

EFFECT OF CITRIC ACID ON EXPERIMENTAL HYPERCHOLESTEREMIA AND ATHEROSCLEROSIS IN RABBITS

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Citric acid in a dose of 960 mg/kg body weight facilitates a more rapid lowering of the cholesterol level in the blood, liver, adrenals, and aorta of rabbits after stopping cholesterol feeding. The activity of certain enzymes of the esterase group in the aorta is also restored to normal. A mixture of carbonates of the alkali and alkaline-earth metals gives the opposite effect.

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There are reports in the literature that in progressive atherosclerosis the intensity of oxidation is diminished and the quantity of incompletely oxidized products in the blood and urine increases. In cases of regression of atherosclerosis, the content of incompletely oxidized products becomes lower. Hence, an increase in the content of incompletely oxidized products may be an index of the activity of atherosclerosis [3]. Disturbance of the tricarboxylic acid cycle always leads to an increased accumulation of ketone bodies, and correspondingly, to disturbance of fatty acid metabolism [4, 5, 9]. The formation and accumulation of ketone bodies are easily suppressed by restoring the normal function of the tricarboxylic oxidative cycle of administration of citrates [2]. The antiketogenic action of citric acid has been demonstrated not only in ketones caused by ammonium salts and butyric acid, but also in diabetic ketoses in man and in alloxan diabetes in rabbits [2].

In the present investigation the effect of citric acid on the regression of cholesterol atherosclerosis was studied in rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 27 male chinchilla rabbits initially weighing 2.3-2.5 kg. In the first period of the experiment (100 days) all the animals received a basic ration consisting of the following components: 50 g hay, 80 g oats, 100 g vegetables, and 200 mg cholesterol/kg body weight. Cholesterol was given to the animals as a fine suspension in starch paste mixed with the food. The cholesterol content in the blood plasma was measured every 20 days for 100 days by the method of Chiamori and Henry [8]. The rabbits developed cholesteremia to an unequal degree, and toward the end of the first period of the experiment the blood cholesterol level varied from 285 to 1169 mg %. To determine the degree of the morphological and biochemical changes in the organs the animals of group 1 were sacrificed after 100 days. Cholesterol in the organs was determined by Abell's method [7], total lipids gravimetrically, and the activity of the enzymes as follows: phosphomonoesterases 1 and 2 (PME-1, PME-2), acetylcholinesterase (ACE), butyrylcholinesterase (BCE), and tributyrinase in the aorta by A. A. Pokrovskii's method [6]. The remaining rabbits were subdivided into 2 groups, with 10 animals in each group, depending on their plasma cholesterol concentration. Cholesterol feeding was stopped after 100 days, and from the following day the animals of group 2 received a basic ration without cholesterol and a mixture of carbonates of alkali and alkaline-earth metals (260 mg/kg) for 50 days.

The rabbits of group 3 (experimental group) received a basic ration: citric acid in a dose of 960 mg/kg, and 260 mg of a mixture of carbonates of alkali and alkaline-earth metals (28 mg NaHCO_3 , 113 mg

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TABLE 1. Effect of Citric Acid on Regression of Experimental Hypercholesterolemia and Atherosclerosis in Rabbits

Group of animals	Basic ration + test preparation	No. of animals	Wt. of animals (in g)	Cholesterol concentration in blood plasma (mg%)	Change in cholesterol concentration in blood plasma after stopping cholesterol feeding (mg%)			Cholesterol concentration			Content of total lipids		
					days of experiment			in liver (mg %)	in adren-als (%)	in aorta (mg/g fresh tissue)	in liver (%)	in adren-als (%)	in aorta (mg/g fresh tissue)
					10th	30th	50th						
1st	Basic ration + cholesterol	7	2477/2948	714 ± 122.3	—	—	—	1010 ± 233.7	17.9 ± 0.5	31.2 ± 4.1	7.9 ± 0.82	55.2 ± 7.7	75.4 ± 6.1
2nd	Basic ration + cholesterol (1st period of expt.)	10	2472/2895	684 ± 106									
	Basic ration + mixture of carbonates of alkali and alkaline-earth metals w/o cholesterol (2nd period of expt.)				393 ± 50.0	172.2 ± 40.4	76.7 ± 11.5	622.4 ± 50.2	17.3 ± 2.7	29.8 ± 4.2	8.1 ± 0.41	53.9 ± 1.5	67.3 ± 6.5
3rd	Basic ration + cholesterol (1st period of expt.)	10	2382/3190	677.5 ± 78.9									
	Basic ration w/o cholesterol + citric acid (960 mg/kg) + mixt. of carbonates of alkali and alkaline-metals (260 mg/kg) (2nd period of expt.)				261 ± 36.1	74.9 ± 6.7	47.5 ± 7.2	495.4 ± 33.3	8.8 ± 1.5	16.5 ± 2.7	6.5 ± 0.34	36.3 ± 3.7	47.0 ± 7.0
		P			<0.05	<0.05	<0.05	<0.05	>0.01	<0.02	<0.01	<0.001	<0.05

