EFFECT OF n-ALKANES ON DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS

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The effect of n-alkanes on the development of atherosclerosis induced by chlolesterol feeding was studied in experiments on rabbits. In doses of 40 and 120 mg/kg n-alkanes led to an increase in the serum cholesterol concentration and the lipid content in the tissues of the aorta and also in the degree of the atherosclerotic changes in the aorta. In some animals receiving n-alkanes with the diet cirrhosis of the liver developed against the background of fatty degeneration. In rabbits receiving n-alkanes the activity of the lipid-mobilizing enzymes was reduced and the content of n-alkanes in the aorta and adipose tissue was increased.

A characteristic feature of the 20th century is the increasing technological use of petroleum products and contamination of the external environment by them.

Assessment of the likely or possible harmful effect of petroleum products on human health requires particular attention to be paid to polycyclic compounds which possess marked carcinogenic properties [4, 5, 14, 15].

The paraffin hydrocarbons, characterized by a degree of chemical resistance, have neither carcinogenic nor acute toxic action. There are only isolated reports in the literature of the discovery of hydrocarbons in the human liver, spleen, and lymph glands [16-18]. However, the biological action of the n-alkanes has been very incompletely studied. The results of investigations in the last 3 years have shown that in the tissues of animals receiving liquid paraffins with their diet there is a tendency for the level of oxygen utilization to fall and for lipids to accumulate in the liver, with an accompanying slight increase in the blood serum cholesterol concentration. By the use of C_{14}-labeled octadecane it has been possible to study both the rate of its absorption by the intestinal mucous membrane and the periods of its deposition in the tissues as well as the dynamics of its metabolism and removal from the body [6].

These investigations suggested that n-alkanes might possibly influence lipid metabolism. A particularly interesting aspect of this problem is the study of the atherogenic properties of the n-alkanes and, in particular, their influence on the course of experimental atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on 60 rabbits kept on the ordinary animal house diet. Atherosclerosis was induced by the method of Anichkov and Khalatov, by feeding the animals with chloesterol (0.2 g/kg body weight) which was administered through a tube as a 10% solution in sunflower oil. The animals were divided into four groups. The rabbits of group 1 (control) received only sunflower oil (2 mg/kg), those of group 2 received sunflower oil and cholesterol (0.2 g/kg), group 3 received sunflower oil, cholesterol, and liquid paraffins in a dose of 40 mg/kg body weight, and the rabbits of group 4 received sunflower oil, cholesterol,

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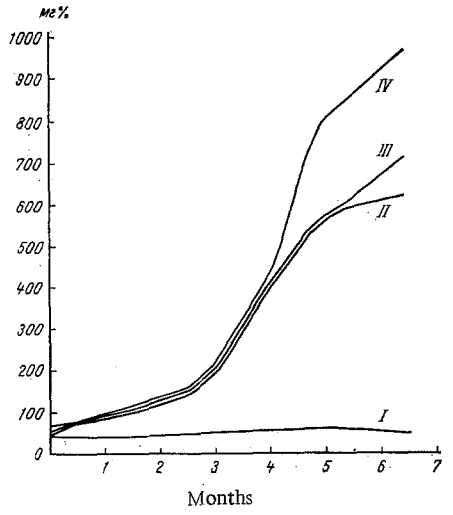

Fig. 1

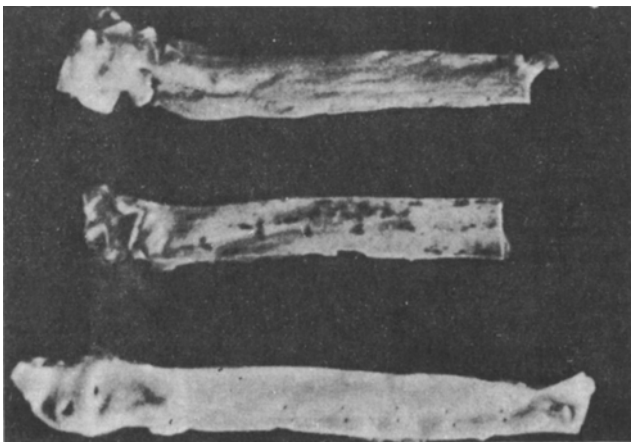

Fig. 2

Fig. 1. Blood serum cholesterol concentration in rabbits receiving a high lipid diet: I) sunflower oil; II) sunflower oil + cholesterol; III) sunflower oil, cholesterol, and 40 mg/kg n-alkanes; IV) sunflower oil, cholesterol, and 120 mg/kg n-alkanes.

