ELECTRON-DENSE STRUCTURES IN MITOCHONDRIA INDUCED BY SHORT-TERM ETHIDIUM BROMIDE TREATMENT

MANLEY McGILL, T. C. HSU, and B. R. BRINKLEY. From The University of Texas Graduate School of Biomedical Sciences at Houston, the Department of Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas 77025, and the Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch at Galveston, Galveston, Texas 77550.

INTRODUCTION

In mammalian cells, ethidium bromide (EB) has been shown to inhibit mitochondrial-associated RNA synthesis (17), to suppress mitochondrial protein synthesis (8, 14), and to inhibit selectively the activity of mitochondrial DNA poly-

FIGURES 1–4 Electron micrographs of Chinese hamster fibroblasts treated for 4 h with EB (10 μg/ml)

FIGURE 1 Survey of fibroblast exhibits three mitochondria containing mitochondrial complexes (arrows). × 5,500.

FIGURE 2 Section reveals three mitochondrial complexes (arrows). In one mitochondrion a divalent cation granule (*G*) can also be seen. × 15,000.

FIGURE 3 Longitudinal section of mitochondrion. The complex appears to be arranged in a helical manner. × 35,000.

FIGURE 4 Cross section of mitochondrion showing complex. × 35,000.

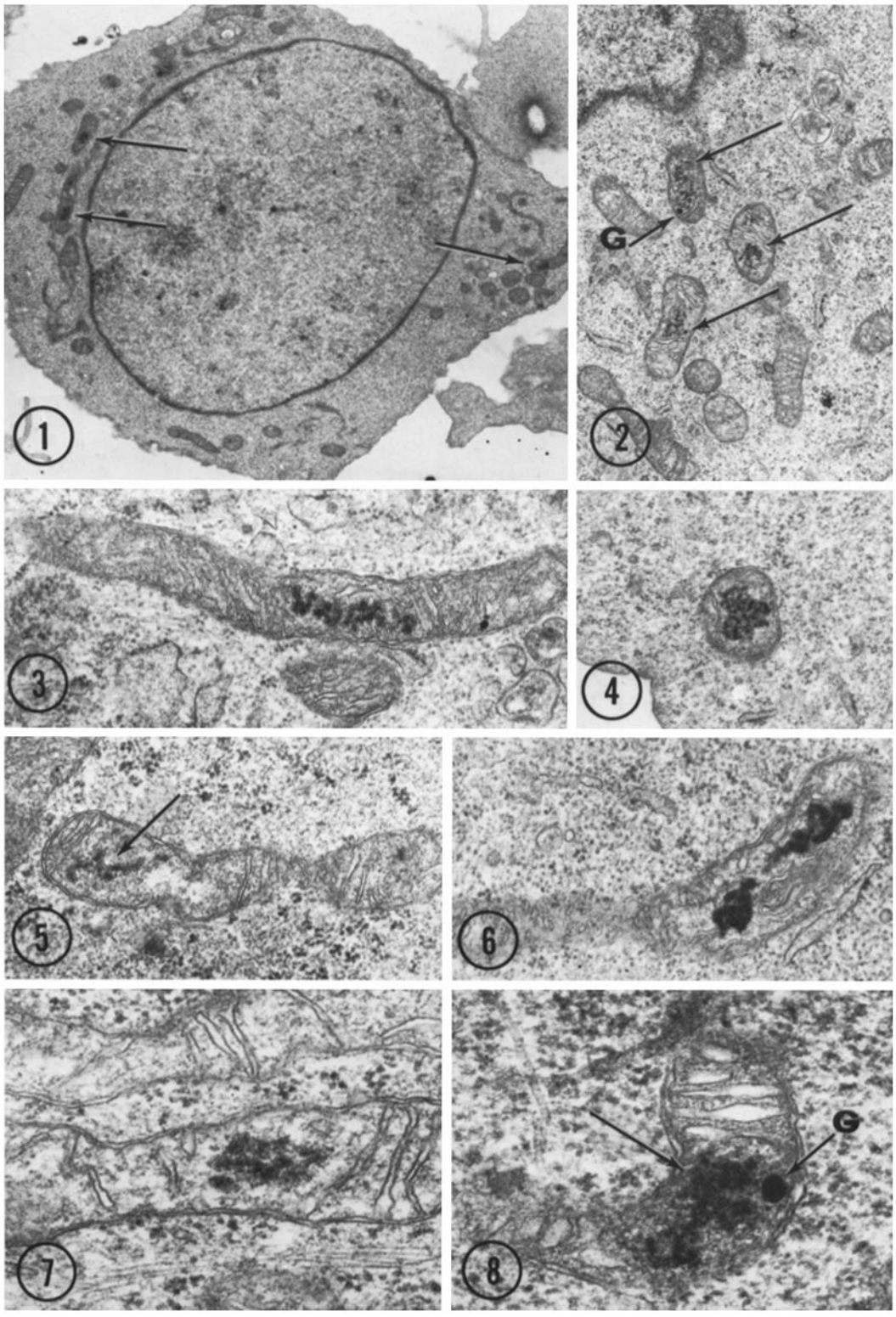
FIGURES 5–8 Sections of mitochondria from cells treated for 8 h with EB (10 μg/ml).

