MICROSPECTROPHOTOMETRIC INVESTIGATION OF DNA CONTENT IN MUSCLE CELL NUCLEI IN THE ZONE SURROUNDING AN INFARCT IN THE RABBIT HEART

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A comparative microspectrophotometric investigation was made of the DNA content in the nuclei of myocytes in the zone of the left ventricle surrounding a myocardial infarct during its development and healing in rabbits (from 12 h to 3 months after ligation of the descending branch of the left coronary artery). A moderate increase in the number of polyploid nuclei (from 2.5% in the control to 6.5% toward the end of the experiment), combined with an increase in the number of amitotically dividing nuclei, were discovered. The results are discussed from the standpoint of the possible participation of mechanisms of polyploidization and amitosis of myocyte nuclei in the intracellular regeneration of the myocardium.

KEY WORDS: microspectrophotometry of DNA; regeneration of the myocardium; polyploidization; amitosis.

Despite many investigations, views on the possibility of activation of DNA synthesis in the nuclei of heart muscle cells under pathological conditions and their role in regeneration of heart muscle and in the development of myocardial hypertrophy in experimental animals and man remain highly contradictory. For instance, a series of biochemical cytophotometric, and autoradiographic investigations revealed no substantial increase in DNA synthesis in the nuclei of rat and rabbit myocardial cells after the production of experimental hypertrophy or infliction of trauma on the myocardium [1, 2, 8, 9, 11]. These results have led several workers to suggest that the genetic apparatus of the myocardial cells of adult mammals is "repressed" [4, 16] and, during hypertrophy of the heart, according to Meerson (1968), "the increase in mass of each cell is not accompanied by DNA synthesis in the nucleus, but develops despite an unchanged number of DNA templates in the genetic apparatus of the cell" [3]. However, in a cytophotometric investigation of the hypertrophied rat heart, Grove et al. [13], in 1969, observed an increase in the number of polyploid muscle nuclei. Investigations showing considerable polyploidization of the nuclei of muscle cells of the hypertrophied human myocardium have also been published [12, 15, 17]. During marked injury to the myocardium and, in particular, in infarction, more marked activation of synthetic processes can be expected in the nuclei of the muscle cells. For instance, Sandritter and Scomazzoni [18] and Fisher et al. [12], in cytophotometric investigations, found nuclei of muscle cells with an interploid content of DNA in the zone around an infarct in the human heart; in their opinion this indicates a regenerative response of the myocardium. In autoradiographic studies DNA synthesis has been observed in muscle cell nuclei in the boundary zone of the infarct [6, 14, 16].

The contradictory nature of existing views on synthetic processes in the nuclei of myocardial muscle cells was the motivation for a microspectrophotometric analysis of DNA in muscle cell nuclei in the zone around the infarct in the left ventricle of rabbits at various times after the production of experimental ischemia.

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TABLE 1. Distribution of Nuclei (in %) of Muscle Cells of Rabbit Heart by DNA Content at Different Times after Myocardial Infarction (in conventional ploidy units) (M ± m)

Time of experiment	Class of ploidy					Accumulation index of DNA
	2c	3c	4c	6c	8c	
Control	96,0± 1,38	1,5± 0,86	2,0± 0,99	0,5± 0,5	—	2,07
12 h	96,0± 1,38	1,5± 0,86	2,5± 1,10	—	—	2,06
24—48 h	92,0± 1,92	4,5± 1,47	2,5± 1,10	0,5± 0,5	—	2,10
7 days	93,5± 1,74	3,0± 1,21	2.5± 1,10	1,0± 0,7	—	2,12
13 days	94,0± 1,68	2,0± 0,99	3,5± 1,30	0,5± 0,5	—	2,11
1—2 months	94,0± 1,68	1,5± 0,86	3,0± 1,21	1.0± 0,7	0,5± 0.5	2,14
3 months	92,0± 1,92	1,5± 0,85	4,0± 1,39	1,5± 0,86	1,0± 0,7	2,31

TABLE 2. Number of Amitotically Dividing Nuclei (in %) of Rabbit Heart Muscle Cells during Formation and Healing of Infarct (M ± m)

Time of experiment	Number of paired nuclei	Total number of amitotically dividing nuclei
Control	0,49± 0,11	0,78± 0,14
12 h	0,53± 0,17	0,88± 0,22
45—48 h	0,82± 0,21	1,91± 0,33
7 days	1,05± 0,25	2,16± 0,36
13 days	1,89± 0,36	2,94± 0,44
3 months	0,73± 0,23	1,39± 0,31

