

THE IMPORTANCE OF PROLONGED FUNCTIONAL STRESS
OF THE NERVOUS SYSTEM IN THE DEVELOPMENT
OF LIPOIDOSIS IN THE AORTA AND MYOCARDIAL
LESIONS IN RABBITS

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The successes in the study of atherosclerosis have become possible due to the creation of a model of that illness in rabbits [1]. Studies of this disease in animals and simultaneous morbid anatomical studies on human subjects enabled N. N. Anichkov [2, 3], K. G. Volkova [3, 4], V. D. Tsinzerling [12], and other Russian scientists as well as a number of foreign authors [16 et al.] to study the morphogenesis of atherosclerosis and the importance of exogenous cholesterol in its development. Exogenous cholesterol, however, does not play the same role in human pathology as in the reproduction of the disease in animals. The studies of S. S. Khalatov [8, 9], N. T. Shutova [13, 14, 15], P. D. Gorizontov [5], and other authors who underlined the role of endogenous cholesterol in the development of cholesterol-emia were of great importance for the understanding of the pathogenesis of atherosclerosis. According to these authors, endogenous cholesterol-emia develops in case of disorders of the nervous system. Reports have been published according to which atherosclerosis occurs more frequently and takes a more severe course in persons engaged in mental work [7] or with a history of prolonged psychical traumas [6].

Comparative Data Concerning the Total and Free Blood Cholesterol Level, Aorta Lipoidosis, and
Cardiosclerosis During Functional Stress of the Nervous System (Without Administration of Cholesterol)

Animal	No. of rabbit	Average blood cholesterol level				Number of conditioned stimulations	Positive responses (in %)	Lipoidosis of the aorta	Cardiosclerosis	Duration of the observation (in months)
		control estimation		during functional stress of the nervous system						
		total cho- lesterol (in mg %)	free cho- lesterol (in %)	total cho- lesterol (in mg %)	free cho- lesterol (in %)					
Experimental rabbits	11	32.6	30.3	33.8*	38.0	1800	80.9	-	+	23
	13	36.7	25.1	46.7	33.6	1938	83.6	+	+	23
	15	44.4	25.1	51.1	33.6	1512	90.0	+	+	23
	17	80.0	27.0	72.2	27.1	1578	97.8	-	-	23
	19	22.2	22.7	23.0	43.0	1872	76.4	-	-	14
	22	68.5	20.6	35.6	42.0	1662	59.3	+	-	14
Control rabbits	12	31.4	28.0	Not investigated				-	-	23
	14	25.3	29.0					-	-	23
	16	31.4	26.1					-	-	23

* The findings refer to the average cholesterol level before the development of neurosis (see text).

In the present study an attempt was made to establish the influence of prolonged functional stress of the higher parts of the nervous system upon cholesterol and lipoprotein metabolism in the development of atherosclerosis.

METHOD OF EXPERIMENTS

Two series of experiments were carried out on ten experimental and nine control rabbits.

In the first series the total cholesterol was investigated in the serum of the experimental rabbits No. 1, 2, 3, and 4 during functional stress of the nervous system. Later the experimental rabbits (No. 1, 2, 3, and 4) and the control rabbits (No. 5, 6, 7, and 8) were given, against a background of stress of the nervous system, cholesterol in milk for 70 days (0.5 g daily). After an interval of three months the experimental rabbits No. 1 and 4 were given, against a background of stress of the nervous system, and the control rabbits No. 9 and 10 were given 19 egg yolks each for 38 days.

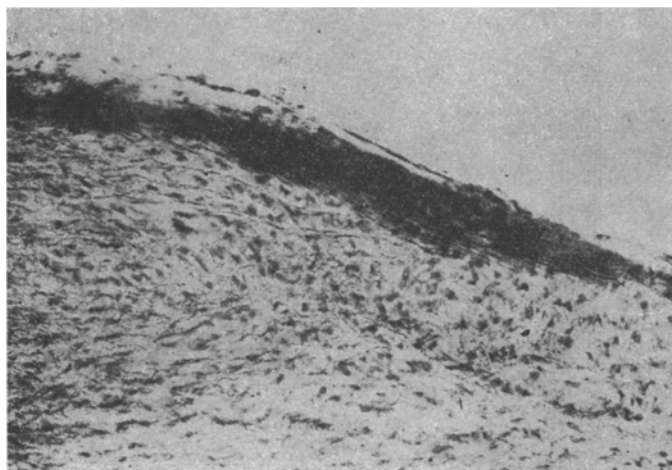


Fig. 1. Aorta wall of a rabbit (No. 13) which had not been given cholesterol with the food. In the abdominal part diffuse deposits and small droplets of lipids can be seen under the endothelium which in some places infiltrate the media. Microphotograph. Staining with Sudan III-haematoxylin. Magnification 180 \times .