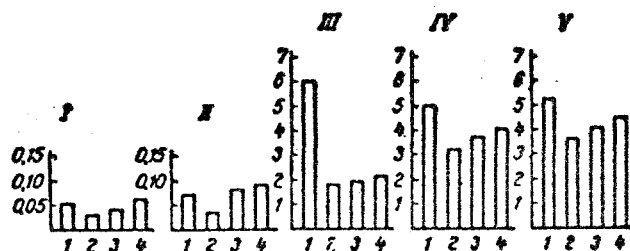


Fig. 1. Activity of certain enzymes in the aorta of control and experimental rabbits. I) Phosphomonoesterase-1; II) phosphomonoesterase-2; III) acetylcholinesterase; IV) butyrylcholinesterase; V) tributyrinase; 1) rabbits not receiving cholesterol with diet; 2) rabbits receiving cholesterol with diet for 100 days; 3) rabbits receiving basic ration for 50 days after stopping cholesterol feeding; 4) rabbits receiving basic diet and citric acid for 50 days after stopping cholesterol feeding. Vertical axis: enzyme activity (in μ moles substrate decomposed by 1 g fresh tissue/min).

K_2CO_3 , 107 mg $CaCO_3$, and 20 mg $MgCO_3$). The ratio between the various elements (sodium, potassium, calcium, and magnesium) present as carbonates was determined from the content of these substances in the natural products.

EXPERIMENTAL RESULTS

The results of biochemical investigations of the blood plasma and internal organs of the animals of group 1 are given in Table 1 and Fig. 1. In the animals of this group a high content of cholesterol and total lipids was observed in the liver, adrenals, and aorta. The atherosclerotic changes in the aorta varied in degree and were assessed from 1+ to 5+ [1]. The total lipid content in the aorta, calculated per gram fresh weight of intima and plaques, reflected the degree of involvement of the aorta. The intima of the aorta of most animals was rough throughout its extent and contained a large number of yellowish-white irregularly shaped plaques which had merged with one another over large areas. A decrease in the activity of the enzymes PME-1, PME-2, ACE, BCE, and tributyrinase was observed in the wall of the aorta. Results showing the effect of citric acid and carbonates of alkali and alkaline-earth metals on regression of the hypercholesteremia and atherosclerosis in the rabbits are given in Table 1 and Fig. 1.

Feeding with citric acid after stopping addition of cholesterol to the diet accelerated regression of the cholesteremia, and in the first 10 days a statistically significant decrease in the blood cholesterol concentration took place. After 30 days, the cholesterol level returned to the upper limit of normal, and by the end of the experiment it was within normal limits. The cholesterol content in the control animals still remained high after 30 days (92.9 to 440 mg %), and not until after 50 days did it reach a mean level of 76.7 mg %. The content of cholesterol and total lipids in the organs of the animals receiving citric acid was much lower than in the controls. The atherosclerotic changes in the aorta were more marked in the control animals (3+) than in the experimental (2+). The total content of lipids in the aorta of these animals bore a definite relationship to the macroscopic severity of the atherosclerosis.

A more rapid recovery of the activity of the enzymes PME-1 and PME-2, ACE, BCE, and tributyrinase was observed in the rabbits receiving citric acid. In the animals of group 1, the activity of these enzymes was sharply lowered, especially in rabbits with a high blood cholesterol (800-1090 mg% or more). The atherosclerotic changes in the aorta of the animals were very severe over wide areas (from the arch of the aorta to its bifurcation). In most of the experiments the correlation between a high blood cholesterol level and the severity of the atherosclerotic changes in the aorta was more or less complete.

Citric acid in a dose of 960 mg/kg accelerates the decrease in blood cholesterol level during regression of alimentary hypercholesteremia and atherosclerosis in rabbits, and also lowers the content of cholesterol and total lipids in the organs. In addition, it facilitates the more rapid recovery of activity of certain enzymes of the esterase group in the wall of the rabbits' aorta. A mixture of carbonates of alkali and alkaline-earth metals has the opposite effect.

LITERATURE CITED

1. L. I. Grebennik, E. Ya. Levashova, and N. G. Shakhnazarova, *Farmakol. i Toksikol.*, No. 5, 590 (1962).
2. M. F. Gulyi, *Biochemistry of Lipid Metabolism* [in Russian], Kiev (1961), pp. 136, 139, 141.
3. N. S. Zanozdra, D. A. Nuzhnyi, and I. Yu. Ivanina, In: *Atherosclerosis* [in Russian], Leningrad (1965), p. 80.
4. M. A. Kolomfichenko, M. F. Gulyi, and G. I. Dubravina, *Ukr. Biokhim. Zh.*, No. 1, 69 (1952).
5. L. Sh. Levina, *Ukr. Biokhim. Zh.*, No. 4, 478 (1952).
6. A. A. Pokrovskii (Editor), *Textbook of the Study of Diet and Health of the Population* [in Russian], Moscow (1964), p. 144.
7. L. L. Abell et al., In: *Standard Methods of Clinical Chemistry*, Vol. 2, New York (1958), p. 26.
8. N. Chiamori and R. J. Henry, *Am. J. Clin. Path.*, 31, 305 (1959).
9. L. H. Edson, *Biochem. J.*, 29, 2082 (1935).