ROLE OF THE FALL IN LIPOLYTIC ACTIVITY OF THE WALL OF THE AORTA AND THE PATHOGENESIS OF ITS LIPOID INFILTRATION

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Considerable attention has recently been paid to the role of disturbances of metabolism of the blood vessel wall in the pathogenesis of lipoidosis and atherosclerosis of this structure.

Zemplenyi and Grafnetter [7] found a relationship between the species and age predisposition to atherosclerosis and the lipolytic activity of the aorta. In species of animals susceptible to experimental atherosclerosis (rabbits, guinea pigs, fowls) the lipolytic activity of the aorta is lower than in albino rats, which are resistant to this disease. A fall in lipolytic activity is also observed during aging. S. M. Leites and Chou-su [1] showed that states favoring the development of atherosclerosis (stress, diabetes mellitus, hypoxia) are accompanied by a fall in the lipolytic activity of the aorta. A similar phenomenon was found by F. L. Leites in relation to hypothyroidism [2].

We were interested to study the changes in the lipolytic activity of the aorta during reproduction of alimentary lipoidosis. We also attempted to discover the effect of a fall in the lipolytic activity of the aorta, caused by repeated states of stress, on the degree of experimental lipoidosis in its wall.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male albino rats weighing from 220 to 300 g. The animals received the ordinary laboratory diet ad lib. On alternate days, after receiving no food for 18 h, 13 rats received a 10% solution of cholesterol in apricot oil in a dose of 1 ml/100 g body weight through a gastric tube; 5 h later they received their ordinary diet. Four hours after receiving cholesterol in oil in the same dose, 8 other rats were kept outstretched on the operating table on their backs for 1 h (stress, as defined by Selye). Stress was produced by the same method on alternate days in 5 rats (after fasting for 22 h), but without the preliminary administration of cholesterol in oil. The control group consisted of 14 rats. The animals were sacrificed by decapitation 25-30 days after the beginning of the experiment and at the end of an 18 h period of fasting.

The lipolytic activity of the part of the aorta from the middle of the ascending division to the diaphragm was determined by a modified Zemplenyi and Grafnetter's method [7]. The aorta was stripped of its adventitia and finely minced; 45-50 mg of the mince was mixed with an apricot oil emulsion (2 ml apricot oil to 0.2 ml "Tween 80" in 100 ml of 1% albumin solution in Sorensen's phosphate buffer at pH 7.4) and emulsified at 5000 rpm in a homogenizer for 20 min. The resulting emulsion was allowed to stand overnight in a separating funnel at room temperature; the lower homogeneous layer was used, and its pH was adjusted to 7.4 with 1 N NaOH solution. The total lipid concentration in the resulting emulsion was determined by a modified Kunkel's method [6] and its value was 600-800 mg %. The mixture was incubated for 150 min in the water bath of a Warburg's apparatus at 37°, with constant agitation. The intensity of lipolysis was determined from the difference between the concentration of nonesterified higher fatty acids (NEFA) before and after incubation and expressed in μeq/ml/g tissue. The NEFA were estimated by Dole's method [5]. Two series of experiments were carried out.

The selection of material for histological investigation was made on the basis that in albino rats with lipoidosis caused by various factors and with experimental atherosclerosis, the area of the aorta most severely affected is its

Lipolytic Activity of the Aortic Wall of Rats (in μeq/ml NEFA/g tissue) Fed on Cholesterol Solution in Apricot Oil (A), in a State of Repeated Stress while Receiving this Diet (B) or while Receiving an Ordinary Diet (C)

First series of experiments			Second series of experiments			
normal	A	B	normal	A	B	C
14,8	13,2	13,6	11,2	8,6	5,2	8,4
14,4	11,2	8,8	11,7	9,0	7,3	10,4
15,2	9,2	6,4	10,4	9,6	7,6	7,6
14,4	9,2	—	9,6	8,0	8,8	5,6
14,0	8,8	—	10,4	8,5	4,0	6,4
14,4	—	—	9,2	7,6	—	—
10,6	—	—	—	11,2	—	—
12,4	—	—	—	7,6	—	—

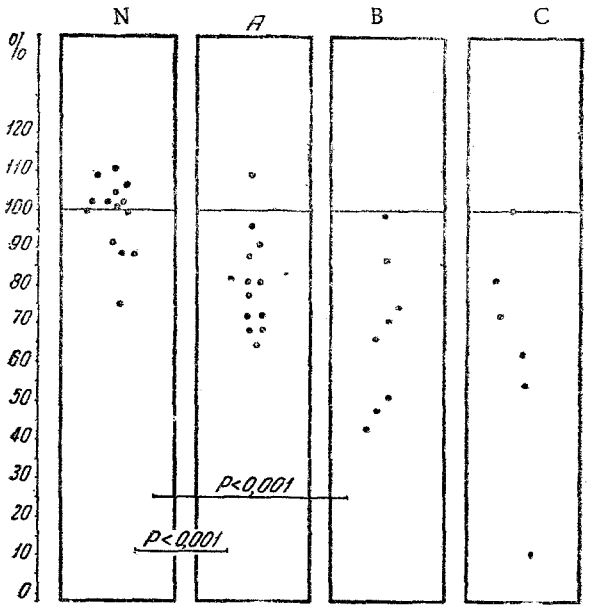


Fig. 1. Effect of feeding cholesterol in apricot oil and repeated states of stress on the lipolytic activity of the aortic wall in rats, expressed as a percentage of the lipolytic activity of normal rats. A) Fed on cholesterol in oil; B) fed on cholesterol in oil + repeated stress; C) repeated stress + normal feeding.

origin at the base of the aortic valves and the surrounding zone [3, 4]. For this reason the origin of the aorta with the aortic valves, and also certain parts of the myocardium and coronary arteries, from each animal were investigated histologically. Besides the ordinary histological methods (staining with hematoxylin-eosin and for lipids with Sudan IV), examinations for anisotropic lipids were made with the polarization microscope and the Schultz test for total cholesterol was performed. In a representative selection of sections from each experimental group, taken from the aortic valves and the origin of the aorta, the activity of the lipolytic enzymes – nonspecific esterase and lipase – was investigated (the Gomori and Gomori-Mark modifications of the Nachlas-Seligman method).

