Deoxyribonucleic Acid Synthesis in the Heart Mitochondria after Acute and Exhaustive Exercise

In a recent report[1] it was shown that, in dogs submitted to acute and exhaustive exercise by means of forced swimming, a marked increase in the mitochondrial mass of the myocardium could be observed. The same experiment, repeated in rats, indicated that, in association with the increase in the mitochondrial mass, there appeared evidence of mitochondrial DNA synthesis. Six adult rats of the Wistar strain were forced to swim for 90 min in a tank filled with warm water. At the start of the experiment the animals were injected i.p. with 250 μc of tritiated thymidine (Schwarz Biores., sp. activity 6 c m/Mol). As controls 6 animals not submitted to exercise were injected with the same dose of radioactive material. At the end of the exercise all the rats were killed with a blow on the head, the heart was exposed and small pieces of each cavity were fixed in 1% OsO_4 in phosphate buffer[2] and processed for electron microscope study. The remainder of the heart was homogeneized in 0.25 M sucrose in a tissue grinder with a Teflon pestle and the mitochondrial fraction was collected according to FERNANDEZ MORAN's technique[3]. The mitochondrial fraction was suspended in 0.25 M sucrose, extended with a fine brush on albuminized slides and covered with a thin film of Parlodion. The mitochondrial smears were mounted with Kodak AR-10 stripping film. After a 4-week-exposure the slides were processed for radioautography. A fraction of each mitochondrial pellet was examined with phase-contrast and electron microscopy in order to control the purity of the preparations.

The electron micrographs of the heart muscle cells of the animals submitted to exercise showed the existence of intramitochondrial fibres. The fibres were generally seen as thick and straight rods from which emerged thin fibrils (Figure 1). The length of the fibres was variable, and generally only one was seen per mitochondrion. The general appearance of the fibres was similar to that previously reported in mitochondria from other kinds of cells[4-6] and considered to be DNA. In the control animals no intramitochondrial fibres were found.

The radioautographs of the smears of the mitochondrial fraction of the animals submitted to exercise showed a marked activity indicated by the number of silver grains

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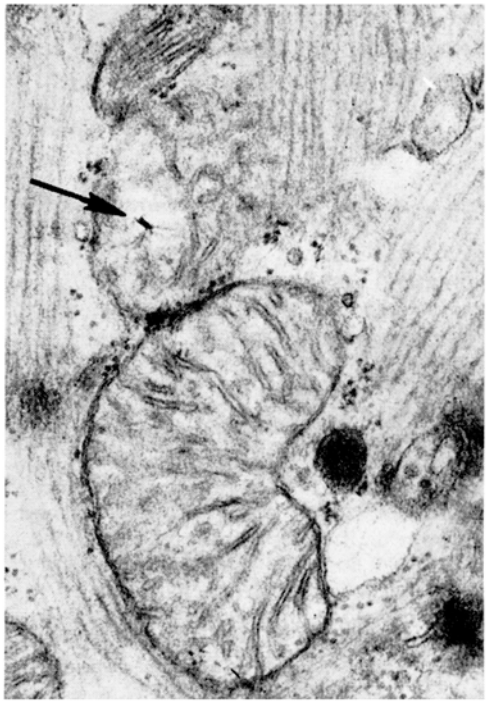

Fig. 1. Electron micrograph of heart muscle cells mitochondria after exercise. In 2 of them thick fibres may be seen in the matrix (arrows). Uranium acetate stain. × 58,000.

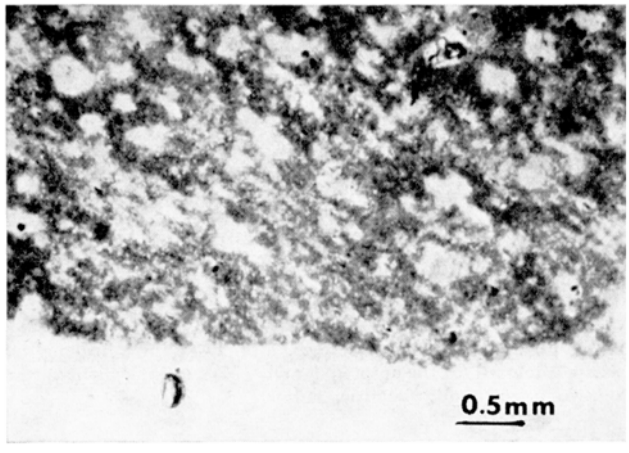

Fig. 2. Autoradiograph of mitochondria after exercise. The emulsion, exposed for 4 weeks, shows great activity on the applied mitochondria. × 160.

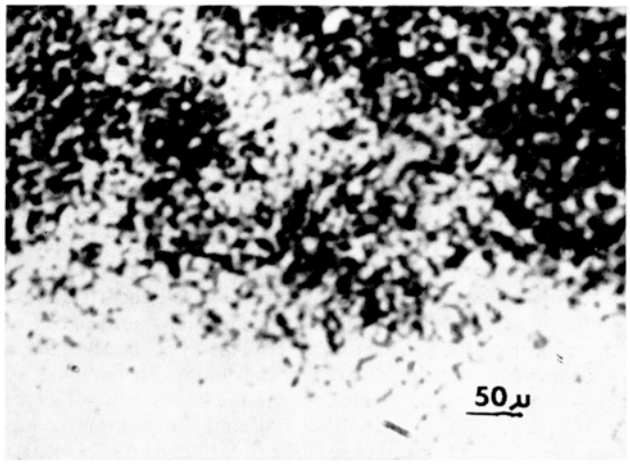

Fig. 3. Higher magnification of the autoradiograph of Figure 2 showing silver grains on many mitochondria visible in the background. × 1250.

on the areas of the applied mitochondria (Figures 2 and 3). In the control animals no difference with the background could be found. The phase-contrast and electron microscope study of the pellets processed for radioautography showed that no intact cells or nuclei were present (Figure 4).

