MITOCHONDRIA IN LIVING CELLS: AN ANALYSIS OF MOVEMENTS*

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PLATES 100 AND 101

Motion pictures of mitochondria remind us that these cellular organelles are active, changing systems and not the apparently immutable structures one may study in sections of fixed cells. The movements of mitochondria have a particular fascination when one considers what is known of these "power plants of the cell" (1) with their content of respiratory enzymes and their apparent prime function of synthesizing adenosinetriphosphate used by the cell to support other syntheses or contractile movement. Perhaps the exchange of material suggested by Frederic (2) to occur during the occasional attachment of mitochondria to the nuclear membrane may involve ATP provided by the mitochondria to the nuclei, which need it at least for the synthesis of diphosphopyridinenucleotide (3), which may be given in return by the nuclei to the mitochondria.

Our immediate interest in mitochondria arose from a study of the effects of 6-mercaptopurine, an antagonistic analogue of physiological purines and nucleic acids and of considerable interest in experimental cancer chemotherapy (4).

An interesting effect of 6-mercaptopurine in mouse tissue cultures is the inhibition of cell division, almost complete in the case of sarcoma 180 but less marked with fibroblasts of embryonic skin cultures. The mitotic inhibition caused by 6-mercaptopurine can be alleviated to some extent by a variety of physiological purines, nucleosides, and nucleotides, but most effectively in the case of sarcoma 180 by coenzyme A (5). This substance, the coenzyme of biological acetylations or 2-carbon transfers (6), is involved in a number of the enzyme systems localized in mitochondria, including the Krebs cycle and the systems for fatty acid synthesis and degradation. Cultures treated with co-

* This investigation was supported in part by an institutional research grant from the American Cancer Society, and in part by research grant C-678(C7) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

‡ Fellow of the Damon Runyon Memorial Fund. Deceased January 26, 1956.

§ With the technical assistance of Mrs. Marilyn Mendelson, Mrs. Ruth Jacobowitz, and Mr. Peter Menegas.

enzyme A have high mitotic incidence, in line with the finding (7) that coenzyme A is highly beneficial to tissue cultures and prolongs their life in incomplete media.

Fibroblasts examined under the phase contrast microscope present striking differences after treatment with 6-mercaptopurine and coenzyme A (8). The control fibroblasts contain rod-like or filamentous mitochondria and a moderate amount of fat. After treatment with 1 mM 6-mercaptopurine, the fibroblasts are almost devoid of fat and contain mitochondria that are shorter and more numerous than in the control cells. Simultaneous treatment with 0.2 mM coenzyme A prevents the appearance of the 6-mercaptopurine effects on lipogenesis and mitochondrial morphology and maintains mitosis at its control incidence. Treatment with 0.2 mM coenzyme A alone produces very actively dividing cultures of rather fatty cells in which the mitochondria are abnormally elongated and reduced in number per cell.

These effects are all noted after 24 hours' exposure to the agents. We applied time-lapse cinephotomicrography to the problem in a search for early changes.

Materials and Methods

Small bits of mouse embryonic skin were planted in a thin chicken plasma clot on a large No. 0 coverslip 43 × 50 mm. This coverslip was sealed with paraffin-vaseline to a Maximow slide in which the depression had been cut through to the other side to produce a circular chamber 35 mm. in diameter and 6 mm. deep. Some fluid medium of chick embryo extract, horse and human sera, and balanced salt solution (8) was placed on the explant, which was then incubated as a hanging drop culture after the other side of the chamber was sealed with a second coverslip. After 2 days of incubation, the coverslip with its culture was transferred to a perforated stainless steel slide about 1 mm. thick, a similar chamber was constructed with the aid of another coverslip, and the chamber was filled with Gey's solution. The culture vessel was then placed on the stage of a phase contrast (dark medium) microscope within an incubator at 37°C., and the culture cells were examined under oil immersion. Time-lapse motion pictures were made with a 16 mm. movie camera that made one photograph with about a 2-second exposure every 4 seconds. A Wratten B58 green filter and glass water cell were used, and the illumination with a tungsten ribbon-filament lamp was intermittent.

