THE EFFECT OF VITAMIN B_{12} ON THE REVERSE DEVELOPMENT OF CHOLESTEROL ATHEROSCLEROSIS IN RABBITS

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Ever since a group of Leningrad pathologists [2,3,7] showed that the reverse development of atherosclerosis was possible in man, many experimental works have been conducted in order to find a way of hastening this process by using substances which prevent the development of experimental atherosclerosis. Since the degree to which experimental atherosclerosis develops depends to a considerable extent on the blood cholesterol level, the substances first tested were those lowering this level.

The works of several authors [6, 7, 9, 11] have demonstrated that thyroidin (dessicated thyroid preparation) has an effect notably accelerating the reverse development of cholesteremia and experimental cholesterol atherosclerosis in rabbits.

When no preparations are used, a period of 6-8 (McMillan, Horlick, Duff) or more (T.A. Sinitsyn) months is required for this process in rabbits and 8-9 months (Horlick and Katz) in chicks, but with the use of thyroidin, the initial signs of reverse development are apparent $1\frac{1}{2}$-$2\frac{1}{2}$ months after the final cholesterol feeding [6].

A combination of thyroidin and estrogens was found to be especially efficient in lowering the cholesterol level and accelerating the reverse development process of atherosclerosis [11]. Heparin, which according to the data of several authors [5, 8, 13], inhibits the development of experimental atherosclerosis, does not significantly affect the reverse development of cholesteremia. The authors differ in their evaluations of heparin's effect on the reverse development of atherosclerosis; acceleration of this process was macroscopically observed by Horlick and Duff in experiments on rabbits, but Akira Horita and Lomis did not observe this phenomenon in experiments on chickens.

Choline's effect on the development of experimental atherosclerosis and the resorption of atheromatous plaques is debatable. It has recently been found that certain vitamins of the B group, particularly vitamin B_{12}, take part in the synthesis of choline and methionine and possibly in the transfer of methyl groups.

At the suggestion of A.L. Myasnikov, Active Member of the AMN SSSR, we studied the effect of vitamin B_{12} on the formation and reverse development of atheromatous elements in the aorta of rabbits, reproducing cholesterol atherosclerosis by the method of Academician N.N. Anichkov. We reported the results of our study investigating vitamin B_{12}'s effect on the development of experimental atherosclerosis in rabbits at the IXth session of the AMN SSSR Institute of Therapy in February, 1958. We demonstrated that the level of cholesterol in the blood did not increase as much in animals which received vitamin B_{12} along with the cholesterol as in the animals given cholesterol alone, so that the phospholipid-cholesterol coefficient figures for the rabbits given vitamin B_{12} were higher, and there was considerably less development of atherosclerosis. Our data were confirmed by the works of Yu.G. Grigorov [4], and Chakravarti and Mukerji [10].

This article presents the results of research on the processes of reverse development of experimental atherosclerosis under the influence of vitamin B_{12}.

EXPERIMENTAL METHODS

The experiments were performed on 24 rabbits of the same breed, sex and age, weighing 1.5-2 kg. Along with their usual food, all the rabbits received a 10% solution of cholesterol in sunflower oil for 105 days in a daily dose of 0.2 g cholesterol per 1 kg of animal weight. We then stopped the cholesterol feeding and divided the rabbits into three groups. The six rabbits of the first group were killed to determine the degree of atherosclerosis development in them at this time; this group served, so to speak, as a control for the other two groups. The remaining 18 rabbits were divided into two groups, identical as to number of animals and blood cholesterol level, forming the second and third groups; these animals were given the usual food ration for 112 days. The nine rabbits of the second group served as the control for the rabbits of the third, or experimental, group, which received a daily subcutaneous injection of 30 μg vitamin B_{12}. Every 25 days throughout the experiment on the

rabbits (217 days), we investigated the cholesterol content of the blood serum according to the Engel'gardt-Smirnova method and determined the lipoid phosphorus. Bloor's method was used to extract the lipoid phosphorus from the blood serum, and the sulfite-hydroquinone method was used for the actual phosphorus determination. The numerical value obtained for the lipoid phosphorus was multiplied by the coefficient 25; the obtained sum of the phosphatides was conditionally assumed to be lecithin, and the lecithin-cholesterol coefficient was computed.

