METABOLISM OF THE NUCLEIC ACIDS, PHOSPHOPROTEINS, AND PHOSPHOLIPIDS OF THE LIVER IN EXPERIMENTAL ATHEROSCLEROSIS

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The process of atherosclerosis is accompanied by a depression of the level of protein synthesis in the organs and tissues [4,5] and of the synthesis of phospholipids in the liver [18], by depression of the tissue respiration in the liver, the brain tissue, the heart, the kidneys, and the spleen [8.9], and by a lowering of the activity of many enzyme systems [6,7,11-13,16,17,19,20]. These changes may be explained by a disturbance of the metabolism of nucleic acids, the main biological function of which is to take part in the biosynthesis of proteins and enzymes.

Information has been obtained concerning the lowering of the intensity of the metabolism of nucleic acids and other phosphorus compounds with age [10], and the decrease in the content of RNA in the liver in experimental hypercholesteremia [14]. There are no details in the literature of the rate of nucleic acid metabolism in atherosclerosis.

The object of the present investigation was to study the rate of metabolism of nucleic acids, phosphoproteins, the phospholipids of the liver, and the phospholipids of the blood serum in experimental atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on 86 rabbits weighing 3.0-3.5 kg, of which 47 were experimental and 39 control. Experimental atherosclerosis was produced in the first group by feeding them on cholesterol with grated carrot or beetroot in a dose of 0.5 g/kg body weight daily for 3.0-3.5 months. To determine the rate of metabolism of phosphorus compounds, the animals were given P^{32} intramuscularly in the form of the disubstituted sodium phosphate in a dose of 0.2 μCi/g body weight. The animals were sacrificed at different times after injection of the isotope: 15 min, and 1, 3, 12, and 72 h. Pieces of the liver were frozen under a jet of carbon dioxide. At the same times, blood was taken for investigation of the total cholesterol, the phospholipids, and the fractions of lipoproteins in the serum. The severity of the atherosclerotic changes in the aortas was determined macroscopically.

The phosphorus fractions of the liver were separated in accordance with the scheme suggested by G. E. Vladimirov, T. N. Ivanova, and N. I. Pravdina [2]. Weighed samples of frozen pieces of liver (each 5 g) were homogenized with 50 ml of a 10% solution of trichloroacetic acid, cooled to 0°. Extraction of the acid-soluble phosphorus and centrifugation were carried out at 0° in a refrigeration centrifuge. The residues were washed ten times with 5% trichloroacetic acid solution, and the lipids were extracted from them successively with acetone, boiling with a mixture of ethanol and ether (3:1), methanol and chloroform (1:1), and ether. The nucleic acids were extracted from the residues with a 10% NaCl solution at 100° and precipitated with a mixture of a 0.1 M solution of lanthanum citrate and a 1 M solution of malonic acid. The DNA and RNA were separated after hydrolysis for 18 h with a 0.3 N solution of NaOH at 37° by acidification of the hydrolyzate with a 64% solution of $HClO_4$. The residue obtained after extraction of the nucleic acids was hydrolyzed with a 1 N solution of NaOH for 20 H at 37°, and the phosphorus of the phosphoproteins precipitated from it by a magnesia mixture [15].

The lipids were extracted from the blood serum by boiling with 20 volumes of a mixture of ethanol and ether (3:1) for 30 min. Cholesterol was determined by the reaction with acetic anhydride and sulfuric acid, and the fraction of lipoproteins was separated by electrophoresis on paper.

