PROLIFERATION OF THE RAT MYOCARDIUM AFTER EARLY POSTNATAL INJURY

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A small quantity of 96% ethanol was injected into the left ventricle of noninbred albino rats aged 7, 14, and 30 days, causing focal necrosis of the myocardium. The animals were killed 2-30 days after the operation. The mitotic index (MI) of the muscle nuclei was determined in different parts of the heart: in the left and right atria and their auricles, the trabecular and compact myocardium and the subepicardial zones of both ventricles, and also in the zone around the focus. Proliferation of myocytes in the compact myocardium was found to be inhibited and was not resumed after injury to the left ventricle. In all other zones, in which mitoses were present in the control animals, MI of the myocytes increased after trauma to the heart. The later the injury was inflicted, the less marked the rise in MI. In animals aged 7 days, MI on the 4th day after the operation reached 320-500% of the control, and on the 7th day it reached 120-380%. MI in the group of animals undergoing the operation at the ages of 14 and 30 days was increased only in some "high-mitosis" rats and not in all parts of the heart. The ability of connective tissue to replace the defect increases with growth of the heart.

KEY WORDS: injury to the myocardium; proliferation of cardiomyocytes; age changes in response of the myocardium.

It was shown previously that the myocardium of day-old rats, in which the level of proliferation of the myocytes is relatively high, responds in a distinctive manner to injury [1, 2]. Since proliferation of the cardiomyocytes in rats gradually subsides during the first month of life, a special object of this investigation was to discover how the response of the myocardium to trauma changes at different stages of this period. Accordingly age differences in healing of the focus of injury and the character of proliferative activity of the cardiomyocytes in the whole heart were studied.

EXPERIMENTAL MATERIAL AND METHOD

An injection of 96% ethanol [7] in a dose of 0.02, 0.04, and 0.09 ml was injected into the left ventricle of noninbred rats aged 7, 14, and 30 days respectively. The animals were killed 2, 4, 7, 14, and 30 days after the operation at 10 a.m. Intact rats of the same age acted as the control to each group. Each group consisted of 8-10 rats. The heart was fixed in Carnoy's fluid and paraffin sections 5-7 μ thick were stained with hematoxylin-eosin. Changes in the histological picture of the pathological focus were noted. The mitotic index (MI) was determined in 3000-4000 muscle cells in different parts of the heart: the left and right atria and their auricles, the trabecular and compact myocardium and subepicardial zones of both ventricles, and also in the zone around the focus of injury: altogether eight indices for the control heart and nine for the heart of the experimental animals (Fig. 1). The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Mitotic activity of the myocytes decreased with age in the myocardium of the control animals (aged 9, 11, 16, 18, 21, 28, 37, 44, and 60 days), but at different rates in different parts of the heart (Table 1).

Mitoses ceased first of all (by the 11th day of life) in the compact myocardium of both ventricles, but in all other parts of the heart proliferation continued until the 44th day. No mitoses were found in any of these parts of the heart in rats aged 60 days.

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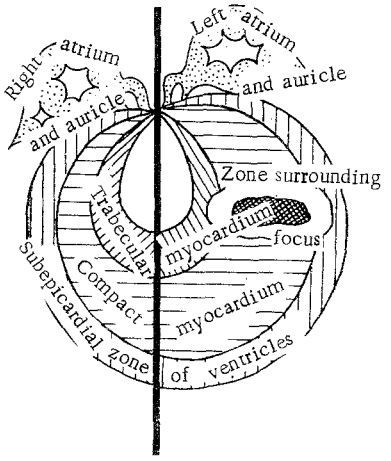

Fig. 1. Regions of the heart in which the mitotic index was determined (scheme).

TABLE 1. MI (in $^0/_{00}$) in Different Parts of the Heart of Control Animals (M ± m)

Age, days	Atria and auricles		Subepicardial zone of ventricle		Compact myocardium of ventricle		Trabecular myocardium of ventricle	
	left	right	left	right	left	right	left	right
9	3,3±1,22	2,1±0,5	5,5±0,9	3,5±0,7	3,4±1,3	3,3±1,0	3,5±1,0	2,7±0,6
11	1,0±0,4	0,5±0,1	1,2±0,5	—	—	0,4±0,1	0,9±0,4	1,4±0,3
16	0,9±0,4	1,2±0,7	1,0±0,5	1,8±0,9	—	0,2±0,1	0,6±0,3	0,8±0,5

Mitotic activity in the injured heart of animals of all age groups changed in the same way as in animals injured at the age of 1 day [6]. On the 2nd day after injury MI in all parts of the heart was sharply reduced, later it rose to exceed the control level, and finally it was extinguished. The later the injury was inflicted, the less clear the rise in MI.

Muscle fibers in the focus of injury in the animals of all age groups developed coagulation necrosis and were replaced by granulation tissue. Sometimes the replacement was incomplete, so that finely granular cell debris could be seen in the mature scar in a dense connective-tissue capsule. The muscle fibers in the zone adjacent to the wound appeared hypertrophied, and pairs and chains of nuclei were common in them.

