

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<sup>4-12</sup>. In a recent paper VOGEL and KEMPER<sup>13</sup> showed evidence that in extra-

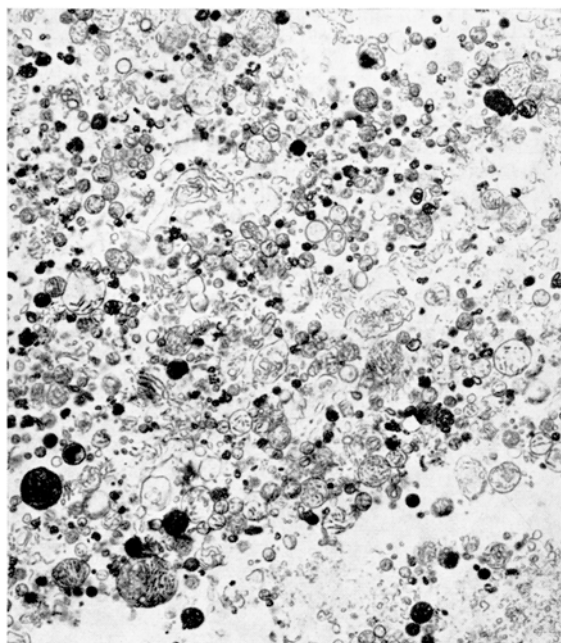


Fig. 4. Low power electron micrograph of one of the mitochondrial pellets employed for the autoradiographs. Most of the pellet appears to be composed of mitochondria.  $\times 1800$ .

cellular 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.

*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 son capaces de una activa síntesis de ADN como respuesta a requerimientos funcionales agudos.

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<sup>7</sup> N. KISLEV, H. SWIFT and L. BOGORAD, *J. Cell Biol.* 25, 327 (1965).

<sup>8</sup> J. A. PARSONS, *J. Cell Biol.* 25, 735 (1965).

<sup>9</sup> E. B. MOURAD, *J. Cell Biol.* 24, 267 (1965).

<sup>10</sup> F. L. SCHUSTER, *Expl Cell Res.* 39, 329 (1965).

<sup>11</sup> F. S. VOGEL, *Lab. Invest.* 14, 1849 (1965).

<sup>12</sup> G. CORNEO, C. MOORE, D. RAO SANADI, L. I. GROSSMAN and J. MARMUR, *Science* 151, 687 (1966).

<sup>13</sup> F. S. VOGEL and L. KEMPER, *Lab. Invest.* 14, 1868 (1965).

## Distribution of Tetracycline-<sup>73</sup>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<sup>1-8</sup>. 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<sup>9</sup> and MALEK<sup>10</sup> have also stated that TTC fluorescence is greatest in the most necrotic regions of tumors. In support of their observations MILCH<sup>11</sup> 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<sup>12</sup> 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<sup>++</sup> 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<sup>++</sup> 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<sup>13</sup> obtained from donors of the same strain. The trypsinized cells ( $6.05 \times 10^6$  cells/0.1 cm<sup>3</sup>) were suspended in 0.2 cm<sup>3</sup> 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  $\mu$ c of tritiated TTC (sp. act.  $1.47 \times 10^{11}$