EFFECT OF ENZYMES OF MUCOPOLYSACCHARIDE METABOLISM AND THEIR METABOLITES ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

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The effect of β-glucuronidase and of a preparation of chondroitin sulfate, consisting of a mixture of sulfonated mucopolysaccharides, on the development of aortic lipoidosis was investigated in animals with experimental cholesterol atherosclerosis. Prolonged administration of these substances was found to increase β-glucuronidase activity in the aortic wall. Parallel accumulation of acid mucopolysaccharides was observed in the intima and the inner layers of the media, accompanied by increased lipoidosis. The results of these experiments indicate that a disturbance of mucopolysaccharide metabolism plays an active role in the development of experimental atherosclerosis.

Recent investigations have shown that the state of vascular permeability, which is determined by the metabolic activity of the ground substance and, in particular, by the dynamic state of the mucopolysaccharides, plays an important role in the onset and progress of atherosclerosis. Among the many enzymes of the vascular wall, the enzyme system of the glycosidases, which catalyze the breakdown of mucopolysaccharides, perform an important function. Among the glycosidases with their different actions, the enzyme with β-glucuronidase activity, regulating the metabolism of the glucuronic acids which are components of the chondroitin sulfates [3, 4, 5], is of special interest.

The object of the investigation described below was to examine the effect of β-glucuronidase and of a preparation of chondroitin sulfate on the onset and course of experimental atherosclerosis.

EXPERIMENTAL METHOD

Three groups of chinchilla rabbits were used in the experiments. Throughout the investigation one group of animals was kept on a standard high-cholesterol (0.25 g/kg body weight) diet, the animals of group 2 received cholesterol and also β-glucuronidase (Schuchardt, 1 mg enzyme corresponds to 250 units of activity) by intraperitoneal injection in a dose of 30 mg twice a week. Some of the animals of the last group were sacrificed 40 days after the beginning of the experiments, while the rest, receiving the atherogenic diet, continued to receive β-glucuronidase up to 100 days. The animals of group 3, also receiving the high-cholesterol diet, received injections of chondroitin sulfate (a mixture of sulfonated mucopolysaccharides) in a daily dose of 20 mg for 40 days.

β-Glucuronidase activity was investigated in the tissue extracts and serum and calculated in units of optical density measured relative to phenolphthalein liberated as a result of the reaction [2].

For the morphological investigation of the aortas standard staining methods for lipids, acid and neutral mucopolysaccharides, and elastic tissue were used.

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TABLE 1. β-Glucuronidase Activity in Blood and Aorta of Animals

Experimental conditions	Period of experiment (in days)	No. of animals	Aorta		Blood	
			$M \pm m$	P	$M \pm m$	P
Cholesterol	40	14	0,129±0,003	—	0,211±0,004	—
Cholesterol +β-glucuronidase	40	14	0,108±0,006	<0,01	0,194±0,006	<0,005
Cholesterol	100	8	0,120±0,0082	—	0,232±0,0187	—
Cholesterol +β-glucuronidase	100	8	0,167±0,0113	<0,01	0,175±0,0128	<0,05
Cholesterol	40	16	0,127±0,006	—	0,205±0,003	—
Cholesterol +chondroitin sulfate	40	16	0,148±0,005	<0,01	0,180±0,004	<0,01

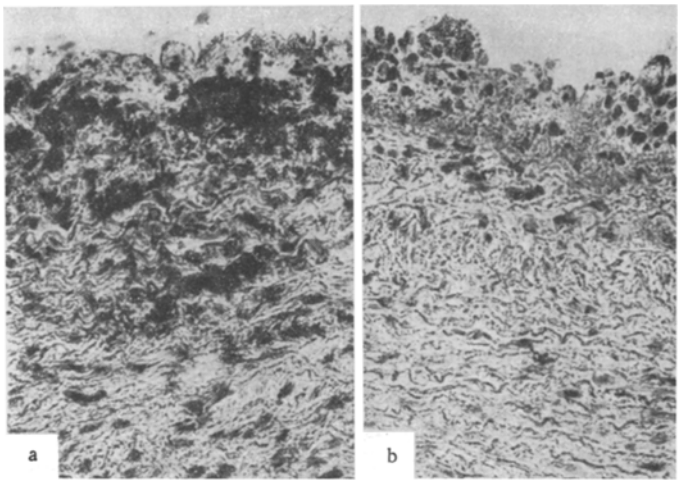

Fig. 1. Lipids in the rabbit aorta: a) marked lipoidosis of intima and media of a rabbit receiving cholesterol and β-glucuronidase; b) lipoidosis in a rabbit receiving cholesterol. Stained for lipids, 265 ×.

