ORIGIN OF ANICHKOV'S CELLS IN THE MYOCARDIUM

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UDC 611.127-018.13:611.127-018.15

KEY WORDS: Anichkov's cells; myocardium; chromatin; mitosis.

Nuclei of characteristic structure (the chromatin distributed as an indented band along the nucleus) have been described in both muscle and connective-tissue cells of the heart, which have been called Anichkov's myocytes or Anichkov's cells. Since these cells are frequently found in foci of myocardial damage, the idea arose that they participate in regeneration as "myocytes," but later this was recognized to be untrue. Similar nuclei also have been found in cells of other organs (skin, thyroid gland) [1-3, 8].

The ultrastructure of Anichkov's cells is evidence of the normal state of the organelles. These cells can incorporate [^{3}H]thymidine [3]. The causes of appearance of Anichkov's cells have not yet been explained. According to my own observations on normal and damaged rat myocardium, the origin of Anichkov's cells is closely connected with foci of proliferation of heart cells or, more exactly, with regions where increased mitotic activity, observed for several days, declines. For example, in the process of normal postnatal development of the rat heart, concentrations of Anichkov's cells can be found on the 3rd day of life in the compact myocardium of both ventricles, on the 5th-8th days in the trabecular myocardium, and on the 21st-28th days in the subepicardial zone of the left ventricle, i.e., in zones of the heart where, according to my previous observations [5], the mitotic index of the cadriomyocytes falls (Fig. 1).

With these facts in mind an investigation was carried out to discover the relationship of Anichkov's cells to proliferation of heart cells and the stage of the cell cycle at which they arise.

EXPERIMENTAL METHOD

[^{3}H]Thymidine with specific activity of 1.3 Ci/mmole was injected intraperitoneally in a dose of 0.5 µCi/g body weight into 44 rats aged 7-8 days. The animals were decapitated after 2, 4, 6, 9, 12, 18, 24, 30, 36, 42, and 48 h. The hearts were subjected to the usual histologic treatment. Dewaxed sections 5-7 µ thick were coated with type M emulsion and, after exposure for 6 weeks, they were developed with amidol developer and stained with hematoxylin. Because of the very weak intensity of the background, nuclei with two or more grains of silver could be taken as labeled. Since, according to data in the literature [7], parameters of mitotic cycles of cardiomyocytes and connective-tissue cells are identical, muscle and connective tissue cells were not counted separately. Grains of silver were counted above labeled nuclei of Anichkov's cells and also above nuclei of cells dividing for the first time after injection of thymidine (9 h after injection), stages of mitosis before and after karyokinesis (prophase + metaphase and anaphase + telophase) being counted separately. At each time of the investigation the number of labeled mitoses and the number of labeled Anichkov's cells as percentages of 100-200 cells were determined. Because of self-absorption of radiation by the preparation [4], counting was done in the top layer of the section.

The DNA content in the nuclei of Anichkov's cells was determined in dissociated cells stained by Feulgen's method [6]. Optical density of the nuclei was measured on an SEM-3 scanning microspectrophotometer (Opton, West Germany). Lymphocytes in blood films from the same animals served as diploid control.

Laboratory of Growth and Development, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 358-360, March, 1984. Original article submitted May 18, 1983.

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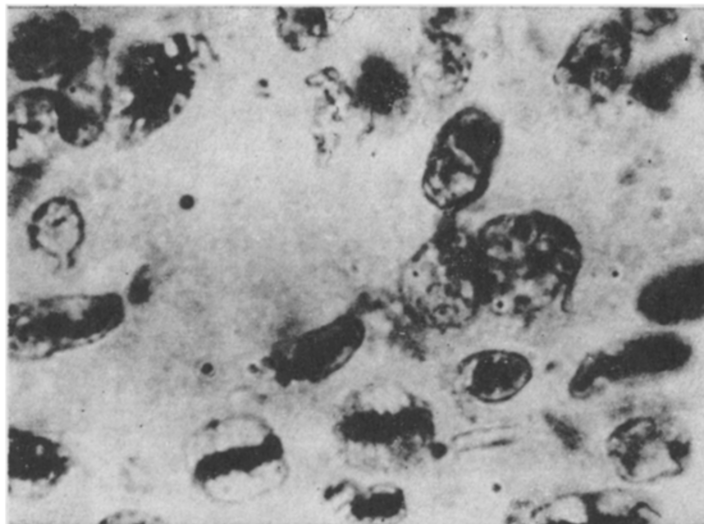

Fig. 1. Anichkov's cells in trabecular myocardium of right ventricle of 8-day rat. Hematoxylin. 1100 ×.