A period of 30-60 days during which the animals were subjected to experiments, in which a conditioned reflex based on protective inhibition of breathing [10] was evoked, was regarded as a state of stress of the nervous system. Periods of functional stress alternated with periods of rest. 90-100 experiments were carried out with each experimental rabbit. The observation was continued for 12 months. The nervous function was evaluated by the number of positive responses given to the conditioned stimulus in relation to the total number of stimuli.

In the second series of experiments stress of the nervous system was produced by a similar method in six rabbits (No. 11, 13, 15, 17, 19, and 22). The observation was continued for 14-23 months in the course of which 180-250 experiments were carried out with each rabbit. During this period the total cholesterol, cholesterol bound to esters and the free cholesterol, the protein fractions and the lipoproteins in the blood serum were estimated twice monthly. The rabbits were given no cholesterol with the food. Proteins and lipoproteins were separated by paper electrophoresis: the protein was stained with amidoschwarz 10 B, the lipoproteins with Sudan black [11]. Three control rabbits (No. 12, 14, and 16) were used to estimate the indices characterizing the lipoprotein metabolism under normal conditions. Towards the end of the investigation the blood pressure was measured (by the acute method) in three experimental and in two control rabbits. All experimental rabbits of the first and second series and the seven control rabbits were killed by air embolism; the heart and the aorta were fixed in 10% formalin. Pieces of the heart were embedded in paraffin and the sections were stained by the method of van Gieson, Heidenhain, Mallory, Weigert, and were impregnated with silver according to Foot. The other part of the sections of the heart and the aorta were cut on the freezing microtome and stained for fat with Sudan III.

EXPERIMENTAL RESULTS

First Series. During the state of functional stress of the nervous system the total cholesterol of the blood in the rabbits decreased in all experimental rabbits by 24-45%. During the rest period the cholesterol returned to the original level and in some animals (Nos. 2 and 3) even exceeded that level by 60-70%.

Moderate feeding of cholesterol during functional stress of the nervous system did not cause a rise in the cholesterol level in three rabbits (Nos. 1, 2, and 4); whereas in one experimental rabbit (No. 3) and in six control rabbits (Nos. 5, 6, 7, 8, 9, and 10) the cholesterol level in the blood increased 10-15 times, reaching 200-600 mg.

Second Series. During the first three days of functional stress of the nervous system the total blood cholesterol increased by 20-30%; including both the level of cholesterol bound to esters and of free cholesterol; the free cholesterol fraction increased to a greater degree. By the 10th-12th day of functional stress of the nervous system the total cholesterol was lower than the original level; by the 30th-60th day the free cholesterol constituted 40-60% of the total cholesterol, in other words showed a relative increase.

Throughout the whole experiment (14-23 months) the average proportion of free cholesterol in per cent was higher in the rabbits than during the periods of rest and higher than in the control animals (see Table).

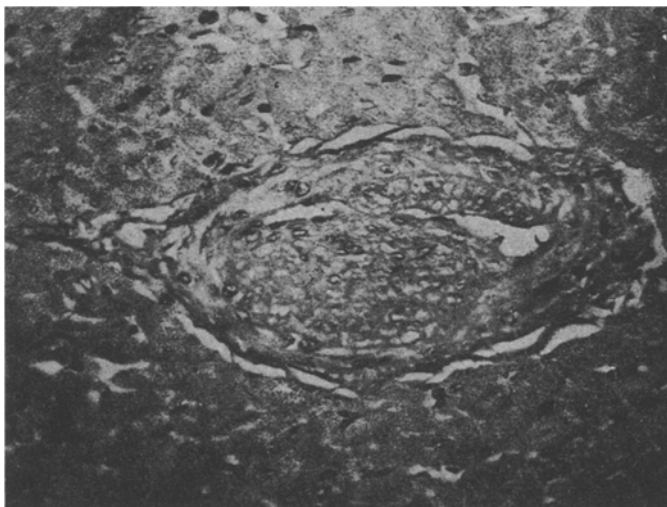


Fig. 2. Section through an artery in the papillary muscle in a rabbit (No. 1). Thickening of the arterial intima on two sides. The lumen of the blood vessel is narrowed. In the thickening course collagenous fibers can be seen among which some histiocytes and fibroblasts occur. At the sites adjacent to the thickening the media is atrophic. Microphotograph staining by van Gieson's method. Magnification 420 \times .

