

THE STATE OF THE GROUND SUBSTANCE OF THE ARTERIAL WALLS IN EXPERIMENTAL ATHEROSCLEROSIS

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Opinions differ on the role of the ground substance of the arterial wall in the development of atherosclerosis. Some authors, on the basis of the study of postmortem material and experimental data, support N. N. Anichkov's view that lipids are deposited in the unchanged arterial wall; the ground substance merely adsorbs them actively [1, 6-8]. In their opinion the increase in the content of acid mucopolysaccharides in the lipoid stains and plaques takes place secondarily, following changes in the connective tissue. Other authors [2, 4, 5, 9] consider that the deposition of lipids is preceded by the accumulation of acid mucopolysaccharides in the blood vessel wall, formed as a result of disturbance of metabolism and an increase in the permeability of the vessel wall. Finally, the view is held [10] that the connection between the changes in the mucopolysaccharides and the accumulation of lipids in the vessel wall has not yet been fully explained.

The object of the present investigation was to study the role of the ground substance of the arterial wall in the genesis of atherosclerosis by examining the arteries at the period preceding the morphological manifestation of atherosclerosis, and in the various stages of development of this process.

EXPERIMENTAL METHOD

The investigation was conducted on adult dogs in which atherosclerosis was produced by the method of Steiner and Kendall.

The animals were subdivided into 4 groups. Group 1 (control) consisted of 4 dogs receiving a normal diet; group 2 of 4 dogs receiving a normal diet plus cholesterol in a dose of 0.5 g/kg body weight and methylthiouracil in a dose of 1 g daily (the experiments continued for 4, 6.5, and 9.5 months); group 3 of 5 dogs receiving an ordinary diet plus cholesterol in a dose of 2 g/kg body weight and methylthiouracil in a dose of 2.5-3 g/kg body weight daily (the experiments continued for 6, 8, and 8.5 months); group 4 of 5 dogs receiving a normal diet plus 0.5 g cholesterol and 1 g methylthiouracil daily for the first 5-9 months, followed by 2 g cholesterol and 2.5-3 g daily (these experiments continued for 10.5, 11.5, 12.5, and 17 months).

After the end of the experiment the dogs were killed with ether or by air embolism. All arteries visible to the unaided eye were studied macroscopically. Some vessels were totally stained with Sudan III. Histological studies were made of all portions of this aorta, and the pulmonary, carotid, iliac, and coronary arteries. The material was fixed in 10% neutral formalin and in Carnoy's fluid. Sections were stained with hematoxylin and by Van Gieson's method. Mucopolysaccharides were detected by the PAS reaction, with control treatment with amylase, acid mucopolysaccharides with alcian blue (control sections were treated with hyaluronidase and lidase), and ribonucleic acid was detected by Brachet's method (control sections were treated with ribonuclease). Lipids in the frozen and embedded sections were stained with Sudan black.

EXPERIMENTAL RESULTS

In the dogs of the control group the intima of the arteries appeared smooth and shining to the naked eye. Treatment of total preparations with Sudan III revealed no deposition of lipids. The histological structure of the

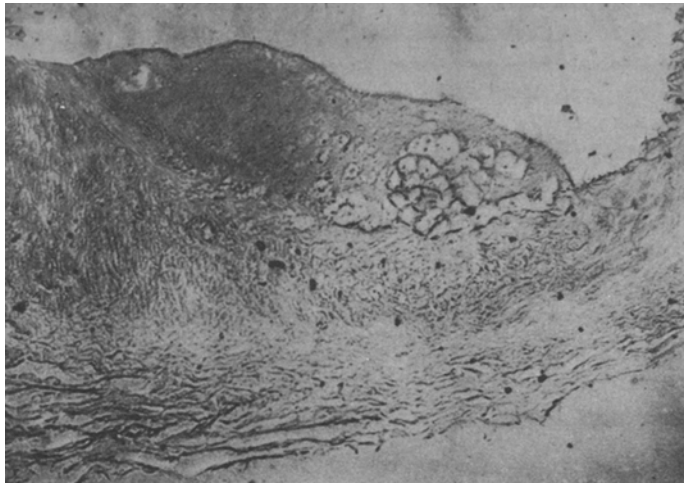


Fig. 1. Increase in content of acid mucopolysaccharides in a plaque in the aorta. Group 4. Duration of experiment 17 months. Alcian blue. Low power.