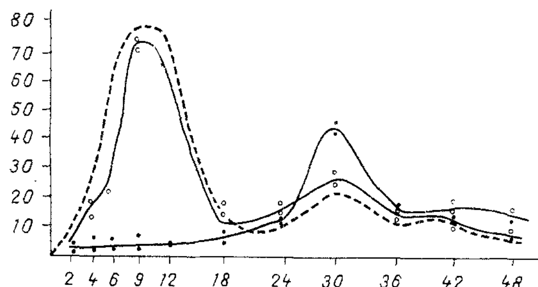

Fig. 2

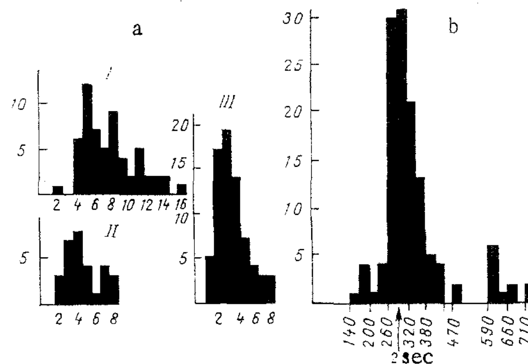

Fig. 3

Fig. 2. Curves of labeled mitoses (empty circles) and labeled Anichkov's cells (filled circles). Broken line shows curve of labeled mitoses taken from [7]. Abscissa, time after injection of thymidine (in h); ordinate, number of labeled cells (in %).

Fig. 3. Proof of postmitotic origin of Anichkov's cells: a) number of grains of silver above prophases and metaphases (I) and above anaphases and telophases (II) (9 h after injection of thymidine), and above Anichkov's cells (III); abscissa, number of grains of silver; ordinate, number of nuclei. b) DNA content in Anichkov's cells; abscissa, optical density (in conventional units); ordinate, number of nuclei.

EXPERIMENTAL RESULTS

The curve of labeled mitoses thus obtained, with a maximum at about the 10th hour after injection of [^{3}H]thymidine coincided on the whole with those given in [7], and were similar for muscle and connective-tissue cells, so that it was possible to disregard the nature of the tissue to which the Anichkov's cells belonged.

The number of labeled Anichkov's cells rose slowly to reach a maximum 30 h after injection of labeled thymidine, evidence that at the time of incorporation of the isotope the future Anichkov's cells were outwardly indistinguishable from other heart cells. The characteristic arrangement of chromatin evidently appears much later than the end of the phase of DNA synthesis (Fig. 2).

The intnesity of labeling of Anichkov's cells (the number of grains of silver above the nucleus was 4.0 ± 0.1) was similar to that for cells passing through mitosis for the first

time after injection of thymidine, i.e., anaphase and telophase 9 h after injection of the isotope (Fig. 3a). More exact evidence that precursors of Anichkov's cells pass through mitosis after doubling their DNA content is given by the results of cytophotometric determination of optical density of the nuclei, which show that most of Anichkov's cells are diploid (Fig. 3b).

The characteristic arrangement of chromatin in the form of a serrated band evidently appears about 20 h after the future Anichkov's cell has completed mitosis (the difference in time between the peaks of labeled mitoses and of labeled Anichkov's cells). Although the causes of this phenomenon and the further fate of the cells await explanation, it can be postulated that the origin of Anichkov's cells is linked with the end of proliferation rather than its beginning.

It must be recalled that not every mitosis in the heart is likely to lead to the formation of an Anichkov's cell. Nevertheless, the easily identifiable Anichkov's cell can serve as marker of mitosis completed 20 h before the material was taken, so that mitotic activity of heart cells at different times can be analyzed in the same preparation.

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ESTIMATION OF CELL AGGLUTINATION BY LASER NEPHELOMETRY

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UDC 616.15-097.34-074:543.436

KEY WORDS: lectins; agglutination; tumor cells; laser nephelometry.

Cell agglutination (CA) can be induced by plant lectins, which bind specifically with glycoproteins on the cell surface. The degree of CA is a characteristic of the state of the cell surface membrane. Changes in CA are observed during tumor development, morphogenesis and embryogenesis, and in various pathologic states [3, 6, 9, 10]. Quantitative estimation of CA is an urgent problem at the present time. Until now a laborious microscopic method has been used to estimate CA [2, 4]. Meanwhile the intensity of agglutination correlates directly with the sedimentation rate of cell agglutinates and translucency of the cell suspension. These parameters form the basis for development of quantitative instrumental methods of estimating CA [7, 8].

The aim of this investigation was to study whether the method of laser nephelometry (LN) can be used for quantitative estimation of agglutination of human tumor cells under the influence of lectins.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 360-362, March, 1984. Original article submitted April 6, 1983.

0007-4888/84/9703-0356$08.50

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