In the usual experiment, motion pictures were made of the same cell both before and after perfusion of the cultures with 6-mercaptopurine or coenzyme A in Gey's solution. The perfusion was carried out by pulling several drops of solution containing the agent into the chamber by applying bibulous paper to the medium at the opposite edge of the coverglass.

The 6-mercaptopurine was kindly furnished by Dr. George H. Hitchings of the Wellcome Research Laboratories, Tuckahoe, New York. The 75 per cent coenzyme A preparations were obtained commercially.

OBSERVATIONS

In the untreated control cells, activities of the mitochondria parallel those described before by a number of authors. There is little, indeed, that one can add to the description given by the Lewises (9) in 1915 or by Bierling (10) in 1954. The mitochondria in untreated mouse fibroblasts after several days in culture are for the most part long filamentous bodies, with a few short dot-like

ones among them. The filamentous mitochondria form a constantly shifting network, with frequent splittings and reunions with other mitochondria. The unions may occur on the sides as well as on the ends. Occasionally a mitochondrion forms a loop by joining its two ends. There may be local changes in thickness along the length of a mitochondrion. While the movement of the fat bodies appears to be passive, as though each were undergoing Brownian motion but vibrating randomly about a more or less fixed point, movement of the mitochondria appears to be of a different nature. Movements of the mitochondria parallel to their long axes may take them considerable distances through the cytoplasm. They tend to move toward the cell periphery or toward the nucleus (9), as though through channels in the cytoplasm or in the hyaloplasmic streams that carry vacuoles to and from the cell center, as Gey, Shapras, and Borysko (11) have seen.

Figs. 1 to 15 illustrate the mitochondrial network near the fat bodies proximal to the nucleus in an untreated fibroblast. Successive photographs in this set were taken 20 seconds apart. The mitochondrion marked by an arrow branches in the course of the sequence to form a triradial figure. Immediately after perfusion of cultures with coenzyme A, mitochondrial movement appears to exceed that in the controls, which is about 100 mμ per second. In the course of an hour after perfusion with coenzyme A, the mitochondria are seen to line up in cell processes and associate terminally into a few long mitochondria. This is illustrated in Figs. 16 to 30. These long forms are not necessarily permanent associations. A second perfusion, this time with 6-mercaptopurine, is followed by a return to an appearance more like that of untreated cells.

In some sequences of cells perfused with 6-mercaptopurine alone, there appears to be less tendency toward terminal association, some of the mitochondria may appear thinner and beaded, and many may be short rods or dots. In some cells perfused with 6-mercaptopurine, little change from control appearances is noted. However, after a repeated perfusion with 6-mercaptopurine, the cells sometimes show some retraction, a tendency toward rigidity of the fat droplets in one group, as though in a gelling matrix, and less activity among the mitochondria.

DISCUSSION

The motion-picture sequences made are all of changes within the first hours after treatment, but they point the way toward the effects noted after 24 hours. Mitochondrial elongation with coenzyme A through terminal association is better expressed in the first few hours than is the shortening and increase in number of mitochondria with 6-mercaptopurine. The 24-hour effects on lipogenesis are not evident in the first-hour pictures.

These mitochondrial effects are by no means unique to the two agents used here. The beading of filamentous mitochondria and their breakdown into a

number of shorter granules have been observed in apparently healthy cells as well as in cells injured with various noxious agents (9, 12, 13, 14). In damaged cells there is in addition often a swelling of the granular mitochondria, as Frederic (15) has portrayed among the effects of dissociating detergents. The damaging agents are of such variety that it is difficult to ascribe any specificity to this mitochondrial change other than a reflection of interference in normal metabolism (13). But the metabolic capabilities of mitochondria are themselves enormous, as is exemplified by the many diverse interactions of mitochondria with other cellular constituents (16), and opportunities for damaging interference are correspondingly multiplied.

