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DYNAMICS OF THE DNA CONCENTRATION IN HEART MUSCLE CELL NUCLEI OF RATS WITH EXPERIMENTAL MYOCARDIAL INFARCTION

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The DNA concentration was determined microspectrophotometrically in heart muscle cell nuclei of rats at different stages of experimental myocardial infarction. In the intact rat heart some nuclei (1.5-1.8%) of myocytes had a tetraploid DNA complement. Myocardial infarction activates the polyploidization of the nuclei of the muscle cells, especially those lying around the area of injury. The highest intensity of polyploidization of the muscle nuclei was found during the first week of myocardial infarction. Later during the experiment the degree of ploidy of the myocytes increased.

KEY WORDS: *regeneration; myocardial infarction; DNA content; polyploidization.*

In the modern view, regeneration of mammalian heart muscle takes place mainly on account of restoration and hyperplasia of the ultrastructures of the muscle cells [3, 6, 7]. It can accordingly be considered that an increase in the functional load on the myocytes would lead to a corresponding increase in all components of the cell and, in particular, to an increase in the quantity of genetic material. One form of increase of the DNA content in the nuclei of muscle cells is polyploidization. For instance, muscle cell nuclei of the human myocardium are diploid at birth but in the adult they are mainly tetraploid, and they reach a high degree of ploidy in the hypertrophied heart [9, 10, 14]. In experimental hypertrophy of the myocardium the number of polyploid nuclei in the rat heart has been shown to increase, although by a lesser degree than in man [11]. Several workers [4, 12, 13] have observed DNA synthesis in some muscle cell nuclei in the zone of the heart surrounding the infarct.

It was decided to study the increase in the DNA content in heart muscle cell nuclei of rats with myocardial infarction and also to ascertain the time of appearance of polyploid

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TABLE 1. Percentage Distribution of Nuclei of Rat Heart Myocytes by DNA Content at Various Times after Infarction (in conventional ploidy units, n)

Stage of experiment (in days)	Zone	Class of ploidy					Index of DNA accumulation
		2n	3n	4n	6n	8n	
C1		98,0±0,81	0,5±0,41	1,5±0,70	—	—	2,03
1	P	98,0±1,40	1,0±1,00	1,0±1,00	—	—	
	I	98,0±1,40	1,0±1,00	1,0±1,00	—	—	2,04
2	P	97,0±1,71	2,0±1,40	1,0±1,00	—	—	2,03
	I	99,0±1,00	1,0±1,00	—	—	—	
3	P	99,0±1,00	—	1,0±1,00	—	—	2,03
	I	98,0±1,40	—	2,0±1,40	—	—	
4	P	95,0±2,19	3,0±1,71	2,0±1,40	—	—	
	I	96,0±1,97	1,0±1,00	3,0±1,71	—	—	2,07
5	P	96,0±1,97	2,0±1,40	2,0±1,40	—	—	
	I	98,0±1,40	—	2,0±1,40	—	—	2,05
7	P	89,0±3,14	4,0±1,97	7,0±2,56	—	—	
	I	95,0±2,19	1,0±1,00	3,0±1,71	1,0±1,00	—	2,14
15	P	89,0±3,14	4,0±1,97	6,0±2,39	—	1,0±1,00	2,18
	I	95,0±2,19	—	4,0±1,97	—	1,0±1,00	
30	P	87,0±3,26	8,0±2,73	4,0±1,97	1,0±1,00	—	2,13
	I	96,0±1,97	3,0±1,71	1,0±1,00	—	—	
90	P	86,0±3,48	6,0±2,39	5,0±2,19	1,0±1,00	2,0±1,40	
	I	93,0±2,56	1,0±1,00	6,0±2,39	—	—	2,23
C2		96,1±1,37	2,1±1,02	1,8±0,94	—	—	2,04

Legend: C1) control animals at beginning of experiment; C2) control animals at end of experiment; zone P) bordering on pathological focus; zone I) intact area of myocardium.

nuclei of myocytes and their topography.

EXPERIMENTAL METHOD

Myocardial infarction was induced in 27 noninbred male albino rats by suturing and ligating branches of the left coronary artery. The animals were killed with ether vapor 1, 2, 3, 4, 5, 7, 15, 30, and 90 days from the beginning of the experiment. Three animals were taken each time; five rats were used as the control, three of them being killed at the beginning of the experiment and two at the end. Pieces of heart excised transversely across the ventricle were fixed in Carnoy's fluid and embedded in paraffin wax. Sections 5 μ thick were stained with hematoxylin-eosin and by Feulgen's method (hydrolysis with 5 N HCl for 60 min at room temperature and staining with Schiff's reagent for 1 h). The DNA content in the nucleus was determined microspectrophotometrically under immersion on a digital integrating cytophotometer [5]. The content of nuclear DNA was determined separately in the muscle cells of the right and left ventricles and the interventricular septum (100 nuclei in each part of the heart) of the control rats, and in the experimental rats a line was drawn with ink on the coverslip dividing the section areas adjacent to and remote from the zone of ischemic damage. In each zone 100 or more muscle nuclei were measured.

The level of ploidy was taken to be the mean DNA content in a haploid set of spermatic nuclei (in conventional units of total optical density) on the basis of 100 measurements made in 5-μ sections through the testes of the same rats.

The DNA "accumulation index" also was determined [1]. This general index is the arithmetic mean weighted content of DNA per nucleus (in conventional units of ploidy).