EXPERIMENTAL METHOD

Ischemia and an infarct of the left ventricle were induced in 20 male rabbits by ligation of the descending branch of the left coronary artery [7]. The animals were killed at various times after the beginning of the experiment (12, 24, 45, and 48 h, 7 and 13 days, and 1, 2, and 3 months). The intact myocardium of the left ventricle of three rabbits of the same weight was investigated as the control. The material was fixed in 10% neutral formalin and embedded in paraffin wax. The Feulgen reaction was carried out in a series of sections 5 μ thick (hydrolysis in 5 N hydrochloric acid for 50 min at room temperature, staining with Schiff's reagent for 1 h). Microspectrophotometry of the sections was carried out with the TsIM-2 numerical frame integrating microphotometer [5]. At each time no fewer than 200 nuclei of myocardial cells from the zone around the infarct and remaining in the zone of the infarct itself (within a radius of 0.5 cm from the focus of necrosis) were measured. Small lymphocytes in sections of spleen tissue from the same animals, mounted in the same block with the myocardium, were used as the standard for the diploid amount of DNA (2 c). As an overall index of the regenerative activity of the tissue, the "accumulation index" of DNA [10] was determined, as the weighted mean content of DNA in the nucleus (the arithmetic mean of the sum of the products of the number of cells and their corresponding units of ploidy). In sections of the same series stained with hematoxylin and eosin, under a magnification of 600 times, the number of amitotically dividing nuclei was counted in the zone around the infarct; the number of double nuclei, of nuclei with a constriction band, and groups of four or more nuclei resembling mulberries in shape. At each time (12 and 45-48 h, 7 and 13 days, and 3 months) 100 fields of vision were investigated; 200 fields of vision were studied in the control material. The mean number of nuclei per field of vision was determined and the number of amitotically dividing myocyte nuclei was expressed as a percentage of the total number of nuclei examined. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Analysis of the distribution of nuclei of the myocardial muscle cells by their DNA content showed changes which were expressed as an increase in the number of polyploid nuclei in the zone around the infarct in the course of its healing in the rabbit myocardium (Table 1). In intact zones of the myocardium only 4.0 ± 1.39 muscle cell nuclei contained more than the

diploid amount of DNA. The same number of polyploid nuclei was observed 12 h after the beginning of ischemia. The first changes in the DNA content were observed after 24-48 h, when the number of paratriploid nuclei was increased to three times the control value. It must also be noted that the number of paired nuclei lying close together, so that it was sometimes difficult to measure the DNA content in each nucleus in isolation, also was increased at these times. The total DNA content in such nuclei varied from diploid to tetraploid, but paratriploid values were more frequently found. At later stages of the experiment (13 days, 1, 2, and 3 months) paired nuclei were arranged more separately from each other and each nucleus contained close to the diploid amount of DNA.

Nuclei in the 3 c range by their DNA content were most frequently found during the first week after the beginning of ischemia, but later their number fell and after 1 month it was close to the control value. The number of tetraploid nuclei was doubled toward the end of the experiment. The number of hexaploid and octaploid nuclei also was increased. For instance, after 3 months their total number was 4% compared with 0.5% in the control ($P < 0.001$). By the end of the experiment the total number of polyploid nuclei in the muscle cells of the zone around the infarct in the rabbit myocardium was increased from 2.5 to 6.5% ($P < 0.05$). However, on the whole their number remained fairly low.

In the absence of mitotic activity of the nuclei, besides polyploidization, another mechanism of regeneration of the myocardium may be by an increase in the number of amitotically dividing nuclei. The number of amitotically dividing nuclei of muscle cells 12 h after the beginning of infarction differed only a little from the control values (Table 2). The number of binuclear cells 45-48 h after the beginning of the experiment was slightly increased, to reach 2.16% ($P < 0.05$) on the seventh day and 2.94% ($P < 0.001$) on the 13th day. By 3 months after the beginning of infarction, the number of amitotically dividing muscle cell nuclei was reduced and was close to the control values. At all periods of the experiment, binuclear cells were distributed chiefly near the focus of destruction. Evidently in rabbits during myocardial infarction the increase in mass and surface area of the nucleus by amitosis and polyploidization is one of the mechanisms of intracellular regeneration of the myocardium under conditions of a sharply increased functional load on the residual muscle fibers.

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