# REPRODUCTION OF ATHEROSCLEROSIS IN RABBITS BY LIMITATION OF MOBILITY*

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The author's clinical observations [2, 3] have shown that by restricting muscular movements, especially by strict confinement to bed, the coronary circulation of some patients with chronic coronary insufficiency is worsened. To analyze the clinical findings, experiments were undertaken on rabbits.

## EXPERIMENTAL METHOD

To restrict the rabbit's mobility, a special cage was built consisting of a floor and a wire framework [5]. When the animals were being fed, blood was taken for estimation of the cholesterol concentration, and during recording of the electrocardiogram (ECG), the conditions of immobilization were not disturbed. At the end of the experiment the arterial pressure was measured. The ECG was recorded before the experiment, at various times during the observations, and at the end of the experiment. Needle electrodes were used. To ensure identical conditions of recording of the ECG and maintenance of the immobilization, each rabbit was placed in a special "jacket" and suspended in a horizontal position. At necropsy of the rabbits sacrificed 14-140 days from the beginning of immobilization, the macroscopic and microscopic changes in the aorta and the microscopic changes in the myocardium were assessed, using the staining methods of Van Gieson and Heidenhain.

Experiments were carried out on 18 male chinchilla rabbits (11 experimental and 7 control animals). The mobility of the experimental animals was considerably restricted, while the control animals were not immobilized – they were kept in ordinary cages. Depending on the duration of restricted mobility, the

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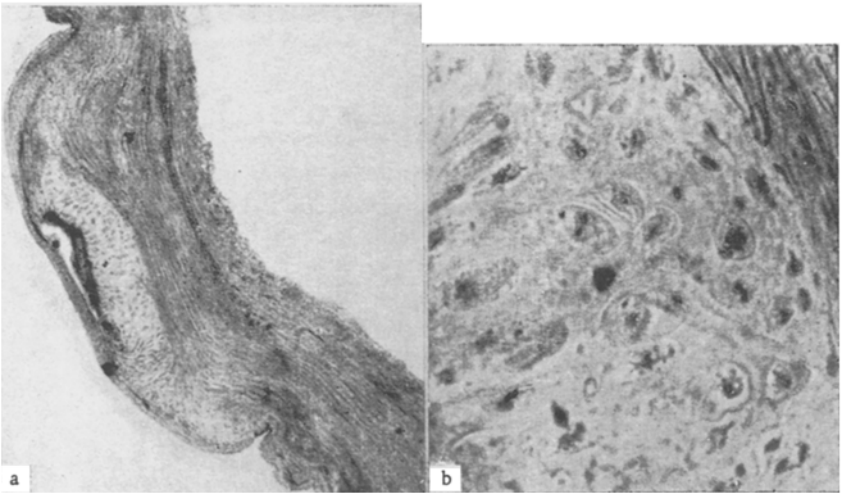


Fig. 1. Area of the arch of a rabbit's aorta 140 days after beginning of immobilization. Explanation in text. Hematosylin-Sudan III. Magnification 50× (a) and 450× (b).

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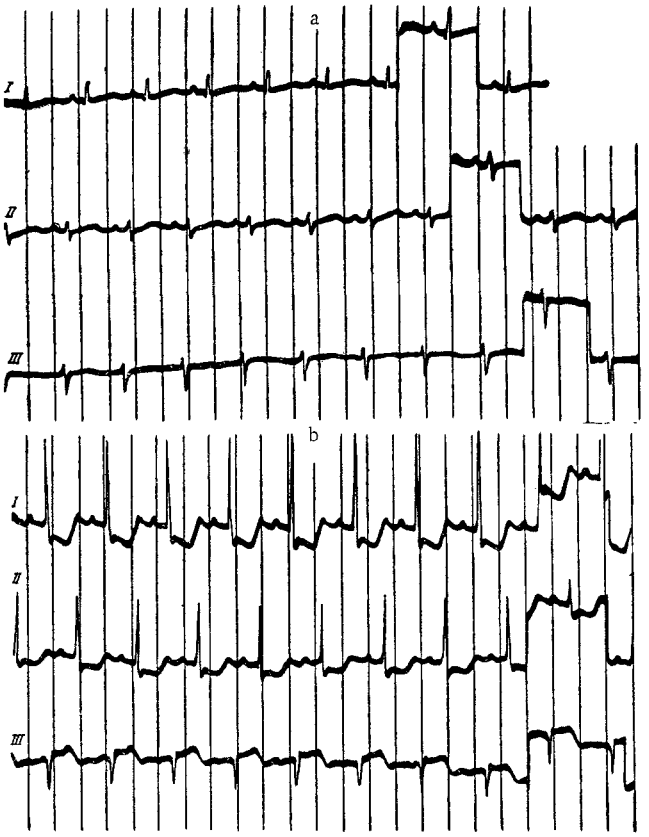


Fig. 2. ECG of a rabbit before (a) and 14 days after (b) immobilization.

experimental rabbits were subdivided into two groups: in the animals of experimental group 1 (4 rabbits) mobility was restricted for 14 days, and in the animals of group 2 (7 rabbits) – for 140 days.

