

EFFECT OF PROLONGED IMMUNIZATION OF RABBITS OF DIFFERENT AGES
WITH HOMOLOGOUS HEART ANTIGEN ON BLOOD LIPID AND LIPOPROTEIN
LEVELS

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During immunization and certain autoimmune processes disturbances of lipid and lipoprotein metabolism are observed [5, 7, 9]. The frequency of discovery of autoimmune reactions of humoral and cellular types to different organ antigens increases during aging [10-12]. Parallel with this, the incidence of atherosclerosis rises. Recently an important role in the pathogenesis of atherosclerosis has been ascribed to immune complexes, which are considered not only to have a direct effect on the vascular wall, but also to be capable of inducing hyperlipoproteinemias. However, information on this question from the age aspect is inadequate.

For the above reasons it was decided to study the character of changes in the blood lipid and lipoprotein levels in young and old rabbits during simulation of an autoimmune lesion of the cardiovascular system.

EXPERIMENTAL METHOD

Experiments were carried out on 26 Chinchilla rabbits aged 8-10 months (young) and 4-4.5 years (old). To obtain a chronic immune lesion of the cardiovascular system, an antigen prepared from homologous heart was injected into the animals for a long time. The rabbit's heart was carefully irrigated through the coronary vessels with cold physiological saline, the muscle tissue was freed from fat and connective-tissue structures, and cut into small pieces with scissors and homogenized at 3000g for 5 min after dilution with physiological saline in the ratio of 1:5. The homogenate was kept for 18-20 h at 4°C and centrifuged for 3-4 min at 1500g. The supernatant, the protein content of which was 15-20 mg/ml, served as the antigen.

Homologous heart antigen in a volume of 1 ml, mixed with an equal volume of Freund's complete adjuvant, was injected weekly for 2.5 months subcutaneously into the medial part of the rabbit's thigh. Animals of the control group received the adjuvant alone, without the antigen, at the same times. The adjuvant was prepared as follows: dry autoclaved BCG vaccine was mixed with lanolin and mineral oil (20 ml mineral oil, 10 ml lanolin, and 150 mg BCG) and thoroughly ground in a porcelain mortar. Before each odd-numbered injection of antigen with adjuvant or of adjuvant alone, a blood sample was taken for determination of autoantibodies (in Boyden's passive hemagglutination test), circulating immune complexes [8], total cholesterol [4], triacylglycerine [6], and total fraction of low- and very low-density lipoproteins (LDL and VLDL respectively) [2]. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

During immunization of the young and old rabbits with homologous heart antigen anticardiac antibodies were found in high titers in the blood serum as early as 3 weeks after the beginning of the experiment. Mean values of the concentrations of anticardiac antibodies, expressed on a logarithmic scale (\log_2 , first dilution of serum 1:10), are given in Fig. 1. With an increase in the number of injections of heart antigen the titers of anticardiac antibodies also increased, and did so more rapidly in young rabbits. By the end of the experiment there was no age difference in autoantibody titer.

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TABLE 1. Time Course of Changes in Blood Lipid and Lipoprotein Concentrations in Young and Old Rabbits during Immunization with Homologous Heart Antigen ($M \pm m$)

Parameter, mg%	Age of animals	Initial value	Number of injections of antigen			
			2	4	6	8
Lipoproteins	Young	226,2 \pm 17,1	212,8 \pm 27,1	469,8 \pm 61,1 ^{b,c}	376,5 \pm 38,6 ^{b,c}	404,3 \pm 50,2 ^{b,c}
	Old	285,6 \pm 27,4	575,2 \pm 91,8 ^{a,b}	673,9 \pm 86,9 ^b	595,9 \pm 47,5 ^{a,b}	368,3 \pm 56,6
Total cholesterol	Young	73,9 \pm 5,2	75,3 \pm 5,7	113,9 \pm 13,9 ^{b,c}	98,3 \pm 11,8	114,9 \pm 12,6 ^{b,c}
	Old	79,6 \pm 5,6	130,3 \pm 14,4 ^{a,b}	193,1 \pm 28,0 ^{a,b}	142,2 \pm 18,9 ^b	119,2 \pm 13,4 ^b
Triacylglycerins	Young	33,9 \pm 3,6	35,0 \pm 3,9	102,4 \pm 14,5 ^{b,c}	104,9 \pm 14,3 ^{bc}	95,6 \pm 12,7 ^{b,c}
	Old	35,0 \pm 3,8	100,1 \pm 20,8 ^{a,b}	167,9 \pm 33,0 ^b	186,5 \pm 26,4 ^{a,b,c}	126,0 \pm 41,5 ^b

Legend. Differences statistically significant ($P < 0.05$): a) between animals of different ages; b) compared with initial values; c) compared with data after second injection of antigen. Each group consisted of not less than 10 animals.

