

keratinocytes. All of the clear cells characteristically lacked desmosomes and showed a general paucity of organelles (Figure 1). The nuclei were round or oval and appeared to be smaller than those of adjacent epithelial cells. A single cleft was observed in the nucleus of a very few cells. The nuclear chromatin was usually condensed peripherally. The cytoplasm was more electron translucent and contained fewer ribosomes which were mostly aggregated in rosette-like polysomes (Figure 2). Although typical bundles of tonofilaments were absent, a few loosely arranged individual filaments with a diameter of 60–80 Å were sometimes observed. An occasional long isolated microtubule was also present in some cells. The cytoplasm contained numerous small smooth-surfaced vesicles, Golgi apparatus and a few isolated cisternae of rough-surfaced endoplasmic reticulum. A majority of the clear cells showed a variable number of dark, membrane-bound granules. These were round, oval or constricted to form an hourglass or bowling pin shape and varied from 0.07 to 0.7 µm in diameter or length. Most of the granules contained an electron-dense, homogeneous, granular core but some of the larger ones showed a heterogeneous internal appearance. The characteristic Langerhans cell granules, which have recently been recognized as cup-shaped or disc-shaped structures¹³, were not seen in the clear cells of the mouse cervix. Membrane-delimited granules with a fine-grained internal substance have also been described, in addition to typical Langerhans

granules, in the clear cells of normal human vagina by HOFFMEISTER and RUPEC¹⁰. These authors have suggested that the granules resemble the premelanosomes present in the cytoplasm of human epidermal melanocytes. Although the precise significance of clear cells in cervical epithelium is unknown, our preliminary studies indicate and increase in frequency of clear cells in chemically induced squamous carcinoma of the mouse cervix. A similar finding has also been reported in in situ carcinoma of the human cervix⁹. However, our studies suggest that the clear cells of mouse cervix might represent a different morphological type of cells than the Langerhans cells of human specimens¹⁴.

Résumé. Un examen au microscope électronique des cellules «claires» de l'épithélium cervical de la souris a révélé qu'il leur manque les granules caractéristiques de Langerhans décrites dans les cellules correspondantes de l'exocol humain.

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¹³ R. W. SAGEBIEL and T. H. REED, *J. Cell Biol.* **36**, 595 (1968).

¹⁴ This work was supported by a grant to M. G. JONEJA from the Ontario Cancer Treatment and Research Foundation.

Seizing Mechanism and Fate of Intranuclear Mitochondria

During a fine-structural study of a transplantable tumor originated from an adrenal cortical carcinoma of the Syrian golden hamster, induced by s.c. injections of urethane given during the suckling period¹, mitochondria and small vesicles were observed in the nuclei of tumor cells. These structures were found in many of the cells at early interphase, which showed dilated perinuclear space and the chromatin tended yet to be disposed throughout

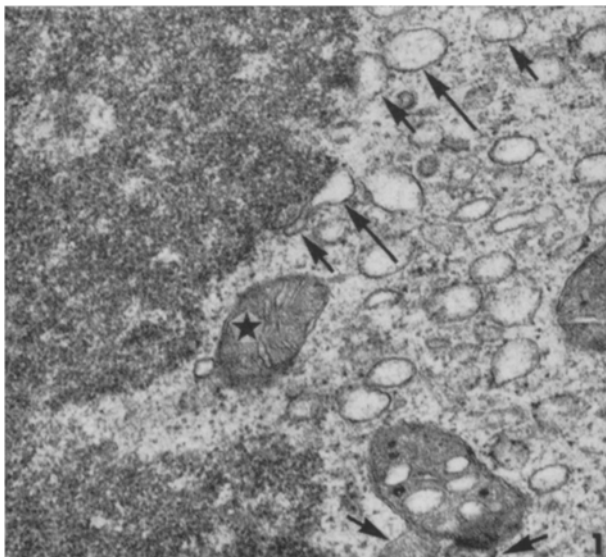


Fig. 1. Portion of a cell, at anaphase-telophase, of a transplantable adrenal cortical carcinoma of the Syrian golden hamster. Vesicles are gathering near a coalescent chromosomal mass and surrounding a mitochondrion (asterisk). Some of them are adhered to both of the structures (arrows) and fusing each other (long arrows). $\times 45,400$.

the nuclei, in larger numbers (Figure 2). In other resting cells these structures were found in smaller number. No nuclear envelope surrounding these mitochondria and vesicles was discernible. Many of these structures could be definitely identified as mitochondria, in that they showed a double limiting membrane and internal cristae. Sometimes swollen and altered mitochondria, which were surrounded only by a single thicker membrane and contained what seemed to represent remnants of cristae, were encountered. Some of them showed a severe decrease in the density in the matrix areas associated with the appearance of fine filamentous components simulating the mitochondrial DNA² and aggregates of electron-dense material (Figure 3). No intranuclear mitochondria were detected in cells at prophase. The small vesicles were probably derived from the endoplasmic reticulum which are very rich in the cytoplasm in this tumor cell line.