EXPERIMENTAL RESULTS

The results of the determination of the lipolytic activity of the aorta are given in the table.

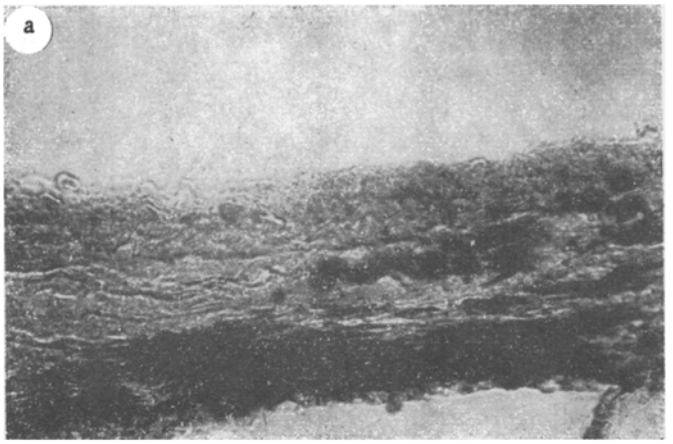
To compare the results of the two series of experiments the lipolytic activity in each was expressed as a percentage of the mean lipolytic activity of the normal rats of this series, taken as 100 (Fig. 1). Feeding the animals on cholesterol in vegetable oil led to a statistically significant fall in the lipolytic activity of the aortic wall.

A more marked fall in the lipolytic activity was observed in the animals receiving cholesterol-lipid loading in association with the background of repeated stress. In some experiments in which stress was produced in animals receiving a normal diet a marked fall in the lipolytic activity was also observed.

During histological investigation of the control rats, in most a very feeble physiological lipoidosis was observed at the base of the aortic valves, where it constitutes a characteristic morphological sign of aging in albino rats.

In the animals receiving cholesterol in vegetable oil a moderate increase in the degree of the above lipoidosis was observed. In these experimental conditions, no difference could be determined histochemically between the activity of the lipolytic enzymes in the groups of intact rats and of rats receiving the cholesterol-lipid loading.

Significant morphological changes were found in the animals receiving cholesterol in apricot oil and subjected to repeated stress. Severe lipoidosis was seen in most of these animals, affecting not only the deep layers of the



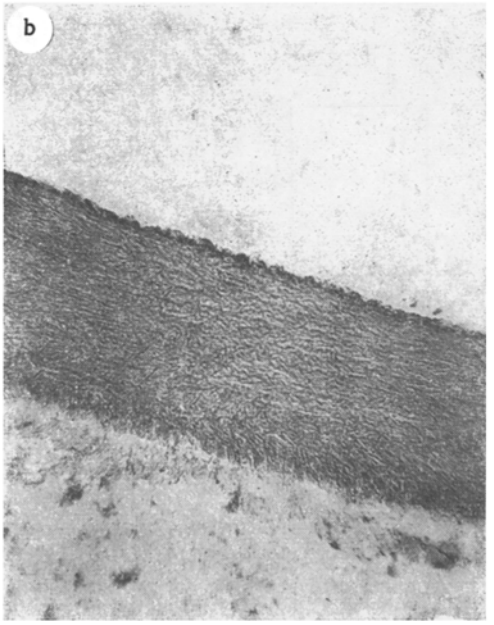


Fig. 2. a) Cells containing nonspecific esterase (black bands and spots) in the aorta and periaortic connective tissue of an intact animal; b) considerable reduction in number of enzyme-containing cells in the aortic wall and perivascular tissue following a combination of repeated stress and cholesterol-lipid loading. Nachlas-Seligman reaction as modified by Gomori. Objective 40, ocular 7.

endocardium forming the base of the aortic valves, but also the subendothelial layer. Larger circumscribed deposits of lipids (including not only neutral fat, but also cholesterol esters, according to the results of polarization microscopy and of the Schultz test) were also observed in the fold forming the base of the aortic valves and origin of the aorta. Large collections of lipids caused elevation of the surface of the endothelium to form characteristic "cushions." In some animals of this group lipoidosis of the coronary arteries was also observed, affecting their larger branches. According to the histochemical findings, the activity of the lipolytic enzymes in the rats of this group was appreciably lower than normal (Fig. 2).

SUMMARY

Biochemical and histochemical methods were used to study the changes occurring in the lipolytic activity of the aorta in albino rats fed on cholesterol and butter and in combining this diet with repeated "stress" conditions. The histochemical method was used to investigate the deposition of lipids in the root of the aorta, aortic valves and the coronary arteties. The cholesterol-fat diet and to a greater extent its combination with "stress" was accompanied by a reduction of lipolytic activity of the aorta. A connection was noted between the fall of lipolytic activity in the aorta and its lipoidosis.

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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.
