ALTERED ACTIVITY OF CERTAIN ENZYMES IN THE AORTA DURING EXPERIMENTAL ATHEROSCLEROSIS

(UDC 616.13-004.6-092.9-07 : 616.132-008.931-074)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 3,
pp. 52-55, March, 1966
Original article submitted November 23, 1964

In the current literature constantly increasing attention is being devoted to studies of enzymatic systems in the arteries. Investigations on atherosclerotic vessels have been carried out relative to the behavior of various component enzymes of the glycolytic, citric acid and pentose cycles and also on proteolytic enzymes and certain other systems [5, 10, 14]. Somewhat less attention has been given to studies of esterase activity.

We have not found any studies reported in the literature dealing with changes occurring in the atherosclerotic vascular walls with respect to such enzymes as specific cholinesterase, aliesterase, and others. We have encountered only a single account of the behavior of nonspecific cholinesterase in the rabbit aorta in atherosclerosis, in which a decrease in enzyme activity was reported after 60 days of feeding the animals cholesterol at the rate of 1 g per day [7]. A series of authors have dealt with the phosphomonoesterase activity in the arterial wall but general agreement is lacking among them [12, 6-8, 9, 15]. Lojda [10] demonstrated great variability in the histochemically observed phosphomonoesterase activity in human arteries. He found increased activity of phosphomonoesterase II in early plaques in the human aorta and very low activity in fibrous plaques.

Because of the major role which esterases play in lipid metabolism, the present work was designed to study a series of esterases in the blood and in the aorta walls simultaneously during the development of experimental atherosclerosis.

METHODS

The experiments were carried out on chinchilla rabbits weighing 1800-3000 g. Experimental atherosclerosis was induced in the animals by giving them cholesterol at the rate of 0.2 g per kg body weight in addition to their regular ration. The rabbits were divided into 5 groups depending on the length of time they were carried in the experiment. Four groups of rabbits received the cholesterol in the form of a 10% solution in sunflower seed oil and differed only in period of observation: the first group (10 rabbits) was examined at 15 days after the start of cholesterol feeding, the second group (10 rabbits) at 30 days, the third group (7 rabbits) at 45 days, the fourth group (11 rabbits) at 90 days. The rabbits of the fifth group (12 animals) received cholesterol for 90-100 days in the same dosage (without sunflower seed oil) as an additive to their regular ration.*

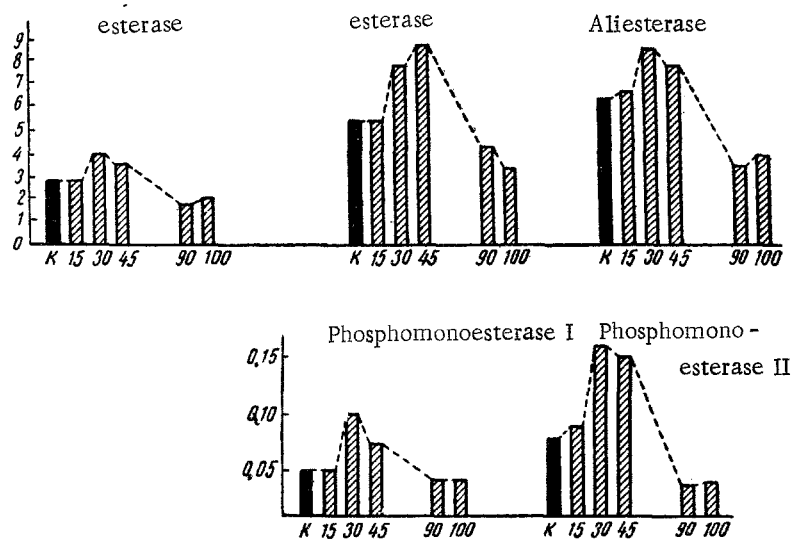
One each of the controls (total of 21 rabbits) and the experimental animals were chosen from the same litter. The lipid changes in the blood serum were followed in the experimental and control rabbits every 2 weeks by determination of cholesterol and phospholipids [1].

At the end of the experiment the animals were decapitated, the aorta removed, and the extent of atherosclerotic changes assessed visually after staining with Sudan dye. A 2% water homogenate was prepared from an intima-media

*Rabbits of the latter group were obtained from the Biochemical Laboratory, All-Union Chemical-Pharmaceutical Research Institute, and for this the authors are indebted to L..I. Grebennik, Director of the Laboratory.

Changes in the Cholesterol and Lecithin Content of Rabbit Blood during the Development of Experimental Atherosclerosis

Measurements	15 Days		30 Days		45 Days		90 Days	
	control	expt.	control	expt.	control	expt.	control	expt.
Cholesterol (mg %)	40 — 70	280 — 500	40 — 60	400 — 900	30 — 60	400 — 900	40 — 65	700 — 1350
Lecithin (mg %)	80 — 100	200 — 325	83 — 100	200 — 500	60 — 100	200 — 400	80 — 100	480 — 600
Lecithin/cholesterol	1,3 — 2,2	0,62 — 0,93	1,4 — 2,4	0,3 — 0,8	1,5 — 2,6	0,36 — 0,75	1,3 — 2,2	0,4 — 0,9



Changes in enzyme activity in the aorta wall tissue from experimental rabbits. The ordinate shows enzyme activity (in μM of substrates split by 1 g of moist tissue per minute); the abscissa shows the days of experiments; K is control.

portion of the aorta.* In this, as well as in the blood, determinations were made of the following enzyme activities: acetylcholine esterase (ACE), butyrylcholine esterase (BCE), aliesterase, phosphomonoesterases I and II (PME-I and PME-II). The enzyme activity and protein content was determined by the micro method devised in our laboratory [2]. Enzyme activity was expressed as the micromolar quantity of substrate split by 1 g of moist tissue or 1 ml of blood per minute. The results were treated statistically.

