MITOTIC ACTIVITY OF CARDIOMYOCYTES DURING GROWTH OF THE MYOCARDIUM AND AFTER ITS INJURY IN DAY-OLD RATS

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Aside from distinct indications of differences in the response of cardiomyocytes in different parts of the heart to injury of the ventricles in adult animals [2, 4, 8, 9, 11, 13, 14], data in the literature on the level of proliferation of muscle cells after trauma to the myocardium at an early age are few in number and contradictory in nature [10]. The facts which exist indicate that the number of mitoses in the zone of myocardium surrounding the focus of injury in newborn animals is not increased [1, 5, 7]. At the same time, we know that postnatal growth of the myocardium in the neonatal period takes place predominantly on account of mitotic division of the cardiomyocytes [3, 6, 9, 14, 15].

The aim of this investigation was to study the degree and topographic features of proliferative activity of cardiomyocytes during early postnatal ontogeny in rats and also to determine the level of mitotic activity of the cardiomyocytes in different parts of the heart after injury to the ventricular muscles in the neonatal period.

EXPERIMENTAL METHOD

The growing myocardium of control intact young rats was studied on the 3rd, 5th, 8th, and 15th days after birth. From eight to 10 rats were used at each time. The heart was fixed in Carnoy's fluid at 10 a.m. Paraffin sections 5-7 μ thick were stained with hematoxylin-eosin. Mitotic activity of the cardiomyocytes was determined in the left and right halves of the heart: in the atria, including the auricles; in the compact myocardium of the two ventricles; in their subepicardial layer; and in the trabecular myocardium – in eight parts of the heart altogether.

In each part the number of mitoses was counted in 3000-4000 cardiomyocytes. The mitotic index was expressed in promille. Focal necrosis of the myocardium was induced in day-old experimental rats by the method of Dusek et al. [12], by injecting 96° ethanol in a dose of 0.01 ml into the wall of the left ventricle by means of a 0.5-ml tuberculin syringe. Altogether over 800 young rats had to be used in the experiments because of the great wastage. Groups of surviving animals, as in the control, consisted of 8-10 individuals. The injured heart was fixed on the 2nd, 4th, 7th, and 14th days after the beginning of the experiment. The mitotic activity of the cardiomyocytes was determined in the same parts of the heart as in the control, and also in the zone of myocardium around the injury, i.e., in nine parts of the heart altogether. The numerical data in the experimental and control series were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

Considerable individual variations in mitotic activity of the cardiomyocytes in different parts of the heart were observed in the growing myocardium of individual animals. Meanwhile, two quite distinct tendencies were noted from the very first days after birth. The first was that the number of mitoses in the left half of the heart was greater than in the right (Table 1). These differences were particularly clear in the compact

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TABLE 1. Mitotic Activity of Cardiomyocytes (in $^0/_{00}$) in Different Parts of the Heart of Control and Experimental Rats (M ± m)

Group of animals	Age, days	Parts of the heart								
		atria, auricles		subepicardial zone of myocardium of ventricle		compact myocardium of ventricle		trabecular myocardium of ventricle		myocardium around focus of injury
		left	right	left	right	left	right	left	right	on left side
Control	3	16,5±2,3	11,3±1,7	12,4±4,2	9,3±1,6	12,27±2,2	8,6±1,8	8,2±1,1	7,6±1,4	—
	5	7,2±1,7	8,0±2,4	7,4±1,6	5,0±1,6	5,8±0,7	4,3±1,0	7,9±1,1	6,0±1,2	—
	8	4,1±1,0	4,7±1,0	6,4±0,9	5,6±1,0	3,7±0,7	4,1±1,1	6,6±1,0	6,8±0,9	—
	15	1,9±0,9	0,9±0,3	1,7±0,3	—	—	—	0,7±0,2	0,5±0,2	—
Experimental	3	1,4±0,4	1,0±0,4	2,1±0,5	2,3±0,1	—	—	2,1±0,4	0,9±0,4	0,7±0,3
	5	17,9±3,9	14,3±3,0	15,6±2,8	9,1±1,6	7,0±2,4	5,8±1,2	9,4±1,8	9,4±2,3	7,1±1,7
	8	10,7±1,8	10,7±2,0	9,8±2,3	6,2±1,8	4,6±1,1	4,9±1,6	7,7±1,1	10,5±1,6	4,8±0,7
	15	14,0±2,0	12,9±2,9	4,6±0,9	3,7±0,6	0,5±0,2	0,4±0,1	1,6±0,5	1,1±0,5	1,1±0,4

