MITOTIC ACTIVITY OF THE RABBIT MYOCARDIUM IN EARLY ONTOGENY AFTER MINOR MECHANICAL INJURY TO THE HEART

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The writer showed previously [5, 6] that after death of a small area of myocardium due to mechanical trauma to the heart in fetuses aged 20–24 days young cross-striated muscle develops in the focus of injury. In newborn rabbits, similar trauma terminates in the formation of connective tissue in the region of injury. It was shown that the undifferentiated muscle cells of the heart do not take part in healing of myocardial injuries, but that proliferation of cardiomyocytes plays the principal role in repair of the heart structure. Nevertheless, in the investigations cited above no attempt was made to study the reason for the difference in the outcome of myocardial regeneration in fetuses and new born rabbits.

In the investigation described below the process of proliferative activity of the heart muscle cells was studied and some principles lying at the basis of myocardial regeneration in fetuses and newborn rabbits were discovered.

EXPERIMENTAL METHOD

Rabbit fetuses and newborn rabbits were used. By the method described previously [5], mechanical trauma was inflicted on the myocardium of the fetuses at the 20th–24th day of intrauterine development. Similar trauma was inflicted on the newborn rabbits on the first day after birth. The number of fetuses and newborn rabbits subjected to trauma was 100 in each group. The times after injury varied in the case of fetuses from 8 h to 11 days, and in newborn rabbits from 1 to 30 days. Mitoses in muscle cells were counted in histotopographical sections stained with hematoxylin and eosin and for neutral mucopolysaccharides close to (in 5000 nuclei) and at a distance from (in 5000 nuclei) the zone of injury. As far as possible animals of the same litter were used, 10 at each time. Statistical analysis of the results was undertaken on the Élektronika-100 and Nairi-K computers by analysis of variance.

EXPERIMENTAL RESULTS

When mitoses in muscle cells are counted, difficulties usually arise in their differentiation from mitoses in connective-tissue cells. Despite this, separate counting of mitoses of these types is possible and has been done several times by a number of workers [2, 4]. The principal features distinguishing mitoses of muscle cells from mitoses of connective-tissue cells are the larger size and the presence of translucency in the cytoplasm of dividing myocytes, and the fact that they contain chromosomes within the boundaries of the muscle fiber (Fig. 1). So far as connective-tissue cells are concerned, their independence during division from muscle fibers, and the presence of basophilia in their cytoplasm are reported in the literature [4]. We used the same distinguishing features in this investigation as previously [4]. An additional criterion for identifying these types of mitoses in some cases was staining for neutral mucopolysaccharides, which gave particularly clear boundaries between muscle cells and enabled the location of the mitosis to be identified more accurately. It was thus shown that as a rule a certain number of grains of glycogen were present in a dividing muscle cell (Fig. 1d). Direct division of nuclei of the myocardium in the normal heart and after various kinds of injuries has been observed by many workers [1, 7]. In the past, great importance was attached to amitosis as one way of division of the myocardial nuclei, which played a definite role in restoration of the structure of the organ at

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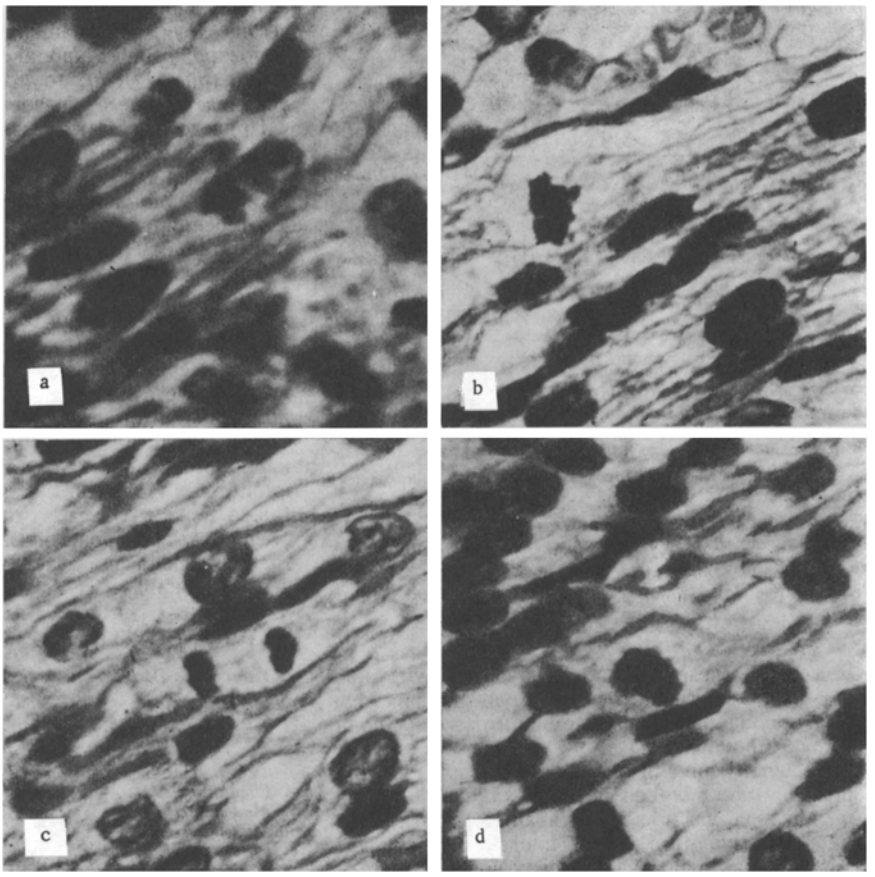

Fig. 1. Mitoses in muscle cells and glycogen grains in cytoplasm of dividing muscle cell (d). a-c) hematoxylin–eosin, d) PAS reaction. 900 ×.

