MORPHOLOGICAL AND HISTOCHEMICAL CHANGES IN THE RABBIT AORTA IN EXOGENOUS AND ENDOGENOUS HYPERGLYCEMIA

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Alimentary hyperglycemia and insulin deficiency in rabbits were accompanied by atherosclerotic lesions of the aorta, the severity of which increased if the same animals were fed with cholesterol. The morphological and histochemical changes found in the wall of the aorta correlate with atherogenic biochemical shifts established previously in the blood serum of animals of the corresponding groups.

KEY WORDS: hyperglycemia; alloxan diabetes; atherosclerosis.

The role of hypercholesteremia in the pathogenesis of atherosclerosis is generally accepted [2, 4]. Experimental, epidemiological, and clinical observations confirm also the concept of the atherogenic action of an excessive intake of simple carbohydrates [3, 5-8]. The writers showed previously [3] that prolonged hyperglycemia in rabbits is accompanied by elevation of the serum levels of cholesterol, triglycerides, and pre-β-lipoproteins, carriers of endogenous triglycerides. Feeding animals with cholesterol against the background of hyperglycemia led to earlier and more severe atherogenic disturbances.

It was interesting to study to what extent the changes mentioned above in the blood serum affect the morphological and histochemical changes in the blood vessel wall.

EXPERIMENTAL METHOD

Experiments were carried out on 43 male chinchilla rabbits (weight 2.5-3.5 kg). The animals were divided into six groups: 1) control, 2) rabbits receiving cholesterol in a dose of 1 g/kg daily for 3 months, 3) rabbits receiving glucose in a dose of 6 g/kg daily for 6 months, 4) rabbits receiving glucose by the same program, followed by glucose and cholesterol in a dose of 1 g/kg each on alternate days for 1 month, 5) rabbits receiving alloxan (5% solution intravenously) in a dose of 130-150 mg/kg body weight, and 6) rabbits with alloxan diabetes receiving cholesterol in a dose of 1 g/kg daily for 1 month.

The severity of the atherosclerotic lesions of the aorta was assessed by direct planimetry [1].

The animals were killed by air embolism. Pieces of the thoracic and abdominal parts of the aorta were fixed in 10% neutral formalin solution and embedded in paraffin wax. The sections were stained with hematoxylin–eosin and with alcian blue for acid mucopolysaccharides (MPS), the PAS reaction was carried out for glycogen and for neutral MPS by Shabadash's method, and a combined staining procedure was used for connective tissue (collagen and elastic fibers). Another part of the material was frozen with solid carbon dioxide, sections were cut to a thickness of 12 μ in a cryostat at $-14°C$, and these were then stained with Sudan III for lipids and reactions were carried out for glutamate dehydrogenase (GD), cytoplasmic and

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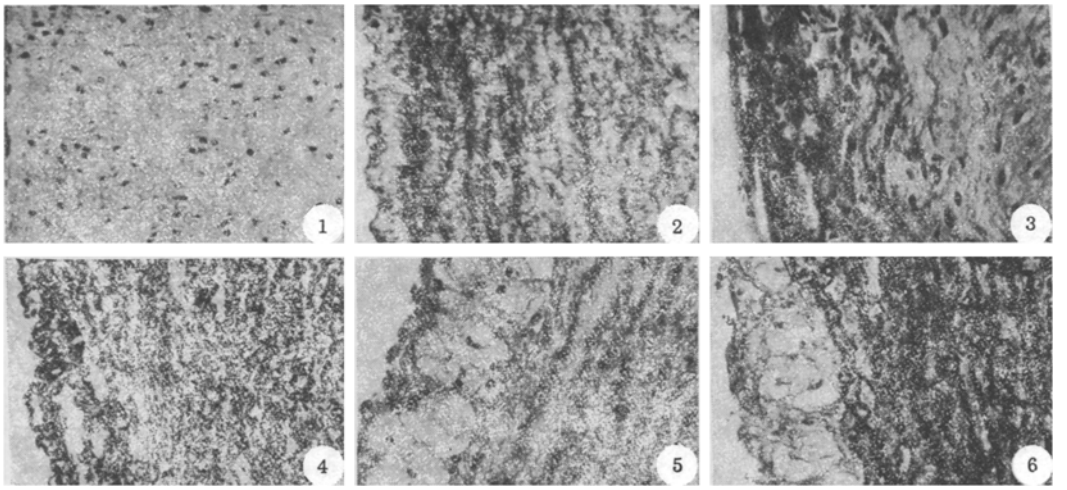

Fig. 1. Morphological changes in rabbit aorta: 1) aortic wall of intact rabbit (hematoxylin–eosin, 250×); 2) slight loosening and thickening of intima of aorta, beneath which is diffusely stained border of PAS-positive substances, in a rabbit receiving glucose (alcian blue + PAS; 250×); 3) aortic wall in rabbit receiving glucose and cholesterol: areas of hyalinosis and sclerosis, in the substance of which are lipoid foci (hematoxylin–eosin, 250×); 4) disorganization of elastic and collagen fibers in media of aorta, with empty spaces between them, in rabbit with alloxan diabetes (combined staining with picrofuchsin–fuchselin for connective tissue; 250×); 5) atherosclerotic changes in aortic wall occupying 70% of its total area, in rabbit with alloxan diabetes receiving cholesterol (hematoxylin–eosin, 250×); 6) aortic wall of same rabbit: loosening and fragmentation of collagen and elastic fibers of aortic wall visible beneath plaque (combined staining with picrofuchsin–fuchselin for connective tissue, 250×).

mitochondrial α-glycerophosphate dehydrogenase (α-GPD), succinate dehydrogenase (SD), and glucose-6-phosphate dehydrogenase (G6PD) by the method of Nachlas et al., with the addition of menadione to the incubation medium in a final concentration of 0.05 M. Sections incubated in medium without substrate acted as the control. Enzyme activity was judged from the density of distribution of diformazan granules formed during the reaction in the cytoplasm of the cells.