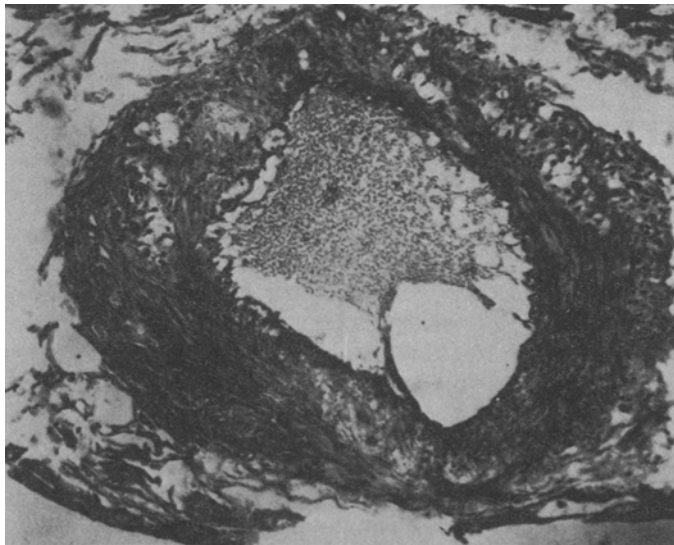


Fig. 2. Increase in content of mucopolysaccharides in the wall of an artery around xanthoma cells. Group 4. Duration of experiment 10.5 months. PAS reaction and Ehrlich's hemotoxylin. Low power.

vessels corresponded to that usually described. Mucopolysaccharides and acid mucopolysaccharides were distributed along the course of the fibrous structures in the intima and in the adjoining third of the media. In the remainder of the media and in the adventitia, Schiff's reagent and alcian blue gave a weak positive reaction in circumscribed areas. The distribution of ribonucleic acid in the wall of the arteries was the same as that of the mucopolysaccharides. In the aorta and the pulmonary and carotid arteries diffuse or localized thickenings of the intima, formed of connective tissue, were constantly found. In the media of the aorta and of the pulmonary and iliac arteries there were a few small scars. These were situated in the inner and middle parts of the media and consisted of hyalinized connective tissue. These changes were attributed to age. Schiff's reagent and alcian blue gave a weak positive reaction in the thickenings of the intima and the scars of the media. Brachet's reaction was also weakly positive in these areas.

In all the dogs receiving cholesterol and methylthiouracil, hypercholesteremia developed. The blood cholesterol concentration, determined by the method of King and Sackett, had a mean value during the experiment of

302-433 mg% in group 2, 348-660 mg% in group 3, and 399-681 mg% in group 4. The initial cholesterol concentration was 86-191 mg%. (These figures were kindly made available by O. E. Tsukershtein, T. A. Panfilova, and M. M. Vol'pe, who carried out the clinical and biochemical investigation of the experimental dogs.)

Morphological examination showed the presence of atherosclerosis of the aorta in all the dogs of group 2; in 2 animals of group 3 (duration of experiment 6 and 8.5 months) atherosclerosis was found in the aorta and pulmonary artery and in 1 dog no changes were present; in group 4 all the animals had atherosclerosis of the aorta and, in addition, atherosclerosis was found in the coronary arteries of 2 dogs (duration of experiment 10.5 and 12 months), in the pulmonary artery of 2 (duration of experiment 12.5 and 17 months), and in the carotid arteries of 1 dog (duration of experiment 10.5 months).

