

## THE EFFECT OF HEPARIN ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

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The reports in the literature on the effect of heparin on the serum cholesterol and phospholipid contents are contradictory. Herzstein (1954) et al. [11], and Raynaud and his co-workers [16] consider that heparin does not affect the level of cholesterol and phospholipids in the blood, whereas other workers [7, 4] have found that heparin raises the serum cholesterol level of patients with xanthomatosis and atherosclerosis.

Graham et al. [10] showed that heparin causes conversion of higher classes of lipoproteins with a flotation rate Sf 10-30 into lower (Sf 5-10) and so on, as a result of which, in the author's opinion, there is inhibition of the development of experimental atherosclerosis in rabbits fed on cholesterol.

No less contradictory are the experimental results [9, 12, 13, 14, 15] on the effect of heparin on the development of atherosclerosis, although the majority of authors conclude that heparin inhibits the development of atherosclerosis in experimental animals.

It is possible that the difference in the experimental results of the various workers is due to the use of different doses of heparin. For this reason we set out to investigate the effect of large and small doses of heparin on the level of the blood cholesterol and phospholipids and on the development of atherosclerosis in rabbits during prolonged feeding with cholesterol.

### EXPERIMENTAL METHOD

Experiments were performed on 53 chinchilla rabbits weighing 2100-2600 g. All the rabbits were kept under identical conditions and on the same diet. Atherosclerosis was produced by N. N. Anichkov's method of feeding the animals for 100 days with a solution of cholesterol in sunflower oil (at the rate of 0.2 g of cholesterol per 1 kg body weight).

Heparin was injected intravenously every day in different doses into the experimental animals (28 rabbits): 15 rabbits received heparin in a dose of 30 mg, seven rabbits received 60 mg and six rabbits 3 mg. The controls (23 rabbits) received cholesterol only.

In order to ascertain the effect of heparin on the content of endogenous cholesterol and lecithin, two rabbits were injected every day with 30 mg of heparin intravenously.

Every 25 days the serum cholesterol content was estimated by the Engel'gardt-Smirnova method [6] and the phospholipids by the sulfite-hydroquinone method [2] with extraction by Bloor's method [8].

When 100 days had elapsed the animals were killed by air embolus of the lungs. At postmortem examination the aorta and heart were extracted. The aorta was fixed in 10% formalin solution and then stained with Sudan III in toto.

The degree of the atherosclerotic changes in the aorta was designated by + signs (0 – no changes; + – slight changes; ++ – moderate changes; +++ – severe changes, and ++++ – very severe changes).

A study of the effect of a single injection of heparin on the blood lipids of rabbits was carried out at various periods of cholesterol feeding. From 5-6 ml of blood was taken from the marginal vein of the rabbit's ear before and 30 minutes, 1-2 and 3 hours after the intravenous injection of heparin.

### EXPERIMENTAL RESULTS

After a single injection of heparin, in not one of the 22 cases was any increase in the cholesterol level observed.

In four cases there was a reduction in the cholesterol content by about 10%, which we regard as within the normal limits of variation of the cholesterol level. In 18 cases, the reduction in the cholesterol varied between 12.5 and 32% of the original value.

The fall in the blood cholesterol content was observed 30 minutes after the injection of heparin and lasted for three hours. In some cases, after an initial fall in the cholesterol level it was observed to rise at the end of the second hour of the experiment, to fall again at the end of the third hour. The effect of a single injection of heparin on the level of the blood cholesterol is shown in the form of curves in Figure 1.

