a combination of at least two risk factors, such as injury to the lung vessel wall and disturbance of lipid metabolism, are necessary before atherosclerotic changes can arise [9].

In hyperlipoproteinemia, atherogenic lipoproteins (low- and very low-density lipoproteins), possessing antigenic properties, circulate in the blood stream and can penetrate into the vessel wall [1, 2, 5, 8].

Until recently the attention of workers studying mechanisms of development of atherosclerosis was directed mainly toward the state of the large vessels. Changes in intramural arteries of organs, their small branches, and the microcirculatory bed of organs have received less study, especially in the early stages of atherogenesis.

The aim of this investigation was to study the effect of hypercholesteremia (HC) on the microcirculatory bed and other components of functional structures [6] of the myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits. Material for investigation was taken 1, 3, 6, 15, 24, and 48 h after administration of a single dose (0.5 g/kg) of cholesterol with the diet (group 1), and on the 2nd and 30th days of keeping the animals on an atherogenic diet, as in N. N. Anichkov's model (group 2). Intact animals and rabbits receiving only carrots (group 3) served as the control. The total number of animals used was 40. Methods of light (staining with hematoxylin–eosin and by Goldman's and van Gieson's methods) and electron microscopy were used. Pieces of the wall of the left ventricle and the left posterior papillary muscle of the heart were taken for electron-microscopic study, fixed for 1 h in 1% buffered osmic acid solution at 0°C, and embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate by Reynolds' method and examined in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

Starting with the first few hours (1, 3, and 6 h) of the experiment structural changes and intra- and extravascular changes were observed in the microvessels of the myocardium. They were most marked 15 and 48 h after the beginning of the experiments, when the blood cholesterol level had increased by 297 and 100%, respectively, above the initial level. Most of the microvessels, mainly venules, were dilated at these times. Signs of edema, a reduction in the number of organelles and in the intensity of micropinocytosis were observed in the endotheliocytes, and numerous cytoplasmic projections were present on the luminal surface; the basement membrane was loose in texture and thickened (Fig. 1). Aggregates of erythrocytes and evidence of stasis were found in the lumen of the microvessels (Fig. 1b), and the interendothelial spaces were widened (up to 20-30 nm) and filled along their whole length with amorphous material with increased electron density. Macrophages, in close proximity to the basement membrane, could be seen in the immediate neighborhood of these spaces, and the phagosomes of these cells contained many large lipid drops (Fig. 1d). During the first 3-6 h changes such as these were found only in individual microvessels and single spaces. After 15 h the number of dilated spaces was appreciably greater and, finally, after 48 h these changes were observed in many microvessels and in most spaces of each individual vessel.

Unique and, evidently, characteristic changes for this type of pathology were discovered in the elastic membranes of the arterioles and perivascular connective tissue surrounding the metarterioles, venules, and capillaries. Small round or oval electron-dense formations of similar type were found in the ground substance of the connective tissue close to the basement membrane, ranging in size from 50-60 to 100 nm; in some places they were grouped into spherical accumulations (Fig. 2a, c).

