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RELATIONS BETWEEN MUSUCLAR AND CONNECTIVE TISSUE COMPONENTS OF THE RAT HEART IN EXPERIMENTAL MYOCARDIAL INFARCTION

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After the first day of experimental myocardial infarction in rats the volume and mean number of connective tissue stromal cells are increased in the "intact" zones of the left ventricle; on the second day of the experiment these indices reach their maximum, and by the 20th day they are equal to the values for the myocardium of control animals. On the third day of the experiment the number of muscle nuclei per standard area of cross section is increased, evidently as the result of amitotic division of the myocyte nuclei, for the number of paired nuclei is increased at the same time.

KEY WORDS: *myocardial infarction; volume of stroma; paired nuclei.*

In the course of histogenesis the muscle cells of the mammalian myocardium lose their ability to divide by mitosis [3, 5]. The subsequent increase in size of the heart, whether the myocardium be normal or injured, in the adult individual is due mainly to hypertrophy of the myocytes and takes place through hyperplasia and hypertrophy of the ultrastructures of the muscle cells [6-8]. At the same time the volume of the nuclei of the muscle cells of the human heart increases, and a high degree of ploidy is reached [9, 13]. A significant increase in the number of muscle nuclei in the hypertrophied myocardium of man and experimental animals also has been discovered [11, 12, 14]. The number of amitotically dividing myocyte nuclei is increased after wounds and infarcts of the myocardium, especially around the zone of damage [2, 4, 10]. In the investigation described below changes in the relations between the muscular and connective tissue component of "intact" zones of the rat heart and the number of paired myocyte nuclei at different stages of myocardial infarction were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 24 noninbred male albino rats weighing 90-100 g. In 18 rats a myocardial infarct was produced by suture and ligation of the rat coronary artery. The animals were killed with ether 1, 2, 3, 5, 7, 15, 30, and 90 days after the beginning of

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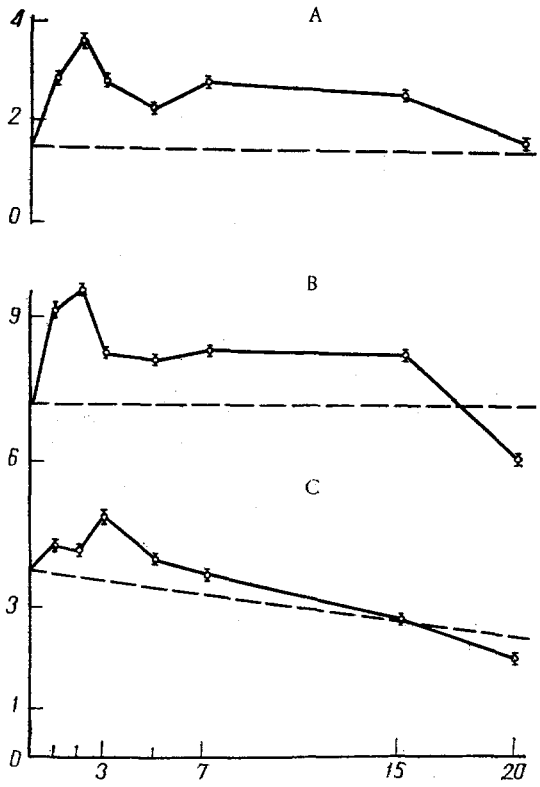

Fig. 1. Dynamics of change in volume of connective tissue stroma of heart (A) and mean number (per standard area of section) of connective tissue (B) and muscle (C) nuclei at different stages of myocardial infarction. Broken line: indices in control. Ordinate: A) area, in %: B and C) number of connective tissue and muscle nuclei, respectively; abscissa: stage of infarct, in days.

TABLE 1. Number of Amitotically Dividing Nuclei of Muscle Cells of the Rat Heart after Infarction and Healing

Stage of experiment, days	Number of paired nuclei, %	Total number of amitotically dividing nuclei, %
Control 1	0,45±0,15	0,84±0,21
1	0,31±0,12	0,57±0,17
2	0,26±0,11	0,67±0,18
3	1,23±0,26	1,67±0,30
7	1,07±0,25	1,38±0,29
15	1,22±0,35	1,52±0,39
30	1,35±0,40	1,83±0,47
90	1,43±0,48	2,28±0,60
Control 2	0,63±0,25	1,05±0,33

Legend. Control 1 and control 2 represent intact animals killed at beginning and end of experiment, respectively.

the experiment. Six intact rats, killed at the beginning, on the 20th day, and at the end of the experiment, served as the control. The hearts were removed, cut across horizontally at the level of the middle third, fixed in Carnoy's fluid, and embedded in paraffin wax. Sections 5 μ thick were stained by Van Gieson's method.

By means of a modified ocular grid [1] and the "point counting" method the area of the connective tissue stroma of the heart was determined and expressed as a percentage (deducting

the area occupied by the tissue spaces) for each stage of myocardial infarction. Under an immersion system 25 measurements were made of longitudinal and transverse sections through muscle fibers. The number of nuclei was counted in the same area of the ocular grid and the mean number of nuclei of muscle and connective tissue cells per standard area of section (0.0036 mm^2) was calculated.