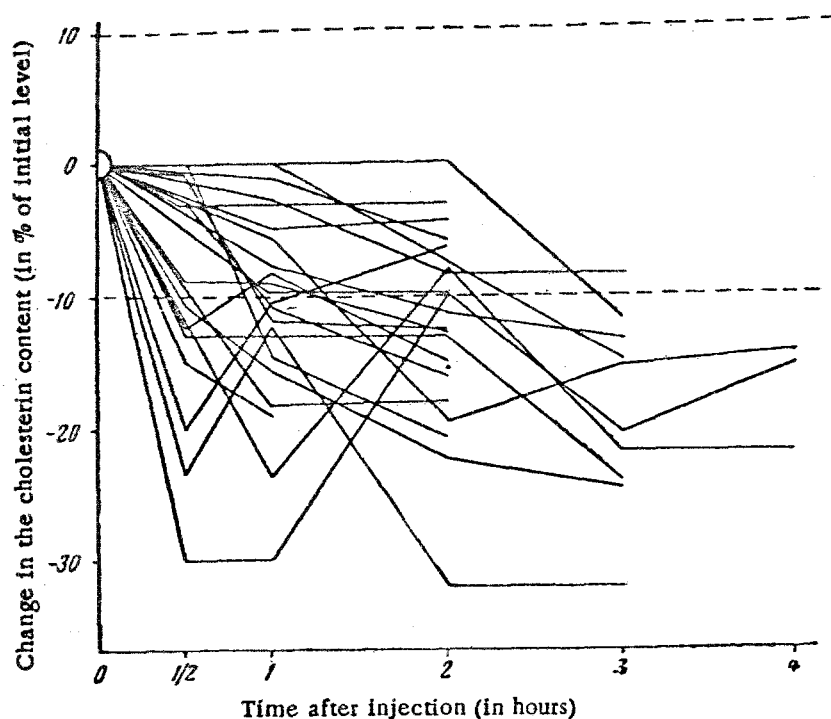


Fig. 1. Change in the blood cholesterol of rabbits after a single intravenous injection of heparin. The curves represent the changes in the cholesterol content in percentages of the initial level; the dotted lines show the normal limits of variation of the blood cholesterol.

Mention must be made of the relation between the degree of the reduction in the cholesterinemia and its initial level: the higher the initial blood cholesterol level the greater the degree of its fall. The magnitude of the fall in the blood cholesterol level did not depend on the dose of heparin used.

The lecithin content fell after injection of heparin (Figure 2) to approximately the same extent as the cholesterol content, so that the value of the coefficient lecithin/cholesterol was practically unchanged.

Thus, a single injection of heparin into rabbits with alimentary hypercholesterinemia led to a reduction in

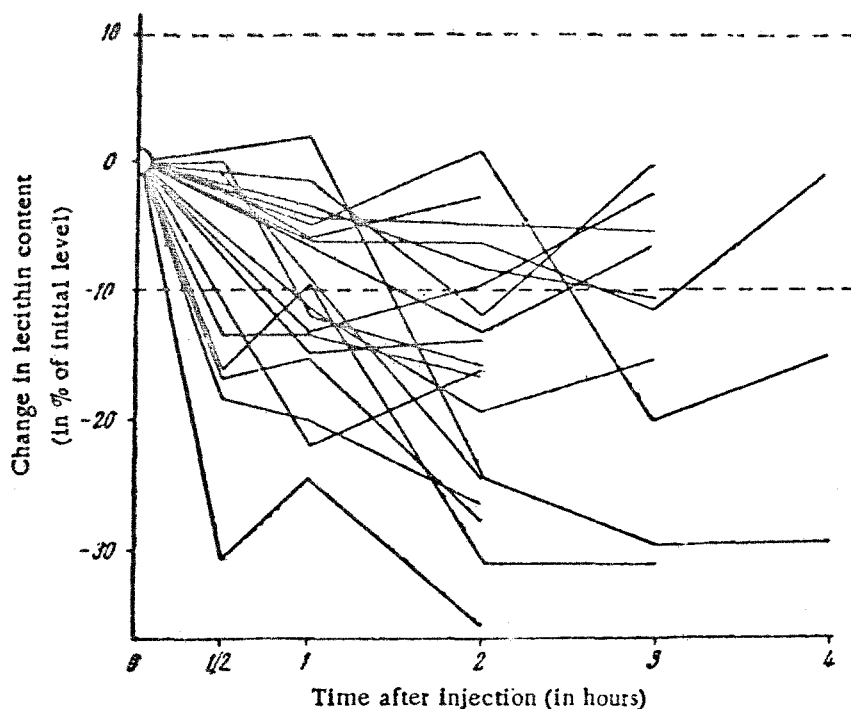


Fig. 2. Change in the blood lecithin level of rabbits after a single intravenous injection of heparin. The curves represent the changes in the lecithin content in percentages of their initial value; the dotted lines show the normal limits of variation of the lecithin level.

the level of cholesterol and lecithin in the blood without any essential change in the lecithin/cholesterol ratio.