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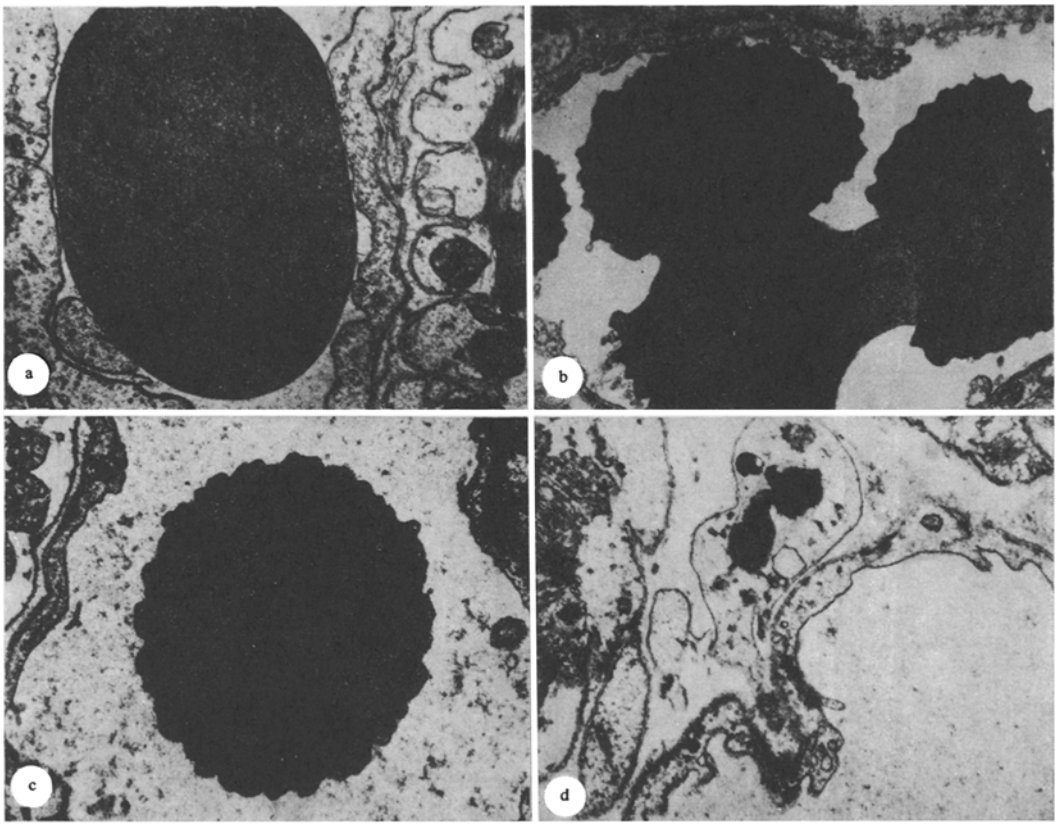

Fig. 1. Microvessels in myocardium of a rabbit with experimental HC. a) After 15 h of experiment: hydration of endotheliocytes, reduced micropinocytosis, subsarcolemmal edema of cardiomyocytes, unwinding of myofilaments with partial destruction. Erythrocyte with smooth surface can be seen in capillary lumen; b) after 48 h of experiment: stasis of erythrocytes with altered surface in capillary; c) after 15 h of experiment: erythrocyte with altered surface in lumen of dilated venule; d) after 48 h of experiment: interendothelial spaces filled with amorphous material with increased electron density, lipid inclusions present in macrophage. Magnification: a, c) 13,000 × b, d) 9750 ×.

About 60 to 70 round bodies were counted in each of these groups (more than 300 around the venules). In some cases these formulations were closely connected with the fibrous structures of the connective tissue, and the electron density of the latter was increased (Fig. 2d). It is important to note that the small formations could merge together to form larger figures with complex shapes. This process was most marked in the inner eleastic membrane of the aterioles (Fig. 2b). The length of these figures was about 1 μ and their width 0.35-0.5 μ. These structures were found much less frequently in the pericapillary connective tissue. It is important to note that changes of this sort were found in the surface of most erythrocytes, in the form of multiple (26-30) round and pointed, regularly alternating projections (Fig. 1a, b, c); they effectively increased the diameter of the erythrocytes (to 7.6 μ from the normal 5 μ). Subsarcolemmal edema, focal degenerative changes, death of individual myofilaments, and the presence of sarcoplasmic inclusions (lipid drops, secondary lysosomes, etc.), together with destructive changes in the mitochondria, were observed in the myocytes (Fig. 1a).

In animals put onto an atherogenic diet (2nd-30th days) changes in the capacitive component of the microcirculatory bed increased: Its tone was high and there was resistance to the blood flow in vessels of venous type. At these times structural changes could be observed in the small intramural arteries of the myocardium, which were converted into arteries of closing type, with the formation of so-called Conti's pillows, followed by deposition of lipids in these formations, i.e., the formation of atherosclerotic plaques.

