PATHWAYS OF STRUCTURAL COMPENSATION IN THE INJURED MYOCARDIUM OF NEWBORN RATS

V. N. Galankin and B. V. Vtyurin

UDC 616.127-001-092.9-053.31-003.96

It is concluded from a study of the mitotic activity of heart muscle cells from rats whose myocardium was injured immediately after birth that regeneration does not take place by hyperplasia of the muscle cells. The electron-microscopic findings suggest that even in early ontogeny the restoration of function of the damaged myocardium takes place chiefly by intensification of intracellular hyperplastic processes, i.e., by hypertrophy of the cells.

Hypertrophy is known to play an important role in the compensation of disturbed myocardial function. The possibilities for hyperplastic intracellular processes in the adult human myocardium are very great; in some cases the weight of the human heart may exceed 1000 g [3], and occasionally it may reach 2340 g [4]; this is considered to take place mainly on account of hypertrophy of the cells. Hypertrophy can occur in newborn infants with congenital defects [2].

The object of this investigation was to determine the moment of development at which intracellular hyperplastic processes begin to play a role in the compensation of disturbed myocardial functions and their relationship to mitotic cell division of the muscle cells after death of a localized area of the myocardium in early ontogeny.

EXPERIMENTAL METHOD

Experiments were carried out on the myocardium of newborn rats. A measured burn of the anterolateral wall of the left ventricle was inflicted on 16 rats which were killed 1, 3, and 6 days after the operation. The myocardium of 16 rats from the same litters as the experimental animals and sacrificed simultaneously with them, acted as the control. The heart was embedded in paraffin wax and histological sections were stained with Heidenhain's iron-hematoxylin. The number of mitoses of the muscle cells in the myocardium of the left ventricle was counted in day-old rats under normal conditions and also in the injured myocardium – around the site of injury (excluding the zone of reactive changes) and some distance from it. The number of mitoses in 5000 muscle cells was counted in each of these areas. Statistical analysis was carried out on the M-220 computer. The absolute number of mitoses of the muscle cells in 300 fields of vision in each case was analyzed in the myocardium of rats (12) aged 3 and 6 days.

Pieces of myocardium were excised for electron-microscopic investigation 24 h after injury from the region of damage (1-1.5 mm away from the visible boundary) and also from the myocardium of the left ventricle some distance from the site of injury (3 animals). The myocardium of 3 intact day-old rats acted as the control. The material was fixed in OsO_4 and embedded in Araldite. Ultrathin sections were cut on the LKB-4800A ultratome, stained with lead salts, and studied in the IEM-100V electron microscope.

EXPERIMENTAL RESULTS

The mitotic activity of muscle cells was at a minimum in the region of injury 24 h after the operation $1.675 \pm 0.411‰$, difference from the control significant). Some distance from the site of injury it was higher

Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A.I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 5, pp. 110-113, May, 1974. Original article submitted July 20, 1973.

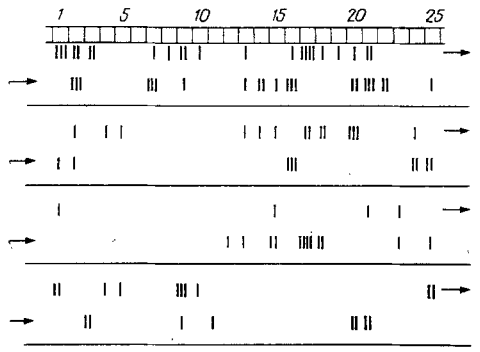

Fig. 1. Scheme of distribution of mitoses in muscle cells in myocardium of newborn rat. Number of mitoses shown by bars in corresponding neighboring fields of vision (squares). Horizontal lines separate individual observations.

Fig. 3. Electron micrograph of myocardium in region of injury 24 h after burning of the heart in a newborn rat: destruction of cristae, clearing of the matrix, and swelling of the mitochondria can be seen; ribosomes are in intimate contact with the newly formed myofilaments (40,000×).

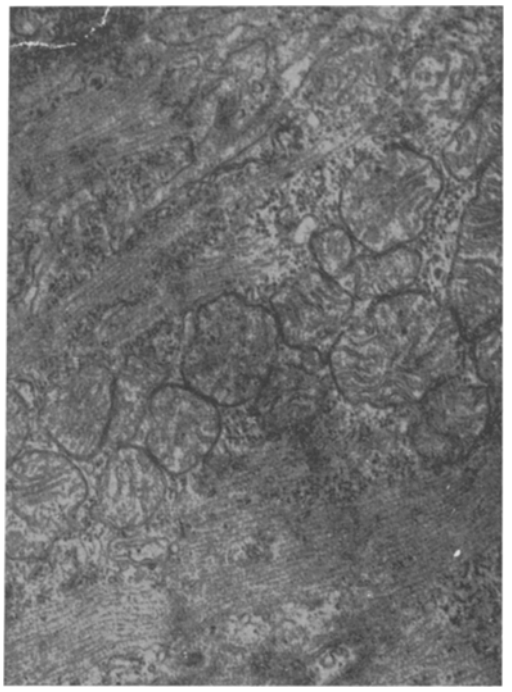

Fig. 2. Electron micrograph of myocardium some distance from site of injury 24 h after burning of the newborn rat heart: groups of ribosomes are formed into chains, continued as bundles of myofilaments not yet forming differentiated myofibrils (30,000×).

