PATTERN OF DEVELOPMENT OF CONNECTIVE TISSUE IN THE AORTIC WALL OF RABBITS WITH EXPERIMENTAL ATHEROSCLEROSIS

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KEY WORDS: autoradiography; modified lipoproteins; cell proliferation; collagen synthesis.

The basis of atherosclerotic plaques consists of foam cells and connective tissue. Whereas sufficient attention has been paid [2] to the processes of foam cell formation, due to interaction between modified lipoproteins (LP) and monocytes—macrophages and settled cells of the vascular wall, the desmoplastic reaction developing in response to deposition of excessive amounts of apo-B,E-containing LP in the intima of arteries have virtually not been studied. Yet we know that more than 40% of the volume of fibrous atherosclerotic plaques is accounted for by connective tissue [1]. Evidence has been obtained that collagen of atherosclerotic plaques is synthesized by the smooth-muscle cells (SMC) of the vascular wall [8]. On this basis it has been suggested that atherosclerosis be regarded as a disease of the mesenchymal cells of the arterial wall [5]. Under normal conditions SMC have been found to synthesize mainly type III collagen, which accounts for about 70% of the total volume [6], or even 100% [4], whereas in atherosclerotic plaques about 65% of the collagen is of type I [7], which is known to be synthesized mainly by fibroblasts. Problems relating to the mechanisms of collagen production in atherosclerosis and the nature of the cells involved in this process remain unsolved.

The aim of this investigation was a combined autoradiographic study of the structural and functional characteristics of cells involved in collagen synthesis during the development of experimental hypocholesterolemia in rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 24 rabbits weighing 2.8-3 kg, kept on an atherogenic diet (0.2 g cholesterol in 5 ml of sunflower oil per os, through a tube, 5 times a week) or 4, 6, 8, 12, and 16 weeks. Lipid stains and atherosclerotic plaques were investigated. Unchanged areas of the vascular wall taken at the same times during the experiment served as the control. An electron-microscopic autoradiographic study was made of the aorta of 13 rabbits, using ^{3}H-thymidine (specific activity 25 Ci/mole) in a dose of 20 μ Ci/g body weight given 5 times in the course of 24 h. To study collagen synthesis, histoautoradiography and electron-microscopic autoradiography were used. Experiments were carried out on 11 animals. Histoautoradiography was used to investigate 40 pieces of aorta taken from five animals (one rabbit for each time), all of which had previously received a simultaneous intravenous injection of ^{3}H-proline (specific activity 4 Ci/mmole) in a dose of 6 μCi/g body weight, and labeled precursor of collagen synthesis. Electron-microscopic autoradiography was carried out on six animals (20 pieces taken from one aorta were tested), using ^{14}C-hydroxyproline in a dose of 20 μCi/ml medium (medium 199 + 10% bovine serum), in which the material was incubated for 30 min at 37°C during ventilation with CO_2/O_2. The pieces of tissue were then washed with several portions of Hanks's solution at 4°C and fixed. In parallel tests pieces of tissue were taken from the same sites of the aortic wall and, before incubation with the isotopes, they were predigested with collagenase (type I, activity 200 unit/mg), in the proportion of 10 ml medium 199 to 1400 unit collagenase, with the addition of antibiotics and albumin, at pH 7.4 and 37°C for 6 h, to serve as the control for specificity of ^{14}C-hydroxyproline incorporation. In the experiments with histoautoradiography the material was fixed in Bouin's

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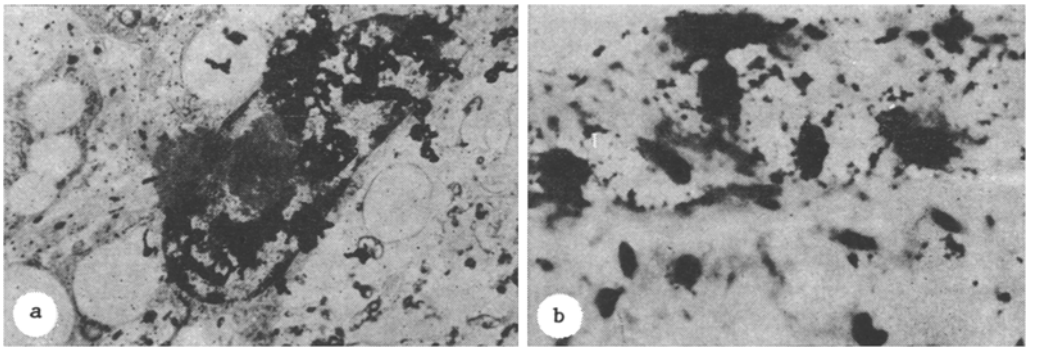

Fig. 1. Lipid stain in rabbit aorta. a) Incorporation of ^{3}H-thymidine into nucleus of muscle cell. Electron-microscopic autoradiographs. 46,000×; b) Accumulation of ^{3}H-proline in zone of lipid stain. Hematoxylin and eosin. 1200×.

