EFFECT OF PRECEDING HYPERFUNCTION AND HYPERTROPHY OF THE HEART ON INJURY TO THE MYOCARDIUM ARISING IN EXPERIMENTAL MYOCARDITIS

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In human pathology, forms of myocarditis of different etiology frequently develop against the background of existing heart defects, hypertension, and so on, i.e., against the background of prolonged hyperfunction and hypertrophy of the heart.

Previous investigations have shown that the contractile power of the myocardial tissue of the left ventricle is disturbed both in animals with prolonged hyperfunction and hypertrophy of the heart and also in animals with experimental myocarditis [1,4]. However, the problem of how the developing myocarditis influences the structure of the hypertrophied heart maintaining compensatory hyperfunction for long periods of time, has not been investigated.

In the present investigation, to study this problem, experimental myocarditis was reproduced in animals with experimental coarctation of the aorta and a hypertrophied heart.

EXPERIMENTAL METHOD

Coarctation of the aortic orifice was reproduced in female rabbits weighing 2.0-2.5 kg by the technique described previously [1]. On the 45th day after the operation, the animals were sacrificed in acute experimental conditions. Myocarditis was also reproduced in female rabbits by single and repeated intravenous injections of theophylline (1%, 2 ml/kg) and adrenalin (0.2 ml of a 1:1000 solution). Five series of experiments were performed. Experiments were carried out on 41 rabbits, 16 of which were investigated histologically.

In the experiments of series I, experimental myocarditis was reproduced in eight hitherto intact rabbits; the animals were sacrificed four days after injection of theophylline and adrenalin (single acute myocarditis). In the experiments of series II, experimental myocarditis was also reproduced once in seven hitherto intact animals sacrificed on the 30th day after injection of theophylline and adrenalin (single chronic myocarditis). In series III myocarditis was reproduced twice in seven hitherto intact animals. The interval between the first and second injection of the substances causing myocarditis was one month; the animals were sacrificed four days after the second injection of these substances (repeated myocarditis). In the experiments of series IV, experimental coarctation of the aortic orifice was reproduced in ten rabbits; the animals were then sacrificed on the 45th day. In the experiments of series V, nine animals were subjected to the combined procedure (stenosis and myocarditis), for which purpose myocarditis was reproduced once on the 45th day after coarctation of the aorta, i.e., against the background of hyperfunction and hypertrophy of the myocardium; the animals were sacrificed on the 4th day after injection of theophylline and adrenalin.

In all the series of experiments, a histological investigation was performed; pieces from the same parts of the myocardium (the wall of the left ventricle with the papillary muscle) were taken for this purpose. Sections cut on a freezing microtome were stained with Sudan III, hematoxylin-eosin by Van Gieson's method, and with toluidine blue for the metachromasia reaction.

EXPERIMENTAL RESULTS AND DISCUSSION

In single acute experimental myocarditis (see figure, a), no essential changes were found in the muscle fibers. In the connective-tissue stroma of the myocardium of the left ventricle of the rabbits' heart isolated small focal clusters of cells were present, consisting mainly of histiocytes and lymphocytes. Both in the clusters of cells and in other areas of the myocardium the intermuscular septa of the stroma were slightly thickened and showed basophilic staining properties, indicating the accumulation of mucoid substances in these areas.