Fig. 2. Aortas of rabbits receiving a high lipid diet. From top to bottom: sunflower oil; sunflower oil + cholesterol; sunflower oil, cholesterol, and 40 mg/kg n-alkanes (stained with Sudan).

and liquid paraffins in a dose of 120 mg/kg. The doses of n-alkanes used (calculated per kg body weight) were below the recommended doses of mineral oil for use in constipation [3]. Purified paraffins with a solidification point of 17°C were used. The chief components of these paraffins were n-alkanes: C_{15} (8.6%), C_{16} (11.9%), C_{17} (13.8%), C_{18} (13.1%), C_{19} (12.4%), C_{20} (10%), and C_{21} (7.9%). The total content of C_{12}-C_{25} n-alkanes was 98.8%. The content of aromatic hydrocarbons was below 0.1%. During the experiment frequent checks were made of the animals' body weight and the concentrations of cholesterol and β-lipoproteins in their blood serum. The experimental and control animals were sacrificed at the same time (6-6.5 months later) when manifestations of severe hypocholesterimia were present. Lipids were extracted by Folch's method [20]. The total lipid content was determined gravimetrically. The fraction of n-alkanes was separated as described previously [6] and their content was determined by gas chromatography. The serum cholesterol concentration was determined by Elk's method in the writers' modification. Several indices of lipid metabolism and enzyme activity were determined in the tissues: total cholesterol [3], esterified cholesterol [2], phospholipids [19], lipid-mobilizing lipase [7], butyrylcholinesterase [8], tributyrinase [9], alkaline phosphatase [10], ketose-1-phosphate aldolase [11], and alanine aminotransferase [12]. The significance of differences between the groups was assessed by Student's criterion. The aorta, taken from its point of bifurcation, was investigated by direct planimetry as described by Avtandilov [1]. The aorta and organs were studied histologically.

EXPERIMENTAL RESULTS AND DISCUSSION

No differences were found in the changes in weight of the experimental and control animals. A sharp increase in the blood serum concentrations of cholesterol and β-lipoproteins, typical of this model, was observed. Additional administration of n-alkanes in a dose of 40 mg/kg revealed only a tendency toward an increase in the cholesterol concentration. With an increase in the dose of n-alkanes to 120 mg/kg, the increase in the hypercholesteremia was significant (Fig. 1). A tendency was also observed for the serum β-lipoprotein concentration to rise in the rabbits receiving n-alkanes.

A study of the dynamics of the increase in the serum cholesterol concentration thus revealed a significant influence of n-alkanes on lipid metabolism, and under the conditions of Anichkov's experimental model this can be interpreted as a sign of an additional atherogenic action. This conclusion was fully confirmed by morphological evaluation of the severity of the atherosclerotic changes. The area and severity of the structural changes in the aorta was found in groups of rabbits receiving n-alkanes together with the oily

TABLE 1. Planimetric Assessment of Atherosclerotic Changes in the Rabbits' Aorta

Group of animals	Lipids administered	Structural modifications of the aorta		
		degree	stage	atheroma index
1	Sunflower oil	Absent		0 (0+0+0+0)
2	Sunflower oil and cholesterol	II, slight*	Fibrous plaques	8,5 (6,5+2,0+0+0)
3	Sunflower oil, cholesterol, and 40 mg/kg n-alkanes	V, very severe†	The same	86,7 (2,9+84,8+0+0)
4	Sunflower oil, cholesterol, and 120 mg/kg n-alkanes	The same	» »	84,3 (3,5+79,8+0+0)

*Less than 1/8 of area of intima of aorta affected.
†More than 1/2 of area of intima of aorta affected.

