AN ELECTRON-AUTORADIOGRAPHIC STUDY OF DNA SYNTHESIS IN MITOCHONDRIA OF CARDIAC MYOCYTES DURING PHYSICAL EXERTION

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DNA synthesis in mitochondria of heart muscle cells was studied by electron autoradiography. After a functional load (swimming for 2.5 h) the number of grains of silver above the changed mitochondria was increased, reflecting of DNA synthesis in the mitochondria, i.e., regenerative processes in these organelles.

It has now been proved that hyperplasia of the mitochondria in the cardiac myocytes can take place in principle and that the process is influenced by the pattern of physical exertion [1]. However, many aspects of the molecular mechanism of physiological and reparative regeneration of the mitochondria still remain unexplained. One such aspect is the synthesis of mitochondrial DNA (M-DNA). Experiments in vitro [5, 6, 7] have shown that M-DNA synthesis can be demonstrated by electron autoradiography. The synthesis of M-DNA under pathological conditions has been studied by Green and Bahr [3]. In an electron-autoradiographic investigation of the mitochondrial fraction of liver (the exact nature of the cells from which the mitochondria came is not clear) they found no changes in M-DNA synthesis 48 h after partial hepatectomy in rats. So far no electron-autoradiographic study has been made of tissue sections from animals exposed to various factors, despite the obvious importance of such work, especially in relation to the heart and brain, organs in which the effects of injury are compensated by intracellular regeneration of the ultrastructures.

The object of the present investigation was to determine the dynamics of M-DNA synthesis in the myocytes of the heart after a single physical exertion.

EXPERIMENTAL METHOD

Mice weighing 26-28 g swam in water at 32°C for 2.5 h. Thymidine-H^3 (specific activity 4.9 Ci/mmole) was injected into the animals (two mice at each time) 24, 48, and 72 h after the end of swimming. The total dose of thymidine (18 μCi/g) was given in four intraperitoneal injections at intervals of 1 h. The heart was fixed in 1% OsO_4 solution 1.5 h after the fourth injection, dehydrated in alcohols, and embedded in Araldite. Sections about 1000 Å thick were stained with lead citrate and uranyl acetate, a carbon film was applied, and they were covered with a monolayer of type M emulsion. After exposure for 3 months the preparations were developed in D-19 developer and examined in the IEM-100B microscope. M-DNA synthesis was shown by the distribution of silver grains above the mitochondria. If a grain lay completely or partly above a mitochondrion, the latter was taken as labeled. The number of labeled mitochondria in an area of 80,000 μ^2 was determined for each animal.

EXPERIMENTAL RESULTS

A feature which distinguished the structure of the myocytes of the experimental animals from that of the myocytes of the control animals (which did not swim) was the swelling of the mitochondria and damage

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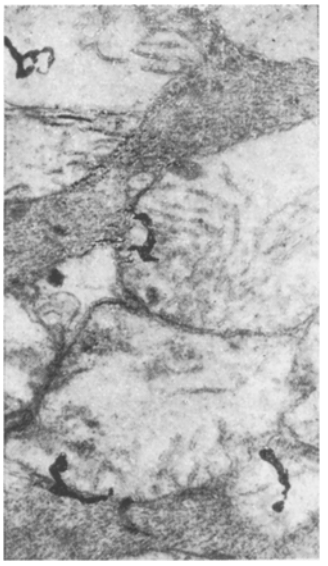

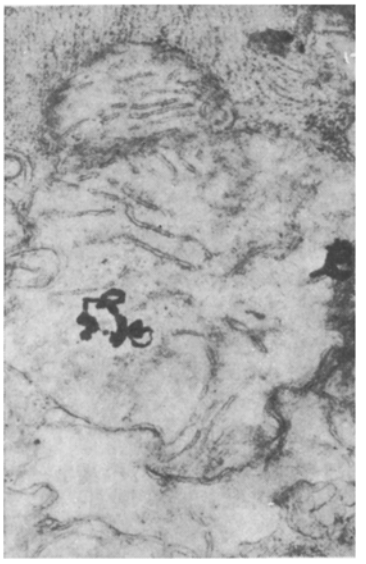

Fig. 1 Fig. 2

Fig. 1. Mitochondria of mouse heart muscle cells 24 h after physical exertion. Grains of silver can be seen above changed mitochondria. Electron autoradiograph, 20,000 ×.