## EXPERIMENTAL RESULTS

In experimental group 1, two rabbits (Nos. 1 and 3) died 7.5 days from the beginning of immobilization. In experimental group 2, 1 rabbit (No. 9) died 15 days after the beginning of immobilization and 1 rabbit (No. 5) died 7 days after discontinuing immobilization.

In all the experimental rabbits macroscopic changes were found in the aorta: roughness of the endothelium and the formation of tubercles. Roughness of the endothelium was conventionally denoted by one plus sign, changes in the aorta with a moderate number of tubercles – by two plus signs, and changes in the aorta with numerous tubercles – by three plus signs. In experimental group 1, roughness of the endothelium (+) was found in three rabbits and tubercles (++) in one rabbit. In experimental group 2, changes corresponding to ++ were found in 4 rabbits, and corresponding to +++ in 2 rabbits. In animal No. 9 of this group, which dies 15 days after the beginning of immobilization, the aorta and heart were not investigated.

On histological investigation of sections of the aorta (stained with hematoxylin and Sudan III) during the first weeks of restricted mobility of the rabbit, metachromatic staining of individual areas of the aortic wall was found in the subendothelial zone. The slightly thickened elastic fibers were most brightly stained. At some places in the subendothelial region, signs of infiltration were observed.

Fourteen days after the beginning of immobilization of the rabbits, the zone of metachromasia in the region of the internal elastic membrane and the signs of subendothelial infiltration increased considerably in intensity. Signs of sudanophilia were observed in the subendothelial region, and here and there intracellular deposition of lipids. In the cells of the endothelial layer, the first signs of fatty inclusions were seen.

A further increase in the intensity of proliferation of the subendothelial cells was observed 140 days after the beginning of immobilization (Fig. 1a, b). The subendothelial metachromatic zones were more ex-

tensive; lightly stained areas of cells with numerous fatty inclusions developed beneath these zones, towards the muscular coat. At some places above the tubercles, detachment of the endothelium from the subendothelial layer of cells was observed. In some places the subendothelial layer was thinner. Evidently in areas such as these, the endothelium later ruptured and an ulcer was formed.

Atherosclerosis of the cornary arteries was not found in a single rabbit.

In the experimental animals and in one control rabbit (with paralysis of the hind limbs) changes were observed in the myocardium: signs of capillary stasis, disappearance of the nuclei and cross striation of the fibers, foci of necrosis and proliferation of connective tissue. The appearance of capillary stasis was conventionally described by one plus sign, disappearance of the nuclei and the cross striation of the fibers in a small area of myocardium by two plus signs, disappearance of the nuclei and cross striation of the fibers over a wide area by three, and changes in the myocardium with foci of necrosis by four plus signs.

In experimental group 1 histological changes in the myocardium corresponding to + were observed in one rabbit, corresponding to ++ in two rabbits, and to +++ in one rabbit. In experimental group 2, changes corresponding to ++ were found in only two animals, and in all the others the changes corresponded to +++.

The ECG at the end of the experiment was recorded before discontinuing immobilization and then at intervals of 3-10 days until the rabbits were sacrificed. The data of the ECG corresponded mainly to the microscopic changes in the myocardium. In all the rabbits of the experimental groups except 1 signs of coronary insufficiency were observed on the ECG taken before discontinuing immobilization: changes in the amplitude of the R wave, displacement of the RS-T interval from the isoelectric line, and deformation of the T waves (Fig. 2).

In all the experimental rabbits one month after the beginning of immobilization the blood cholesterol level rose to 164-242 mg % (before the beginning of restriction of movement it was 36-84 mg %).

No appreciable difference was found between the arterial pressure of the rabbits of the experimental and control group.

Hence, during the first week of immobilization the rabbits developed trophic changes in the aortic wall (the pre-lipoid stage of atherosclerosis). By the end of the second week these changes gradually led to the formation of atherosclerotic tubercles. It is interesting that the initial changes in the aorta appeared when the blood cholesterol level was normal or even slightly below normal.

The changes in the myocardium were evidently associated principally with neuro-humoral disturbances in the body. It was found in acute experiments on animals [1] that muscular movement, by stimulating the proprioceptors, leads to the reflex dilation of the coronary arteries. In conditions of restriction of muscular movements, the reflex influences from the proprioceptors of the muscles on the coronary vessels are evidently considerably diminished, and this may lead to a decrease in the blood supply to the myocardium. In the present experiments indirect evidence of this was given by the electrocardiographic results. However, even during immobilization of animals the negative influence of the catecholamines on the myocardium cannot be ruled out: Raab and co-workers [6] showed that a lack of physical activity leads to the relative pathogenic predominance of the influence of the sympathetic nervous system on the myocardium.

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