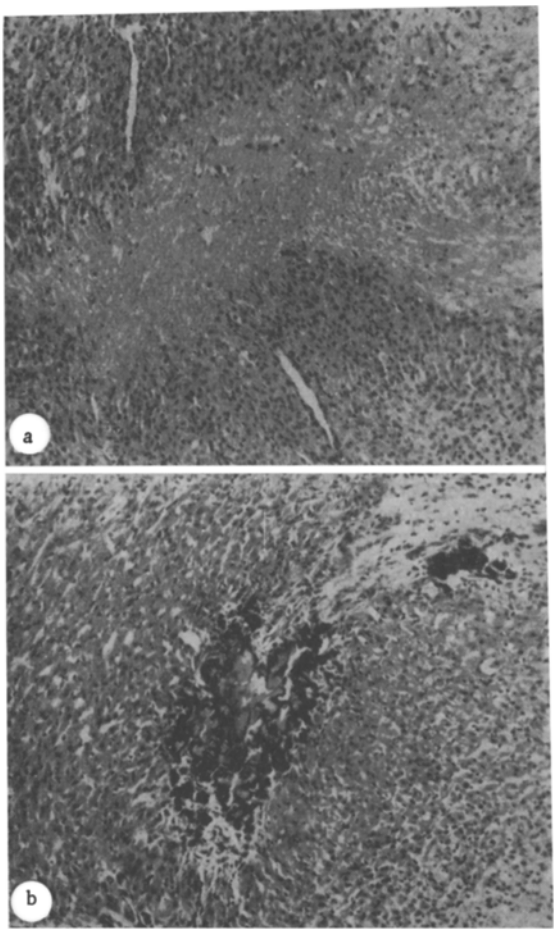

Fig. 1. Necrotic focus in left ventricle of myocardium of newborn rat on 2nd (a) and 14th (b) days after injury. Hematoxylin-eosin, 200×.

myocardium on the 3rd day after birth of the rats ($P > 0.99$). This can evidently be attributed to the fact that immediately after birth the hemodynamic load on the left side of the heart is sharply increased. In consequence of this the left ventricle grows more rapidly than the right, and the width of its wall soon becomes appreciably greater than the width of the wall of the right ventricle. The second tendency was a more frequent appearance of mitoses in the myocytes of the atria and auricles, the subepicardial layer of the ventricles, and the trabecular myocardium than in the compact myocardium. This was seen most clearly in rats approaching 2 weeks of age.

On the whole, the mitotic activity of the cardiomyocytes in all parts of the heart was sharply reduced as early as on the 5th day after injury, and by the age of 2 weeks it did not esceed 1-2% (Table 1).

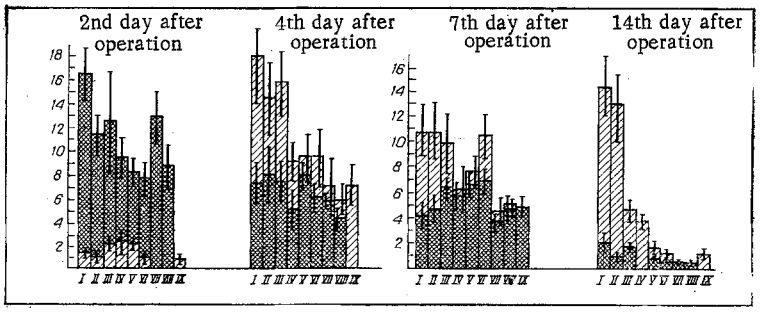

Fig. 2. Mitotic activity of cardiomyocytes during growth of the heart and after its injury in the neonatal period on the 2nd and 4th days after trauma (a) and on the 7th and 14th days (b). Cross-hatched columns indicate different parts of the intact heart, obliquely shaded columns – injured heart. Abscissa, parts of the heart in which mitoses were counted: I and II) left and right atria and auricles; III and IV) left and right subepicardial zones of ventricular myocardium; V and VI) compact myocardium of left and right ventricles; VII and VIII) trabecular myocardium on left and right sides; IX) myocardium around focus of injury; ordinate, mitotic index (in $^0/_{00}$).