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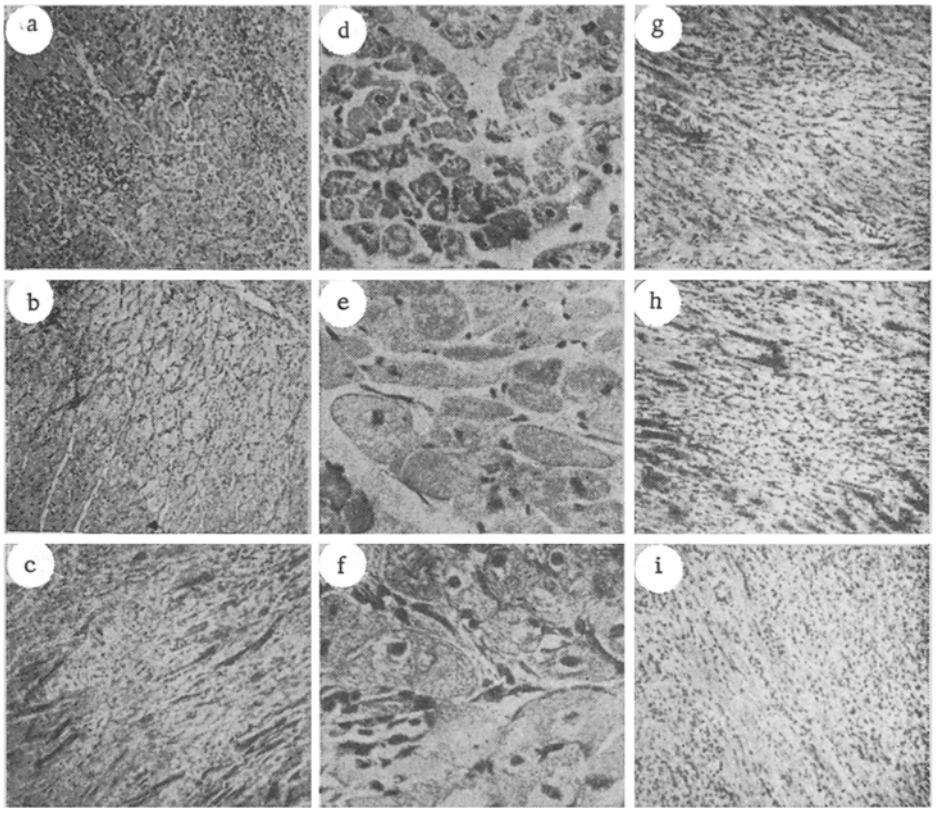

Structure of the myocardium in various pathological states. a) Single acute myocarditis. Focal inflammatory changes. Hematoxylin-eosin. Magnification 10 × 10; b) single chronic myocarditis. Thickened intermuscular septa. Van Gieson's stain, magnification 10 × 10; c) repeated experimental myocarditis. Diffuse fibrosis with inflammatory changes. Hematoxylin-eosin. Magnification 10 × 10; d) wall of left ventricle of an intact rabbit's heart. Muscle fibers and stroma of the myocardium a are unchanged. Hematoxylin-eosin. Magnification 10 × 40; e,g) experimental stenosis. Hypertrophy of muscle fibers. Hematoxylin-eosin. Magnification 10 × 40. Fibrosis of stroma of myocardium. Van Gieson's stain. Magnification 10 × 10; f,h,i) combined procedure (stenosis and myocarditis). Hypertrophy, vacuolation of muscle fibers, pycnosis of their cell nuclei. Van Gieson's stain. Magnification 10 × 40. Focus of granulation tissue with cell proliferation. Van Gieson's stain. Magnification 10 × 10. Diffuse inflammatory changes. Van Gieson's stain. Magnification 10 × 10.

The observed changes, taken as a whole, indicate that the doses of the pharmacological preparations used led to the development of a moderate focal myocarditis, as other authors [5,6] have described.

It has previously been shown that in this pathological state the relative weight of the left ventricle is increased by 15% compared with the weight of the left ventricle in intact animals, while the maximal attainable intensity of function of the structures (IFS), characterizing the contractile function of the heart muscle, is lowered by 18% [1].

In the case of single chronic myocarditis (see figure, b), changes in the muscle fibers were almost absent. In the connective-tissue stroma of the myocardium, only very slight thickening of the intermuscular septa was observed in the subepicardial layer and in the region of the papillary muscle.

In the pathological state described above, the relative weight of the left ventricle likewise was increased by 15%, and the contractile function of the heart muscle was lowered by 16% [1].

In the case of repeated experimental myocarditis (see figure, c), initial necrobiotic changes were found in the muscle fibers of the wall of the left ventricle (homogenization of the cytoplasm, pycnosis of the nuclei). In the

connective-tissue stroma of the myocardium, the appearance of a diffuse fibrosis was noted. The inflammatory changes, in the form of clusters of histiocytes and lymphoid cells, likewise became diffuse in character.

As previously shown, in repeated myocarditis the relative weight of the left ventricle increased by 69% compared with the weight of the left ventricle in the intact rabbit, while the contractile function of the myocardial tissue was lowered by 40% [1].

Comparison of the structural changes in the heart muscle during single acute and single chronic myocarditis and the changes described in repeated myocarditis shows that, in the latter, both the inflammatory and the sclerotic changes were more marked, and this was manifested by more marked functional disturbances. The impression was gained that the heart muscle had become less resistant to the subsequent administration of theophylline and adrenalin, leading to more severe structural changes.