FIGURE 5 Mitochondrial complex (arrow) located in a cristae-free area. Note also the dense ribosomes in the cytoplasm. × 34,000.

FIGURE 6 Condensed mitochondrial complex typically seen in 8-h-treated cultures. Note the disorder of the few cristae present. × 34,500.

FIGURE 7 A swollen mitochondrion with complex located in a large cristae-free area. × 50,000.

FIGURE 8 Mitochondrion with prominent cristae contains a complex (arrow) and divalent cation granule (*G*). × 54,000.


1
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merase (9). EB binds to DNA and RNA and its strong interaction with DNA involves intercalation of the drug between adjacent base pairs of the DNA double helix (16). The affinity of EB for deoxynucleoprotein (DNP) is much reduced compared to that of purified DNA (1), and with selective deproteinization of DNP an increased interaction of EB with DNP was observed. Nuclear DNA synthesis is unaffected by low concentrations of EB (5, 12) so the affinity of EB for DNA and not for DNP might explain the specific inhibitory effects of the drug on mitochondrial DNA in the mammalian cell cytoplasm.

Ultrastructural studies of mammalian cells treated with EB at concentrations of 1.0–2.0 μg/ml from 1 to 8 days have consistently shown swollen mitochondria with abnormally arranged or lost cristae (8, 11, 12, 15). In addition to the usual structural changes induced by the drug, we have recently observed electron-dense bodies appearing in mitochondria within 12 h after treatment with higher concentrations of the drug. The present report describes the fine structure of these bodies as well as other changes induced by EB.

MATERIALS AND METHODS

Chinese hamster fibroblasts (strain Don) were seeded into plastic flasks (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.) and grown in modified McCoy's 5a medium supplemented with 20% fetal calf serum. All cultures were incubated at 37°C overnight or until uniform but nonconfluent monolayers were produced. The medium was removed, replaced with fresh medium containing EB at a concentration of 10 μg/ml, and reincubated at 37°C for 4, 8, or 24 h. After each time period cultures were rinsed with phosphate buffer, fixed in 1% osmium tetroxide for 1 h (10), and prestained in aqueous uranyl acetate for 20 min. After rapid dehydration in a graded series of ethanol, the cells were flat embedded (3) in Epon 812. After polymerization at 60°C for 48 h, the culture flasks were broken away from the Epon and appropriate cells were located with a phase-contrast microscope. The cells were cut out of the Epon plate, mounted on a blank Epon capsule, and trimmed for sectioning on an LKB Ultrotome III. Sections were picked up on Formvar-coated, 200-mesh copper grids (Belden Mfg. Co., Chicago, Ill.), stained with alcoholic uranyl acetate for 5 min, and poststained with lead citrate (7) for 2 min. The sections were examined in a Philips EM 201 electron microscope operated at 60 kV.

RESULTS AND DISCUSSION

After 4 h of exposure to EB interphase cells exhibited many mitochondria each containing an electron-dense structure similar in appearance to the condensed chromatin located at the periphery of the nucleus (Figs. 1–8). This structure can be seen in cross sections (Fig. 4) as well as in longitudinal sections (Fig. 3), and always appeared in a cristae-free area, presumably where the mitochondrial DNA is located (15). For convenience we shall hereafter refer to this structure as the "mitochondrial complex." In many longitudinal sections (e.g., Fig. 3) this complex displayed a helical arrangement. Other than the presence of this complex, the mitochondria from 4-h-treated cultures appeared similar to those seen in untreated cells (Fig. 14). Furthermore, such complexes were not found in the mitochondria of mitotic cells. After 8 h, however, the mitochondria appeared swollen (Figs. 5–8), and many of the complexes were more condensed (Figs. 6 and 8). The frequency of appearance of this complex in thin section was reduced after 8 h. Also, structures resembling divalent cation granules (13) appeared more numerous in the mitochondria of the 8-h samples (Fig. 8).

