

EFFECT OF MALIC ACID AND CARBONATES  
OF ALKALI AND ALKALINE-EARTH METALS  
ON DEVELOPMENT OF ALIMENTARY  
HYPERCHOLESTEREMIA AND LIPOIDOSIS  
OF THE AORTA IN RABBITS

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Against the background of an atherogenic diet malic acid lowers the level of cholesterol and total lipids in the liver, adrenals, and aorta of rabbits, while carbonates of the alkali and alkaline-earth metals give the opposite effect.

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Disturbance of oxidation-reduction reactions in the Krebs cycle leads to the accumulation of ketone bodies [5]. This does not take place if normal function of the Krebs cycle is restored by administration of citric acid [1].

It was decided to investigate the effect of another intermediate compound of the Krebs cycle, malic acid, given with the diet in large doses, on cholesterol metabolism [4].

EXPERIMENTAL METHOD

Experiments were carried out in the summer on 24 male rabbits weighing 2.7-2.8 kg. For 100 days the animals received a basic diet consisting of: 50 g hay, 80 g oats, 100 g vegetables, and cholesterol in a dose of 200 mg/kg body weight as a suspension in starch gel, given with the diet [3]. The content of cholesterol in the blood [7] and in certain organs [6] was determined. Total lipids were determined gravimetrically. Control animals (group 1) received only the basic diet; experimental animals of group 2 received malic acid in addition to the basic diet in a dose of 1 g/kg, and those of group 3 received a mixture of carbonates of alkali and alkaline-earth metals with the diet in a dose of 500 mg/kg ( $\text{NaHCO}_3$ , 83 mg;  $\text{K}_2\text{CO}_3$ , 218 mg;  $\text{CaCO}_3$ , 91 mg;  $\text{MgCO}_3$ , 108 mg). The relative proportions of the salts corresponded approximately to those in natural food products contained in the animals' daily ration [2].

EXPERIMENTAL RESULTS

The experimental results (Table 1) showed that by the end of the experiment a significantly ( $P < 0.05$ ) higher increase in the blood cholesterol concentration was observed in the rabbits of group 3 than in the controls. The lowest blood cholesterol concentration was found in the rabbits of group 2 ( $P < 0.002$ ). The concentration of cholesterol and total lipids in the organs was sharply increased in animals of all groups. However, in the rabbits of group 2 they were found in lower concentrations. The concentration of cholesterol and total lipids in the aorta of the animals of all groups corresponded to the severity of the macroscopic picture of atheromatosis. Atherosclerotic changes in the aorta of the rabbits of group 3 were assessed as from +++ to +++++, and in rabbits of group 2 from + to +++.

Macroscopic examination of the aorta of the control animals revealed numerous small and large plaques, some of which had joined together. Histological examination of pieces of the aorta showed that the tissue of the atheromatous plaques consisted mainly of xanthoma cells. Diffuse infiltration with lipids was found in the wall of the large vessels branching from the aorta. In rabbits of group 2, the changes in

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TABLE 1. Effect of Malic Acid and Carbonates of Alkali and Alkaline-Earth Metals on Development of Alimentary Hypercholesteremia and Lipoidosis of the Aorta in Rabbits (M $\pm$ m)

Group and number of animals	Weight of animals (in g)		Cholesterol concentration in blood plasma (in mg%)		Cholesterol concentration			Concentration of total lipids		
	beginning of expt.	end of expt.	1st day of expt.	100th day of expt.	in liver (in mg%)	in adrenals (in %)	in aorta (in mg/g fresh tissue)	in liver (in %)	in adrenals (in %)	in aorta (in mg/g fresh tissue)
1-я (8)	2 755	2 800	45.4 $\pm$ 3.6	661.3 $\pm$ 33.0	969 $\pm$ 165.9	16.7 $\pm$ 0.53	19.1 $\pm$ 2.5	8.9 $\pm$ 0.91	37 $\pm$ 3.2	50.5 $\pm$ 4.1
2-я (8)	2 766	2 818	59.9 $\pm$ 3.1	P < 0.002 450.3 $\pm$ 45.0	P < 0.05 682 $\pm$ 143.6	P < 0.05 13.6 $\pm$ 1.5	P < 0.001 12.2 $\pm$ 1.8	P < 0.001 7.2 $\pm$ 0.61	P < 0.2 33.2 $\pm$ 4.1	P < 0.01 384.4 $\pm$ 5.5
3-я (8)	2 708	2 923	61.8 $\pm$ 5.1	P < 0.05 769 $\pm$ 69.1	P < 0.05 1164 $\pm$ 134.5	P < 0.02 17.6 $\pm$ 1.17	P < 0.05 21.7 $\pm$ 2.3	P < 0.05 11.2 $\pm$ 6.5	P < 0.2 38 $\pm$ 2.1	P < 0.02 65.8 $\pm$ 3.6

the aorta were much less severe: solitary, very small atherosclerotic plaques were observed on the inner surface of the aorta, together with large areas free from pathological changes. In the rabbits of group 3, atheromatosis of the aorta was much more severe than in the controls. In most animals of group 3, multiple large plaques, irregular in shape and joined together, were found on the inner surface of the aorta (from the arch to the bifurcation). Histological examination showed that the plaques consisted of cells staining intensely with hematoxylin, and of xanthoma cells containing lipids. The number of elastic fibers was reduced, and the fibers present were broken and fragmented (shown by staining with orcein). Macroscopic examination of the liver of the animals of groups 1 and 2 showed no changes. The liver in the animals of group 3 was friable, clay-colored, and its weight and volume were increased by 2-3 times compared with normal. Microscopic examination revealed droplets of neutral fat in the liver of the animals of groups 1 and 2, located in individual polygonal cells in the peripheral zones of the hepatic lobules. In the animals of group 3, diffuse infiltration of the polygonal cells of the liver with fat was observed.

In the rabbits of groups 1 and 3 the adrenals, aorta, and thyroid gland were enlarged by 50-100% compared with normal. In the animals of group 2 these organs were hardly distinguishable in weight from the organs of healthy rabbits. Malic acid in a dose of 1 g/kg body weight, when added to an atherogenic diet, has a beneficial effect, lowering the level of cholesterol and total lipids in the blood and organs, while carbonates of the alkali and alkaline-earth metals increase the cholesteremia starting from the 40th day after the beginning of the experiment.

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