therefore cannot be ruled out that both H_2O_2 and NaClO can modify the anticataract agents as they penetrate into the anterior chamber of the eye and the various layers of the lens.

The use of the technique of screening quenchers of singlet oxygen described above is therefore suitable only for preparations which do not react with H_2O_2 or with hypochlorite. The list of such preparations includes Senkatalin and Baineiting. A different plan of investigation is needed for the other preparations.

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EFFECT OF PROTAMINE ON BLOOD LIPOPROTEINS IN A MODEL OF ATHEROSCLEROSIS

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A high content of protamines, which are proteins with marked alkaline properties, is found in male generative cells, where they replace the histones of chromatin during maturation of spermatozoa. As a pharmacological preparation, protamines isolated from fish sperm are wisely used in clinical practice for the treatment of bleeding. It has been suggested that alkaline proteins, including protamines, can prevent the development of atherosclerosis [6, 8]. However, the effect of protamine in models o the early stages of atherosclerosis in studies of the protein—lipid composition of lipoproteins, has been found to be ambivalent. The changes found have been regarded by some workers as promoting, but by others as preventing atherogenesis [4, 5].

The aim of this investigation was to compare the action of protamine on quantitative changes in lipoproteins in the early and late periods of hypercholesterolemia (HChE).

EXPERIMENTAL METHOD

The experimental animals were 40 rabbits weighing about 3 kg, divided into six groups. Animals of group 1 received cholesterol in their diet daily for 1 month in a dose of 0.5 g/kg body weight. Animals of group 2, besides cholesterol, received a 1% solution of protamine (10 mg/kg) by intraperitoneal injection. Animals of group 3 received cholesterol for

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TABLE 1. Number of Lipoprotein Particles (10^{14}/ml Blood Serum) in HChE and under the Influence of Protamine ($\bar{x} \pm s\bar{x}$)

Experimental cond.	HDL	LDL	VLDL
Control	57±2,8	9,8±0,7	1,1±0,2
Protamine	60±2,1	11,4±1,0	1,6±0,2
HChE 1 month	243±11*	10,3±0,9	3,3±0,6*
HChE 7 months	62±3,6	77,5±4,8*	7,0±0,7*
HChE 1 month + protamine	291±13**	22,4±0,2**	8,9±1,1**
HChE 7 months + protamine	125±6,8**	60,2±3,4**	2,8±0,3**

Legend. Here and in Table 2 asterisk indicates changes are statistically significant compared with control, two asterisks — the same, under the influence of protamine, compared with level characteristic of HChE. $\bar{x}$, Arithmetic mean, $s\bar{x}$, standard error of arithmetic mean.

TABLE 2. Relative Content of Cholesterol and Protein of Lipoprotein Fractions in HChE and under the Influence of Protamine (in % of total blood serum cholesterol, ($\bar{x} + s\bar{x}$)

Experimental conditions	Cholesterol			Protein			Protein/ cholesterol in blood serum	Total cholesterol in blood serum mg/ml
	HDL	LDL	VLDL	HDL	LDL	VLDL		
Control	10,0±0,9	48±3	9,5±0,9	150±9	121±8	17±1,1	81,7	0,8±0,1
Protamine	8,9±0,7	56±5	13,3±1,1	180±15	160±11	30±1,8	94,1	0,7±0,1
HChE 1 month	3,7±0,2*	55±5	8,7±0,6	232±14*	46±3*	19±1,3	39,4	2,2±0,3*
HChE 7 months	1,5±0,1*	20±3*	39,5±4,1*	8±0,3*	50±4*	6±0,3*	4,8	15,0±1,3*
HChE 1 month + protamine	2,2±0,2*	48±3	17±1,5*	102±7	36±3*	19±1,2	14,1	5,9±0,6*
HChE 7 months + protamine	5,1±0,4**	43±4**	36±3,3*	42±3**	93±5**	6±0,3	10,7	6,3±0,6**

7 months. Group 4 consisted of animals kept on an atherogenic diet, and which received protamine 1 month before the end of the experiments. There were also two control groups. The animals of one of them received physiological saline, the other received protamine in the same dose as the experimental animals, for 1 month. Preparative ultracentrifugation of the lipoproteins, determination of the concentrations of the proteins and cholesterol, and other preparative and analytical methods were described previously [11, 15]. Data in the literature on molecular weight of lipoproteins were used in the work: high-density lipoproteins (HDL) $2.67 \cdot 10^5$ daltons; low-density lipoproteins (LDL) $2.7 \cdot 10^6$ daltons, very low density lipoproteins (VLDL) 10^7 daltons [9]. After determination of the mean protein content by weight in each fraction, the number of lipoprotein particles in 1 ml of blood serum was calculated. The distribution of protein and cholesterol in the lipoprotein fractions studied was not expressed in absolute terms, but in relative values, namely percentages of the total blood serum cholesterol, for it is this parameter that is considered to be most informative when characterizing the functions of atherogenic and antiatherogenic fractions of lipoproteins [14]. The results obtained were subjected to statistical analysis by the usual methods.

EXPERIMENTAL RESULTS

As the data in Table 1 show, in the initial period of HChE a considerable (by 4.3 times) increase was observed in the number of HDL particles. The concentration of VLDL in the blood serum also increased. This can be explained by the action of cholesterol itself on stimulation of synthesis of apoB, in the composition of VLDL, in the liver and its secretion into the blood stream [10]. The LDL concentration was unchanged in the early stages of HChE.