Consequently, in the early stages of experimental HC unusual ultrastructures were found in the elastic membranes of the ateriole, the amorphous material, and fibrous structures of the perivascular connective tissue of the venules and capillaries of the myocardium of rabbits. By their electron density and other distinguishing features they were characteristic of lipids at this level. The appearance of amorphous material of increased electron density in the widened interendothelial spaces of the exchange microvessels may evidently be connected with lipid transport; a certain proportion of the lipids under these circumstances undergo phagocytosis by macrophages.

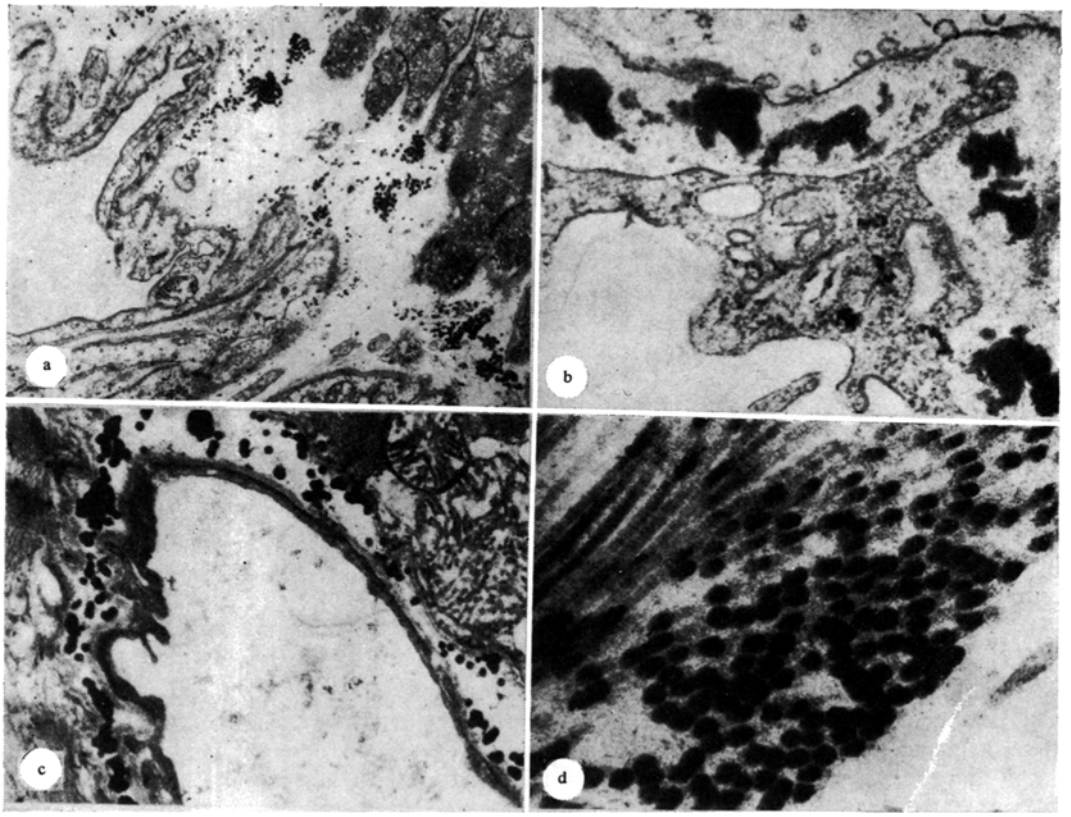

Fig. 2. Vascular and extravascular changes in myocardium of rabbit with experimental HC. a, b) After 15 h of experiment. a) Multiple uniform electron-dense formations visible in perivascular space; b) confluent electron-dense structures of unusual shape visible in elastic membrane of ateriole. c, d) After 48 h of experiment. c) Similar structures located in amorphous material of perivascular connective tissue; d) connection of electron-dense particles with collagen fibrils of connective tissue. Magnification: a) 9750 X, b, d) 53,000 X, c) 26,000 X.