The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The great majority of myocardial muscle cell nuclei of the control rats killed at the beginning of the experiment belonged to the diploid class and only 1.5 ± 0.7% of nuclei had tetraploid values of DNA content (Table 1). In the control animals killed at the end of the experiment the relative percentages of the ploidy classes were not significantly changed.

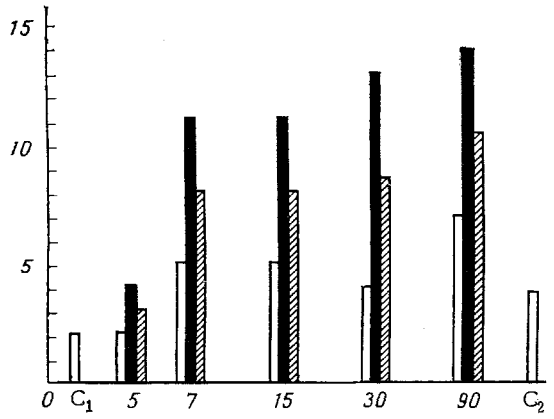

Fig. 1

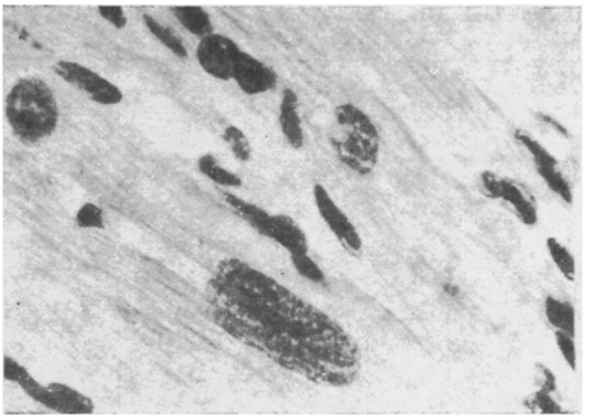

Fig. 2

Fig. 1. Topography of distribution of myocyte nuclei containing more than the 2n quota of DNA at different stages of the experiment. Abscissa, stages of experiment (in days); ordinate, number of nuclei (in %); black columns represent zone bordering on pathological focus; unshaded columns intact areas of left ventricle; obliquely shaded columns mean values for myocardium as a whole.

Fig. 2. Polyploid muscle nucleus. Myocardium of rat 3 months after production of infarct. Hematoxylin-eosin, 1080×.

The number of polyploid nuclei in the muscle of the right and left ventricles and the ventricular septum of the intact heart did not differ significantly, and for that reason only two zones were distinguished in the rats with myocardial infarction: a zone bordering on the pathological focus and a distant zone (sometimes tiny foci of inflammatory infiltration and of scar tissue were observed in it). During the first 3 days of the investigation the DNA content in the nuclei of the heart muscle cells was substantially unchanged and the number of diploid and tetraploid nuclei fluctuated within the control range. After the fourth day of the experiment an increase in the number of nuclei containing over 2n of DNA was observed. The process of polyploidization of the myocyte nuclei developed fairly quickly (Fig. 1), and on the seventh day of the experiment the number of polyploid nuclei was more than 3 times greater than in the control ($P < 0.01$). Toward the end of the experiment the number of muscle nuclei in the heart of the experimental rats with a DNA content of more than diploid increased considerably to 10.5 ± 2.17%, compared with the level of 3.9 ± 1.37% in the control animals of the same age ($P < 0.01$).

As a result the polyploid nuclei were larger than the diploid. Both polyploid and diploid nuclei could be observed in the same muscle fiber. At all stages of the experiment, in muscle fibers bordering on the pathological focus the number of polyploid nuclei was statistically significantly greater than in distant areas of the myocardium (Fig. 1). The phenomenon of preferential polyploidization of the myocyte nuclei in the zone bordering on the infarct and of the muscle fibers preserved in the focus of cardiosclerosis may, in the writers' opinion, be explained by the additional hemodynamic pressure exerted on the affected area. Indirect evidence of an increase in the functional load on the myocardium was given by increased activity of various oxidoreductases in the muscle cells of the zone bordering on the infarct [2]. Klinge's [12] suggestion that "necrohormones" act as stimulators of synthetic activity in muscle cell nuclei in the marginal zone of an infarct evidently requires further detailed analysis, although Shumeiko et al. [8] isolated an ischemic toxin, affecting myocardial electrical activity, from the anoxemic heart.

During healing of the myocardial infarct, nuclei with a high degree of ploidy (6n and 8n) appeared in the muscle cell nuclei of the rats, but no such nuclei were ever found in the myocardium of the control rats. Whereas on the 7th day of the experiment only one nucleus with a DNA quota of 6n was found, on the 15th day of the experiment octaploid nuclei also were encountered, and toward the end of the experiment the number of nuclei in the 6n-8n range reached 1.5 ± 0.8%. These huge nuclei (Fig. 2) were usually situated around the postinfarct scars or in muscle fibers remaining intact in the scar tissue. With a change in the DNA content in the paired nuclei it was found that each nucleus contained the same quantity of DNA, close to the diploid quota.

The over-all dynamics of the quantity of genetic material was reflected by the DNA accumulation index, which rose appreciably in the heart after the 7th day of infarction and continued to rise at the subsequent stages of the experiment (Table 1).

Activation of the process of polyploidization of the muscle nuclei in myocardial infarction can thus be regarded as a type of compensatory reaction arising in connection with the increased functional load on the residual myocytes, especially on those lying in the zone bordering on the infarct.

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