The primary substrate inducing morphological changes in arteries characteristic of atherosclerosis is cholesterol, which is transported by lipoprotein particles [9]. After 1 month of keeping rabbits on an atherogenic diet the total blood serum cholesterol as a rule is 3-4 times higher than initially [6, 12]. Against this background there is a decrease in the fraction of cholesterol of HDL relative to the total pool, without any change in the composition of the apoB-containing

lipoproteins (Table 2). The protein concentration, calculated relative to total blood serum concentration, in HDL compared with the control was increased by 82%, whereas in LDL it was reduced by about the same amount (75%). Distinct morphological changes were not found in the aorta at this time of the investigation. This observation is in agreement with data in the literature, showing that in rabbits after being kept on an atherogenic diet for 3-4 weeks only solitary lipid stains can be observed [2].

It can be concluded from the results that 1 month after the beginning of cholesterol feeding of animals, despite a marked increase in its concentration in the blood, no signs of atherogenesis are present. Conversely, in the system of blood lipoproteins, a shift is observed in what is considered to be the antiatherogenic direction. Accordingly it is important to note that experimental atherosclerosis does not develop uniformly. This is reflected in the presence of periods of reduction of the index of aortic damage and the minima on the curve describing the time course of changes in cholesterol concentration, occurring the 3rd-4th week after the beginning of the experiments [1, 7]. It can accordingly be postulated that in the course of HChE and atherogenesis there is a period of induction of adaptive mechanisms, probably connected with activation of the blood cholesterol transport system.

During long-term HChE an extremely sharp increase was observed in the number of LDL and VLDL particles (by 7.0 and 6.4 times respectively). The number of HDL particles did not differ from the control. Meanwhile the fraction of cholesterol of HDL in the total blood serum pool was reduced fivefold. The level of LDL cholesterol also, was lowered, evidently on account of VLDL cholesterol, for its fraction under these circumstances was increased fourfold (Table 2) Long-term HChE was characterized by a relative decrease in protein in all lipoprotein fractions. The sharpest fall of this parameter was found in HDL (by 142%). These changes in the parameters studied are evidence of the atherogenicity of the blood lipoprotein system [9]. The morphological picture of the arteries corresponded to this state of affairs. The whole inner surface of the aorta was covered by fibrous plaques. Nodular structures formed by confluent plaques could be observed.

These results are evidence that injection of protein into intact animals caused no significant changes in the parameters studied with the exception of a small increase in the relative content of VLDL proteins. Against the background of HChE for 1 month no statistically significant differences were found in the relative content of HDL or of VLDL proteins, compared with the control. The content of LDL proteins was lower than in the control and did not differ from the level characterizing short-term HChE. As has already been stated, in the initial period of HChE the content of HDL and VLDL was increased compared with the control. The action of protamine against this background led to a further increase in the number of these particles. The content of LDL particles also was more than doubled. Thus it can be tentatively suggested that protamine, in the initial period of HChE, gives rise to additional activation of the blood cholesterol transport system.

Under conditions of long-term HChE the action of protamine led to an increase in the relative content of HDL cholesterol compared with the characteristic level of HChE. The LDL cholesterol level also was increased. Compared with the control, the concentration of LDL cholesterol was unchanged but that of HDL cholesterol remained low. The level of VLDL cholesterol was just as high as during long-term HChE. The relative content of HDL and LDL proteins was increased compared with HChE, but that of VLDL was unchanged.

The animal may possibly possess mechanisms connected with the function of nonlipoprotein blood serum proteins, which counteract the development of atherosclerosis. The presence of negative correlation was suggested previously between the change in the total cholesterol concentration and the total blood serum proteins [3], is demonstrated particularly clearly under conditions of cholesterol withdrawal after keeping animals for 3 months on an atherogenic diet. The cholesterol concentration. in this case was reduced by 48% over a period of several days, whereas the protein concentration was increased by 38% [6]. It is interesting to note that the same relationship was observed during the action of protamine. The increase in the relative content of total blood serum proteins after injection of protamine, against the background of long-term HChE, was accompanied by a corresponding decrease in the cholesterol concentration (Table 2).

By contrast with the uniform pattern of change in the number of lipoprotein particles induced by protamine against the background of HChE for 1 month, in the case of long-term HChE concentrations of LDL and VLDL fell by 23% and 60% respectively. Compared with the level during HChE the concentration of HDL particles was considerably increased — by 101%. Thus the action of protamine in experimental atherosclerosis s causes an increase in the content of antiatherogenic HDL and a decrease in the content of atherogenic apoB-containing fractions. In other words protamine probably is able to counteract the development of two types of dyslipoproteinemias (hyper-β- and hypo-α-lipoproteinemia), which are regarded as independent risk factors of atherosclerosis and ischemic heart disease [13].

The results of this investigation, showing the stimulating effect of protamine in the early stages of HChE and its regulatory action on blood serum lipoproteins during long-term HChE, suggest that protamine and alkaline proteins similar to it in structure possess antiatherogenic properties.

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MECHANISM OF HYDROGEN PEROXIDE INDUCED OXIDATION OF OXYHEMOGLOBIN

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Autooxidation of oxyhemoglobin (HbO_2) into methemoglobin (MtHb) is accompanied by the formation of superoxide anion-radicals [3, 11], from which H_2O_2 is formed by spontaneous dismutation, and also under the influence of superoxide dismutase (SOP) [9]. Both O_2 and H_2O_2 can induce oxidation of hemoglobin, but there is reason to suppose that H_2O_2 has the most powerful destructive action on hemoglobin [6, 11].

Incidentally, in the literature on the study of oxidative destruction of hemoglobin through the action of H_2O_2, most attention has been paid to direct interaction of the active center of hemoglobin with H_2O_2 [6, 7]. Yet hydrogen peroxide can oxidize the amino groups of proteins, including SH-groups [14], and this can cause structural damage to the protein

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