ELECTROPHORETIC INVESTIGATION OF HEART MUSCLE PROTEINS IN EXPERIMENTAL ATHEROSCLEROSIS

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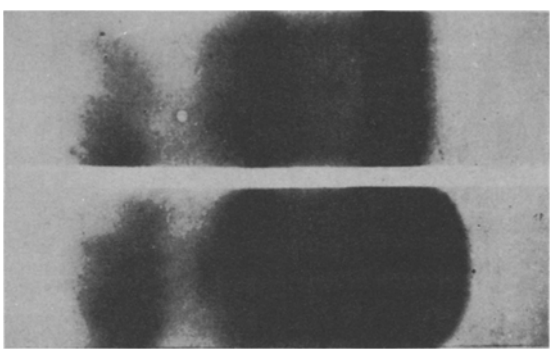
Numerous investigations have shown that in atherosclerosis in man and animals not only the lipid, but also the protein metabolism is affected, as revealed by changes in the protein composition of the blood [1, 2, 6, 13, 15, 8, 16]. The protein metabolism of the organs and tissues in this disease has been studied less intensively. M.G. Kritsman and M. V. Bavina [8, 9, 10], using isotopes as indicators, found an increase in the intensity of protein biosynthesis in the organs of rabbits in the initial period of development of atherosclerosis, followed by a considerable decrease in its intensity as the disease continued to develop. Beside inhibition of protein synthesis, these workers also observed increased protein breakdown [11].

V. A. Shalimov [14] observed a decrease in the concentration of free sulfhydryl groups in the tissue proteins (heart muscle, brain, liver, kidneys) in experimental atherosclerosis. D. G. Grigor'yan and N. A. Nazarenko [7], by means of the complement fixation reaction, found that specific changes developed in human heart muscle in atherosclerosis. M. F. Bondarenko [3] used the method of electrophoresis to investigate the protein composition of the heart muscle in rabbits with experimental anoxia and found a decrease in the myosin content together with an increase in the myogen. M. F. Bondarenko and M. E. Raiskina [4] discovered changes in the protein functions of the heart muscle during stimulation of Pavlov's augmentor nerve. M. F. Bondarenko and F. Z. Meerson [5] observed changes in the content of protein fractions in stages 1 and 2 of compensatory hyperfunction of the heart.

Since no information concerning electrophoretic changes in the heart muscle proteins in atherosclerosis is available in the literature, we investigated this problem.

EXPERIMENTAL METHOD

Experiments were conducted on rabbits in which atherosclerosis was induced by Anichkov's method. The animals were given cholesterol in a daily dose of 0.02 g/kg body weight for 120-130 days. The serum cholesterol was determined by the Engel'gardt-Smirnova method. After the experimental animals had developed a stable hypercholesteremia (750-930 mg %), they were sacrificed and the severity of the atherosclerotic changes in the aorta was determined macroscopically and assessed by a four-point system. Healthy rabbits of the same breed and weight (2.5-3.0 kg) acted as controls.



Electrophoresis of serum proteins of a healthy rabbit (below) and of a rabbit with experimental atherosclerosis (above). MA) Myoalbumin; K_1 and K_2) proteins possessing phosphorylase activity; 1, m, n) myogen.

The heart was freed from connective and adipose tissue. The muscle tissue (the experiments were carried out on tissue from the left ventricle) was sectioned on a freezing microtome (thickness of sections 30-45 μ). Proteins were extracted from the muscle tissue by means of a buffered phosphate solution at pH 7.45 for 2 h in the cold. The extracts were separated by centrifugation for 20 min at 6000 rpm and at a temperature of 0-2°. The total protein concentration was

TABLE 1.* Content of Protein Fractions in Heart Muscle of Healthy Rabbits

Index	Rabbit No.						Mean data	
	25	26	27	29	30	31		
Myoalbumin	23,38	19,30	19,27	21,79	15,68	20,05	19,91	
Proteins possessing phosphorylase activity:								
K_2	12,63	15,36	9,32	15,20	14,56	14,74	13,64	36,42
K_1	18,13	20,05	28,01	22,05	29,97	18,51	22,79	
Myogen n	26,75	28,04	28,94	27,50	25,55	25,59	27,06	
Myogen m	12,05	9,15	9,42	7,50	9,17	9,31	9,43	
Myogen 1	7,16	7,10	5,04	5,96	6,27	11,16	7,11	

* All the figures given in Tables 1 and 2 are means of two parallel electrophoretic investigations.

TABLE 2. Content of Protein Fractions in Heart Muscle of Rabbits with Experimental Atherosclerosis*

Index	Rabbit No.								Mean data	
	1 4+	2 1½+	16 3½+	18 not det.	11 4+	22 2½+	12 1½+	13 4+		
Myoalbumin	17,06	20,48	19,00	20,10	19,98	13,41	21,48	23,54	19,38	
Proteins possessing phosphorylase activity:										
K_2	17,51	12,17	15,81	11,60	17,61	16,05	18,58	10,83	15,02	46,19
K_1	34,64	31,48	27,95	30,93	24,78	35,98	36,58	27,05	31,17	
Myogen n	19,32	24,20	27,80	28,07	30,04	26,68	15,15	25,85	24,64	
Myogen m	11,45	9,36	10,83	9,3	7,65	7,49	6,1	9,15	8,92	
Myogen 1	—	2,30	—	—	0,86	—	1,62	3,50	1,03	

* The number of + signs denotes the degree of atherosclerotic change in the aorta.

determined by Lowry's method. The proteins of the extracts were separated by electrophoresis using horizontal apparatus.* Electrophoresis was carried out in phosphate buffer (pH 6.4, ionic strength 0.058) for 18-19 h at a voltage of 250 V. Extracts were applied in a volume of 0.04 ml to the middle of the band (width of band 42 cm).