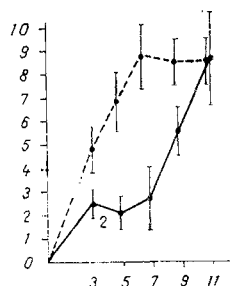


Fig. 1. Time course of antiheart autoantibodies in rabbits of different ages during immunization with homologous heart antigen. Abscissa, time of immunization (in weeks); ordinate, \log_2 of autoantibody titer. 1) Young animals, 2) old animals.

It will be clear from Table 1 that immunization of the rabbits caused considerable disturbances of their blood lipid and lipoprotein concentrations. The VLDL and LDL concentrations increased after the fourth injection of antigen in the young rabbits and remained at a high level during the subsequent weeks of the experiment. In the old animals hyperlipoproteinemia appeared earlier (after the second injection of antigen) and remained at a high level during the subsequent periods, and then fell a little toward the end of the experiment. The lipoprotein concentration was higher in the animals of the older age group in the interval between the second and sixth weeks after the beginning of injection of antigen.

The time course of changes in the concentrations of total cholesterol and triacylglycerins largely resembled that of the lipoproteins. These parameters also were characterized by a more rapid rise in the old immunized animals than in the young. At the same time, immunization was found to have a more marked effect on the blood triacylglycerin level regardless of the rabbits' age. The total cholesterol concentration was less prone to changes, especially in the young animals. Disturbances of this kind in the blood serum lipid spectrum indicate predominance of VLDL, which are the principal transport form of endogenous triacylglycerins. Electrophoresis of lipoproteins also revealed a more regular rise in the VLDL level than in the LDL level in the serum of rabbits immunized with homologous heart antigen. Unlike in these animals, in rabbits of different ages in the control group, which received adjuvant alone without antigen, no significant deviations were observed in the blood lipid and lipoprotein levels.

Considering the character of VLDL metabolism it can be tentatively suggested that the substantial accumulation of these substances in the blood of immunized animals observed in these experiments is connected with activation of their formation in the liver or with depression of activity of lipoprotein lipase, an enzyme which degrades VLDL [1]. Participation of the second mechanism in the pathogenesis of autoimmune hyperlipoproteinemias is discussed in the literature [5]. From these standpoints it becomes clear why there was a greater increase in the blood VLDL concentration of the older rabbits in these experiments, for it has been shown that even under normal conditions they have a lower lipoprotein lipase level and a slower rate of VLDL catabolism compared with these same parameters in younger animals [3].

The results of this investigation thus confirm the validity of views on a possible role of immune mechanisms in the genesis of disturbances of lipid and lipoprotein metabolism. During aging, conditions favoring the greater development of autoimmune reactions and an increase in the frequency of hyperlipoproteinemia arise, and this correlates with the known increase in the severity of manifestations of atherosclerosis in the blood vessels with age.

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DISULFIDE REDUCTASE ACTIVITY IN THE REGENERATING MOUSE LIVER

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Changes in the concentrations of thiol groups [6] and cyclic nucleotides have been demonstrated in dividing liver cells. The study of the activity and control of disulfide reductase enzymes during division is important. Great interest is attached to cCMP, whose regulatory effects are almost completely unstudied.

The aim of the present investigation was to study the total disulfide reductase activity (DSRA) of enzymes capable of reducing the artificial substrate 5,5'-dithio-bis-2'-nitrobenzoate in subcellular fractions of regenerating liver. In parallel experiments activity of this individual enzyme glutathione reductase (GR) was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 129 (CBA × C57BL)F₁ mice of both sexes weighing 18-22 g and aged 3-5 months. Under superficial ether anesthesia two thirds of the weight of the liver was removed from the animals of the experimental group [7]. Laparotomy was performed on the control animals. The nuclear fraction was obtained by centrifugation of homogenates at 900g for 10 min and mitochondria by centrifugation at 9000g for 20 min. DSRA was determined by the method in [2], GR activity as in [11], and protein by Lowry's method.

EXPERIMENTAL RESULTS

DSRA and GR activity of the homogenates and mitochondrial fractions showed no significant change at the times tested. Changes in DSRA also were not found in the nuclear fraction (Table 1). Phasic changes in DSRA were found in the cytosol fraction. A small decrease in this parameter was observed in the experimental group 14 h after the operation compared with the control (by 18%). DSRA of the experimental samples increased appreciably (by 32%) after 45 h.

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