The exaggerated elongation of filamentous mitochondria under the influence of coenzyme A is paralleled by similar changes noted by others. The Lewises early observed (9) that two granules might fuse together, that rods might unite to make threads, and that some cells might show a collection of all their mitochondria into several very large forms. The parallel observation by Frederic (15) is particularly noteworthy. He found that metabolically stimulatory 2,4-dinitrophenol causes formation of very long mitochondrial filaments through the terminal association of shorter rods. Mitochondrial movement is more rapid in cells treated with dinitrophenol, just as in those treated with coenzyme A, and mitoses are completed more rapidly. There is a difference, however, in that mitochondria under longer dinitrophenol treatment become thin and the cells degenerate after some hours.

Mitochondria also lengthen in the early stages of anaerobiosis preceding their breakdown into granules (17). It is of considerable interest that the elongate mitochondria stain more intensely with Janus green B, whereas the granular mitochondria of later anaerobiosis stain feebly or not at all (17). The shortened and thickened mitochondria formed under the damaging influence of the mitotic inhibitor, trihydroxy-*N*-methylindole, also fail to stain with Janus green B (18). Staining with Janus green B is also poor in the shortened and poorly mobile mitochondria of cultured cells one day after treatment with phenylurethane (15). According to the studies of Lazarow and Cooperstein (19), staining of mitochondria with Janus green B results from the failure of reduction of the dye by reduced flavoproteins because of the activity of the cytochrome oxidase system in the mitochondria. Chèvremont-Comhaire and Frederic (20) suggest that trihydroxy-*N*-methylindole is a preprophasic inhibitor because it modifies the succinic dehydrogenase-cytochrome oxidase system of the mitochondria, and that the oxidation-reduction system of the mitochondria plays an important role in the preparation for mitosis.

Our own observations fit into this general pattern. Mitochondrial shortening accompanies mitotic inhibition by 6-mercaptopurine, while mitochondrial elongation accompanies increase in mitotic incidence under the influence of coenzyme A. Mitochondrial movement is greater under the influence of sub-

stances or conditions that cause mitochondrial elongation, while mitochondrial shortening and slowing also go together.

Observations of this sort have suggested to Frederic and Chèvremont (21) that mitochondrial movements are not only the result of passive movement by cytoplasmic currents, as Zollinger (22) and Gey, Shapras, and Borysko (11) have maintained, but are also the result of local modifications of surface tension produced by metabolic exchanges between the mitochondria and the surrounding cytoplasm. Our own results are not considered contradictory to this hypothesis. Mitochondria shortened under the influence of phenylurethane are still moved about in the cytoplasm, but they retain a rigid, "fixed" aspect (15). Some rigidity of mitochondria is also seen in some of our sequences with 6-mercaptopurine, but here the apparent gelation of the ground cytoplasm complicates the interpretation. The free bendings of the mitochondria upon themselves would represent in part the effect of metabolic interchanges between local mitochondrial regions and cytoplasm and in part the effect of conflicting hyaloplasmic currents (11). Detergents bring an early stop to this active deformation of the mitochondria without interfering in cytoplasmic flow (15). Denaturation of the mitochondrial proteins might "fix" them so that local changes in hyaloplasmic flow no longer distorted the mitochondria. It would be expected that the passive movement of mitochondria in cytoplasmic currents would be greater under conditions of more active metabolism in the ground cytoplasm itself, for this must be the source of much of the cytoplasmic flow.

SUMMARY

Time-lapse cinephotomicrography of mouse embryonic fibroblasts before and shortly after perfusion of tissue cultures reveals that the elongation of mitochondria caused by coenzyme A results from the terminal association of many shorter rods into a smaller number of long filaments. These are not permanent associations, but they reflect an exaggeration of the cohesive tendency of mitochondria, which in untreated cells is counterbalanced by frequent disjoinings and breakings of the anastomotic network.