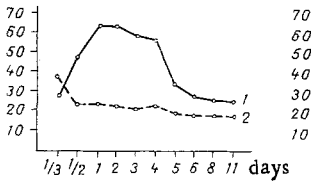

Fig. 2.

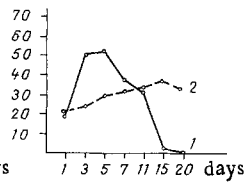

Fig. 3.

Fig. 2. Number of mitoses (1) and of binuclear muscle cells (2) in rabbit fetuses. Here and in Figs. 3–5: abscissa, time after operation (in days); ordinate, number of dividing muscle cells.

Fig. 3. Number of mitoses (1) and binuclear cells (2) in new born rabbits.

the cellular level. It has now been shown that direct division does not exist and that so-called binuclear myocardial cells arise mainly in early ontogeny as a result of incomplete mitoses [3]. The binuclear cells found in mammals are mainly polyploid. The formation of binuclear cells in postnatal ontogeny thus leads to polyploidy of the myocardial, and this, together with hypertrophy of the myocytes, lies at the basis of growth and differentiation of heart muscle tissue in mammals after birth [3]. Analysis of the proliferative activity of heart muscle cells, in the present investigation, emphasized the unique relationship between the number of mitoses in muscle cells and the number of binuclear cells (Figs. 2 and 3). As Figs. 2 and 3 show, in rabbit fetuses, with a high mitotic index, the number of binuclear myocytes was small. Since, in the modern view,

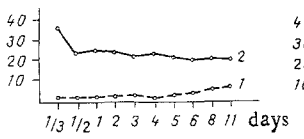

Fig. 4.

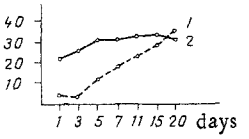

Fig. 5.

Fig. 4. Number of binuclear cells in normal heart (1) and after mechanical trauma to heart (2) in rabbit fetuses.

Fig. 5. Number of binuclear cells under normal conditions (1) and after mechanical trauma to the heart (2) in newborn rabbits.

binuclear myocytes arise as a result of incomplete mitoses [3], it is logical to suggest that if in rabbit fetuses many mitoses in myocytes were incomplete, the number of binuclear myocytes ought to depend directly on the number of mitoses in muscle cells. Since this was not observed in the present case, it can be safely concluded that most mitoses in myocytes completed in the myocardium of rabbit fetuses go on to division, and only a small proportion of mitoses results in the formation of binuclear cells, i.e. in polyploidy of the myocardium.

In newborn animals the relationship between mitoses in muscle cells and the number of binuclear myocytes was of a different character. The number of mitoses in myocytes was relatively small, but at the same time the number of binuclear cells was large. Consequently, in newborn rabbits mitoses in myocytes mainly went on to polyploidy of the myocardium and not to proliferation of cardiomyocytes.

Previously the writer stated that after mechanical trauma to the myocardium in fetuses and newborn rabbits mitotic activity of the myocytes was activated [6]. This effect was much more marked in fetuses than in newborn rabbits. However, as the results of the present investigation show, it is not only this which determines the difference in the character of regeneration in fetuses and newborn rabbits. The data given above are evidence that it depends above all on how mitoses are realized in muscle cells. In fetuses they mainly end in proliferation of myocytes, and complete cellular regeneration of the myocardium takes place at this age. In newborn rabbits mitoses in myocytes mainly go on to polyploidy of the myocardium, and complete cellular regeneration does not take place at this age.

It was shown in [6] that trauma inflicted on the fetal myocardium leads to the earlier appearance of cross-striation in fibers of the newly formed tissue. In addition, if curves characterizing the number of binuclear myocytes in the myocardium of fetuses and newborn rabbits under normal and experimental conditions are compared (Figs. 4 and 5) it will be seen that trauma intensifies the process of formation of binuclear cells.

These facts are evidence that minor trauma to the myocardium in fetuses and newborn rabbits leads to activation of polyploidy, i.e., ultimately it activates differentiation of the rabbit myocardium in early postnatal ontogeny.

Analysis of the proliferative activity of the myocardium in fetuses and newborn rabbits thus shows that the majority of mitoses in myocytes in fetuses terminate in proliferation of the heart muscle cells. This evidently lies at the basis of the complete cellular regeneration of the myocardium in this stage of ontogeny. In newborn rabbits mitoses in muscle cells go on mainly to polyploidy and not to proliferation of cardiomyocytes. These results also are evidence that minor trauma to the heart induces activation of myocardial differentiation in fetuses and, to a greater degree, in newborn rabbits.

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