Fig. 2. Two grains of silver above a mitochondrion with clear matrix and deformed cristae. Electron autoradiograph, 30,000 ×.

TABLE 1. Distribution of Grains of Silver in Autoradiographs

Criterion	Control		After exertion (in days)		
	without thymidine	with thymidine	1	2	3
Mean number of labeled mitochondria in area of 80,000 μ^2	2	12	170	20	10
Mean number of grains above other cell structures in area of 80,000 μ^2	3	5	28	7	6

to their internal structure: disturbance of the regular arrangement of the cristae, an increase in the distance between them, rupture and deformation of the cristae, and the appearance of large space, not filled with cristae, inside the mitochondria (Fig. 1). This type of structure of the mitochondria was observed in the animals sacrificed 24 h after swimming. No appreciable disturbances of the structure of the mitochondria could be observed in material fixed on the 2nd and 3rd days after physical exertion.

Labeled mitochondria were comparatively rare and in most cases the label consisted of only one grain of silver. Of course grains of silver can appear in photographic emulsion not only because of radioactive emission from the section, but for many other reasons besides (the emulsion background). For instance, in the autoradiographs examined in these experiments isolated grains of silver also were found above the bundles of myofibrils, but they were far fewer in number than above the mitochondria. In order to determine whether the appearance of grains above the mitochondria was due to the presence of radioactive material (thymidine-H^3) in them or whether the grains represented the background due to random causes, two types of comparisons were therefore made: 1) the number of labeled mitochondria in sections through the heart of the experimental and control animals was compared with their number in sections of the heart of a mouse not receiving thymidine; 2) the number of grains of silver above the mitochondria was compared with their number above the other structures of the cell in the same section. The results are given in Table 1.

The following conclusions can be drawn from a comparison of the data given in Table 1: the number of labeled mitochondria in preparations obtained from the experimental and control animals receiving

thymidine-H^3 was appreciably greater than in preparations obtained from an animal not receiving thymidine-H^3; in the animals receiving thymidine-H^3 the density of the grains above the mitochondria was greater than above the remaining territory of the cell where, it is now considered, DNA is not synthesized. The inclusion of the nuclei in this territory in the myocytes was based on data in the literature and on the authors' observations showing the absence of DNA synthesis in the overwhelming majority of nuclei. However, in preparations obtained 24 h after exertion very occasionally it was possible to find nuclei with a few grains whose appearance might have been caused by the slow synthesis of nuclear DNA.

The increase in the density of distribution of grains of silver observed above the mitochondria by comparison with the background (mitochondria of an animal not receiving thymidine-H^3, parts of the cell not synthesizing DNA) suggests that the autoradiographs studied in fact reflect the synthesis of M-DNA. Considerable intensification of M-DNA synthesis was found 24 h after physical exertion. Later still the rate of M-DNA synthesis was not significantly different from the control. The increase in the rate of M-DNA synthesis coincided with the time of the severest disturbances of structure of the mitochondria. It must also be noted that not only were more labeled mitochondria found at that time, but mitochondria containing not one, but 2 or 3 grains of silver, also were more frequent (Fig. 2). Because of the simultaneous recording of considerable disturbances of the structure of the mitochondria and the increase in the rate of M-RNA synthesis, the latter can be regarded as a compensatory reaction reflecting accelerated regeneration of the mitochondria, i.e., intracellular regeneration.

According to some reports, M-DNA codifies the components forming the inner membrane of the mitochondria [2, 4]. Consequently, the increase in the rate of M-DNA synthesis may be aimed at restoring the internal structure of partially destroyed mitochondria. The intensification of M-DNA synthesis may also provide for the more rapid formation of mitochondria de novo by division. Most probably both these processes take place: the formation of new mitochondria and the restoration of their internal structure.

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