(on the average by $1.38 \pm 0.274‰$), but it was not as high as mitotic activity in the same part of the myocardium of the intact rats ($4.333 \pm 0.847‰$). Mitoses in the muscle cells were irregularly distributed in the myocardium of the newborn rat (Fig. 1). Counting them (the bars in Fig. 1) in consecutive fields of vision (squares) showed that fields with increased mitotic activity alternated more or less uniformly with fields in which it was absent or considerably reduced. This was presumably evidence of functional and proliferative heterogeneity of the myocardium at the time of investigation. Fields with increased mitotic activity were presumably mainly zones of growth, while fields with reduced activity were zones performing mainly a specific function.

Electron-microscopic investigation of the myocardium of the normal newborn rat shows definite heterogeneity of the ultrastructure of the muscle cells forming the different parts. Some muscle cells contain many myofibrils lying close together, in which relatively

TABLE 1. Number of Mitoses in Myocardium of Left Ventricle of Injured and Intact Rat Hearts

Age of animal (in days)	Experimental newborn rats	Control rats
3	300/47 300/131 300/125	300/120 300/170 300/125
	Total 900/303	Total 900/415
6	300/78 300/48 300/122	300/75 300/79 300/93
	Total 900/248	Total 900/247

Note. Number of fields of vision in numerator, number of mitoses in denominator.

clearly defined m- and z-bands, having numerous cristae in their mitochondria, can be seen. Usually it is impossible to observe any considerable number of free ribosomes in these cells and the clusters of ribosomes are comparatively small. In other cells the myofibrils are thinner, consist of fewer myofilaments, often with an irregular course, and their organization reflects the lower degree of differentiation than in the cells described above. Collections of ribosomes between the myofibrils, sometimes large, are a special feature of these cells. Many large inclusions of lipids, varied in shape, are formed in both types of the cells described above. These electron-microscopic differences between the muscle cells of the normal myocardium of the young rats, like the irregular concentration of the mitoses, presumably reflect variation in the functional state of the muscle cells.

In the myocardium of the left ventricle of the newborn animals undergoing the operation cells in which the distribution of the ribosomes deserves attention were frequently found some distance from the focus of injury. These ribosomes often covered considerable areas and, in addition, the groups were of different sizes. Usually these groups were found close to the myofibrils and the ribosomes in them were formed into chains, which continued as bands of myofilaments, often not yet forming definitive myofibrils. Chains of ribosomes could be seen inside a fully formed myofibril, in which they were arranged parallel to its length (Fig. 2). Mitochondria were seen among the formed myofibrils and cell granules.

In the region of injury the ultrastructure of the muscle cells had much in common with that found in areas remote from the injury. However, in this case changes were also present in the mitochondria, with fragmentation of the cristae and loss of detail of their internal structure. Compared with other areas, polymorphism in the size of the mitochondria was more marked and irregularly shaped nuclei appeared (Fig. 3), while lysosomes were found in some cells. No lipid inclusions were seen in cells from different parts of the injured myocardium.

On the basis of these light and electron-microscopic observations the following conclusions can be drawn concerning processes taking place in the normal and injured myocardium of day-old rats.

Cells with relatively few ribosomes and with a distinct structure of the myofibrils for that age evidently reflect a state characterized chiefly by the contractile function in the normal myocardium at the time of investigation. The reduced contractile functions and correspondingly increased synthetic function were reflected by the appearance of cells with larger areas of ribosomes and with less highly differentiated myofibrils. Lipid inclusions in cells of different parts can evidently be regarded as an indicator of the existing energy reserves in the normal myocardium.

After trauma to part of the myocardium the deficit of function in areas remote from the injury is caused by intensification of intracellular hyperplastic processes. The number of diffusely scattered ribosomes, often in intimate connection with the newly formed myofilaments (Fig. 3), was increased in many of the cells. Disappearance of the lipid inclusions characterizes the energy mobilization of the myocardium, in which the proliferative activity of the muscle cells is reduced.

At the site of injury the functional loads are increased still further compared with the preceding areas and the degree of cell proliferation is reduced. The contribution of intracellular regenerative processes increases correspondingly. These are characteristic of the pathohistological phenomenon of hypertrophy of the heart muscle cells.

The processes described above develop toward the end of the first day after injury. Later the mitotic activity of the muscle cells in the injured hearts rises, but, in absolute terms, according to the results of these investigations (Table 1), above the level of activity existing in intact hearts of young rats from the same litters as the experimental animals. Similar results were obtained by Akhabadze and Olenina [1] with young rats whose parentage was not specified.

Structural compensation in the myocardium with a focus of injury thus takes place, even during the period of mitotic activity of the muscle cells, largely on account of intracellular regenerative processes.

LITERATURE CITED

1. L. V. Akhabadze and N. T. Olenina, Krovoobrashchenie, No. 3, 50 (1972).
2. V. N. Galankin, Arkh. Pat., No. 1, 43 (1971).
3. J. C. Doane and N. J. Skwersky, Am. Heart, J., 28, 816 (1944).
4. T. E. Lowe and E. W. Bate, Med. J. Aust., 1, 618 (1948).