Our own observations and a survey of the literature suggest that elongate mitochondria with rapid movement and high metabolic activity tend to accompany proliferation in tissue cultures, and that mitotic inhibition of cultured cells may go together with short, slow mitochondria of low metabolic activity.

The movement of mitochondria may be both active, reflecting metabolic exchanges with the cytoplasm, and passive, the result of hyaloplasmic currents.

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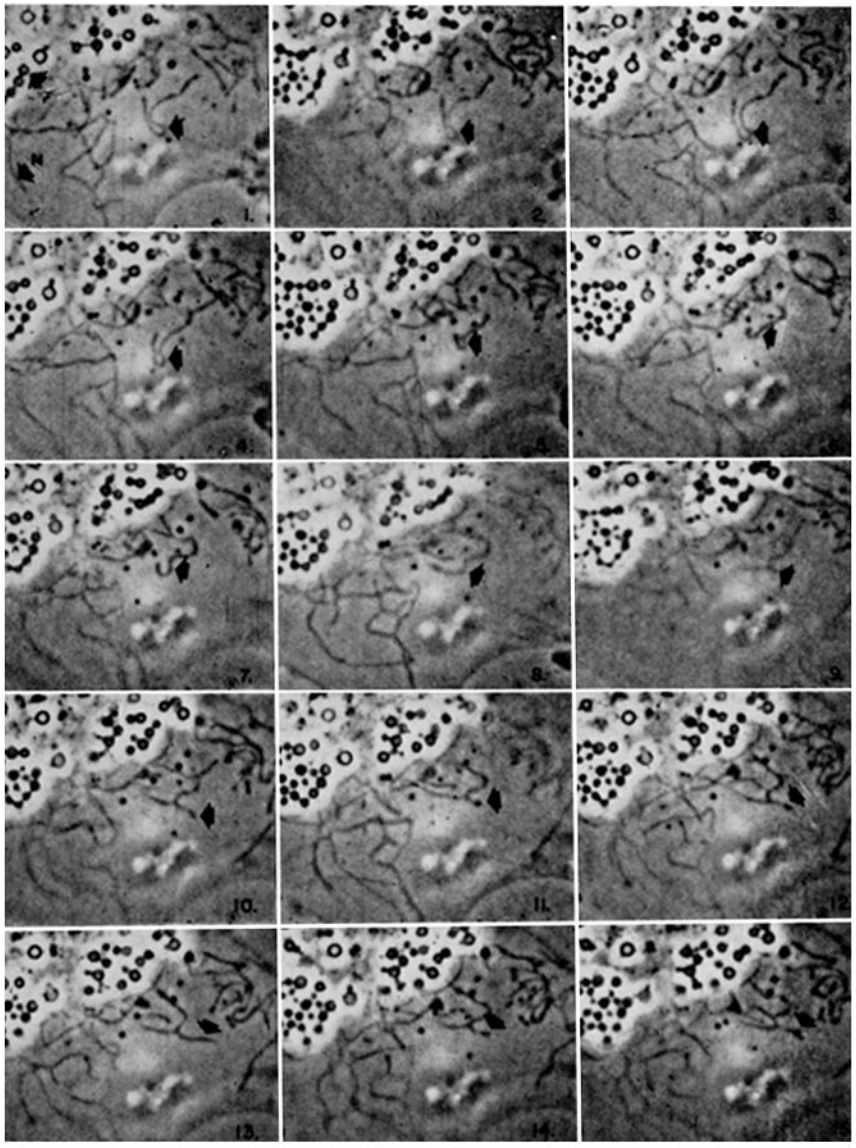
EXPLANATION OF PLATES

