4. N. B. Simon, S. S. Timoshin, and S. A. Alekseenko, Byull. Éksp. Biol. Med., No. 11, 1371 (1976).
5. S. S. Timoshin, "The effect of pyrogenal on mitotic activity of cells of some types of epithelium," Candidate's Dissertation, Khabarovsk (1970).
6. S. S. Timoshin and S. A. Alekseenko, Byull. Éksp. Biol. Med., No. 1, 74 (1974).
7. S. S. Timoshin and S. A. Alekseenko, in: Some Aspects of Mechanisms of Adaptation under Normal and Pathological Conditions [in Russian], Khabarovsk (1975), p. 36.
8. S. S. Timoshin and N. B. Murzina, Byull. Éksp. Biol. Med., No. 8, 248 (1978).
9. I. V. Uryvaeva and V. M. Faktor, Tsitologiya, No. 9, 1100 (1974).
10. V. Wilfried, Microsc. Acta, 74, 36 (1973).

MECHANISMS OF POLYPLOIDIZATION OF MOUSE CARDIOMYOCYTES

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Most ventricular cardiomyocytes in mice aged 5-6 days are polyploid cells. By this time 60% of the cardiomyocytes have become binuclear and a further 10% have become mononuclear polyploid cells. Binuclear cardiomyocytes are formed as a result of acytokinetic mitosis, mononuclear tetraploid cells as a result of termination of mitosis in the initial phases.

KEY WORDS: cardiomyocytes; polyploidization.

Polyploidy is considered to be a feature which distinguishes the cardiomyocytes of primates from those of other mammals so far studied [14, 15], although it is now more than 10 years since Rumyantsev et al. [5, 7] found a certain number of polyploid cells in the rat myocardium. The study of polyploidy has been hindered by the fact that work has had to be done on tissue slices, when it is impossible to estimate the number of binuclear cells and determination of mononuclear polyploid cells (by DNA cytophotometry) gives rise to considerable distortion. The development of methods of dissociating the myocardium into single cells [2, 3, 10] has changed opinions on the composition of the cardiomyocyte population. Most cardiomyocytes in the myocardium of the adult mouse and rat have been found to be binuclear, i.e., definitely polyploid with respect to their combined genome. The present writers have shown [1] that with respect to DNA content some cardiomyocyte nuclei are tetraploid or octaploid. The object of this investigation was to study the origin of polyploid (mono- and binuclear) cardiomyocytes.

EXPERIMENTAL METHOD

Thymidine-^{14}C was injected subcutaneously in a dose of 1.5 μCi/g (specific activity 52 mCi/mmole) into 28 (CBA × C57BL/6)F_1 mice aged 3-4 days. The animals were killed after 1-36 h and the heart was placed whole in 10% formalin in Sorensen's buffer (pH 7.0). Next, in accordance with the method suggested in [2, 3], a cell suspension was prepared from the ventricles by dissociating them in KOH. One drop of suspension was placed on a slide. For DNA photometry, the Feulgen reaction was carried out on preparations of isolated cells (hydrolysis in 5N HCl for 10 min at 37°C, treatment with Schiff's reagent for 60 min at room temperature). The preparations were then coated with type M emulsion (Photographic Chemical Research Institute) and exposed for 14 days. The DNA content in the nuclei of the cardiomyocytes was measured on a Vickers M-86 microdensitometer. Before photometry, the preparation was drawn and the position of labeled cells marked on the drawing; the label was then removed. During photometry cells which had incorporated thymidine were located on this drawing. The mitotic index and the number of binuclear and labeled cells were determined by examination of 2000-3000 cardiomyocytes from each animal, in films stained by Giemsa's method. To determine the frequency of labeled mitoses at least 20 mitoses, usually 50-80, were found.

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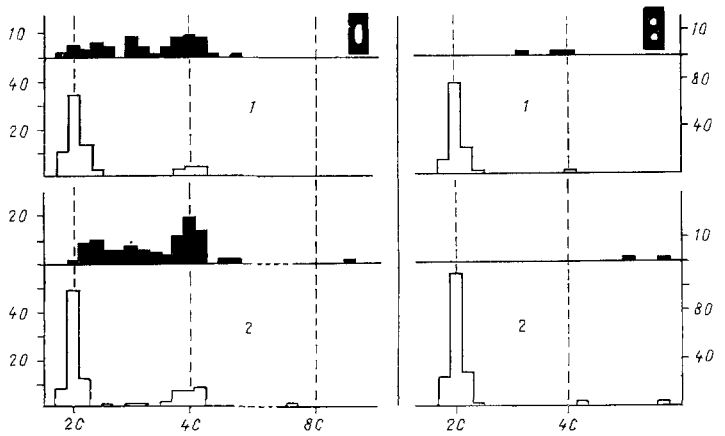

Fig. 1. DNA content in synthesizing (black columns) and unlabeled (white columns) cardiomyocytes from mice aged 3-4 days. Abscissa, DNA content (in C ploidy units); ordinate, number of nuclei measured 2 h (mouse No. 1) and 3 h (mouse No. 2) after injection of thymidine-^{14}C. Left side of figure relates to mononuclear, right side to binuclear cells.