RESULTS

The total cholesterol of the blood in the experimental animals increased greatly with time, and by the end of the 90-day period it reached 17-33 times the normal (see table). The lecithin level also rose sharply but to a lesser extent. As a consequence the lecithin/cholesterol ratio in the experimental rabbits decreased in comparison with the controls. There were no statistically significant changes in the blood levels of the investigated enzymes during the development of atherosclerosis. However, during this same period there was ample evidence of change in most of these enzymes in the aorta tissue.

It is of the greatest interest that definite changes in the activity of specific enzymes can be demonstrated to occur in the aorta wall during the development of experimental atherosclerosis (see figure). At first, excessive loading of the rabbits with fat and cholesterol resulted in an increased activity of the various esterases. A small increase occurred in ACE activity (26%) and a large increase was found in PME-I and PME-II activities (respectively 100 and 107%). By the 90th and 100th days of the experiment the activity of all the enzymes had fallen sharply (compared with the controls): ACE to 70%, BCE to 62%, aliesterase to 61.5%, and PME-I and PME-II to 77 and 40% respectively of the control levels.

*After isolating the intima-media portion of the aorta, this was divided into samples weighing 50-70 mg and these were thoroughly ground in the deep-freeze (−70°). Then the tissue samples were further disintegrated by means of a glass homogenizer at 0° for 3 min.

There was no correlation between the changes in enzyme activity in the aorta wall with those in the blood. The observed drop in esterase activity of the aorta wall coincided with the appearance of atheromatous plaques in the aorta intima, i.e., with the development of experimental atherosclerosis.

In intrepreting the changes in enzyme activity it should be borne in mind that aliesterase and perhaps cholinesterase play a role in lipid metabolism [3] and also that elevation in phosphomonoesterase activity in the vascular walls has often been observed in conjunction with the development of young connective tissue [4]. It was of interest to us also that M. K. Malinow and M. A. Fernandez [11] have observed a parallelism between the intensity of PME-I activity in the vascular walls in various animal species and their resistance to factors which promote experimental atherosclerosis. The increased enzyme activity we have noted appears to us to be a self-initiated protective adaptation response on the part of the organism. The organism struggles to deal with excessive lipid loading and the elevated esterase activities in the vascular wall, aliesterase in particular, facilitate the cleavage of lipids. However, later on, this temporary elevation in enzyme activity is replaced by a depression. Consequently, the first plase of the organism's reaction to conditions of the experiment is the stage of adaptation to loading with lipids; later a stage appears which is characterized by exhaustion of the adaptive mechanisms,and this tends to promote the development of the pathological process.

Thus, it was found that the activity of specific and nonspecific cholinesterase, aliesterase, PME-I and PME-II in the rabbit aorta, during the development of experimental atherosclerosis, are elevated in the early stages (30 and 45 days) and are depressed by the end of a 3-month period. There is no correlation between changes in enzyme activity in the aorta and changes in the blood.

LITERATURE CITED

1. Textbook on Research in Nutrition and Public Health. Edited by A. A. Pokrovskii, [in Russian], Moscow 1964, p. 159.
2. Ibid., pp. 103, 144, 170, 201.
3. J. W. Clitherow, M. Mitchard, and N. J. Harper, Nature, 199 (1963), p. 1000.
4. B. S. Gould, Vitam, and Horm., 18 (1960), p. 89.
5. E. Kask, Angiology, 13 (1962), p. 35.
6. J. E. Kirk and J. Geront., 14 (1959), p. 181.
7. S. D. Kobernick and Y. Hashimoto, Lab. Invest., 12 (1963), p. 638.
8. E. Levonen, J. Raekallio, and U. Uotilia, Nature, 188 (1960). p. 677.
9. Z. Lojda and T. Zempleni, In the book: Current Problems in Cardiology, I. I. Speranskii (Ed.), [in Russian], Moscow (1960), p. 261.
10. Z. Lojda, Csl. Morfol., 10, (1962), p. 46.
11. M. R. Malinow, M. A. Fernandez, et al., Nature, 183 (1959), p. 1262.
12. H. Schlief, C. G. Schmidt, and H. J. Hillenbrand, Z. ges. exp. Med., Bd. 123, S. 491 (1954).
13. L. B. Sorensen and J. E. Kirk, J. Geront., 11 (1956), p. 28.
14. T. Zemplenyi, J. Atheroscler. Res., 2 (1962), p. 2.
15. T. Zemplenyi, et al., Ibid., 3 (1963), p. 50.