PLATE 100

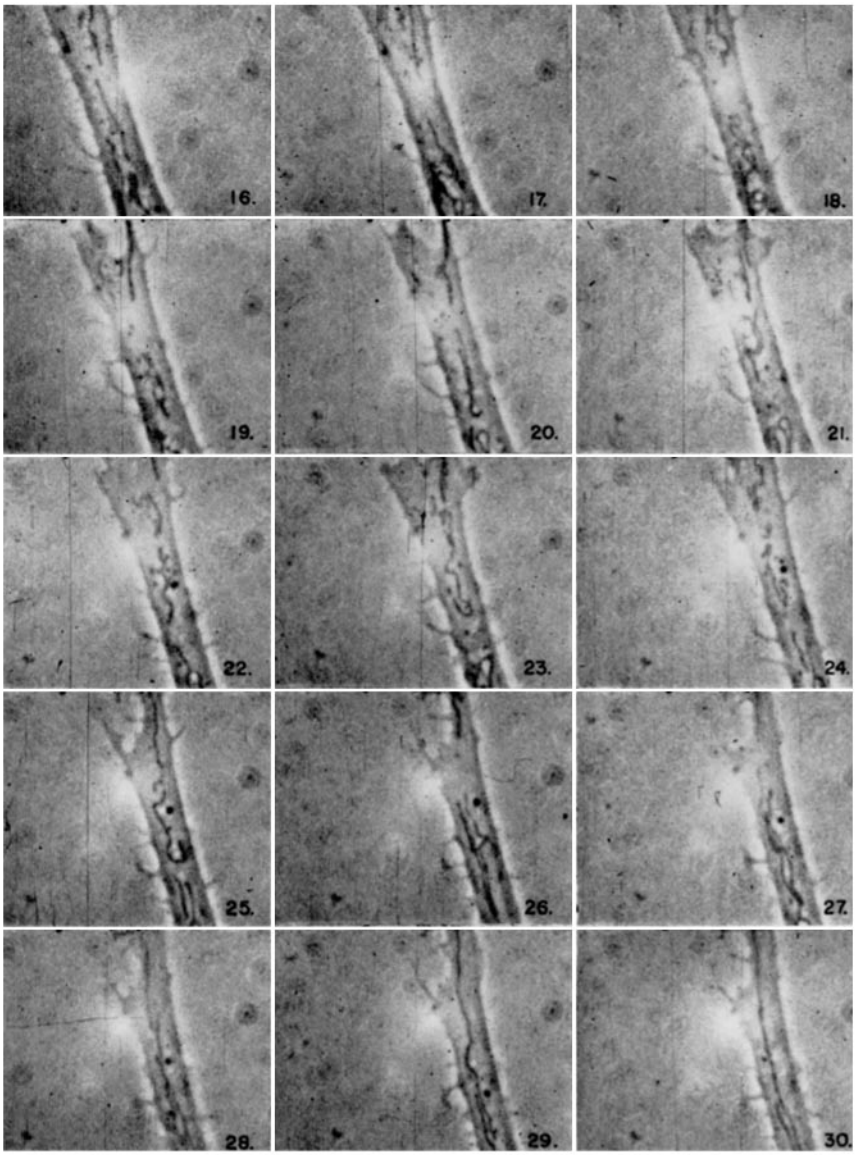
FIGS. 1 to 15. Portion of an untreated embryonic mouse fibroblast from a film sequence with 20 seconds between successive photographs. *F*, fat bodies; *M*, mitochondria. Note shape changes and branching of mitochondria marked with arrow. Phase contrast, × 970 originally, here enlarged to 3500.



(Tobioka and Biesele: Mitochondrial movement)

PLATE 101

FIGS. 16 to 30. Cytoplasmic process of an embryonic mouse fibroblast in culture about 1 hour after perfusion with 0.2 mM coenzyme A, from a film sequence with 1 minute between successive photographs (2 minutes each between Figs. 22 and 23, 23 and 24, 25 and 26, and 26 and 27). The mitochondria associate end-to-end in this sequence into 2 elongate forms. Phase contrast, × 970 originally, here enlarged to 3500.



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