EXPERIMENTAL RESULTS

In 10 control rabbits the wall of the aorta was uniform in thickness and had a smooth intima; the epithelial cells, arranged in a regular layer, were separated by an elastic membrane, and the media and adventia were well developed. Elastic fibers stained intensely and uniformly and were moderately tortuous in their course. The ground substance could be seen between the elastic and collagen fibers and the smooth-muscle cells of the inner third of the media. No lipids were seen in the wall of the aorta. Smaller amounts of PAS-positive substances than of acid MPS were present in the surface layers of the intima and in the media (Fig. 1).

In all five animals receiving cholesterol (group 2) the aorta showed atherosclerotic changes consisting of lipid strains and strips (Avtandilov's stage I). The atherosclerotic index reached 17.5-18.1 (degree III). Histological investigation showed marked thickening of the intima of the aorta and swelling of the endothelium. Many xanthoma cells, arranged in groups, and pale honeycombed formations, surrounded by delicate bands of collagen fibers, which bounded the plaque externally, could be seen in the widened subendothelial layer. Staining with Sudan III revealed large amounts of fat in the lipoid plaque. Most of the lipids were concentrated in the widened subendothelial layer, in its ground substance, and in the large xanthoma cells. In the free spaces between the cells lipids were present as large localized accumulations and tiny dust-like particles in the ground substance. The content and distribution of PAS-positive substances were irregular, and they were especially numerous in the ground substance and the smooth-muscle cells of the media. Acid MPS were seen in large amounts at the base of the plaque, where proliferation of collagen fibers and elastic membranes were observed.

Of the six rabbits which received glucose (group 3), solitary projecting lipid plaques located in the thoracic part of the aorta (stage I) were found in only one, and the atherosclerotic index was 14.6 (degree I). Microscopically the intima of this aorta was slightly loosened in structure and thickened beneath the swollen epithelium, the cytoplasm of which was vacuolated; single cells with lipid inclusions were present in this area. The walls of the other aortas were of uniform thickness with a smooth shining intima. In sections stained with hematoxylin–eosin it had its usual structure. In nearly all aortas of the animals of this group a narrow diffusely stained border of PAS-positive materials could be seen in the whole length of the intima beneath the endothelium.

In five of the six rabbits receiving glucose and cholesterol (group 4) atherosclerotic changes of stage I were seen in the aorta (lipid stains and stripes), and changes of stage II (fibrous plaques) in one rabbit. Projecting lipoid plaques were more frequently located in the arch of the aorta, less frequently in the thoracic and abdominal portion. Lipoid plaques in the ascending aorta were close to each other and had a tendency to fuse together. The atherosclerotic indices reached 17.5 (degree I-III). The microscopic picture of the aortic wall was characteristic of the above-mentioned stages of atherosclerosis.

Of 11 rabbits with alloxan diabetes (group 5), only in one (severe diabetes) did the aorta show atherosclerotic changes in the form of lipid stains and fibrous plaques (stage II), with an atherosclerotic index of 6.6 (degree I). Microscopically the fibrous plaques consisted almost entirely of fibers of ground substance and coarsely honeycombed structures. In the region of the plaque there were few collagen fibers. In all cases, including mild forms of diabetes, disorganization of the elastic and collagen fibers of the media, between which neutral and a smaller amount of acid MPS accumulated, was a noteworthy feature.

In all ten animals with alloxan diabetes and receiving cholesterol (group 6), marked atherosclerotic lesions were present in the aorta: the atherosclerotic index varied from 3.1 (degree I) to 70 (degree V); the atherosclerotic changes reached stages I and II. Microscopically the plaques consisted of loosened and fragmented collagen and elastic fibers and connective-tissue cells; they contained considerable amounts of fat in the ground substance and in the cytoplasm of the macrophages. Acid and, to a lesser degree, neutral MPS accumulated in the ground substances of the plaques and in the regions surrounding them. Focal infiltration of the intima with dust-like particles and tiny droplets of fat also was observed.

Histochemical investigation of the activity of oxidative enxymes in the endothelial cells of the aorta showed that in rabbits with exogenous and endogenous hyperglycemia (groups 3 and 5) activity of GD and cytoplasmic α-GPD was above the control level, whereas SD activity was almost normal. In the animals of groups 4 and 6 (receiving cholesterol against the background of hyperglycemia) activity of GD and cytoplasmic α-GPD was much higher than in the intact rabbits; SD activity also increased. Activity of mitochondrial α-GPD was very low in the animals of all groups.

Morphological and histochemical changes in the aortic wall of rabbits with exogenous and endogenous hyperglycemia and also in rabbits receiving cholesterol correlated completely with the atherogenic shifts in lipid metabolism in the animals of the corresponding groups [3].

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