Prolonged administration of heparin was well tolerated by the animals. No difference was observed in the weight of the rabbits of the control group receiving cholesterol alone, and the animals of the experimental group receiving cholesterol and heparin. One rabbit only, receiving heparin in a dose of 60 mg, died on the 27th day of the experiment from gastrointestinal hemorrhage.

Towards the end of the experiment all the animals of the control group developed hypercholesterinemia, sometimes reaching very high figures. In rabbit No. 3, for instance, the blood cholesterol level rose to 2560 mg%. The average value of the hypercholesterinemia in this group was  $1071.5 \pm 113.3$  (Figure 3). Besides the development of hypercholesterinemia, in all the rabbits the blood lecithin content also rose. Its average value was  $630.7 \pm 65.7$ . The degree of increase in the blood phospholipid content was considerably less than that of cholesterol, so that the lecithin/cholesterol ratio fell from 2.3 to  $0.59 \pm 0.028$  (average values).

Prolonged administration to rabbits of both large and small doses of heparin did not prevent the development of hypercholesterinemia. In the group of rabbits receiving small doses of cholesterol and heparin the average level of the blood cholesterol was higher ( $1295 \pm 209$ ) than in the control group (Figure 3). The average values of the blood lecithin content were  $690 \pm 95.7$ . The value of the lecithin/cholesterol coefficient in these rabbits was the same as in the animals of the control group.

In rabbits receiving large doses of heparin (60 and 30 mg), the average level of the blood cholesterol at the end of the experiment was  $980.7 \pm 51.1$  and  $749 \pm 36$  (see Figure 3), i.e., somewhat lower than in the control group, but the difference was not significant. Injection of large doses of heparin did not affect the rise in the blood lecithin content. The average lecithin level was  $688 \pm 75.1$  and  $474 \pm 49.6$ . The mean values of the lecithin/cholesterol ratio in these groups of rabbits were slightly higher than in the control group ( $0.7 \pm 0.09$  and  $0.75 \pm 0.06$ ), but this difference also was not significant.

The administration to rabbits of heparin alone in a dose of 30 mg intravenously had no effect on the level of endogenous cholesterol and lecithin.