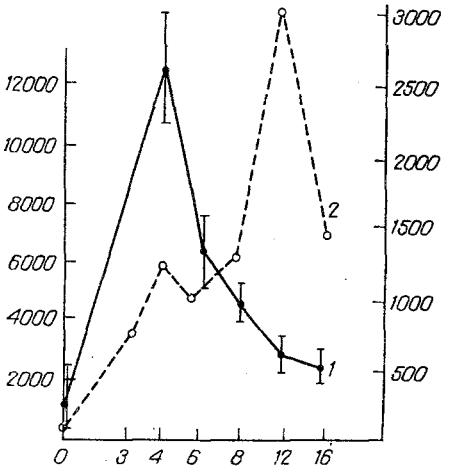

Fig. 2. Time course of cell proliferation in zone of atherosclerotic lesions of the aorta. Abscissa, duration of experiment (in weeks); ordinate; on left) total proliferative activity of cells (index of labeled cell nuclei), on right) plasma cholesterol level (in mg %). 1) Plaque cells; 2) plasma cholesterol.

fluid, and Ilford K-5 photographic emulsion was used in a dilution of 1:2; the specimens were exposed for 2 weeks and counterstained with hematoxylin and eosin. The intensity of protein synthesis was estimated quantitatively by the PLAG-method on the MIA-11 apparatus (probe 2, 20 probings with each block). Differences were considered to be significant for which $p < 0.05$ and $t > 2.00$. In the experiments with electron-microscopic autoradiography the material was fixed in 2.5% glutaraldehyde by the method described previously [3]; Ilford L-4 photographic emulsion was used in a dilution of 1:9, and applied to ultrathin sections cut on the LKB-III ultratome, and the samples were exposed for 40 days. Material was examined in the JEM-100B and EMV-100L microscopes.

Histologic sections also were stained with Oil Red O and hematoxylin and eosin, and by Van Gieson's and Foot's methods to study the structure of the lipid stains and plaques. The plasma cholesterol level was determined with the Technicon AA-II automatic analyzer.

EXPERIMENTAL RESULTS

Incorporation of ^{3}H-thymidine into the nuclei of monocyte–macrophages, endothelial cells, and SMC was observed in the newly formed lipid stains (4 and 6 weeks of the experiment; Fig. 1a). The writers previously showed that the first foci of proliferation are connected with sites of deposition of apo-B-containing LP [3]. After 4 weeks of the experi-

