CHANGES IN THE SOLUBLE LIVER PROTEINS IN THE EARLY STAGES OF DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

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In atherosclerosis, besides disturbances of lipid metabolism, considerable protein changes are also observed in a number of organs, especially the liver. In experimental atherosclerosis, protein synthesis is depressed and protein breakdown stimulated [7, 8], while the character of these changes depends on the duration of feeding the animals with cholesterol: at the beginning of feeding synthesis is increased, but when a marked degree of atherosclerosis is present it is depressed [9]. All the serum albumins and some of the serum globulins are synthesized in the liver [6, 23, 26, 27], so that the changes in the protein metabolism and in the composition of the blood proteins in atherosclerosis [1-3, 11-15, 17, 19, 21, 22, 24, 25] may be associated to some extent with a disturbance of the liver function.

A factor of great importance to the study of the liver proteins is the development during recent years of methods of extraction of soluble proteins (i.e., proteins soluble in water or in saline solvents) from organs, and of methods of their electrophoretic separation. It was found by means of these methods that the serum albumins account for 2.5-6.5% of the total amount of liver proteins, and the serum globulins 3-7% [5, 6, 10, 18, 20, 28]. The greater part of the liver proteins is composed of proteins belonging to the liver itself.

There are no reports in the literature of changes in the soluble proteins of the liver in atherosclerosis. The liver is known to be concerned in the metabolism of the serum proteins and changes in the liver proteins are important in the subsequent disturbance of other forms of metabolism [16]. The object of the present investigations was to study the changes in the soluble proteins of the liver in the early stages of the development of experimental atherosclerosis before the appearance of its first macroscopic signs in the aorta.

EXPERIMENTAL METHOD

Observations were made on 50 chinchilla rabbits weighing 2.5-3.0 kg. A control group consisted of 10 rabbits not receiving cholesterol. The remaining 40 rabbits received cholesterol, dissolved in sunflower oil, in a dose of 0.2 g/kg body weight, daily with their diet: 10 rabbits for 5, 10-15, and 10-30 days respectively, and 10 rabbits for for 45 days. At the beginning and end of the experiments the protein content of the serum, the protein fractions, and the cholesterol were determined.

The liver was freed from blood by perfusion with physiological saline, after which pieces of liver were frozen to −30° and dried to constant dry weight in vacuo. The soluble protein of the liver was extracted with phosphate buffer, pH 7.4. The dried piece of liver, together with the phosphate buffer, were frozen for 3-4 h at −30°, and then thoroughly ground in a mortar at room temperature. This procedure was repeated 5 or 6 times, after which the homogenate was centrifuged in the cold for 30 min at 3500 rpm. To the residue an approximately equal volume of phosphate buffer was added and the suspension was kept in a refrigerator at 2-4° for 24 h, after which it was again centrifuged with cooling. The residue was allowed to stand with phosphate buffer at 2-4.° for a further period of 3-4 h. The supernatant fluid obtained after three successive extractions of the soluble liver protein was pooled and treated with ether. A yellowish opalescent solution was obtained, the protein concentration in which was determined by Lowry's method.