EXPERIMENTAL RESULTS

The level of cholesterol and phospholipids in the blood serum increased considerably in almost all the rabbits during the cholesterol feeding period, and the phospholipid-cholesterol coefficient decreased.

Towards the end of the 105-day cholesterol feeding period, the average cholesterol content of the rabbits' blood serum was 1125 mg% in the first group, 787 mg% in the second, and 740 mg% in the third. The average content of phospholipids was 745 mg% in the first group, 533 mg% in the second group, and 652 mg% in the third. The phospholipid-cholesterol coefficient was 0.65 in the first group, 0.77 in the second, and 0.61 in the third.

During the period of reverse development, the cholesterol and phospholipid levels decreased in both the second, or control, group and the third, or experimental, group.

At the end of the experiment, 112 days after the final cholesterol feeding, the average level of blood cholesterol was 285% in the control group of rabbits, but 142 mg% in the animals which had received vitamin B_{12}; the average content of phospholipids was 191 and 131 mg% respectively, and the phospholipid-cholesterol coefficient was 0.88 and 1.34.

On the basis of the average figures at the end of the experiment, therefore, vitamin B_{12} can be said to have a marked effect on the reverse development of the cholesterol and phospholipid content of the blood (Fig. 1). It should be emphasized, however, that this effect of vitamin B_{12} is not immediately evident.

If the curves of the reverse development of cholesteremia and the phospholipid level, which we constructed from the average levels of these substances during the experiment, are compared, it is evident that the phenomenon of the decrease in the level of blood lipids effected by vitamin B_{12} was not clearly apparent until the 4th month after the final cholesterol feeding. The curves for the first three months were about the same.

The degree to which aortic atherosclerosis had developed was estimated by the weight planimetric method after total staining of the aorta with sudan III; according to this method, the degree to which the aorta is affected by the various atheromatous elements (thick plaques, thin plaques, lipoid spots) is expressed in percent of the

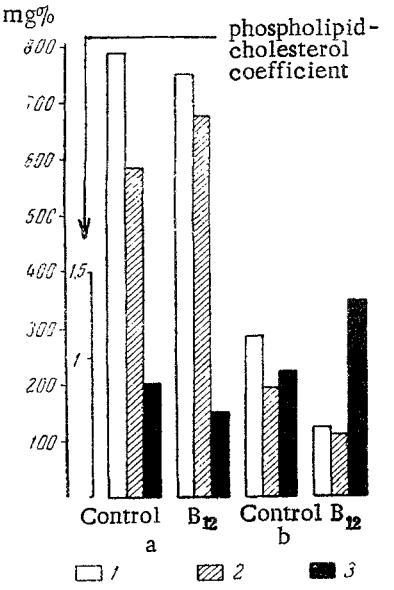

Fig. 1. Average levels of the cholesterol and phospholipid content of the blood and values of the phospholipid-cholesterol coefficient for the rabbits of the second (control and third experimental groups at the end of the cholesterol feeding period and at the end of the experiment. a) At end of cholesterol feeding period; b) at end of experiment; 1) Cholesterol level; 2) phospholipid level; 3) phospholipid-cholesterol coefficient.

total area of the aorta. The sum of all the elements, expressed in percent, is the overall degree of aortic atheromatosis.

The graphs on the left side of Fig. 2 show the degree of aortic atheromatosis in each animal of the three groups, and those on the right show the average values. First of all, one can see from Fig. 2 that the degree of aortic atheromatosis was was considerably higher in the rabbits of the second group, which were fed their customary diet after the end of the cholesterol feeding period, than it was in the rabbits of the first group, which were killed at the end of the 105-day cholesterol feeding period, even though the average blood cholesterol level of the first group at the end of the feeding (1125 mg%) significantly exceeded that of the second group (787 mg%). After the cholesterol feeding was stopped, therefore, intense atherogenesis, which the reverse development processes could not affect in so short a time (112 days), occurred in the rabbits of the second group.