During the stress of the nervous system (30-60 days) the proportion of albumin in the blood serum decreased and the β - and γ -globulin fraction increased. For example, in the control group the average albumin level reached $58.5\% \pm 0.67$, in the experimental group (during the actual experiment) $51.4\% \pm 1.9$ ($td = 3.50$, in other words the difference between the values was statistically significant)*; in the control group the level of the β -globulins

* $td = (M_1 - M_2) / (\sqrt{m_1^2 + m_2^2})$

reached on the average $12.5\% \pm 0.33$, in the experimental group it increased to $15.7\% \pm 0.76$ ($td = 3.89$, i.e., the difference was statistically significant); in the control group the γ -globulins reached on the average $16.9\% \pm 0.67$, in the experimental group an increase of the γ -globulins to $20.7\% \pm 0.67$ could be observed ($td = 4.0$, i.e., the difference was statistically significant).

The level of α -lipoproteins and β -lipoproteins in the experimental rabbits No. 19 and No. 22 decreased during the functional stress of the nervous system, which lasted 30-60 days. During the rest interval the level of α -lipoproteins and β -lipoproteins increased. Towards the end of eight months' work with continued stress of the nervous system the reaction was reversed and an increase in the level of β -lipoproteins and neutral fats could be observed (colored spot in the region of the γ -globulins). In experimental rabbits No. 11, 13, 15, and 17 lipoproteid investigation results came after 17 months' work. In addition, the strain on the nervous system was watched for growth of β -lipoprotein and neutral fats.

Towards the end of the investigation a neurosis developed in experimental rabbit No. 11: during that period the blood cholesterol level increased in this rabbit within seven days from 25 to 420 mg% out of which 300 mg% consisted of free cholesterol. The level of β -lipoproteid and neutral fats showed a similar marked increase. The blood pressure in the experimental rabbits and the control rabbits varied between 98 and 112 mm Hg, i.e., the blood pressure was normal.

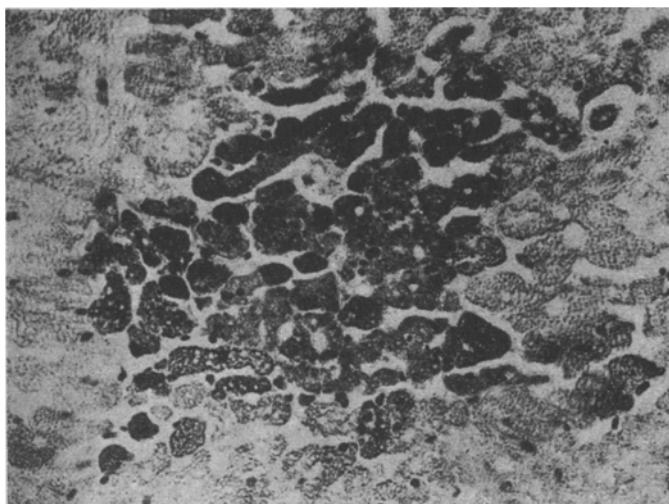


Fig. 3. Section through the papillary muscle of a rabbit (No. 11). Part of the muscle in a state of necrosis. Vacuoles of different size can be seen in the muscle fibers. Microphotograph staining by Heidenhain's method. Magnification 380 \times .

Histological investigation revealed in two experimental rabbits (Nos. 1 and 4) which had been given cholesterol with the food and had a normal blood cholesterol level the presence of lipoidosis of the aorta. In experimental rabbit No. 3 and in two control rabbits in which the blood cholesterol level had varied between 300 and 600 mg% lipoidosis of the aorta could also be observed. In the aorta of the experimental rabbits the lipids were situated along the internal elastic membrane under the endothelium in the shape of diffuse deposits and small droplets.

In the group of experimental rabbits which had never been given cholesterol but the nervous system of which had been exposed to prolonged functional stress lipoidosis of the aorta also developed in three out of six animals (see Table).