What can be said about the nature of the ultrastructures discovered? Carbohydrate–protein complexes (proteoglycans) are known to be among the important components of the amorphous material and fibrous structures of connective tissue. Some of them, especially glycosaminoglycans (GAG), are able to form insoluble complexes with atherogenic lipoproteins (ALP) in the vessel wall [3, 5, 7]. Accumulation of GAG in the large vessels is regarded at the present time as a protective-adaptive response to endothelial injury and to the increased inflow of ALP into the vessel wall [5]. This inflow of ALP undoubtedly took place in the present experiments. It is also supported by data in the literature. For instance, immunomorphologic studies have revealed ALP on the elastic membranes of large vessels and the appearance of antibodies against elastin of the vessel wall in the early stages of atherogenesis [1].

Analysis of our own data and of information in the literature thus suggests that the ultrastructures described are complexes of ALP with GAG.

The changes described above in the erythrocyte surface are evidently also connected with the action of HC. It is stated in the literature that in HC cholesterol can penetrate into erythrocyte membranes and damage them [4].

On the whole, the structural changes found in the microcirculatory bed of the myocardium of the experimental animals and the intra- and extravascular changes are evidence that HC leads to marked disturbances of permeability and of the rheologic properties of the blood. Microcirculatory disturbances and the development of degenerative changes in the cardiomyocytes are evidently results of these changes. Injury to the microcirculatory bed and other components of functional structures of the myocardium evidently plays the initial role in the development of coronary atherosclerosis.

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AGE DIFFERENCES IN THE ATHEROGENIC EFFECT OF DIFFERENT FORMS OF HYPERLIPOPROTEINEMIA ON THE AVIAN VASCULAR WALL

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The development of atherosclerosis and of its clinical manifestations is linked with the existence of risk factors, among the most important of which are disturbances in lipid and lipoprotein metabolism [2, 8]. It is generally considered that those types of hyperlipoproteinemia (HLP) that are characterized by an increased blood level of low-density lipoproteins (LDLP) and very low-density lipoproteins (VLDLP), namely types II, III, and IV, are the most atherogenic. HLP due to the accumulation of chylomicrons (CM) in the blood do not give rise to any significant atherosclerotic lesions in blood vessels [9, 11]. The character of the atherogenic effect of different types of HLP has not been studied from the age aspect, although we know that the number of persons with disturbances of lipid and lipoprotein metabolism increases with age [6, 13], as also does mortality from cardiovascular diseases of atherosclerotic origin [3]. HLP obtained in individuals following administration of cholesterol and intramuscular injection of estrogenic preparations provide convenient experimental models for the study of differences in the effects of HLP of different origin [12].

The object of this investigation was an experimental study of the atherogenicity of different types of HLP in young and old cocks.

EXPERIMENTAL METHOD

Cocks of the Russian White breed, aged 5-6 months and 3.5-4 years, were used. HLP and atherosclerosis were induced in the birds by peroral administration of cholesterol (CS) in a dose of 2 g/kg body weight (50% solution in sunflower oil) for 4 months or by intramuscular injection of the estrogen diethylstilbestrol propionate (DESP) as a 0.5% oily solution in a dose of 5 mg/kg body weight 3 times a week for 4-5 months. In order to identify the character of the disturbances in the lipid and lipoprotein composition of the blood, total CS, triacylglycerols (TG), the total TG-rich fraction of lipoproteins (CM), VLDLP and LDLP (total lipoprotein fraction – TLPF), and the lipoprotein spectrum were determined on the basis of the technical recommendations of Klimov and Ganelina [4]. To measure the content of total CS and TG in the aortic tissue, a total lipid extract was first obtained [10]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Administration of CS or DESP caused inequal changes in the blood lipid and lipoprotein levels in young and old cocks (Table 1). The absolute content of TLPF, total CS, and TG increased more in the birds receiving DESP, irrespective of age. Analysis of the lipid and lipoprotein spectrum shows that hyperlipidemia and HLP in birds of this group were due to a predominant increase in the content of TG and CM, respectively. The total CS/TG ratio was much lower in the birds after injection of DESP than in normal birds, thus confirming that they had indeed developed hypertriacylglycerolemia.

Peroral administration of CS caused hypercholesteremia, an increase in the total CS/TG ratio, and accumulation of LDLP and VLDLP in the blood. In this case a higher level of HLP was found in birds aged 3.5-4 years than in those aged

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