BIOSYNTHESIS OF PROTEINS OF THE BLOOD SERUM, LIVER, AND AORTA IN EXPERIMENTAL ATHEROSCLEROSIS

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Previous investigations conducted at the Institute of Therapy have shown that in atherosclerosis the protein metabolism is considerably modified [2-5]. During the study of protein synthesis in these investigations, the total tissue proteins were used in the form of a complex.

To obtain more detailed information of the character of protein biosynthesis in atherosclerosis, in the present investigation the synthesis of individual protein fractions in the serum, liver, and aorta was studied by means of labeled amino acids. The state of metabilism in these tissues is, of course, of great importance to the development of atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits weighing 2.5-3 kg. Experimental atherosclerosis was produced by N. N. Anichkov's method, by feeding the animals with cholesterol (0.1 g/kg body weight for 3 months). Healthy rabbits were used as controls.

To investigate the biosynthesis of the serum and liver proteins, 1 h before sacrifice the rabbits received an intraperitoneal injection of a solution of glycine-1-C^{14} in a dose of 20,000 pulses/min/g body weight.

The radioactivity of the serum protein fractions, separated by paper electrophoresis, was determined with a gas-flow counter.

The biosynthesis of the proteins of the nuclei, mitochondria, and microsomes of the liver cells was studied.

The nuclei of the liver cells were isolated by Chauveau's method as modified by V. A. Gvozdev [1]. The liver was washed with physiological saline and a 0.25 M solution of sucrose. The liver tissue was then homogenized in a mixture of solutions of sucrose (2.2 M) and $CaCl_2$ (0.0018 M) in a Waring homogenizer, and the homogenate was filtered through two layers of gauze and centrifuged with cooling for 1 h at a speed of 17,000 rpm. The residue containing the nuclei was suspended in a mixture of solutions of sucrose (0.25 M) and $CaCl_2$ (0.0018 M) and centrifuged at 1000 rpm, after which the residue of the nuclei was resuspended in a 0.25 M solution of sucrose.

The mitochondria of the liver cells were isolated by the method of Hogeboom and Schneider [7]. A piece of washed liver was homogenized in a 0.25 M solution of sucrose in a glass homogenizer, and the homogenate was filtered through two layers of gauze and flannel, after which it was again homogenized in the same solution. A 0.34 M solution of sucrose was poured into centrifuge tubes, and an equal volume of homogenate was poured as a separate layer above the solution and the samples were centrifuged for 10 min at 2500 rpm. The supernatant fluid was then centrifuged at 5000 rpm in a cooling centrifuge, and the residue was suspended in a 0.25 M solution of sucrose and centrifuged with cooling for 10 min at 11,000 rpm. The residue of mitochondria thus obtained was resuspended in sucrose solution.

The supernatant fluid obtained after centrifugation of the mitochondria at 5000 and 11,000 rpm was collected separately; this was the microsome fraction consisting of microsomes and soluble liver protein.

The isolation of the nuclei and mitochondria was verified under the microscope; the nuclei were stained with hematoxylin and the cytoplasmic particles with eosin.

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The radioactivity of the subcellular fractions of the liver was determined after treatment by a method described earlier for the total serum protein [3-5].

For the investigation of the synthesis of the aortic proteins, control rabbits and animals receiving cholesterol for 100 days were given an intraperitoneal injection of methionine-1-C^{14} in a dose of 30,000 pulses/min/g body weight 24 h before sacrifice. The aorta was ground to powder in liquid nitrogen. The soluble proteins of the aorta were extracted for 24 h in physiological saline and centrifuged with cooling. Most of the lipids were removed from the extract by means of ether. After extraction of the soluble proteins from the residue of aortic tissue, the collagen was extracted with a hot 0.3 M solution of trichloroacetic acid [9]. The residue of the aorta after extraction of the soluble protein and collagen contained elastin.

The radioactivity of the proteins of the subcellular fractions of the liver and of the aortic proteins was measured in a gas-flow counter, and the protein content was estimated by Lowry's method.

EXPERIMENTAL RESULTS

The incorporation of glycine-1-C^{14} into the serum protein fraction took place irregulary. If the total radioactivity of the serum proteins was taken as 100, in the control animals the albumins contained 32%, the α_1-globulins 10%, the α_2-globulins 18%, the β-globulins 21%, and the γ-globulins 19% of the total radioactivity.

In the rabbits with experimental atherosclerosis by comparison with the control the incorporation of glycine-1-C^{14} into the albumins was reduced on the average by 10%, into the α_1-globulins by 19%, into the α_2-globulins by 20%, into the β-globulins by 29%, and into the γ-globulins by 16%. Hence, the decrease observed previously in the biosynthesis of the total serum proteins in experimental atherosclerosis [4] was due to an irregular decrease in the synthesis of all the serum protein fraction.

In the liver, glycine-1-C^{14} was incorporated into the microsomes to a greater degree (in the control rabbits on the average 46% of the total radioactivity of the liver) and into the nuclei (mean 34%) and the mitochondria (mean 20%) to a lesser degree. The fact that incorporation of glycine into the microsomes was maximal was presumably because the synthesis of the tissue proteins takes place mainly in the microsomes.

In experimental atherosclerosis the incorporation of glycine-1-C^{14} was reduced (by comparison with the controls) both into the microsomes (on the average by 28%) and into the nuclei (on the average by 34%) of the liver cell. The incorporation of glycine into the mitochondria was unchanged. Hence, the decrease in the synthesis of the total liver proteins in experimental atherosclerosis was due to a decrease in the biosynthesis of protein in the nuclei and the microsomal fraction.

The study of the biosynthesis of proteins in the aorta is of the greatest interest from the point of view of the pathogenesis of atherosclerosis. The incorporation of methionine-1-C^{14} was greatest into the soluble protein of the aorta, and in the control rabbits it amounted on the average to 56% of the total radioactivity of the aorta. The collagen of the aorta contained on the average 12%, and the elastin 32% of the total radioactivity of the aorta.

In experimental atherosclerosis the incorporation of labeled methionine into collagen decreased (on the average by 23%) and its incorporation into the soluble protein and elastin was unchanged.

The decrease in the biosynthesis of the collagen of the aorta in atherosclerosis, revealed in these experiments, at first glance contradicts the observations indicating that the collagen content is increased in the aorta in atherosclerosis [10]. This contradiction is explained by the fact that incorporation of amino acids takes place mainly into procollagen, and it falls considerably as the collagen ages [6]. The content of procollagen in the aorta falls in atherosclerosis [8], and this is evidently explained by a decrease in its biosynthesis.

SUMMARY

Labelled glycine-1-C^{14} and methionine-1-C^{14} were used to study the biosynthesis of protein fractions of the blood serum, proteins of the subcellular fractions of the liver and fractions of aorta proteins in rabbits with experimental cholesterol atherosclerosis. It has been found that in atherosclerosis there is an unequal decrease in the biosynthesis of all protein fractions of the blood serum. The decrease in the biosynthesis of liver proteins in experimental atherosclerosis is due to a decrease in protein synthesis in nuclear and microsome fractions of the liver cell. In experimental atherosclerosis there is a decrease in the synthesis of collagen and no change in the biosynthesis of soluble proteins and elastin of the aorta.

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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 the first issue of this year.