Giant mitochondria have been seen in L cells

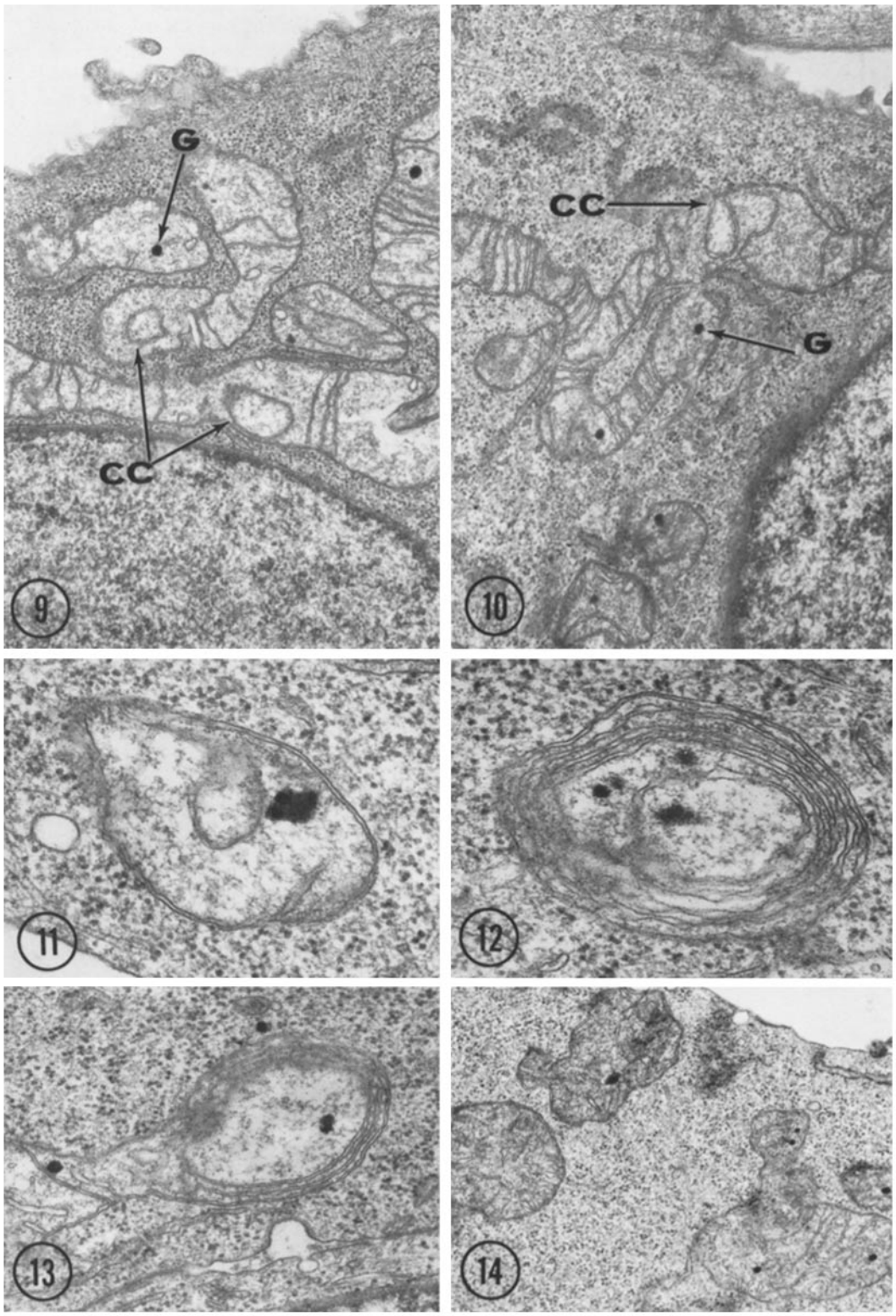
FIGURE 9–13 Fibroblasts maintained for 24 h in EB (10 μg/ml).

FIGURES 9 and 10 Giant mitochondria with large open areas contain circular cristae (*CC*) and numerous divalent cation granules (*G*). × 24,000.

FIGURE 11 Cross section of mitochondria reveals a dense body thought to be a condensed mitochondrial complex. × 52,000.

FIGURES 12 and 13 Mitochondria displaying multiple membranes. Note the large, dense ribosomes in the cytoplasm. × 50,000.

FIGURE 14 Thin section of normal, untreated fibroblast. × 22,500.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14

following prolonged treatment with EB (15), and we observed them in Chinese hamster fibroblasts after 24 h (Figs. 9 and 10). These mitochondria have large cristae-free areas and contained numerous divalent cation granules. In addition, dense bodies which were believed to be condensed forms of the complexes seen in earlier samples are shown in Figs. 11 and 12. Similar dense bodies have been reported in L cells treated with 1.0 μg/ml EB for 3 days (15). These dense bodies are clearly different from those shown in Fig. 2–8. At 24 h no dispersed complexes could be found. Another striking feature of the 24-h-treated cells was mitochondria with multiple membrane complexes forming myelin-like figures (Figs. 12 and 13). However, mitochondria with this configuration were not common.

At present no concrete evidence has been obtained to identify the molecules involved in the mitochondrial complexes. Since a number of investigators have shown that EB has affinity for circular DNA (1, 2, 12, 14) and for RNA (4, 16), it is highly possible that these complexes represent nucleic acids of the mitochondria, particularly DNA, supercoiled by EB. Electron microscope autoradiography, enzyme digestion experiments, and chemical isolation are being planned to gain further information regarding the chemical nature of this structure.

Previous workers (5, 6, 8) have shown that EB suppresses the formation of the protein complex responsible for cristae formation but not the protein synthesis necessary for an outer membrane. Therefore, the resultant giant mitochondria have few cristae and in some cases possess a multiple membrane complex. Nass (11) demonstrated that this effect was reversible. When EB was deleted from the medium, cellular growth resumed and mitochondria with normal size and morphology reappeared. If the mitochondrial complex observed in the present study indeed represents the condensed mitochondrial genome, it is not unreasonable to postulate that DNA replication, RNA transcription, and protein synthesis of this organelle are impaired, thus giving rise to abnormal mitochondria.

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