Extracts of healthy muscle and heart muscle of rabbits with atherosclerosis were subjected to electrophoresis simultaneously in each apparatus. Staining for protein was by means of acid blue-black dye [16] for 20 min. A solution of acetic acid and phenol was used to remove dye not bound with protein. Dye was eluted from the stained paper strips after electrophoresis by means of 0.1 N NaOH solution. The relating proportions of the protein fractions were determined photometrically (FEK-M apparatus with a red filter). An area of the paper not containing protein was used as a control sample. The serum proteins were also investigated by electrophoresis on paper with buffer at pH 8.6 (Michaelis's buffer).

EXPERIMENTAL RESULTS

The results given in the figure and in Tables 1 and 2 show that no new fractions were found in the rabbits with experimental atherosclerosis not present in normal animals. On the contrary, electrophoresis of the heart extracts of rabbits with atherosclerosis showed in most cases disappearance of the 1-myogen fraction.

Whereas in the extracts of the hearts from healthy rabbits the mean content of the 1-myogen fraction was 7.11% (Tables 1 and 2), in atherosclerosis this fraction could not be detected by electrophoresis. The mean values for the 1-myogen fraction were no higher than 1.03%. The concentration of proteins possessing phosphorylase activity rose to 46.19% in atherosclerosis, whereas in normal animals its mean value was 36.42%. The myoalbumin fraction in the animals with atherosclerosis remained at the normal level.

* We used apparatuses made in the experimental workshops at the A. A. Bogomolets Institute of Physiology, Kiev.

TABLE 3. Content of Serum Protein Fractions of Normal Rabbits and Rabbits with Experimental Atherosclerosis

Index	Rabbit No.							Normal (mean)
	2	16	18	11	22	12	13	
Albumina	47,31	51,69	55,21	45,90	38,08	45,01	46,12	54,4
Globulins: α	11,79	18,67	12,74	16,09	15,48	14,58	16,61	15,42
β	11,72	12,36	9,42	17,41	15,82	14,80	13,93	12,11
γ	29,18	17,28	22,63	20,60	30,99	25,61	23,34	17,77

In nearly every case a correlation was observed between the changes in the protein fractions of the heart muscle and the severity of the changes in the aorta. The changes in the serum protein fractions in rabbits with experimental atherosclerosis are shown in Table 3.

Comparison of the results in Tables 2 and 3 shows that the changes in the protein metabolism of the heart muscle of rabbits with atherosclerosis did not always correspond to the changes in the protein composition of the blood serum. A similar pattern was also observed in relation to the cholesterol concentration. A higher serum cholesterol level sometimes corresponded to less marked changes in the protein fractions of the heart muscle and, conversely, when the cholesterol level was comparatively low, more considerable changes were seen in the protein metabolism of the heart muscle.

SUMMARY

A method of electrophoresis on paper was used to study the proteins of the heart in experimental atherosclerosis. In this condition a change in the protein metabolism, as demonstrated in the cardiac muscle, manifested by an increase in proteins possessing phosphorylase activity (K_1, K_2) and a decrease in the myogen fraction.

LITERATURE CITED

1. M. V. Bavina and M. G. Kritsman, Dokl. Akad. Nauk SSSR, 88, 2 313 (1953).
2. M. V. Bavina and M. Yu. Melikova, Atherosclerosis and Coronary Insufficiency [in Russian], p. 143, Moscow (1956).
3. M. F. Bondarenko, Byull. éksper. biol., 11, 43 (1954).
4. M. F. Bondarenko and M. E. Raiskina, Byull. éksper. biol., 8, 39 (1956).
5. M. F. Bondarenko and F. Z. Meerson, cited by F. Z. Meerson in: Compensatory Hyperfunction and Failure of the Heart [in Russian], p. 144, Moscow (1960).
6. D. G. Grigor'yan, Proceedings of an All-Union Scientific Conference on Experimental Spa Therapy and Physiotherapy [in Russian], p. 78, Moscow (1962).
7. D. G. Grigor'yan and N. A. Nazarenko, Byull. éksper. biol., 1, 75 (1964).
8. M. G. Kritsman and M. V. Bavina, Atherosclerosis [in Russian], p. 127, Moscow (1953).
9. M. G. Kritsman and M. V. Babina, Dokl. Akad. Nauk SSSR, 94, 4, 721 (1954).
10. M. G. Kritsman and M. V. Bavina, Atherosclerosis and Coronary Insufficiency [in Russian], p. 126, Moscow (1956).
11. M. G. Kritsman, M. V. Bavina, and B. S. Sukhareva, Abstracts of Proceedings of the 10th Scientific Session of the Institute of Therapy AMN SSSR [in Russian], p. 14, Moscow (1959).
12. Z. A. Likhareva, Proceedings of an All-Union Scientific Conference on Experimental Spa Therapy and Physiotherapy [in Russian], p. 194, Moscow (1962).
13. T. N. Lovyagina, Theses of a Symposium on Atherosclerosis [in Russian], p. 11, Kuibyshev (1959).
14. V. A. Shalimov, Proceedings of an All-Union Scientific Conference on Experimental Spa Therapy and Physiotherapy [in Russian], p. 323, Moscow (1962).
15. A. B. Shakhnazarov, G. V. Troitskii, L. S. Tarasova et al., Vrach. delo, 1, 87 (1959).
16. N. L. Yastrebtsova, K. A. Gornak, E. A. Kyandzhuntseva et al., Atherosclerosis (Problems in Pathology and Pathogenesis) [in Russian], p. 117, Leningrad (1961).