TABLE 2. Content of Total Lipids in Rabbits' Aorta

Group of animals	Lipids administered	Total lipid content (in % of weight of fresh tissue)
1	Sunflower oil	3,98±0,21
2	Sunflower oil and cholesterol	9,20±0,39
3	Sunflower oil, cholesterol, and 40 mg/kg n-alkanes	10,70±0,54
4	Sunflower oil, cholesterol, and 120 mg/kg n-alkanes	12,30±0,72

*P < 0.05 compared with group 2.
†P < 0.01 compared with group 2.

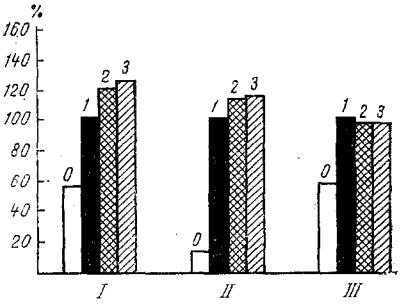

Fig. 3. Effect of n-alkanes on indices of lipid metabolism in the rabbits' liver: O) sunflower oil; 1) sunflower oil + cholesterol; 2) sunflower oil, cholesterol, and 40 mg/kg n-alkanes; 3) sunflower oil, cholesterol, and 120 mg/kg n-alkanes; I) total lipids; II) total cholesterol; III) fraction of cholesterol esters.

solution of cholesterol (Table 1). The greater part of the changes in these animals consisted of fibrous plaques which, forming continuous bands, covered practically the whole surface of the aorta (Fig. 2). Lipoid stains occupied a comparatively small area, evidence of a combination of sclerotic changes with lipoid infiltration. The aortas of the animals receiving sunflower oil and cholesterol were affected mainly with lipoid stains, with no particularly marked fibroblast response. The incidence of lesions in the aorta in animals receiving cholesterol and sunflower oil was 70%, and this was raised to 90% by the addition of n-alkanes.

A marked increase in the content of total lipids was observed in the tissues of the aorta in rabbits receiving sunflower oil and cholesterol (by 2.3 times). On the addition of n-alkanes the increase in the total lipid content was more marked still, especially if the dose was 120 mg/kg (Table 2). The marked atherogenic action of n-alkanes during the production of experimental atherosclerosis by Anichkov's method can thus be regarded as firmly established. Feeding with cholesterol and sunflower oil also induced a sharp increase (by more than 80%) in the content of total lipids in the rabbits' liver. On the addition of n-alkanes the increase in the total lipid content in the liver was more marked still and was directly dependent on the dose of n-alkanes. The increase took place through further accumulation of cholesterol in the tissues (Fig. 3).

Histological examination showed that in animals receiving n-alkanes not only was there a more marked anisotropic fatty infiltration of

the liver, but in individual cases the picture of portal cirrhosis also was observed, with extensive proliferation of the interstitial tissue. The manifestations of fatty degeneration of the liver correlated with the tendency toward a decrease in activity of the lipid-mobilizing enzymes (lipase and tributyrinase) in the liver. Alkaline phosphatase activity in the liver was significantly increased. The addition of n-alkanes caused no significant changes in the activity of ketose-1-phosphate aldolase, butyrylcholinesterase, and alanine aminotransferase in the blood serum and liver or of lipase in the adipose tissue.

The results of these experiments suggest that n-alkanes lead to systematic disturbances of several indices of lipid metabolism. A fact to be clearly noted was the marked accumulation of n-alkanes in the aorta and adipose tissue, where their content was increased by more than 500% over that in the tissues of the control animals.

There is therefore reason to suppose that an important factor in the mechanism of the atherogenic action of n-alkanes observed during the production of experimental atherosclerosis by the method of Anichkov and Khalatov is the accumulation of n-alkanes in the tissues, their slight inhibitory action on biological oxidation, and the inhibition of enzyme systems mobilizing lipids.

The practical significance of these results is not yet sufficiently clear. Investigations in this direction are in progress.

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