After injection of 96° ethanol into the wall of the left ventricle a necrotic focus measuring from 40×10^3 to 120×10^3 μ^2 in area was formed in the wall of the left ventricle and very closely resembled a myocardial infarct such as could be induced in adult rats by ligation of a branch of the coronary arteries [10, 11, 14]. However, by contrast with adult animals, in day-old rats on the 2nd day after trauma the inflammatory reaction around the focus of injury was extremely poorly developed or, in some cases, completely absent (Fig. 1a). Furthermore, in young rats, both in the healthy myocardium around the focus of injury and in the remainder of the heart, mitotic activity of the cardiomyocytes was extremely depressed compared with the control (Table 1).

However, by the 4th day the protective reaction of the heart to injury was more complete: A leukocytic barrier could be clearly seen around the focus of necrosis, and mitotic activity of the cardiomyocytes in all parts of the heart was much higher than at the previous time of investigation and in the control (Fig. 2a; $P = 0.99$). Mitoses were particularly numerous in the cardiomyocytes of both atria, the subepicardial layer of the ventricles, and the trabecular myocardium.

Meanwhile, the number of mitoses in cardiomyocytes surrounding the focus of injury did not significantly exceed the number of mitoses in the compact myocardium of the left ventricle of control 5-day-old rats. The mitotic activity of the cardiomyocytes around the necrotic focus likewise was not increased at later times of observation (on the 7th and 14th days after the operation). On the contrary, often it was equal to the control values or could even be lower (Table 1). Growth of scar tissue and resolution of the necrotic masses took place quite slowly. The necrotic debris could often be clearly observed even 2 weeks after injury to the heart (Fig. 1b). The reason for this could possibly be that the number of mitoses in all the remaining parts of the injured heart was higher than in the control on the 8th and 15th days of life (Fig. 2b).

Three main conclusions can be drawn from the results of this investigation. First, in different parts of the heart the degree of mitotic activity among the cardiomyocytes varies. This is confirmed also by data in the literature obtained previously with the aid of radioactive thymidine [6, 9]. Second, the ability of the cardiomyocytes to undergo mitotic division during postnatal growth of the heart muscle does not enable perfect repair to take place if a defect of the muscle is formed. Just as in adult animals, the necrotic part of the myocardium in newborn rats is ultimately replaced by a scar. Third, in young animals, just as in adults [2, 4, 9, 11, 13, 14], after injury to the ventricular myocardium the mitotic activity of the cardiomyocytes is considerably increased, not in the zone of the myocardial defect, but in other parts of the heart (atria and auricles, subepicardial layer of the myocardium of both ventricles, trabecular myocardium). This fact is evidence that the heart responds at any age to any local trauma as a single entity.

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BIOCHEMICAL AND IMMUNOLOGIC CHARACTERISTICS OF SERUM PROTEINS AFTER BONE TRAUMA IN RATS

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The role of the blood proteins in mechanisms of posttraumatic regeneration of bone tissue has been inadequately studied. It has been suggested that serum proteins and blood mucopolysaccharides, which are detectable in callus, promote regeneration of bone tissue [2, 3, 5-7] by stimulating proliferation of bone cells [8, 9]. Nevertheless, the particular features of posttraumatic dysproteinemia and the role of the protein–carbohydrate complexes of the blood in the formation of the organic matrix of regenerating bone still remain inadequately studied.

The object of this investigation was a quantitative study of the protein, lipoprotein, and protein–carbohydrate components of blood and callus in rats in the acute stage of bone trauma.

EXPERIMENTAL METHOD

Experiments were carried out on 145 Wistar rats weighing about 100 g. Bilateral closed fractures of the femoral diaphyses were produced under pentobarbital anesthesia (20 mg/kg). Blood was taken for testing individually from each animal on the 1st, 2nd, 3rd, 5th, 7th, and 10th days after trauma. Total serum protein was determined refractometrically and protein fractions by electrophoresis on paper (Whatman No. 3 MM, barytal buffer, pH 8.6). After electrophoresis the strips, stained with bromphenol blue, were examined on the 301E Statron (East Germany) integrating densitometer. The seromucoid content was determined by the method in [4] on the SF-16 spectrophotometer at 280 μ. Quantitative immunoelectrophoresis of blood serum antigens and antigens of a saline extract of callus obtained 7 days after infliction of the fracture was carried out with the aid of antinormal rabbit serum [1]. After immunoelectrophoresis the strips were stained for proteins with Coomassie blue and for lipoproteins with Sudan black B. At each time blood from seven or eight experimental and/or seven or eight control rats (without fracture) was investigated. To obtain protein

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