CA content dropped significantly within the first 24 hours, and such a decrease persisted even on the 7th day after the operation.

Thus, CA (mainly NA), are delivered to the OM during the period of basal secretion and are expended during induced secretion. In this respect the neurotrophic maintenance of the OM shows a similarity with another organs, where CA supplies are replenished during the normalization of activity [4].

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Effect of Eicosapentaenoic Acid and the Calcium Antagonist Isradipin on Lipid Metabolism and Erythrocytes in Cholesterol-Fed Rabbits

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Hypercholesterolemia is considered to be a risk factor for atherosclerosis. Fat of arctic animals (seals and others) containing a high concentration of eicosapentaenoic acid (EPA) is known to possess a cholesterol-lowering effect [1]. This property allows it to be used in different states accompanied by pronounced hypercholesterolemia, in particular in atherosclerosis. There is no agreement regarding the antiatherogenic effect of dihydropyridine calcium antagonists. Several data demonstrate that such an effect does exist [5], while others show no positive effect of these drugs on the level of blood serum lipids [6]. Of particular interest is the calcium antagonist isradipin, which is successfully employed for the treatment of arterial hypertension and coronary heart disease due to its pronounced hypotensive and vasodilating effects. One of the possible mechanisms of EPA action is a change of the lipid metabolism in cell membranes, as has been shown in erythrocytes [4]. Whether isradipin exhibits such an effect is not understood.

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TABLE 1. Changes in Cholesterol and FFA Blood Concentration in Rabbits with Experimental Hypercholesterolemia Treated with EPA and Isradipin *(M±m)*

Dietary group	Cholesterol		FFA	
	number of animals	concentration, mmol/l	number of animals	concentration, mmol/mg protein
1. Standart diet	17	1.7±0.1	17	9.4±0.8
2. Atherogenic diet	18	7.6±0.3	18	18.5±1.1
3. Atherogenic diet + EPA	17	6.7±0.1	17	17.8±1.2
4. Atherogenic diet + minimal dose of isradipin	7	2.4±0.5	5	8.1±2.1
5. Atherogenic diet + middle dose of isradipin	7	2.3±0.2	5	12.1±1.8
6. Atherogenic diet + maximal dose of isradipin	7	5.7±0.8	5	10.9±1.1

The aim of the present study was to compare the effects of EPA and the dihydropyridine calcium antagonist isradipin (Sandoz) on lipid metabolism and erythrocytes in cholesterol-fed rabbits.

MATERIAL AND METHODS

The experiments were carried out on 48 male Chinchilla rabbits with the initial weight 3.0-3.5 kg. Experimental atherosclerosis was induced by daily feeding of a 10% cholesterol suspension in sunflower oil during 3 months (0.2 g cholesterol per kg body weight). The animals were divided into six groups. The 1st group (n=11) consisted of intact animals maintained on a standard diet. The 2nd group (n=10) was the control group, which received a cholesterol-enriched diet. The 3rd group (n=12) received a cholesterol-enriched diet supplemented with seal fat containing 25% EPA (provided by the Institute of Biological Problems of the North, Magadan) in a dose of 0.5 ml per kg body weight. The rabbits of the 4th, 5th, and 6th groups (n=7 for each) received a cholesterol-enriched diet simultaneously with isradipin administration in three different doses: 0.625, 1.25, and 1.875 mg.

Blood for the experiments was obtained from the marginal ear vein. The erythrocytes were washed three times with isotonic phosphate buffer. The physico-chemical state of the erythrocyte membrane was analyzed after Mochenova *et al.* [2] as follows: a 5% erythrocyte suspension was incubated at 37°C for 15, 30, 45, and 60 min in a medium containing 5mM kalmycin and 0.2 mM $CaCl_2$, and then the hemoglobin concentration in the supernatant was determined. Hemolysis was estimated as a percentage of the 100%-hemolysed probe. Cholesterol content was measured by the enzymatic method with Boehringer kits; free fatty acids (FFA) were assayed after Prokhorov *et al.* [3].