To determine the number of amitotically dividing nuclei of muscle cells, and the number of paired nuclei, nuclei with constriction bands, and nuclei resembling mulberries in shape, were counted in 100 fields of vision (magnification 1350 times) of sections of the same series, stained with hematoxylin–eosin. The number of paired nuclei and the total number of amitotically dividing nuclei of heart muscle cells were expressed as percentages of the total number of all myocyte nuclei counted.

All the numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Planimetry of sections through the wall of the left ventricle of the hearts of the intact rats showed that the stroma occupies 1.5 ± 0.1% of the volume of the myocardium and the number of nuclei of the connective tissue cells was 65.4 ± 2.0% of the total number of nuclei counted. The area occupied by the stroma and the mean number of connective tissue cells per area of one ocular grid showed no significant change in the control animals killed at or 20 days after the beginning of the experiment (Fig. 1A, B), whereas the mean number of muscle nuclei contained in the same area (Fig. 1C) decreased ($P < 0.001$) with an increase in the age of the rats, as a result of an increase in the volume of the muscle cells.

The volume of the stroma in the "intact" zones of the heart 24 h after the beginning of myocardium infarction was almost doubled compared with the control ($P < 0.001$). There was a parallel increase in the number of connective tissue cells. On the second day of the experiment the total volume of the connective tissue cells and their number reached a maximum. In the later stages of myocardial infarction the volume occupied by the connective tissue stroma of the heart decreased, to reach the control level by the 20th day of the experiment, whereas the mean number of cells per standard area of section at this stage was actually less than in the control animals of the same age ($P < 0.001$).

The mean number of muscle nuclei per standard area of section showed a small increase 24 h after the beginning of the experiment, and on the third day of myocardial infarction it reached 4.9 ± 0.2 compared with 3.9 ± 0.2% in the myocardium of the control rats ($P < 0.01$). In the later stages of the experiment the number of muscle cell nuclei contained in the same area of section decreased, especially rapidly in the second and third weeks of the experiment.

Paired nuclei were found also in the control myocardium, in which their number increased insignificantly with age (Table 1). On the third day of myocardial infarction the number of binuclear muscle cells was more than twice that in the control ($P < 0.01$). Paired nuclei, incidentally, were particularly numerous in myocytes bordering on the focus of injury. The number of paired nuclei continued to increase in the later stages of the experiment, but the differences between the numbers of paired nuclei in the heart of the rats in the third month of the experiment and in the myocardium of the control animals of the same age were not statistically significant.

The increase in the number of muscle nuclei in the "intact" myocardium of rats with experimental infarction was thus probably the result of amitotic division of the nuclei, for mitotic division figures were not found in the myocytes of the left ventricle.

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ELECTRON-MICROSCOPIC STUDY OF THE MOUSE MYOCARDIUM IN EXPERIMENTAL COXSACKIE A13 VIRUS INFECTION

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Diffuse degenerative-proliferative myocarditis is described in adult BALB/c mice infected with Coxsackie A13 virus. A marked tendency was observed for sclerotic processes to develop 30-60 days after infection; this may lie at the basis of the reduced functional activity of the myocardium and may lead to the development of cardiomyopathy.

KEY WORDS: *Coxsackie viruses; myocarditis; ultrastructural changes.*

The Coxsackie viruses play an important role in human pathology. They are the most cardiotropic of all known viruses. It was formerly considered that they are pathogenic only for newborn rodents. However, it has now been shown that adult mice can also develop Coxsackie infection, sometimes in chronic forms [8-10, 12].

The object of this investigation was to study ultrastructural changes accompanying the development of acute and chronic myocarditis in adult mice infected with Coxsackie A13 virus.

EXPERIMENTAL METHOD

Male BALB/c mice aged 2 months were infected intraperitoneally with 0.3 ml of a culture of Coxsackie A13 virus in a titer of $10^{-5.5}$-$10^{-7.2}$ TCD_{50}/ml. Material was taken 1, 7, 30, and 60 days after infection. Intact mice and animals receiving an injection of culture fluid served as the control. At each stage of the investigation material was taken from the left ventricle of five mice, in the region of its apex, for light and electron microscopy. The subsequent processing of the material was carried out in the usual way.

EXPERIMENTAL RESULTS

Congestion of the vessels and foci of recent hemorrhages, and perivascular and irregularly distributed edema of the stroma were discovered in the hearts of the mice 24 h after infection; histiocytes, fibroblasts, and lymphocytes appeared in the intermuscular bands of connective tissue. This diffuse response of the stroma was found in all the mice. Small perivascular collections of histiocytes and lymphocytes were seen. The myocardium of the mice contained many foci of necrobiotic myocytes and solitary muscle fibers which stained more intensely with acid dyes.

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