Histological examination of the intima and media of the aorta, and the pulmonary and carotid arteries of all the dogs revealed age changes analogous to those in the animals of the control group. The earliest atherosclerotic changes in the dogs of all the groups took the form of lipid infiltration of the aorta and of the pulmonary, carotid, and coronary arteries. Lipids were deposited diffusely in the intima in the form of dustlike droplets. Corresponding areas of the media were also infiltrated with lipids. The distribution of the mucopolysaccharides, acid mucopolysaccharides, and ribonucleic acid in the areas of lipid infiltration in some arteries was the same as in the animals of the control group. In most vessels in these areas Schiff's reagent and alcian blue gave a more intensive color reaction than in the remainder of the vessel wall. Brachet's reaction was also more intensive in the foci of lipid infiltration than in the unchanged parts of the wall. In the dogs of groups 3 and 4, besides lipoidosis, other signs characteristic of the later stages of atherosclerosis were present. For instance, in the subepicardial branches of the coronary arteries some absorption of lipids was taking place, as shown by the accumulation of xanthoma cells in the media. The reaction for mucopolysaccharides and ribonucleic acid was more intensive in these areas than in the unchanged wall. Acid mucopolysaccharides were detected mainly around the xanthoma cells. In the aorta and coronary arteries plaques consisting of xanthoma cells and loose connective tissue also were found. In the aorta the plaques were situated in the intima and, partly, in the media. In the coronary arteries they lay mainly in the media, between the separated muscle fibers. Schiff's reagent and alcian blue gave a more intensive reaction in the plaques than in the unchanged vessel wall. Mucopolysaccharides were distributed uniformly in the plaque, but acid mucopolysaccharides reacted most intensively around the xanthoma cells (Figs. 1 and 2). Small plaques of loose connective tissue with slight infiltration with lipids were found in the pulmonary artery. The reaction for mucopolysaccharides and acid mucopolysaccharides in these plaques was more intensive than in the neighboring parts of the vessel wall.

Hence, in all the dogs receiving cholesterol and methylthiouracil hypercholesteremia was observed, and in 11 animals this was accompanied by atherosclerosis, localized in the aorta, and the pulmonary, carotid, and coronary arteries. The development and spread of atherosclerosis in different parts of the arterial system depended on the dose of cholesterol and methylthiouracil and on the duration of their administration. The individual susceptibility of the animals also played a part. Various stages of atherosclerosis were observed, both early (lipoid stains with a diffuse deposition of lipids or with absorption of lipids and clustering of xanthoma cells) and later — plaques. Besides the intima, the media of the investigated arteries was also affected, with marked absorption of lipids and an ill defined fibroblastic reaction, i.e., the characteristic changes of experimental atherosclerosis in dogs [1, 4, 6].

These observations confirm the data given in relation to the topography of the mucopolysaccharides in the normal arterial wall. It was also observed that the distribution of ribonucleic acid in the vessel wall was the same as that of mucopolysaccharides. Histological and histochemical investigations of the ground substance of the vessel wall of dogs receiving cholesterol and methylthiouracil showed no differences in the quantity and topography of the mucopolysaccharides, the acid mucopolysaccharides, and the ribonucleic acid in the wall of the different arteries in areas where no atherosclerotic changes were present. In the areas of lipid infiltration of the intima and media, the tissue stained more intensively with Schiff's reagent and alcian blue. In the newly formed, loosely built atherosclerotic plaques Schiff's reagent and alcian blue also gave a more intensive color reaction than in the intima and media outside the pathological focus. The reaction with alcian blue was most marked around the xanthoma cells. The reaction for ribonucleic acid was also more intensive and extensive in the lipid patches and the plaques than in the normal arterial wall. This shows that the content of acid mucopolysaccharides and ribonucleic acid was increased in the areas of deposition of lipids in the arterial wall. The accumulation of acid mucopolysaccharides and ribonucleic acid took place secondarily, after the deposition of lipids. This was demonstrated by the absence of changes in the mucopolysaccharides and ribonucleic acid in the vessel wall of the dogs with hypercholesteremia but

without morphological manifestations of atherosclerosis and in the dogs with atherosclerosis but without lipid deposits. After enzymic treatment of the sections, the reactions of Schiff and Brachet, and with alcian blue were negative. This shows that the substances accumulating in the lipid patches and plaques were mucopolysaccharides, acid mucopolysaccharides, and ribonucleic acid.

SUMMARY

Twelve dogs with experimental atherosclerosis induced after Steiner and Kendall's method were carried out to study the state of the intermediate substance of the arterial wall. The application of histochemical methods made it possible to show that in experimental atherosclerosis the deposition of lipoids is followed by an increase in the amount of acid mucopolysaccharides and ribonucleic acid in the lipid patches and plaques. Prior to deposition of lipoids, the intermediate substance shows no visible morphological changes in spite of the presence of hypercholesterolemia.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
