PREVENTION OF EXPERIMENTAL ATHEROSCLEROSIS BY PARENTERAL INJECTION OF ATHEROGENIC LIPOPROTEINS INTO NEWBORN RABBITS

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Injection of atherogenic lipoproteins from adult rabbits with atherosclerosis into newborn rabbits prevented the development of atherosclerosis in these animals when fed with cholesterol in adult life.

Particular attention has recently been paid to low-density lipoproteins (β-lipoproteins), as the principal carriers of cholesterol, in the genesis of atherosclerosis. It has also been reported that in atherosclerosis β-lipoproteins are deposited in the walls of the aorta and coronary arteries [3-6].

The autoantigenicity of β-lipoproteins and their localization in the walls of affected arteries perhaps reflect the participation of an immunopathological mechanism in the development of atherosclerosis.

It was therefore decided to study the effect of injection of atherogenic lipoproteins into newborn rabbits on their resistance to the subsequent development of experimental atherosclerosis induced by an atherogenic diet.

EXPERIMENTAL METHOD

Newborn rabbits were injected with β-lipoproteins, isolated by the method Klimov et al. [2] from the serum of adult rabbits with atherosclerosis induced by N. N. Anichkov's method, by daily administration of 0.5 g cholesterol in oil through a tube for 4 months. The blood concentration of β-lipoproteins in the donor rabbits was not less than 3000 mg%. Lipoproteins were injected into the young rabbits during the first 6-10 h after birth. Sixteen animals (without distinction of sex) were injected with the resulting β-lipoproteins in the following concentrations: 13 animals (6 rabbits from litter No. 1, 7 rabbits from litter No. 2) each received β-lipoproteins equivalent to 65 mg protein, 3 rabbits (litter No. 3) each received 10 mg in a solution of 0.5% NaCl in a dose of 2-3 ml per rabbit, with the addition of 1000 units of the potassium salt of benzylpenicillin (1 ml intraperitoneally, the rest subcutaneously into the femoral and dorsal regions). On the second day after birth, the rabbits received a second injection as follows: animals from litter No. 1 received 65 mg protein, from litter No. 2 30 mg, from litter No. 3 10 mg each. On the 7th day, all the rabbits received a third injection of β-lipoproteins: 30 mg, 30 mg, and 10 mg respectively. After the end of the experiment, the aorta was removed from the rabbits and fixed in 10% neutral formalin and then stained in toto with Sudan III. The degree of severity of the atherosclerotic lesions (the "atherosclerotic index") was then determined planimetrically by Avtandilov's method [1]. Sections were stained with Sudan III and hematoxylin-eosin.

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TABLE 1. Indices of Development of Atherosclerosis in Rabbits

Animal No.	Atherosclerotic index (in %)	Degree of increase	
		cholesterol	β-lipoproteins
	Expt.		
466	0	3,8	2,7
470	0	0,8	1,6
472	0	3,0	2,3
462	0,5	3,8	3,3
	Control		
118	2,7	10,0	6,5
119	17,5	11,6	15,0
115	7,5	10,0	11,0
112	20,0	22,5	23,7

[1]Number of times by which final concentration of cholesterol and β-lipoproteins in blood serum exceeds initial level.

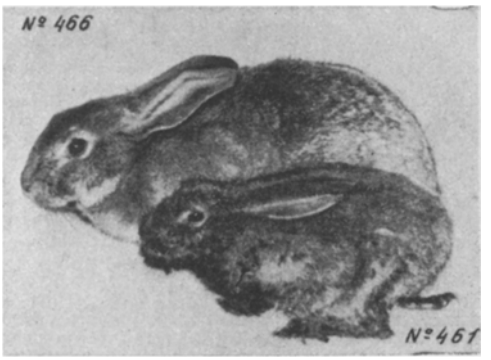

Fig. 1. Rabbit No. 466, receiving an average dose of antigen; rabbit No. 461, receiving a course of immunization equivalent to 150 mg protein and dying from pneumonia. Age of animals 4 months, weight 2100 and 400 g respectively.

EXPERIMENTAL RESULTS

Twelve of the 16 experimental newborn rabbits survived after the end of immunization. On the 30th day after birth they were removed from their mothers and transferred to an ordinary diet. Most animals from litters Nos. 1 and 2, which were injected with a large dose of antigen (150 and 120 mg protein each, equivalent to about 750 and 600 mg β-lipoproteins respectively), showed considerable retardation in growth and weight during development (Fig. 1) compared with the rabbits from litter No. 3, which received only 30 mg protein. After 4 months, only 1 rabbit (No. 462) survived in the group of rabbits taken from litters Nos. 1 and 2, the rest having died from pneumonia and atrophy of the thymus. Animals from litter No. 3 (Nos. 466, 470, and 472) and rabbit No. 462 were transferred to the "atherogenic" diet on attaining a weight of 2 kg. These animals ("prepared"), together with normal rabbits of the same weight, were given 0.5 g cholesterol in oil daily for 3 months (the concentrations of β-lipoproteins and total cholesterol in the blood serum of all these animals were determined every month).

During feeding of the "prepared" rabbits with cholesterol, they developed moderate hypercholesteremia (Table 1). Only in 1 rabbit (No. 462), which recieved a large dose of antigen, was an increase in the serum cholesterol concentration observed, and this had fallen appreciably by the end of the experiment. The concentrations of cholesterol and β-lipoproteins in the "unprepared" animals was 3-8 times higher before the end of the experiment, compared with the initial level, than in the "prepared" rabbits (Table 1). Macroscopic and microscopic examinations of the "prepared" rabbits revealed no atherosclerotic changes in the aorta, no fatty degeneration of the liver, and no changes in the other parenchymatous organs, whereas in the control animals severe atherosclerotic lesions were present, such as usually develop under these experimental conditions (Table 1).

Parenteral injection of atherogenic homologous lipoproteins into newborn rabbits thus prevented the development of experimental atherosclerosis. The experimental results also showed that a moderate dose of antigen (7-15 mg) must be used in order to obtain this effect.

LITERATURE CITED

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