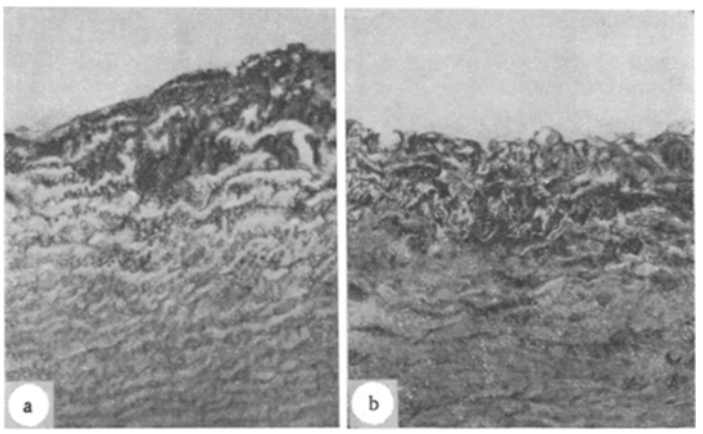

Fig. 2. Acid mucopolysaccharides in rabbit aorta: a) marked accumulation of acid mucopolysaccharides in intima and subintimal space in aorta of a rabbit receiving cholesterol and chondroitin sulfate; b) aorta of rabbit receiving cholesterol. Hale's method, 265 ×.

EXPERIMENTAL RESULTS

β-Glucuronidase activity in the blood and aorta of the animals sacrificed after administration of the enzyme for 40 days was lower than in the control group (Table 1). After 100 days of the experiment its activity in the blood of the animals remained appreciably lower than in the control, whereas its activity in the vessel wall was considerably increased (Table 1). The same result was observed in animals receiving injections of chondroitin sulfate.

Morphological investigation of the aorta of animals receiving a high-cholesterol diet showed that by the 40th day of the experiment they had developed marked lipoidosis of the intima. Lipoidosis of the intima was not found in the aortas of five of the seven animals receiving injections of β-glucuronidase, despite the fact that they had received cholesterol with their diet for the same period of time, while in the other two only single droplets of lipids were deposited in the ground substance which was only slightly sudanophilic. In both cases, focal accumulation of acid mucopolysaccharides of the chondroitin sulfate A and C types was observed in the intima and in the subintimal zones of the media.

A completely different picture was found on morphological examination of the aortas of animals receiving injections of β-glucuronidase for 100 days and also of animals receiving injections of chondroitin sulfate. In both groups much more severe lipoidosis of the intima was found than in the control (Fig. 1). The intima of the aorta was thickened and looser in structure, and the endothelial cells appeared swollen, with rounded nuclei. In some zones of the intima, marked proliferation of the endothelium was observed with the formation of cushion-like thickenings consisting of pale "foam" cells. The character of the lipoidosis was that of a drop-like deposition of lipids inside the endothelial cells and in the ground substance of the intima. The spread of lipid infiltration to the inner layers of the media along the course of the elastic membranes could be seen in many parts. The elastic membranes were considerably altered, and, on the whole, the media showed marked destruction: areas of intact fibers alternated with extensive foci of granular degeneration. The ends of the fragmented elastic membranes were twisted, their fibers were separate, and in some places they were thicker and in others thinner than normal. Considerable accumulation of acid mucopolysaccharides was found in the intima, the subintimal space, and the inner layer of the media, in the form of foci and pools between the fibers of the stroma, which were apparently forced apart by these foci of mucoid edema. These changes were particularly marked in animals receiving chondroitin sulfate (Fig. 2). The main mass of mucopolysaccharides in these foci had the chemical nature of chondroitin sulfate B; diffuse saturation of the intima took place with chondroitin sulfates of types A and C. In the subintimal space and inner layers of the media, accumulation of chondroitin sulfates of the B type also was observed.

On staining by the PAS method, a diffuse, moderate accumulation of substances with a bright crimson color, uniformly permeating the media, could be seen.

In experimental cholesterol atherosclerosis, the course of the lipoidosis was thus influenced by β-glucuronidase. The activity of the enzyme in the aortic wall could be changed by administration either of the enzyme itself or of one of the products of mucopolysaccharide metabolism (a mixture of chondroitin sulfates). Activity of β-glucuronidase in the aorta was increased parallel with the increase in lipoidosis. Meanwhile an accumulation of acid mucopolysaccharides was observed in the intima and in the subintimal layer of the media.

Since the degree of permeability of the vascular wall for the ingredients of the plasma is determined by the state of the ground substance [1], it can be assumed that β-glucuronidase exerts its effect through changes in mucopolysaccharide metabolism.

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