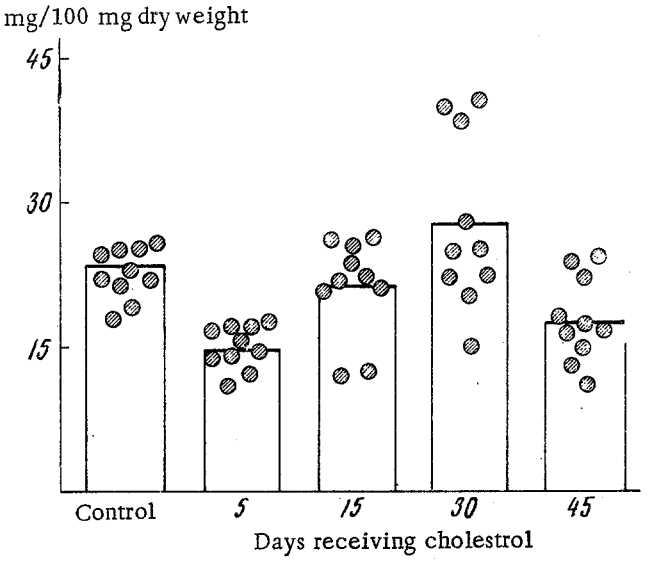

Fig. 1. Changes in content of soluble protein in the liver of rabbits receiving cholesterol for 5, 15, 30, and 45 days. The columns denote the mean value for the corresponding group of animals, the shaded circles-individual rabbits.

Electrophoresis of the proteins of the liver and serum was carried out on paper in a veronal-medinal buffer, pH 8.9, for 16-18 h at a voltage of 200 V. The paper strips were stained with the dye Amidoschwarz 10B and then cut up into separate fractions, eluted with a 0.1 N solution of NaOH in a 50% solutinn of methanol, and examined colorimetrically with a red filter. The total protein extracted from the liver was expressed per 100 mg dry weight of the organ. The fractions of the soluble protein of the liver were expressed in absolute numbers, because its total content varied considerably in the course of the experiment.

EXPERIMENTAL RESULTS

It is clear from Fig. 1 that in the rabbits which received cholesterol for 5 days the liver contained much less soluble protein than in the healthy animals. However, in the rabbits which received cholesterol for a longer period (15 or 30 days), the content of soluble protein in the liver was greater than in the animals receiving cholesterol for only 5 days. In the rabbits investigated 45 days after the beginning of cholesterol feeding the content of soluble pro-

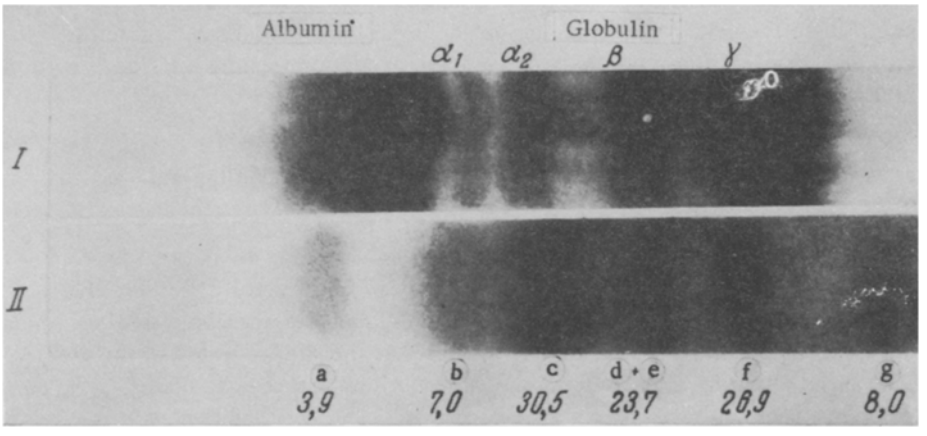

Fig. 2. Electrophoresis of serum protein (I) and soluble liver protein (II). The values of the fractions are means obtained with 10 healthy rabbits and expressed in % of total protein concentration.