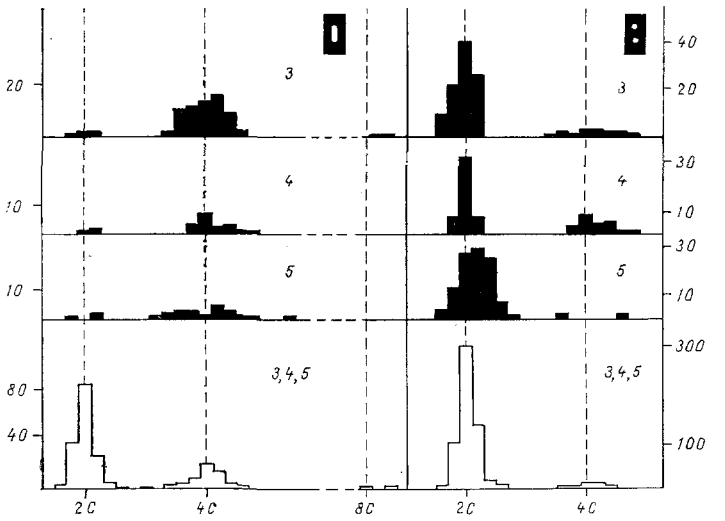

Fig. 2. DNA content in postmitotic (black columns) and unlabeled (white columns) cardiomyocytes: 36 h (mice Nos. 3 and 4) and 32 h (mouse No. 5) after injection of thymidine-^{14}C into mice aged 3-4 days. Unlabeled cells of three animals shown in combined histogram (Nos. 3, 4, and 5). Abscissa and ordinate as in Fig. 1.

EXPERIMENTAL RESULTS

The ventricular myocardium of mice aged 3-5 days contained many binuclear cardiomyocytes. Their number increased in the course of 2 days from 40% on the 3rd day to 60-70% by the 6th day. The mitotic index on these days showed only a small change from 1 to 14‰, with a mean value of about 5‰. On average 10% of the cardiomyocyte nuclei (from 5 to 14% in individual animals) were labeled 1-3 h after injection of thymidine.

DNA photometry in the nuclei of the mononuclear cardiomyocytes showed that tetraploid and octaploid cells are present in mice as early as at the age of 3-5 days (Figs. 1 and 2). They were much less numerous than in adult animals. Binuclear cardiomyocytes mainly contained two diploid nuclei, i.e., altogether they were tetraploid (2C × 2). Some binuclear cells were octaploid (4C × 2). The total number of polyploid cardiomyocytes in the ventricle of the mouse heart a week after birth was 60-70%.

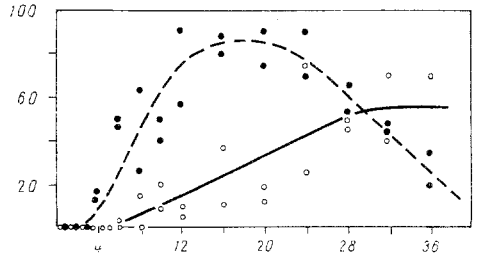

Fig. 3. Curve of labeled mitoses (filled circles and labeled binuclear cells (empty circles) after a single injection of thymidine-^{14}C. Abscissa, time after injection of thymidine-^{14}C (in h); ordinate, percentage of labeled mitoses among all mitoses found (20-80) and percentage of labeled binuclear cardiomyocytes among all labeled cardiomyocytes.

Pulse labeling (1-3 h after injection of thymidine), reflecting DNA synthesis, was found virtually exclusively in mononuclear diploid cardiomyocytes (Fig. 1). Mainly polyploid cells – binuclear cells with diploid nuclei, mononuclear tetraploid cells, and binuclear cells with tetraploid nuclei (Fig. 2), were labeled 32-36 h after injection of thymidine, after the main wave of labeled mitoses had passed (Fig. 3). The dynamics of DNA and thymidine labeling justifies the conclusion that polyploid cardiomyocytes are formed by polyploidizing mitosis. For instance, the precursors of the binuclear cells with two diploid nuclei were undoubtedly mononuclear diploid cells (Fig. 1). Among the postmitotic labeled cells the number of binuclear cells increased sharply whereas the number of mononuclear cells fell. The first labeled binuclear cells began to accumulate only after the first labeled mitoses. The number of labeled binuclear cells reached a maximum after the wave of labeled mitoses had passed (Fig. 3). Binuclear cardiomyocytes appeared, like binuclear hepatocytes [9] and melanocytes [4], as a result of acytokinetic mitosis. The same conclusion was drawn by Rumyantsev [6], who observed labeled binuclear rat cardiomyocytes 24-72 h after injection of thymidine into the animals. Mononuclear tetraploid cardiomyocytes also were formed as a result of incomplete mitosis, but in a different way from tetraploid liver cells. In the liver acytokinetic mitoses alternate with normal mitoses and mononuclear polyploid cells are formed during mitosis of binuclear cells [13]. Binuclear cardiomyocytes in the myocardium practically never succeeded in starting the cycle in the course of the time interval studied (Fig. 1). Consequently, mononuclear tetraploid cells could be formed only from diploid cells. Postmitotic label (Fig. 2) was found in a high proportion of mononuclear tetraploid cardiomyocytes. Klinge [12] observed modified "collapsed" prophases and, less frequently, metaphases in the early postnatal development of the rat myocardium. He also observed defects in the achromatin apparatus. We also observed pictures of marked heterochromatization of mouse cardiomyocytes. Prophase or metaphase block is a likely method of formation of tetraploid mononuclear cardiomyocytes. In the next cycle these cells could be the source of cardiomyocytes of the 4C × 2 and 8C type.

