TISSUE LIPOLYTIC ENZYME ACTIVITY OF SOME INTERNAL ORGANS DURING TREATMENT OF EXPERIMENTAL ATHEROSCLEROSIS WITH PHEPRACET (IEM-366)

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Phepracet produced definite inhibition of activity of the lipolytic enzymes in the pulmonary artery wall and restored the normal cholesterol level in the blood, liver, and aorta and the normal lipid content in the aortic wall (experiments by M. N. Gaziev) in rabbits with experimental atherosclerosis. In animals receiving cholesterol, in the presence of marked atherosclerosis, a considerable increase in activity of these enzymes was observed. Phepracet had no significant effect on the intensity of lipolysis of liver, heart muscle, and adrenal tissues.

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The object of this investigation was to study changes in activity of lipolytic enzymes in the tissues of some internal organs of rabbits during regression of experimental atherosclerosis in animals treated experimentally with phepracet, which has a marked anticholesteremic action.

Phepracet (p-aminophenylacetic acid amide of β-phenylisopropylamine) is an original preparation with an action opposite to that of amphetamine [4]. It has a favorable effect on the course and development of experimental hypercholesteremia in albino mice and rats [2] and on cholesterol atherosclerosis in rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing about 3 kg. Cholesterol (1 mg/kg) was given to 20 rabbits daily for the first two months, and every other day for the third and fourth months. Cholesterol was given as a 20% solution in sunflower oil, mixed with chopped vegetables. Subsequently 13 rabbits acted as controls and the remaining 12 animals received phepracet by subcutaneous injection in a dose of

TABLE 1. Activity of Lipolytic Enzymes in Tissues of Internal Organs of Rabbits During Regression of Experimental Atherosclerosis Under the Influence of Phepracet (M±m)

Animals	NEFA (in meq/liter)	Lipolytic activity of tissue (in meq/ml/g tissue)			
		Pulmonary artery	Liver	heart muscle	adrenals
Control	0,80±0,035	10,0±0,96	29,8±0,59	7,4±0,44	39,4±1,93
		0,001	0,3	0,01	0,005
Treated	0,64±0,04	4,8±0,36	29,6±0,66	7,1±0,39	37,7±1,67
	0,05	0,001	0,9	0,5	0,5
Intact	0,76±0,06	6,0±0,51	29,0±0,63	9,6±0,55	30,9±1,70

Note. Samples for analysis taken from mince of both adrenals and heart muscle weighed 100 mg each, from liver tissue 180 mg, and from pulmonary artery tissue 50 mg.

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25 mg/kg daily for the next four months (these animals are described as treated). Eleven intact rabbits received a normal diet.

The rabbits were sacrificed by air embolism after fasting for 15-17 h. The content of nonesterified fatty acids (NEFA) (by Dole's [5] method) and the lipolytic enzyme activity in the tissues of the liver, adrenals, heart muscle, and wall of the pulmonary artery were investigated (the content of cholesterol and lipids in the aortic wall was determined). The picture of the lesions in the pulmonary artery and aorta was usually identical. The lipolytic (total lipase-esterase) activity in the samples was determined by Zemplenyi's method [8] as modified by Leites and Chou-su [2], based on hydrolysis of substrate Tween-60. We modified this method slightly: instead of diluting the Tween-60 with Sorensen's buffer solution with albumin, we used a 0.02M aqueous solution of $CaCl_2$. Korn [7] and Grafnetter and Zemplenyi [6] mention the possibility of this substitution in their papers. The incubation period was shortened from 150 to 90 min. To verify the validity of this substitution a special series of experiments was carried out. It was also found that the N-heptane (C_7H_{16}) can be replaced by iso-octane (C_6H_{18}). The lipolytic activity of the tissues of certain internal organs of the rabbits was investigated after feeding the animals with cholesterol for four months and then after regression of the atherosclerosis for another four months.

EXPERIMENTAL RESULTS

The experiments showed that lipolytic enzyme activity in the wall of the pulmonary artery of the rabbits receiving phepracet was significantly lower than the animals fed with cholesterol alone (controls). At the same time the lipid content in the aortic wall and the cholesterol level in the blood serum and liver tissue returned to normal* .

The decrease in intensity of lipolysis in the wall of the pulmonary artery by comparison with the results found in the control animals was evidently due to a decrease in the lipid content in the aorta caused by the action of phepracet and due to diminished induction of lipolytic enzymes.

In control animals with marked atherosclerosis (high blood cholesterol and lipid content in the aorta and liver) considerable increase in activity of the lipolytic enzymes was observed in the wall of the pulmonary artery, reaching a maximum in those rabbits with the severest lesions in the aorta. The increase in lipolysis in the wall of the pulmonary artery led in this case to destruction of the lipoprotein complex, as a result of which free cholesterol was liberated and exerted its harmful action.

M. I. Sukasova and co-workers [3], who used the preparation delipin (0.3 g each of ascorbic acid and methionine, 0.03 g luminal, and 0.05 g pyridoxin) prophylactically in experimental atherosclerosis, likewise found a decrease in lipolytic enzyme activity in the aortic wall with obvious inhibition of the development of atherosclerosis compared with the results of investigation of rabbits receiving cholesterol alone.

Administration of phepracet had no significant effect on the course of lipolysis in the liver, heart muscle, and adrenals (Table 1). Judging by the NEFA concentration in the blood serum of the treated animals, phepracet slightly inhibited mobilization and oxidation of fat.

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* Experiments by M. N. Gaziev (Kardiologiya, No. 12, 43, 1967).