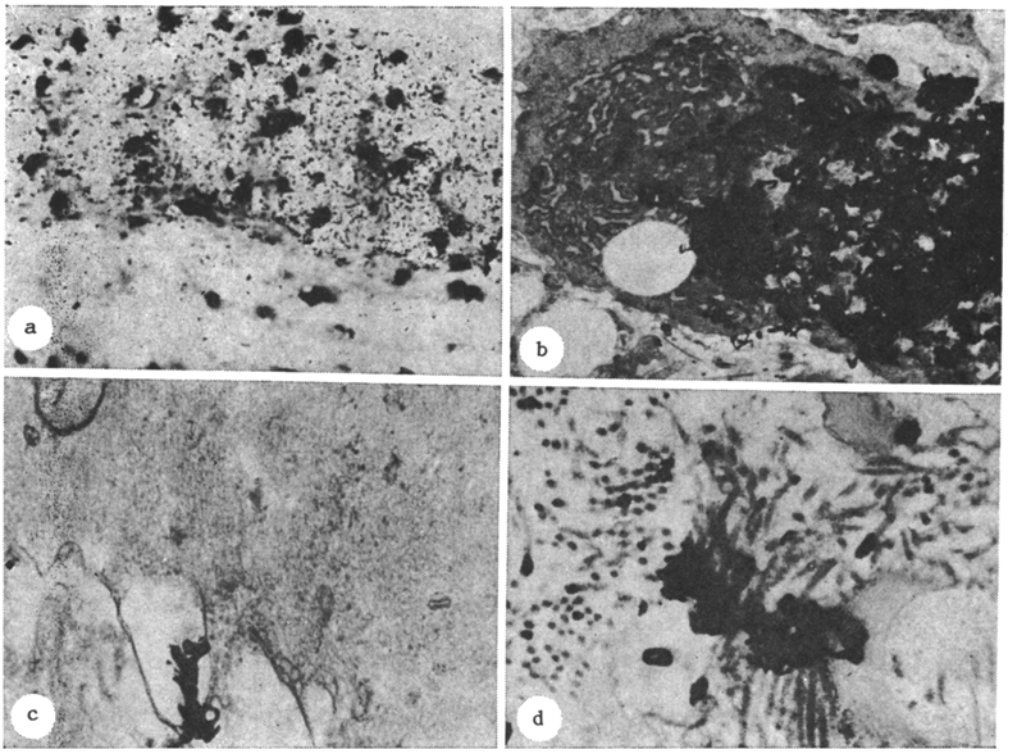

Fig. 3. Desmoplastic reactions in atherosclerotic plaques (autoradiography). a) Accumulation of ^{3}H-proline through full thickness of plaque. Hematoxylin and eosin. 640×; b) Incorporation of ^{3}H-thymidine into nucleus of a fibroblast. 46,000×; c) ^{14}C-hydroxyproline concentration in zone of rarefaction and translucency of hyaloplasm of SMC. 48,000×; d) Incorporation of ^{14}C-hydroxyproline into collagen fibrils surrounding foam cells.

ment, focal accumulation of ^{3}H-proline was observed only in the intima in the zone of SMC proliferation and of serofibrinous edema (Fig. 1b). Quantitative photometry showed that synthesis of fibrillary proteins in the lipid stains was about 10 times greater in intensity than in the unchanged regions of the intima and media of the aorta (Fig. 2). For example, incorporation of ^{3}H-proline in the zone of atherosclerotic changes was 12,463 ± 2041 (relative units), compared with 1205 ± 390 in the media of the aorta. The more marked the process of formation of lipids stained, transformed into atherosclerotic plaques, the greater the incorporation of ^{3}H-proline in the intima (Fig. 3a).

The electron-microscopic investigation showed that in the initial stages of atherogenesis collagen synthesis was connected with activated SMC, migrating into the intima through fenestration of the inner elastic membranes, and taking part in division. The structure of the contractile apparatus in these cells was ill defined, and their cytoplasm contained large numbers of coated secretory vacuoles, the formation of which is linked with the Golgi complex, i.e., a definite phenotype of SMC, actively participating in desmoplastic reactions, was formed. In transverse sections through the growth bulbs of SMC it would be clearly seen that they were filled with a flocculent material, containing very fine fibrils. Collagen fibrils in the extracellular space also were connected with membranes of growth bulbs. The use of ^{14}C-hydroxyproline showed that synthesis of collagen precursors took place in the cisterns of the rough endoplasmic reticulum. Analysis of the autoradiographs revealed the label distributed above the endoplasmic reticulum and Golgi complex, even in cells containing lipid vacuoles in their cytoplasm.

During the development of hypercholesterolemia and atherosclerotic plaque formation (12-16 weeks of the experiment) redistribution of ^{3}H-proline incorporation took place in the zone of the lesions. General quantitative analysis of ^{3}H-proline incorporation into the plaques revealed a decrease of radioactivity with an increase in the duration of the experiment (Fig. 2). At the same time, however, investigation of the atherosclerotic plaques zone by zone revealed a marked increase in isotope incorporation into the peripheral portions

TABLE 1. Intensity of Incorporation of ^{3}H-Proline into Atherosclerotic Plaques of the Rabbit Aorta in the Course of Experimental Hypercholesterolemia (in optical density units; M ± m)

Time of experiment, weeks	Atherosclerotic plaques	
	superficial part	central part
8	5618±782	3976±562
12	3484±1188	3243±830
16	3190±711	1661±303

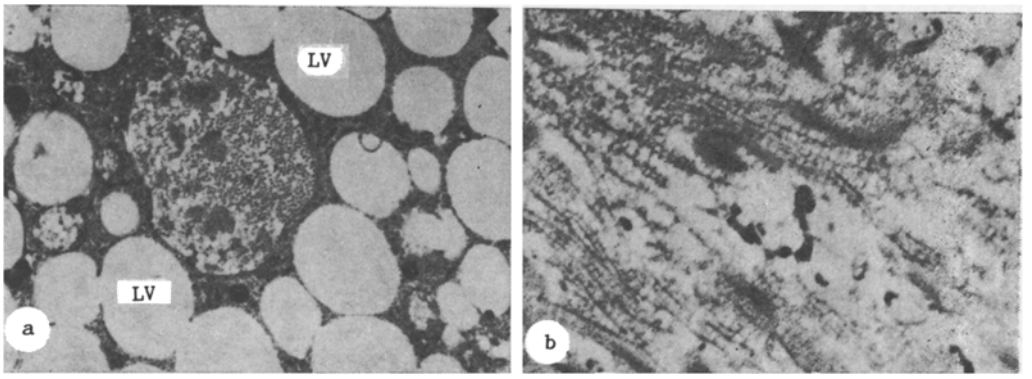

Fig. 4. Ultrastructural features of collagen formation in atherosclerotic plaques. a) Discoid formation of collagen fibrils in cytoplasm of SMC containing lipid vacuoles (LV); b) zebroid bodies surrounding disintegrating fibroblasts at apex of plaque.

