CHANGES IN THE TISSUE-ENZYME PROFILE OF THE RABBIT AORTA IN EARLY STAGES OF EXPERIMENTAL ATHEROSCLEROSIS

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The results of a comparative microspectrophotometric investigation have shown quantitative changes in the tissue profile of activity of oxidative enzymes in the rabbit aorta at the third week of experimental hypercholesteremia. The role of metabolic disturbances and also of accumulation of α-glycerophosphate for lipid synthesis in the aortic wall in the early stages of experimental atherosclerosis is emphasized.

Despite many investigations of atherosclerosis, modern views regarding the metabolism of the vessel well under normal conditions and at various stages of development of atherosclerosis are contradictory. No information concerning the relationship between the degree of spread of atherosclerotic changes and the character of metabolic disturbances in the vessel wall can be found in the literature.

The object of the present investigation was to study quantitative changes in the activity of some oxidative enzymes (glutamate dehydrogenase, connected with protein metabolism, α-glycerophosphate dehydrogenases, connected with carbohydrate and lipid metabolism, and succinate dehydrogenase, participating in the Krebs cycle) in the aorta of rabbits under normal conditions and in the early stages of experimental atherosclerosis.

EXPERIMENTAL METHOD

The experimental animals were 30 sexually mature male chinchilla rabbits. Experimental atherosclerosis was induced by N. N. Anichkov's method by giving the animals cholesterol daily with vegetables at the rate of 0.2 g/kg body weight [9]. The animals were sacrificed after feeding for 4, 8, 12, 16, 24, 32, and 64 days. Atherosclerotic changes in the aorta were assessed by the use of Avtandilov's planimetric ruler [1]. Some of the material was fixed in 10% neutral formalin and embedded in paraffin wax. Sections were stained with hematoxylin-eosin, by Van Gieson's method, and with Sudan black. Another part of the material was frozen with solid carbon dioxide, sections of unfixed tissue were cut to a thickness of 12μ in a cryostat, and tests were made from glutamate dehydrogenase (GDH), cytoplasmic α-glycerophosphate dehydrogenase (α-GPDHC) by Kul'tas's method [7], mitochondrial α-glycerophosphate dehydrogenase (α-GPDHM), and succinate dehydrogenase (SDH) by the method of Nachlas et al. [11], with the addition of menadione to the incubation medium in a final concentration of 0.05 M. Sections incubated in medium without substrate were used as the control.

Allowing for data in the literature [6], the cytophotometric examination of the sections was carried out on an integrating scanning microspectrophotometer designed in the writer's laboratory [2]. The instrument consists basically of a stabilized source of light, a monochromator, a microscope, a table (fitted with a diaphragm, photomultiplier, and telescopic sight) moving in the plane of the enlarged image, an integrator tor, and an electronic recorder. In contrast to instruments described previously, this microspectrophotom-

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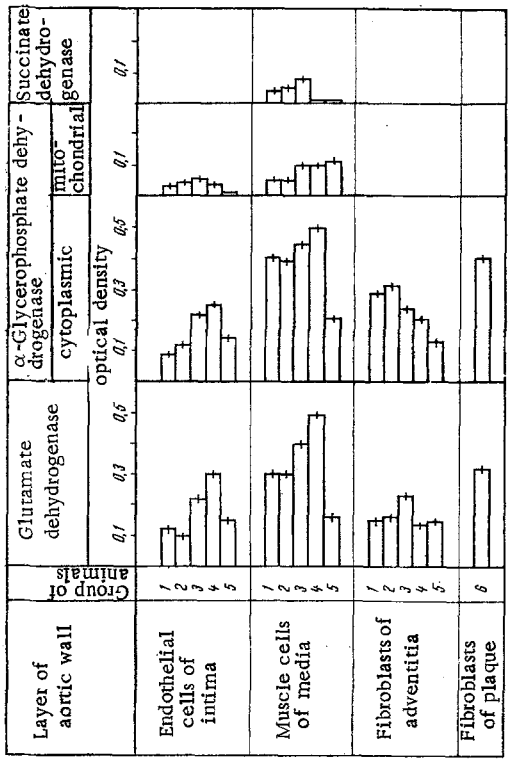

Fig. 1. Changes in tissue-enzyme profile of rabbit's aorta (M ± m). Group 1) control; 2) investigations on fourth day of experiment; 3) on 16th day; 4) on 24th day; 5, 6) on 64th day of experiment.