RESULTS

The 3-month cholesterol-enriched diet led to pronounced hypercholesterolemia in the experimental animals. The changes in the blood cholesterol concentration are presented in Table 1. The EPA-containing diet did not cause significant alterations in the serum blood cholesterol concentration, although cholesterol accumulation was considerably inhibited in comparison to the control group (cholesterol-enriched diet), especially during the first month of the experiment. Isradipin administration in the concentrations used revealed a cholesterol-lowering effect in a dose-dependent manner. The maximum cholesterol-lowering effect was achieved when the middle dose of isradipin was used, while the minimal dose caused less pronounced changes. The maximal dose of isradipin lowered the cholesterol level to a lesser extent

TABLE 2. Ca^{2+} – Kalmycin – Induced Hemolysis in Rabbits with Experimental Hypercholesterolemia Treated with EPA and Isradipin *(M±m)*

Dietary group	Number of animals	Time, min			
		15	30	45	60
1. Standard diet	17	64.6±3.5	73.0±2.3	86.7±2.5	90.8±2.1
2. Atherogenic diet	15	25.6±4.9	42.6±5.8	63.5±6.6	71.3±7.7
3. Atherogenic diet +EPA	17	39.7±3.2	56.2±3.0	65.5±3.3	76.5±2.4
4. Atherogetic diet + minimal dose of isradipin	7	20.5±3.5	40.6±6.4	59.5±6.4	70.2±6.4
5. Atherogenic diet + middle dose of isradipin	7	30.1±10.4	44.5±8.2	61.1±6.1	70.4±7.4
6. Atherogenic diet + maximal dose of isradipin	7	38.1±8.47	54.9±5.6	72.7±4.4	75.9±4.4

than the minimal and the maximal doses, but was more effective than EPA.

The blood plasma FFA content was two times greater in the group which received the cholesterol-enriched diet (see Table 1). In the case of the EPA-containing diet a trend toward a drop in the level of FFA was observed. In rabbits of 3rd, 4th, and 5th groups treated with different doses of isradipin the FFA concentration dropped to near-control values, and was even below this level when the minimal dose of isradipin was used.

The rate of Ca^{2+}-kalmycin-induced hemolysis of the erythrocytes was markedly decreased in rabbits of the 2nd group with pronounced hypercholesterolemia (Table 2). In rabbits of the 3rd group (EPA administration) this index rose but did not return to the control value. Similar results were obtained in the 6th group (the maximal dose of isradipin). The 4th and 5th groups (the minimal and middle doses) did not differ significantly from the 2nd group.

Thus, the effect of seal fat containing 25% EPA consists mainly in changes of the physicochemical properties of the erythrocytes, manifested as hemolysis rate reduction. A hypocholesterolemic effect of seal fat was not detected.

In experimental animals the administration of dihydropyridine calcium channel blockers in doses close to those applied for humans does not make it possible to discriminate clearly the effects resulting from the direct influence on the calcium current inside the cells and the pharmacological and hemodynamic changes induced by these drugs. The fluctuations of FFA concentration in the blood plasma around the control value are possibly to be attributed to both the immediate influence on calcium homeostasis and a drop of the calcium concentration, which may lead to an inhibition of phospholipase activity, a drop of the lipolysis rate, and reduced FFA production.

A long-term atherogenic diet in rabbits induces marked abnormalities in cell cholesterol metabolism and alteration in the calcium mediated physiological response of the cell. Calcium antagonists and EPA are able to normalize this function by regulating both the intracellular calcium concentration (isradipin) and the membrane-bound phospholipase activity, which produce FFA and the lysophosphatide pool.

From the medical point of view, the results obtained in this study suggest that EPA, being a "marine" lipid, may be beneficial in an antiatherogenic diet as a nutritional supplement obtained from marine fishes and animals. The calcium antagonist isradipin in the low and middle doses reduces the cholesterol and FFA blood level in the experimental animals and may be applied in patients with atherosclerosis for normalization of an elevated level of blood lipids and for the prevention of lipid metabolism disturbances.

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