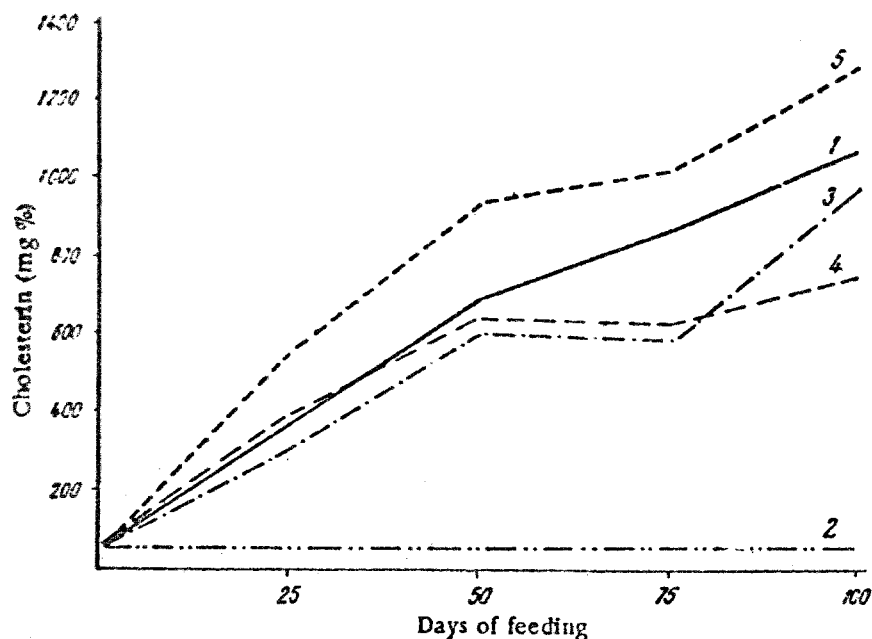


Fig. 3. Development of hypercholesterinemia in various groups of rabbits after prolonged feeding with cholesterol: 1) cholesterol - control; 2) heparin - control; 3) cholesterol + heparin 60 mg; 4) cholesterol + heparin 30 mg; 5) cholesterol + heparin 3 mg.

It should be mentioned that in the control group and in the group of rabbits receiving cholesterol and heparin in a dose of 30 mg, the blood cholesterol content of three animals either did not rise at all (rabbit No. 9) or rose only slightly in the last month of feeding (rabbits Nos. 4 and 52).

There are reports of such "cholesterol resistant" rabbits as these in the articles by S.M. Leites [3], and T.A. Sinitsyna and T.N. Loviagina [5].

Atherosclerotic changes in the aorta were shown in varying degree in all the experiments, except in rabbit No. 9 in which, as mentioned above, no increase in the blood cholesterol level was observed in spite of prolonged feeding with cholesterol.

Large atherosclerotic patches were situated mainly in the region of the arch of the aorta and smaller ones around the orifices of the intercostal arteries. In cases of well-marked atherosclerosis lipid deposits were observed throughout the whole extent of the aorta.

#### The Degree of Atherosclerosis of the Aorta in the Various Groups of Rabbits

Agent applied	Number of rabbits in the group	Degree of atherosclerosis of the aorta				
		++++	+++	++	+	0
Cholesterol (control)	23	2	11	5	5	—
Cholesterol, heparin 60 mg	7	—	1	3	3	—
30 mg	15	—	2	5	7	1
3 mg	6	4	1	1	—	—

On comparing the degree of atherosclerosis of the aorta with the magnitude of the hypercholesterinemia a parallel trend can be observed between them in the majority of cases. However, in some cases, the degree of atherosclerosis of the aorta did not correspond to the height of the hypercholesterinemia. In rabbit No. 82 for instance, with a blood cholesterol level of 1600 mg % the degree of lipoidosis of the aorta was insignificant (+).

while in rabbits Nos. 33 and 34, with hypercholesterinemia of 500 and 580 mg % the degree of atheromatosis of the aorta was very severe (+++).

On comparing the degree of atherosclerosis of the aorta in the different groups of rabbits (see table) it is clear that the giving of small doses of heparin did not prevent the development of atherosclerosis. Of the six rabbits in this group, in four the atherosclerosis of the aorta was very severe (++++).

In more than half the rabbits of the control group (in 13 out of 23) a severe degree of atherosclerosis of the aorta was observed (++++ and +++). In the remaining rabbits the degree of involvement of the aorta was moderate (++) or slight (+). Meanwhile, in the group of rabbits receiving large doses of heparin (60 and 30 mg) in addition to cholesterol, out of 22 animals there was a severe degree (++) of atherosclerosis of the aorta in only three, and the remaining 19 rabbits showed a moderate (++) or slight (+) degree of atherosclerosis.

Administration of large doses of heparin thus inhibited the development of experimental atherosclerosis in rabbits during prolonged cholesterol feeding. This action of heparin is difficult to put down to its effect on the blood lipids.

### SUMMARY

Single administration of various doses of heparin to rabbits with alimentary hypercholesterinemia brought about the decrease in the cholesterol and lecithin blood level to the same degree. The value of the lecithin/cholesterol coefficient remained almost unchanged. Prolonged introduction of low doses of heparin to rabbits fed with cholesterol had no effect on the degree of atheromatosis of aorta and on the blood lipids. Prolonged intravenous introduction of large doses of heparin (30 and 60 mg) inhibited the development of aortic atherosclerosis in rabbits but had no definite effect on the content of the blood serum lipids.

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