eter can scan any part of a cell under visual control in four directions and can yield data for the size and optical density of histological and cytological specimens and their structures. By comparative microspectrophotometry of organs of the control and experimental animal in the same section, it is possible to determine the mean size and area of a cell, and the mean and specific (per square micron of cell area) optical density of diformazan deposits (photometry was carried out in monochromatic light at a wavelength λ -585 mμ).*

RESULTS

On the 4th-16th day of the experiment the initial signs of lipoidosis of the aorta (up to 2-3% of the intima) were discovered. Subsequently (25th, 32nd, and 64th day days) the lipoidosis of the aorta reached 50% of the intima and plaques appeared in its abdominal part. Histological examination of the aortic wall showed the early changes of experimental atherosclerosis in agreement with those described in the literature [3, 4, 10, 12].

Histochemical and microspectrophotometric investigation of activity of oxidative enzymes in the aorta of the control animals showed (Fig. 1) that under normal conditions activity of GDH and α-DPDHC are highest, and activity of α-GPDHM is much lower. SDH activity was extremely low and was found only in the smooth-muscle cells of the media. Activity of the investigated enzymes in the different layers of the rabbits' aorta varied: the highest level was found in the smooth-muscle cells of the media and a somewhat lower level in the fibroblasts of the adventitia and in the cytoplasm of the endothelial cells of the intima.

In rabbits investigated on the fourth, eighth, and 12th days of the experiment, activity of GDH, α-GPDHC and α-GPDHM differed only very slightly from normal, but on the 16th and 24th days, GDH activity in the endothelial cells was increased by 2.5 times and activity of α-GPDHC and α-GPDHM by almost 3 times. In the smooth-muscle cells of the media, the level of GDH activity rose by 1.6 times and that of α-GPDHM by 2 times. Changes in activity of α-GPDHC were negligible in these same cells. Activity of GDH in the fibroblasts of the adventitia was doubled, activity of α-GPDHM remained at the same level while that of α-GPDHC was reduced.

Toward the end of the experiment, on the 64th day, activity of GDH and of α-GPDHC in all layers of the rabbits' aorta was reduced compared with the preceding periods, whereas activity of mitochondrial α-GPDH was unchanged. The decrease in activity of oxidative enzymes in the aortic wall was accompanied by an increase in the area affected by atherosclerosis. It was a very striking fact that SDH activity in the smooth-muscle cells of the media remained unchanged at all times.

Meanwhile high GDH and α-GPDH activity was observed in the fibroblasts of the newly formed atherosclerotic plaques.

The results of this investigation thus showed that cells of each different layer of the aorta under normal conditions possess a definite tissue-enzyme profile, depending on individual quantitative relationships between the conventional indices of their activity.

Experimental hypercholesteremia, after a compensatory phase lasting about two weeks, caused definite disturbances of metabolism in the aortic wall, and the character of the changes in activity of the investigated enzymes in the cells of all three layers of the aorta was different (phase of increased activity).

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For example, whereas by the fourth week of the experiment GDH activity showed a definite increase in the cells of all layers of the aorta and mitochondrial α-GPDH activity was increased only in the endothelial and muscle cells, activity of cytoplasmic α-GPDH was sharply increased in the endothelial cells but only slightly increased in the muscle cells of the media, while in the fibroblasts of the adventitia it was reduced. The extent to which cells of different layers of the aorta participate in the genesis of atherosclerosis thus differs.

The reaction with participation of α-GPDHC is known to be an intermediate stage through which products of glucose metabolism can be incorporated into biosynthesis of lipids. An increase in the activity of α-GPDHC and α-GPDHM indicates accumulation of α-glycerophosphate, an important precursor in lipid synthesis in the cells of the aortic wall [5, 8]. The considerable increase in α-GPDHC activity indicates that the role of carbohydrate participation in lipid synthesis is sharply increased in experimental hypercholesteremia.

The results of this investigation, obtained by comparative microspectrophotometry, demonstrate quantitative changes in the tissue-enzyme profile of the aorta in the early stages of atherosclerosis produced by alimentary hypercholesteremia in rabbits. Hypercholesteremia, a trigger mechanism of a group of metabolic disturbances in the body and, in particular, in the aortic wall induces excess biosynthesis and accumulation of lipids in its structures.

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