and at their apex (Table 1), especially in newly formed plaques (8 weeks of the experiment). Under these circumstances ^{3}H-thymidine was incorporated mainly into fibroblast nuclei (Fig. 3b). Quantitative analysis of the proliferating cells showed that fibroblasts (68% of the total number of labeled and identified cells) divided most actively at the apex of the plaques, 20% of the cells were SMC, and 12% were monocyte–macrophages. Concentration of ^{3}H-proline was observed at sites of fibroblast accumulation. On the histoautoradiographs, accumulation of radioactive label also was observed in the atherosclerotic plaques on connective-tissue structures surrounding foam cells.

The more intensively the course of destruction of the foam cells in the central parts of the plaques, the greater the intensity of collagen synthesis (Table 1). Whereas at the apex and in the peripheral portions of the plaques mainly fibroblasts were involved in collagen synthesis, at the base of the plaques, on the boundary with the media, mainly activated SMC with pale cytolasm took part in this process.

The results of electron-microscopic autoradiography of the atherosclerotic plaques (12-16 weeks of the experiment) enabled the time course of the discharge of synthesized collagen from the cells to be studied. Concentration of ^{14}C-hydroxyproline above the endoplasmic reticulum and Golgi complex, was followed by their concentration above the ectoplasm. During elimination of the synthesized collagen precursor changes were observed in the juxtamembranous regions of the cytoplasm, incorporating ^{14}C-hydroxyproline, and the basement membrane (Fig. 3c). In these regions partial, followed by complete loosening of the structure of the hyaloplasm was observed and the basement membranes appeared fragmented. After elimination of the procollagen, the next stages of the biosynthetic process evidently took place in the zone of the outer cell membrane, which explains the incorporation of ^{14}C-hydroxyproline into the very fine fibrils, connected with the cell membrane (Fig. 3d).

Around the atheroma and disintegrating foam cells, long spindle-shaped cells (probably SMC and fibroblasts) with atypical collagen synthesis were distinguished. The formation of discoid forms of collagen was observed in the zone of the endoplasmic reticulum, surrounded by lipid vacuoles (Fig. 4a). So-called zebroid bodies also were observed among the mature

collagen fibrils, surrounded by a necrotic mass, mainly consisting of disintegrated foam cells (Fig. 4b).

Intensive collagen synthesis is thus associated with the pattern of proliferation of the intimal cells in the zone of the developing atherosclerotic lesions. Cells which have divided not only take up lipids and participate in foam cell formation, but also cause the formation of a connective-tissue stroma of the plaques. Autoradiographic investigation with the aid of ^{3}H-proline and ^{14}C-hydroxyproline showed that collagen synthesis takes place only in the intima, but in the initial stages of atherogenesis, and it is associated with the accumulation of connective-tissue cells. In the initial stages of experimental atherosclerosis SMC of synthetic phenotype are mainly involved in collagen synthesis, whereas in the progressive stages, fibroblasts are the main participants.

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SURFACTANT SYSTEM AND STRUCTURE OF THE RESPIRATORY PART OF THE LUNGS DURING DISADAPTATION AFTER A SINGLE EXPOSURE TO ACUTE PRESSURE CHAMBER ANOXIA

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The lung surfactant undergoes a series of changes in the body. According to Romanova [7], it can be conventionally divided into "immature" surfactant, consisting of the lamellar bodies of large alveolocytes, "reserve" surfactant, in the form of the tubular myelin of the hypophase, "mature" surfactant, which is a phospholipid layer separating the air and liquid phase in the alveoli, and "spent" surfactant which is phagocytosed by alveolar macrophages and eliminated through the bronchi. The alveolar macrophages and Clara cells, which are responsible for catabolism of the surfactant, are considered by Nevodnik, et al. [6] to be cellular components of the "antisurfactant system of the lungs." This subdivision (despite the questionable nature of the terminology) allows a differential approach to the evaluation of the role of changes in the lung surfactant system (LSS) in pathology. Acute anoxic anoxia causes a decrease in activity [4, 8], to a degree which depends on the intensity and duration of anoxia [5]. Electron-microscopic data suggests that the decrease in LSS activity is evidently due to a decrease in the content of lamellar bodies in the large alveolocytes and to disorganization of the surfactant on the surface of the alveoli [3]. Regression of changes in LSS during disadaptation after exposure to anoxia has received little study.

The aim of this investigation was to compare changes in the surface activity of different fractions of surfactant with the structure of the lung parenchyma during disadaptation after exposure to acute pressure chamber anoxia.

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