Besides the general features described above, the reaction of the heart to trauma also had special features in the rats of each age group.

In rats undergoing the operation at the age of 7 days healing took place slowly and granulation tissue did not fill the focus until the 14th day after injury. Infiltration with leukocytes was very slight. This picture resembles the response of the myocardium of day-old rats to trauma [6]. On the 4th day after injury to the animals aged 7 days MI in all parts of the heart where proliferation still continued was significantly higher than in the control ($P = 0.01$), but on the 7th day the significance of differences from the control was reduced in the left subepicardial zone ($2.4 \pm 0.7\%$ in the experiment compared with $1.7 \pm 0.3^0/_{00}$ in the control; $0.01 < P < 0.05$), whereas in the left atrium the difference was not significant: $2.2 \pm 0.06^0/_{00}$ compared with $1.9 \pm 0.9\%$ (Fig. 2).

It is interesting to note that although no mitoses appeared in the compact myocardium of either ventricle, weak mitotic activity was observed in all animals in the zone surrounding the focus, where the myocytes were exposed to stronger stimulation ($1.5 \pm 0.06^0/_{00}$ on the 4th and $1.6 \pm 0.6^0/_{00}$ on the 7th day of the experiment).

Healing of the injury followed a totally different course in animals injured at the age of 14 days. The focus was surrounded by a barrier of leukocytes, chains of polymorphs penetrated deep into the focus between the muscle fibers, and they were followed by fibroblasts. Granulation tissue replaced the damaged muscle by the 7th day after injury. Mitotic activity of the cardiomyocytes was reduced: MI was increased only on the 4th day in the atria of three of the nine animals (2.0, 7.9, and $22.4^0/_{00}$ in the left and 2.5, 4.6, and $3.0^0/_{00}$ in the right respectively), in the subepicardial zone of the left ventricle in two of them (0.5 and $2.8^0/_{00}$, and in the subepicardium of the right ventricle ($1.9^0/_{00}$) and in the zone surrounding the focus ($1.5^0/_{00}$) in one of them.

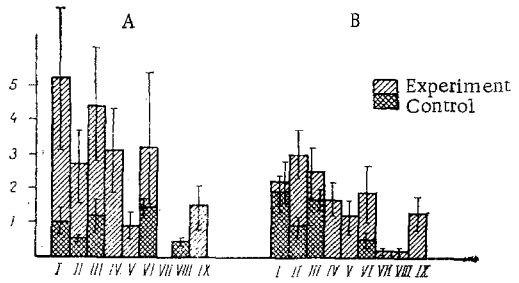

Fig. 2. Mitotic activity in different zones of the heart of rats injured at the age of 7 days. A and B) 4th and 7th days respectively after operation. Abscissa, parts of heart: I, II) left and right atria and auricles; III, IV) left and right subepicardial zones of ventricles; V, VI) compact myocardium of left and right ventricles; VII, VIII) trabecular myocardium of left and right ventricles; IX) zone around focus of injury. Ordinate, mitotic index (in $^0/_{00}$).

According to data in the literature, after diathermy coagulation of the left ventricular myocardium in animals of this age group the index of labeled nuclei in the region near the wound is significantly above normal from the 4th through the 10th day of the experiment [1]. Since, according to our own data, only a few muscle nuclei enter into mitosis, polyploidization of the nuclei probably follows DNA synthesis.

Among the group of animals aged 30 days the abundance of macrophages, quickly eliminating necrotic masses, was a noteworthy feature. On the 4th day after the operation much of the focus was occupied by granulation tissue. Beside the residues of cell debris there were foreign body giant cells. Mitoses were found on the 7th day after operation in the atria of two animals (MI was 9.2 and $2.3^0/_{00}$ in the left and $12.3^0/_{00}$ in the right atrium). Similar results were obtained previously by the present writer in adult rats after the production of an experimental myocardial infarct in the middle third of the left ventricle [5]. Meanwhile, when an infarct extended to the atrium, or when the auricle was directly injured, mitoses appeared in the atrium of all animals and MI could amount to $10\text{-}15^0/_{00}$ [5]. Stronger stimulation is evidently required to intensify proliferation of myocytes in the atria of 30-day-old rats than the local injury to the ventricle used in the present experiments.

During analysis of mitotic activity of the cardiomyocytes during growth of the myocardium and its response to injury a special feature was noted: individual variations in MI were very great. It must be emphasized that each group split up, as it were into two subgroups of "high-mitosis" and "low-mitosis" animals. The same pattern was discovered previously for the normal growing [4] and regenerating [3] mouse liver.

The results obtained indicate that proliferation in the compact myocardium is inhibited in animals undergoing operation at the ages of 7, 14, and 30 days and is not renewed after injury to the left ventricle. However, in zones where mitoses are still found under normal conditions, mitotic activity of the myocytes may be increased after trauma to the heart. The ability of the connective tissue to replace the defect increases with growth of the heart.

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