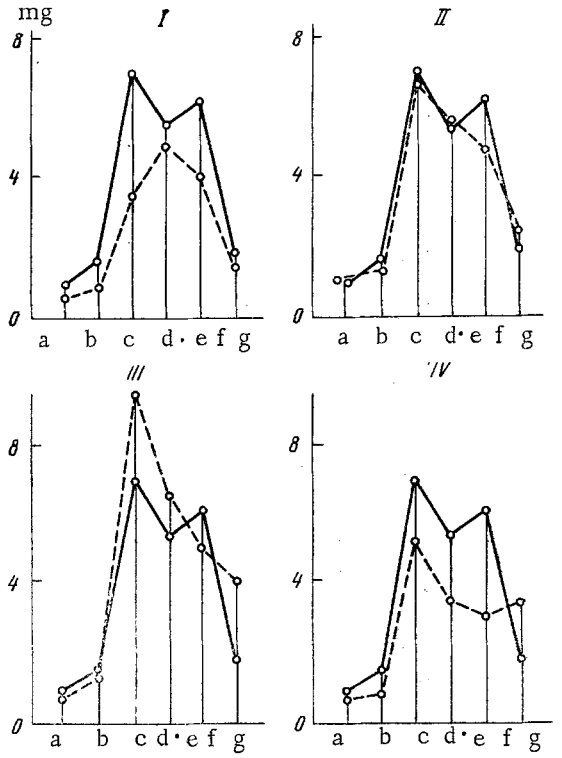

Fig. 3. Changes in fractions of soluble protein of the liver in rabbits receiving cholesterol for 5 days (I), 15 days (II), 30 days (III), and 45 days (IV). The values of the fractions are given in absolute figures. The protein fractions of the control rabbits are denoted by continuous lines, and the fractions of the liver proteins of the animals receiving cholesterol by broken lines.

tein in the liver was low, as in the case of the animals receiving cholesterol for only 5 days.

During the electrophoretic separation of the soluble proteins of the liver six fractions could be clearly distinfuished (Fig. 2). The position of the fractions of the soluble proteins in relation to the position of the serum protein fractions in our investigations corresponded to those reported in the literature. Fraction A of the soluble protein of the liver had an electrophoretic mobility equal to that of serum albumin. Fraction B was situated opposite the serum α-globulins, fraction C between the α- and β-globulins, fraction D + E opposite the β-globulins, and fraction F opposite the γ-globulins, while the mobility of fraction G was less than the mobility of the serum γ-globulins.

The values of the fractions of the soluble proteins of the liver, like the ratios between these fractions, varied considerably in relation to the duration of feeding the rabbits with cholesterol (Fig. 3, Table 1). In the rabbits receiving cholesterol for 5 days the value of all the fractions was smaller than in the rabbits of the control group, so that the decrease in the total amount of soluble liver protein in these animals took place as a result of a more or less uniform decrease in all its fractions. By the 15th day of the experiment the value of nearly all the fractions of the soluble protein except fraction F was nearly normal. In this fraction too, however, the protein content was greater than in the rabbits receiving cholesterol for 5 days only. In the rabbits receiving cholesterol for 30 days a considerable increase was observed in the fractions C, D + E, and G, the value of which was greater than normal. On the 45th day of the experiment the value of fraction G was as high as on the 30th day, but the level of the other fractions of the soluble protein was much lower than that in the healthy animals.

At first (feeding for 5 days with cholesterol), therefore, a decrease was observed in the value of all the fractions of soluble liver protein, and later (feeding for 15 days with cholesterol) their content and relative proportions were restored almost to normal. In the next period (feeding for 30 days) the level of several fractions rose considerably over the normal value, and then fell again on the 45th day; the exception was the fraction G with the lowest mobility in the electric field.

The total serum protein content showed no significant change. The changes in the serum protein fractions also were very slight. All that was noticed was a slight increase in the content of β-globulins and a decrease in the albumin content 15 days after the beginning of feeding the animals with cholesterol. These changes also persisted in the rabbits receiving cholesterol for a longer time (Table 2).

The cholesterol concentration in the serum of the animals receiving cholesterol with their diet showed an increase depending on the duration of cholesterol feeding. In all the animals receiving cholesterol for 45 days, small lipid stains were found in the aorta, especially in the arch of the aorta at the sites of origin of the great vessels, raised above the level of the intima. In the rabbits receiving cholesterol for a shorter period of time, no macroscopic signs of lipidosis of the aorta were seen.