In the rabbits No. 13 and No. 15 the deposits of lipids were situated in the abdominal part of the aorta, under the endothelium along the internal elastic membrane (Fig. 1); in rabbit No. 22 in the beginning part of the aorta also under the endothelium along the internal elastic membrane. The lipids appeared in the shape of diffuse deposits and small droplets. In rabbits No. 13 and No. 22 they infiltrated the media of the aorta adjacent to the internal elastic membrane (see Fig. 1).

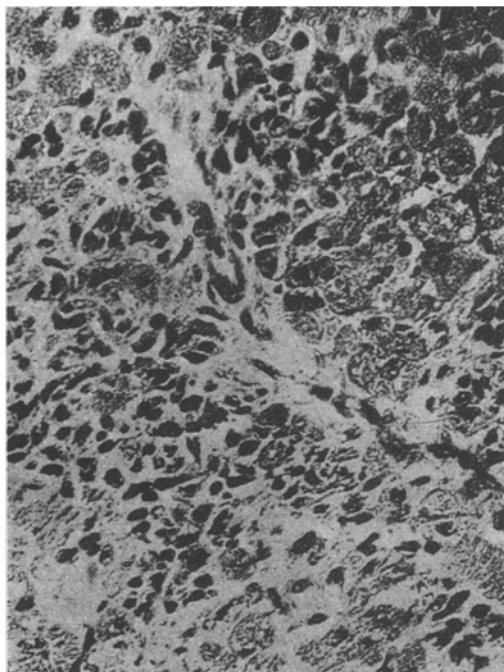


Fig. 4. Section through the left ventricle of a rabbit (No. 11). Accumulation of polyblast elements and fibrocytes which include necrotic muscle fibers. Microphotograph. Staining by van Gieson's method. Magnification 380 \times .

In the rabbits No. 13, 15, and 22 the blood cholesterol level never exceeded 80 mg%; a relative increase in the level of free cholesterol and an absolute increase in the level of β -lipoproteins, and neutral fats could be observed. In three other experimental rabbits (Nos. 11, 17, and 19) and control rabbits (Nos. 12, 14, and 16) no deposits of lipids could be observed in the aorta.

In the coronary arteries of the experimental rabbits (Nos. 1, 2, and 4) which had been given cholesterol and of the experimental rabbits (Nos. 11, 13, and 15) which had not been given cholesterol with the food, thickening of the arterial intima could be observed. In the coronary arteries of the control animals no thickening of the arterial intima could be observed.

Thickenings of the arterial intima were mainly situated in the arterioles and occurred more frequently in rabbits which had been given cholesterol. In sections stained with Sudan III no lipids deposits could be found in the thickenings. Only in one rabbit (No. 15) a yellow stain along the course of the elastic fibers could be seen in a changed area of the arterial intima in a large branch of the left coronary artery. The thickenings were produced by fairly coarse collagenous fibers arranged without any definite order which included some histiocytes and fibroblasts (Fig. 2). In places adjacent to the thickening the media of the blood vessels was atrophic (see Fig. 2), the internal elastic membrane was split up and interrupted. Besides a great number of argyrophile fibers could be seen in the thickenings.

In sections through the myocardium the capillaries were found to be moderately dilated and congested with blood in all experimental rabbits. In the myocardium of the experimental rabbits Nos. 1, 2, 4, 11, 13, and 15* small areas were found in which the muscle fibers were of homogenous appearance or were in a state of necrotic fragmentation.

In the papillary muscles of rabbit No. 11 necrotic areas could be seen (Fig. 3) in other regions of the myocardium accumulations of polyblast elements and fibrocytes, including necrotic fibers, could be seen (Fig. 4).

Analysis revealed that the severity of the myocardial lesions was related to the duration of stress to which the nervous system was exposed and to the character of the conditioned reflex activity. In rabbit No. 11 which was suffering from neurosis the myocardial lesions were most severe of all.

SUMMARY

Lipoidosis of aorta with a thickening of the tunica intima of the coronary vessels and dystrophy of the cardiac muscle was seen to develop in prolonged functional stress of the nervous system (12-23 months) in rabbits which received food with or without cholesterol.

Lipoid deposition into the wall of aorta occurred with the cholesterol blood level equal to 35-70 mg%; however, the content of free cholesterol, β -lipoproteids, and neutral fats was found to be increased in the blood of experimental rabbits. The mentioned lesions in the aorta, the coronary vessels of the heart, and the cardiac muscle are the sequence of prolonged functional stress of the nervous system.

* The state of the myocardium in rabbits Nos. 19 and 22 was not investigated.

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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 this issue.