A different picture was observed in the rabbits of the third group, which were given vitamin B_{12} after the cholesterol was discontinued. As Fig. 2 shows, the

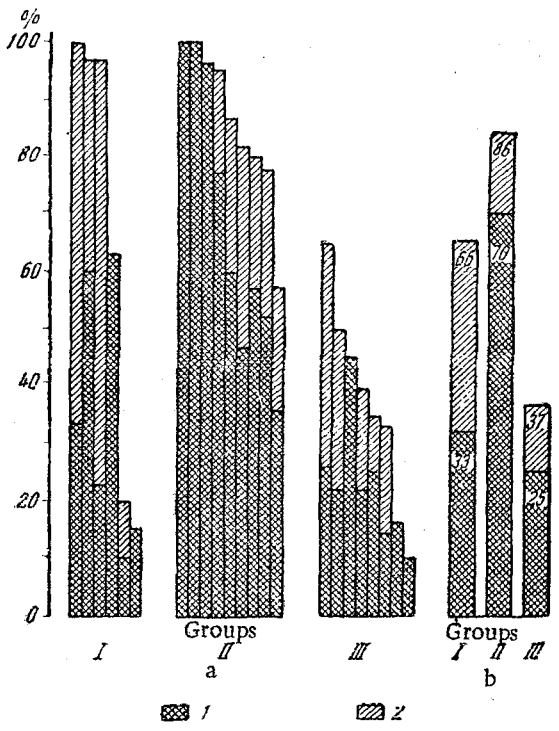

Fig. 2. Degree of aortal atheromatosis. a) Degree of aortal atheromatosis in all three groups; b) average percent of aortal injury in each group; 1) Injury by thick atheromatous plaques; 2) total atheromatosis; I) Rabbits killed after 105-day cholesterol feeding; II) rabbits subsequently fed the usual diet; III) rabbits subsequently given vitamin B_{12}.

degree of aortic atheromatosis in these animals was not only no higher, but definitely lower than in the rabbits of the first group and much lower than the degree of aortic atheromatosis in the second group.

Comparison of the blood lipid level and the degree to which aortic atheromatosis developed in the rabbits of the second and third groups suggests that the considerably lesser degree of aortic atheromatosis observed in the rabbits given vitamin B_{12} cannot be explained solely by the sharper decrease in the blood lipid level of the experimental group as compared with the second group, because, as we have already stressed, this sharper decrease was only clearly apparent towards the end of the experiment. This of course suggested that the reason for the relatively rapid reverse development of aortic atheromatosis observed in the rabbits of the experimental group had to do with the influence of vitamin B_{12} on the process of resorption of lipoid masses from the walls of the aorta. The nine aortas of the rabbits in the second (control) group and the nine aortas of the animals comprising the third (experimental) group were therefore subjected to histological examination. We examined pieces taken from the ascending aorta, the arch of the aorta and the abdominal aorta.

Microscopic examination showed the intima of the aortas from the control rabbits to be considerably thickened by a profuse deposit of fatty substances in the form of large and small drops of fat, which stained red with scarlet red. A large number of fat drops were found in the round cells with well-stained nuclei. In places, fat drops were beginning to pass into the tunica media of the aorta. Staining with picrofuchsin showed a significant number of delicate collagen fibrils in the places of the fat deposits. In the intima of the aortas from the rabbits of the experimental group, which had received vitamin B_{12}, the fat deposits were considerably smaller, and staining with picrofuchsin demonstrated a greater number of collagen fibrils than in the control rabbits. This suggests that the reverse development process of atheromatous plaques was more intense in the experimental rabbits.

SUMMARY

Prolonged vitamin B_{12} administration promotes reverse development of atheromatous elements in the rabbit aorta, affected by cholesterol atherosclerosis. Evidently, accelerated reverse development of cholesterol atherosclerosis is connected with the action of vitamin B_{12} upon the concentration of the lipids in the blood serum.

One cannot exclude, however, the direct activating effect of the vitamin upon the vascular wall elements on which depends the resorption and removal of lipids infiltrating its intima.

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