The presence of DNA in the mitochondria has been demonstrated by morphological and biochemical methods in several plant and animal species[4-12]. In a recent paper VOGEL and KEMPER[13] showed evidence that in extracellular cultures of mushroom mitochondria, active DNA synthesis associated with morphological evidence of mitochondrial replication could be observed. To our knowledge no report exists on the existence of in vivo mitochondrial DNA synthesis in mammalian tissues. The appearance of intramitochondrial fibres tentatively identified as DNA according to morphological criteria, and the captation of tritiated thymidine would indicate that, under the stimulus of acute and exhaustive exercise, the mitochondria of the heart muscle fibres are capable of active DNA synthesis. Whether a relationship exists between the DNA synthesis and the increase in the mitochondrial mass cannot be stated with the present evidence. However, it is tempting to speculate that, as a response to the increase in the energy requirements of the heart muscle submitted to enforced exercise, the mitochondria would reproduce in a short time, and the DNA synthesis would be the first step in this replication.

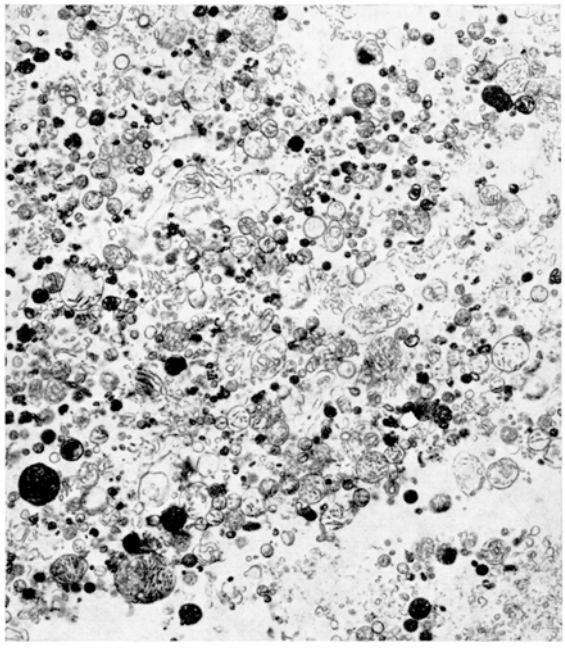

Fig. 4. Low power electron micrograph of one of the mitochondrial pellets employed for the autoradiographs. Most of the pellet appears to be composed of mitochondria. × 1800.

Resumen. Las mitocondrias del corazón de ratas sometidas a natación forzada muestran filamentos considerados morfológicamente como de ADN y captan timidina tritiada. Sobre la base de estas observaciones se sugiere que las mitocondrias miocárdicas con capaces de una activa síntesis de ADN como respuesta a requirimientos funcionales agudos.

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Comisión de Investigación Científica de la Provincia de Buenos Aires, La Plata (Argentina), 5 June 1967.

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Distribution of Tetracycline-7^{3}H in Subcellular Components of Normal and Tumor Tissues of the Albino Rat

The quantitative distribution of tetracycline, 4-dimethylamino-1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 6, 10, 12, 12a-pentahydroxy-6-methyl-1-11-dioxo-2-naphthacen-carboxamid (TTC), in normal cells, tissues and organs has been the subject of a number of investigations during the past 15 years[1-8]. In tumor tissues the uptake of TTC has been described as being limited to anaplastic tissue, as benign tumors do not exhibit TTC fluorescence; RHODES[9] and MALEK[10] have also stated that TTC fluorescence is greatest in the most necrotic regions of tumors. In support of their observations MILCH[11] has proposed that the most necrotic region is the area where TTC is 'activated' to fluoresce by the formation of chelate-type bond with a calcium-polypeptide complex which has been released by lytic action. DUBUY[12] reports that TTC has a selective localization in the mitochondrial and microsomal components of cells, and presumably these are the subcellular components with the greatest amount of Ca^{++} or other metallic cations or polypeptide available for interionic bonding. Since the fluorescent measurement of TTC in tissues may be limited by the amount of Ca^{++} or polypeptide available, we have explored the possibility of utilizing tritium-labeled TTC as a means of quantitation. This paper presents the results of our study on the distribution of this material in various cell fractions of normal and tumor tissue.

Materials and methods. Female Sprague-Dawley rats, 140–150 g, age 50 days, were obtained from a local supply house and maintained on laboratory chow and water ad libitum. After random division into control and experimental groups, half the animals were inoculated with TC sarcoma-line No. 21 cells[13] obtained from donors of the same strain. The trypsinized cells (6.05×10^6 cells/0.1 cm^3) were suspended in 0.2 cm^3 Eagle's solution and injected s.c. at a site lateral to the nipple line while control animals received an equal volume of saline vehicle.

Five days after inoculation, tumors could be palpated at the injection site. At this time 10 animals were each injected with 8.0 μc of tritiated TTC (sp. act. 1.47×10^{11}