Discovery of the radioactive label in single postmitotic diploid cardiomyocytes (Fig. 2) is evidence that only a few cells complete mitosis, increasing the number of diploid cells in the population. However, the main method of postnatal growth of the myocardium is by polyploidization of its cells. It is interesting to note that the further increase in weight of the organ takes place on account of hypertrophy of its cells with an unchanged polyploid genome. Sarkisov [8] argued some years ago in support of this principle of tissue growth. The dimensions of the cells in the mouse myocardium, just as in that of the rat [11], increase in size significantly after they have completed polyploidization. This is an important difference between the biology of the myocardial cells and that of polyploid cells in other organs.

LITERATURE CITED

1. A. M. Aref'eva, Byull. Éksp. Biol. Med., No. 10, 501 (1978).
2. L. N. Belov, M. E. Kogan, T. A. Leont'eva, et al., Tsitologiya, No. 11, 1332 (1975).
3. M. E. Kogan, L. N. Belov, and T. A. Leont'eva, Arkh. Patol., No. 1, 77 (1976).
4. T. L. Marshak, Ontogenez, 5, 192 (1974).
5. P. P. Rumyantsev, Z. Zellforsch., 129, 471 (1972).

6. P. P. Rumjantsev (P. P. Rumyantsev), Int. Rev. Cytol., 51, 187 (1978).
7. P. P. Rumyantsev and V. O. Mirakyan, Tsitologiya, No. 10, 1276 (1968).
8. D. S. Sarkisov, Arkh. Anat., No. 10, 3 (1963).
9. I. V. Uryvaeva and M. A. Lange, Ontogenez, 2, 26 (1971).
10. W. Grabner and P. Pfitzer, Arch. path. Anat. Abt. B, Zellpath., 15, 279 (1974).
11. A. Katzberg, B. Farmer, and R. Harris, Am. J. Anat., 149, 489 (1977).
12. O. Klinge, Fortschr. Med., 88, 101 (1970).
13. C. Nadal and F. Zajdela, Exp. Cell Res., 42, 99 (1966).
14. P. Pfitzer, H. J. Knieriem, and H. Schulte, J. Med. Primatol., 6, 349 (1977).
15. R. Zak, Circulat. Res., 35, Suppl. 2, 17 (1974).

EFFECT OF THE HELIUM-NEON LASER BEAM ON POSTRADIATION REPAIR IN SKELETAL MUSCLE TISSUES

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Three series of experiments were carried out on the rat gastrocnemius muscle. Whole muscles were autografted in series I. Both hind limbs of the animals were irradiated in a dose of 1000 R before autografting of the muscles in series II. In series III x-ray irradiation in the same dose was followed by exposure of the hind limbs for 10 days to the action of a helium-neon laser, after which the muscles were autografted. The process of transplantation regeneration was investigated histologically 2 weeks and 1 and 2 months later. Exposure to the laser beam was shown to stimulate repair in skeletal muscle tissues and to normalize the process of post-traumatic regeneration when depressed by x-rays.

KEY WORDS: transplantation regeneration; x-ray irradiation; postradiation repair; helium–neon laser.

Ionizing radiation in a dose of 1000-2000 R has been shown to depress the ability of skeletal muscles to undergo posttraumatic regeneration for a long time [1, 2, 5, 7, 8]. The writers showed previously that the action of light from a helium-neon laser on a limb previously irradiated (2-3 h before transplantation) with x-rays can restore much of the ability of the gastrocnemius muscle to undergo transplantation regeneration, terminating with the formation of a contractile organ composed of muscle and connective tissue [6]. The mechanism of the stimulating action of red laser radiation on processes of regeneration of irradiated tissues is not yet clear: does recovery of the muscle from the structural changes taking place after transplantation take place purely on account of the more rapid elimination of muscle cells most severely damaged by x-rays, or is intracellular repair from radiation injury stimulated, i.e., does postradiation regeneration affect all the tissues composing the muscle? The investigation described below was carried out to shed light on this basic question.

EXPERIMENTAL METHOD

The method chosen to study this problem was one of those widely adopted in radiobiology to study intracellular repair after exposure to ionizing radiation. Essentially, radiation injury, revealed by inhibition of mitotic activity, chromosomal aberrations, depression of regenerative power, and by other indices, is recorded immediately after irradiation and also when a certain time interval has elapsed, during which the consequences of radiation injury have cleared up. According to the writers' own observations and also to data in the literature, radiation injury to skeletal muscles, reflected as a disturbance of posttraumatic regeneration, is very

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