In experimental stenosis (see figure, e) signs of marked hypertrophy were found both in individual muscle fibers and also in many groups of muscle fibers, situated mainly in the epicardial layer of the wall of the left ventricle. Some of these fibers were vacuolated. The connective-tissue stroma of the myocardium was characterized by the appearance of thickened septa consisting of bundles of collagen fibers, and in the region of the papillary muscle, and in particular in the muscle itself, they formed dense, wide bands (see figure, g), replacing the lost muscle fibers in these areas. In sections treated with toluidine blue, a metachromatic reaction was distinctly seen at the places of the thickenings in the intermuscular septa, especially in the papillary muscle, demonstrating the accumulation of mucopolysaccharides in these areas.

On the whole the changes in the myocardium of the left ventricle of the rabbits' heart in this series of experiments were characterized by the appearance of hypertrophied muscle fibers and by the presence of focal cardiofibrosis of the myocardial stroma. These changes are characteristic of the stages of stable hyperfunction described by F. Z. Meerson.

In the animals subjected only to experimental coarctation of the aortic orifice, the relative weight of the left ventricle was greater than in the control animals by 69%, while the maximal attainable IFS of the myocardium was lowered by 38% in these circumstances.

It is clear from the figure, e, that with the combined procedure (stenosis and myocarditis) the hypertrophy of the muscle fibers in the myocardium of the left ventricle was greater than the hypertrophy of the muscle fibers during aortic stenosis alone, the cytoplasm of the muscle fibers was swollen, and the nuclei were enlarged. This picture in hypertrophied muscle fibers has been described by other authors [2]. In many muscle fibers signs of perinuclear edema, vacuolation of the cytoplasm, and pycnosis of the nuclei were observed. The thickened intermuscular septa, consisting of coarse collagen fibers, were joined together in some places, forming thick bands. Besides the bands of coarse fibers of connective tissue, separate discrete, and also confluent foci of proliferation of young granulation tissues with large numbers of cells (see figure, h) were present in the thickness of the myocardium, although absent in the animals subjected to aortic stenosis only. In sections stained with toluidine blue, the metachromatic reaction of the ground substance of the connective tissue was clearly distinguished at the places where the intermuscular septa were thickened, and especially at the places of proliferation of the young granulation tissue. Besides scar changes in the myocardium, inflammatory changes were also found in its stroma, mainly in the form of generalized or diffuse clusters of histiocytes and lymphoid cells (see figure, i). The walls of the blood vessels of small and medium caliber were thickened, leading to narrowing of their lumen.

On the whole, the combined procedure caused more severe changes in the heart muscles both in the muscle fibers (amounting even to necrosis) and in the stroma (with the development of diffuse cardiosclerotic and inflammatory changes).

In these animals, the weight of the left ventricle was increased by 61% compared with the weight of the left ventricle in intact rabbits, while the contractile function of the myocardial tissue was depressed by 46%.

Hence, the superposition of myocarditis on previously existing hypertrophy of the myocardium led to more severe morphological changes, distinguished from those characteristic of myocarditis alone or of hypertrophy alone by the following: the muscle fibers were more hypertrophied, and more severe necrobiotic changes were observed in them. In the stroma, besides evidence of cardiofibrosis, foci of young granulation tissue and diffuse inflammatory changes developed.

When myocarditis was superposed on the hypertrophied myocardium, the contractile function of the heart was disturbed rather more severely than in the presence of hypertrophy uncomplicated by myocarditis. Taken as a whole,

these results show that the resistance of the hypertrophied myocardium to the injurious action of an excess of theophylline and adrenalin was distinctly lowered. One possible explanation of this phenomenon is that the muscle fibers, hypertrophied by hyperfunction, possess comparatively lower powers of renewal and regeneration of their disturbed structures. This depression of the ability of the genetic apparatus of the muscle fibers to provide for renewal of their structures by means of protein biosynthesis has been demonstrated for the later stages of cardiac hypertrophy [4]. Evidently in these experimental conditions, the superposition of myocarditis in turn accelerates and intensifies the decrease in the rate of protein synthesis, and thus contributes to the more severe changes in the heart muscle after the combined procedure. The intensified proliferative and inflammatory reaction of the connective tissue of the heart observed in this situation could be the result of the more severe injury to the muscle fibers.

The results obtained help to explain the clinically observed fact that when myocarditis is superposed on the hypertorphied myocardium, the course of the disease is often more serious than in primary myocarditis.

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