The phosphorus content of the separated fractions was determined by the Fiske—Subbarow method in Braunshtein's modification [1]. During the determination of the inorganic phosphorus, the phosphomolybdate salt was

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TABLE 1. Content of Cholesterol, Phospholipids, and Fractions of Lipoproteins in Blood Serum of Rabbits

Group of animals	No. of animals	Cholesterol (in mg%)	Phospholipids (in mg%)	Cholesterol/phospholipids	α-lipoproteins (in %)	β-lipoproteins (in %)	β/α-lipoproteins
Experimental	47	415±30.5	388±22.2	1.07±0.140	13.5±1,01	85.9±0.98	6.36±0,54
Control	39	57±2.2	176±8.2	0.32±0.028	43.1±1.70	56.2±1.72	1.30±0.09
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

TABLE 2. Relative Specific Activity of Nucleic Acids, Phosphoproteins, Phosphoproteins of the Liver, and Phosphoproteins of the Blood Serum of Rabbits

Group of animals	Duration of experiment (in h)	No. of animals	DNA	RNA	Phosphoproteins	Phospholipids	Phospholipids of blood serum
Experimental	1/4	6	—	0.089±0.003	11.9±0.56	0.31±0.017	—
Control		6	—	0.091±0.011	14.1±0.75	0.32±0.025	—
Experimental	1	6	—	0.25±0.017	34.6±1.98	0.94±0.051	—
Control		6	—	0.31±0.008	41.5±0.60	1.17±0.060	—
Experimental	3	14	0.28±0.015	0.91±0.036	38.4±2.02	3.51±0.11	0.72±0.048
Control		12	0.31±0.026	1.52±0.150	49.3±1.86	4.46±0.27	1.14±0.054
Experimental	12	8	1.49±0.23	8.3±0.39	76.9±2.68	17.8±1.19	5.25±0.48
Control		8	2.32±0.36	10.7±0.65	50.4±1.46	25.8±1.90	8.88±0.69
Experimental	72	9	3.41±0.39	30.1±2.18	79.2±8.07	91.3±6.46	94.6±8.2
Control		7	4.43±0.61	20.5±2.25	45.4±5.06	61.3±5.50	60.1±8.1

extracted from the acid-soluble fraction and the phosphoproteins with isobutanol [3]. The radioactivity of the phosphorus fractions was measured on a T-25-BFL end-type counter and a type B-2 apparatus.

The specific activity (SA) in pulses per min per microgram P and the relative specific activity (RSA) — the ratio between the SA of the fraction and the SA of the inorganic phosphorus of the liver in percent — were calculated for all the phosphorus fractions. Comparison of the RSA of the phosphorus fractions at various time intervals after injection of the isotope into the experimental and control animals gave information about the rate of metabolism of these compounds.

EXPERIMENTAL RESULTS

As a result of cholesterol feeding the experimental rabbits developed atherosclerosis, as indicated by an increase in the concentrations of cholesterol, phospholipids, and β-lipoproteins in their blood serum, by a decrease in the concentration of α-lipoproteins, and by the higher cholesterol/phospholipids ratio and the higher atherogenic index by comparison with the control animals (Table 1). The changes in the aortas of the experimental rabbits were of different grades of severity — from single to generalized deposits of lipids in the intima.

It is clear from Table 2 that the relative specific activity of the RNA and phospholipids of the liver 1, 3, and 12 h after injection of the isotope had fallen significantly in the experimental rabbits by comparison with the controls. The RSA of the phospholipids of the serum showed a significant decrease 3 and 12 h after injection. This indicated a slowing of the incorporation of P^{32} into these compounds in the experimental animals. The position was reversed after 72 h: the RSA of all the phosphorus fractions in the experimental animals was higher than in the controls. This demonstrated the slower elimination of the label from these compounds.

The more intensively metabolizing phosphoproteins of the liver in the experimental rabbits were characterized by a significant fall in the RSA after 15 min and 1 and 3 h, and by a significant rise after 12 and 72 h by comparison with the controls. The differences in the incorporation of P^{32} into DNA in the experimental and control animals were not statistically significant.

Table 2 shows that the RSA increased in both the experimental and the control series at all the time intervals. This was because the P^{32} content in the inorganic phosphate fraction increased only in the first 3 h, and by 72 h it had fallen to almost 10% of the maximum. The rate of incorporation or elimination of the label could therefore be estimated by comparing the experimental and control values at each time interval.

In rabbits with experimental atherosclerosis, the intensity of metabolism of RNA, phosphoproteins, liver phospholipids, and serum phospholipids is therefore lower than in control animals.

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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.