In the early stages of development of experimental atherosclerosis in rabbits various changes were observed both in the total content of soluble liver protein and in its individual fractions. On the 5th day of cholesterol feeding the content of soluble protein in the liver cell considerably, on account of all its fractions. On the 15th day of cholesterol feeding, however, the total content of soluble protein in the liver not only did not fall, but, on the con-

TABLE 1. Content of Soluble Liver Protein and of its Fractions in Control Animals and in Rabbits Receiving Cholesterol (mean data in mg/100 mg dry weight of liver)

Number of animals	Period of feeding with cholesterol (in days)	Soluble protein	Fraction of soluble protein					
			A	B	C	D + E	F	G
10	Control	23,2±0,8	0,89±0,06	1,65±0,21	7,06±0,40	5,52±0,21	6,23±0,40	1,89±0,15
10	5	15,1±0,7	0,47±0,03	0,86±0,08	3,48±0,61	4,91±0,30	3,97±0,51	1,45±0,30
10	15	21,5±1,5	1,40±0,41	1,23±0,22	6,60±0,62	5,58±0,60	4,67±0,52	2,42±0,31
10	30	27,8±2,5	0,83±0,10	1,41±0,31	9,51±0,80	6,60±0,71	5,10±1,10	4,35±0,72
10	45	17,4±1,4	0,87±0,11	0,90±0,10	5,36±0,11	3,65±0,05	3,07±1,10	3,46±0,41

TABLE 2. Level of Total Protein and Protein Fractions of Blood Serum in Control Animals and in Rabbits Receiving Cholesterol

Number of animals	Period of feeding with cholesterol (in days)	Total serum protein (g %)	Serum protein fractions (in g%)				Albumin-globulin ratio
			albumins	globulins			
				α	β	γ	
10	Control	7,7±0,4	62,6±1,6	11,5±1,4	12,1±1,2	13,8±0,9	1,7±0,1
10	5	8,6±0,2	63,5±2,5	13,0±1,5	11,9±0,6	11,6±0,9	1,7±0,2
10	15	8,6±0,4	58,2±2,0	12,0±1,4	13,6±0,8	12,6±0,9	1,6±0,1
10	30	8,5±0,4	60,8±3,3	10,4±1,3	15,2±1,0	13,6±2,5	1,5±0,2
10	45	7,8±0,4	60,6±2,1	10,8±0,7	14,5±1,1	14,1±1,5	1,5±0,1

trary, returned almost to its normal level. The content of nearly all the fractions of the soluble protein also approached the normal value. On the 30th day of the experiment the content of soluble liver protein rose still further on account of some fractions, which were present in amounts greater than normal. Finally, on the 45th day of the experiment, a decrease in the content of soluble liver protein was again determined, on account of almost all the fractions, similar to that observed on the 5th day of the experiment. However, these periods (the 5th and 45th day of feeding) differed considerably as regards the relative proportions of the individual protein fractions.

Hence, the changes in the total content of soluble liver protein and of its fractions in the early stages of development of experimental atherosclerosis are variable in character and bear no linear relationship with the duration of feeding the animals with cholesterol. A period particularly worthy of mention is that when, after the initial decrease in the content of soluble liver protein, its content was restored, and subsequently actually exceeded the normal level. It may be postulated that this restoration is associated with the activation of the defensive reactions of the organism, eradicating the consequences of the primary metabolic disturbance arising after the administration of large amounts of cholesterol. The activation of the protective reactions and the restoration of the protein structure of the liver may possibly prevent the disturbance of other types of metabolism, for the liver plays an important role in the metabolic processes of the organism and the greater part of the liver protein consist of enzymes. To some extent this may also prevent the development of atherosclerosis of the aorta. During prolonged feeding with cholesterol these defensive mechanisms are apparently depressed, as a result of which the content of soluble liver protein is reduced, the relative proportions of its fractions are changed, and the first macroscopic signs of lipidosis of the aorta appear.

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