INJURY TO CYTOPLASMIC MEMBRANES AND THROMBOPLASTINEMIA IN HYPERCHOLESTEREMIA

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One of the so-called risk factors producing repeated injuries to the endothelium is hypercholesteremia [9].

Setting out from the suggestion put forward by Klimov et al. [4], that "in the case of marked hypercholesteremia oversaturation of lipoprotein particles with cholesterol may even cause detachment of the endothelial membrane where it is in contact with the lipoproteins, i.e., injury to the endothelial cover," the investigation described below was carried out to test this hypothesis experimentally, by looking for degradation products of the plasma membranes in the blood stream. The writers showed previously [3] that 5′-nucleotidase and alkaline phosphatase are indicators of the entry of cell membrane fragments into the blood stream. Accordingly the dynamics of changes in the activity of these enzymes in rabbits with alimentary hypercholesteremia and the effect of experimental treatment with vitamin B_{12}, its coenzyme, and cobalamide on this process were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 67 Chinchilla rabbits of both sexes weighing 2.5-3.0 kg. Cholesterol was administered to the rabbits perorally in a dose of 0.2 g/kg body weight as a 20% solution in sunflower oil; seven rabbits received cholesterol for 10 days and 60 rabbits for 120 days. Administration of cholesterol was stopped after 4 months and the experimental animals were divided into four groups. The rabbits of group 1 were untreated, those of group 2 received daily intramuscular injections of 50 μg vitamin B_{12} for 2 months, the rabbits of group 3 received daily intramuscular injections of 50 μg vitamin B_{12} coenzyme, and the rabbits of group 4 received daily intramuscular injections of 50 μg cobalamide.

Levels of cholesterol, phospholipids [1] and the cholesterol–phospholipid ratio in the blood serum, and 5′-nucleotidase [7] and alkaline phosphatase [1] activity in the blood plasma were determined in the experimental animals.

Blood for investigation was taken from the marginal vein of the ear before cholesterol feeding began, and thereafter monthly for 6 months. To investigate 5′-nucleotidase and alkaline phosphatase activity blood plasma stabilized by the addition of crystalline heparin was used.

The experimental results were subjected to statistical analysis [6].

EXPERIMENTAL RESULTS

No appreciable changes in the activity of the various enzymes was detected in rabbits during the first 10 days of cholesterol feeding. The increase in 5′-nucleotidase activity was comparatively small: initial activity 105.0 ± 8.3 nkat/liter, after 10 days 113.4 ± 26.7 nkat/liter ($P > 0.1$). Alkaline phosphatase activity also was substantially unchanged: initially 384.9 ± 71.6 nkat/liter, falling after 10 days to 296.9 ± 35.8 nkat/liter ($P > 0.2$).

Administration of cholesterol to the rabbits for 4 months caused a characteristic and significant rise in the cholesterol concentration and cholesterol-phospholipid ratio in the blood serum (Table 1).

Hypercholesteremia was accompanied by increased 5′-nucleotidase activity in the blood plasma. The maximal increase was observed 2-4 months after the beginning of cholesterol feeding. The increase in plasma 5′-nucleotidase activity could be the result of severe injury to the outer cell membranes.

A significant increase in alkaline phosphatase activity was observed 2 months after the beginning of cholesterol feeding of the rabbits, but the increase was less than in the case of 5′-nucleotidase activity.

The blood cholesterol concentration 2 months after the end of cholesterol feeding was appreciably lower, but had not yet returned to its initial values. The phospholipid level in the rabbits of this group was depressed, but the cholesterol-

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TABLE 1. Effect of Treatment with Vitamin B_{12}, Its Coenzyme, and Cobalamide on Blood Indices of Rabbit with Alimentary Hypercholesteremia (M ± m)

Index	Initial value		Group of rabbits			
	before cholesterol feeding	before beginning of treatment	1	2	3	4
5'-Nucleotidase activity, nkat/liter	105,0±8,3	560,1±118,4	403,4±73,3 $P_1<0,002$	233,4±63,3 $P_1<0,05$ $P_2<0,05$	198,4±43,3 $P_1<0,05$ $P_2<0,02$ $P_3<0,05$	331,7±86,7 $P_1<0,05$
Alkaline phosphatase activity, nkat/liter	384,9±71,6	465,5±89,5	268,6±62,7	187,9±44,8 $P_1<0,05$ $P_2<0,02$	331,2±89,5	635,6±152,2 $P_3<0,05$
Cholesterol concentration, mg %	7,2±3,3	756,0±8,2	164,3±55,3 $P_1<0,001$ $P_2<0,001$	147,2±35,3 $P_1<0,001$ $P_2<0,001$	133,4±23,3 $P_1<0,001$ $P_2<0,001$	134,3±39,9 $P_1<0,001$ $P_2<0,001$
Phospholipid concentration, mg %	250,0±35,4	229,9±24,2	56,3±7,7 $P_1<0,001$ $P_2<0,001$	82,5±6,9 $P_1<0,001$ $P_2<0,001$ $P_3<0,05$	110,0±12,0 $P_1<0,001$ $P_2<0,001$ $P_3<0,05$	288,0±11,8 $P_1<0,05$ $P_3<0,05$
Cholesterol-phospholipid ratio	0,5±0,1	4,1±0,2	3,4±1,5 $P_1<0,05$	1,9±0,5 $P_1<0,001$ $P_2<0,001$	1,2±0,3 $P_1<0,001$ $P_2<0,001$	1,1±0,5 $P_1<0,001$ $P_2<0,001$

Legend. P_1) Significance of differences compared with initial values, P_2) significance of differences compared with values before beginning of treatment, P_3) significance of differences compared with values for untreated rabbits.

phospholipid ratio was increased. 5'-Nucleotidase activity remained high, evidence of continued severe damage to the outer cell membranes (Table 1).

It will be clear from Table 1 that administration of vitamin B_{12}, its coenzyme, and cobalamide to the experimental rabbits led to a decrease in the blood cholesterol and phospholipid levels, similar to the dynamics of these levels in the rabbits of group 1. However, in rabbits treated with vitamin B_{12} and its coenzyme a significant fall was observed in 5'-nucleotidase activity, evidence of the more rapid normalization of the properties of the cell membranes.

The sharp increase found in plasma 5'-nucleotidase activity in rabbits at the height of hypercholesteremia is direct confirmation of a change in the properties of the outer cell membranes. Excessive intake of exogenous cholesterol with the diet is known to lead to an increase in the total cholesterol content in the plasma membrane [2]. According to data in the literature [10], incorporation of cholesterol into membranes modifies their physical properties. This process evidently lies at the basis of detachment of cell membrane fragments into the blood stream or, at least, detachment of the marker enzyme 5'-nucleotidase, which is lipoportein in nature [5].

That is why in hypercholesteremia the circulation of cell membrane fragments with the properties of tissue thromboplastin presents a risk of thrombus formation through initiation of intravascular blood clotting.

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