The absence of a nuclear envelope surrounding the mitochondria and vesicles indicates that they are not 'nuclear pseudo-inclusion'³. Several possibilities can be suggested to explain their location within the nuclei. It has been assumed^{4–7} that passage through enlarged nuclear pores, incorporation within a pinched-off invagin-

¹ M. MATSUYAMA and H. SUZUKI, *Br. J. Cancer* **24**, 312 (1970).

² H. SWIFT and D. R. WOLSTENHOLME, in *Handbook of Molecular Cytology* (Ed. A. LIMA-DE-FARIA; North-Holland Publishing Co., Amsterdam 1969), p. 972.

³ W. BERNHARD and N. GRANBOULAN, *Expl. Cell Res. Suppl.* **9**, 19 (1963).

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⁵ E. BUCCIARELLI, *J. Cell Biol.* **30**, 664 (1966).

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⁷ A. R. MORALES, G. FINE, R. C. HORN, and J. H. L. WATSON, *Lab. Invest.* **20**, 412 (1969).

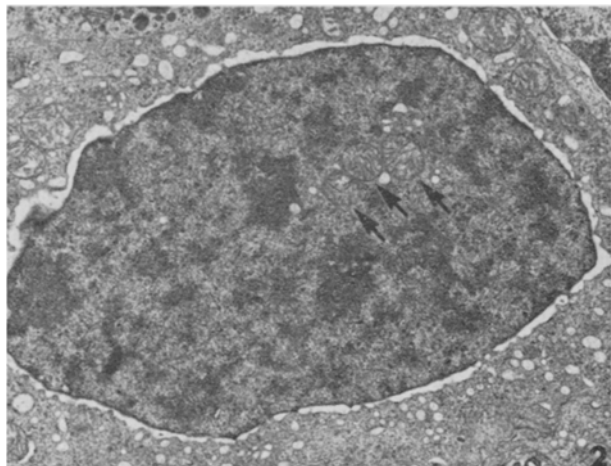


Fig. 2. Cells of the same tumor. A nucleus contains several mitochondria (arrows) and vesicles. The perinuclear space is markedly dilated and the chromatin is not yet reduced to a marginal zone of the nucleus showing that this cell is at early interphase. In the cytoplasm mitochondria and numerous vesicles, the same found in the nucleus, are scattered. $\times 12,790$.

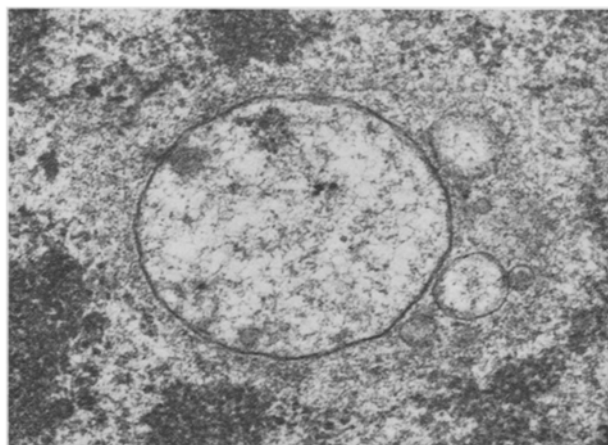


Fig. 3. A swollen and altered intranuclear mitochondria with the single thicker membrane and matrix areas of lesser density, containing prominent fine filamentous components and opaque aggregates. Such a mitochondrion is always separated by a halo area of medium density from the nucleoplasm. $\times 59,680$.

ation, or inclusion within the nuclear envelope at anatelephase may have been induced. We found these structures mostly in the cells at early interphase. Furthermore, an evidence of seizing of the mitochondria by a rapid and abnormal fusion of the numerous, comparatively large vesicles, in which some of them were adhering to the coalescent chromosomal masses and mitochondria, during the course of the reformation of the nuclear envelope⁸ at ana-telophase, was obtained (Figure 1). We, therefore, emphasize the importance of the seizing at ana-telophase as the most likely mechanism. Observation of sequential alterations of the intranuclear mitochondria shows that mitochondria can survive for several hours, and finally go into disintegration, in the abnormal condition of the nucleus, in the resting cell stage. The features of the altered mitochondria closely resemble those of yeast mitochondria under anaerobic conditions and of mitochondria from rapidly growing root tip of the broad bean, *Vicia faba*². It is especially interesting to note that in these cells the mitochondria have prominent DNA filaments. The presence of the intranuclear mitochondria

and their possession of a genetic material, DNA, may influence some of the properties of cancer cells⁹.

Zusammenfassung. In Kernen transplantierte Tumorzellen, die aus adrenocortikalem Carcinom des syrischen Goldhamsters stammen, wurden häufig Mitochondrien und kleine Bläschen festgestellt. Diese Strukturen wurden in Anatelephase von Kernen umgeben und innerhalb eines Zellenzyklus werden die eingekreisten Mitochondrien allmählich im Kern aufgelöst.

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⁸ E. ROBBINS and N. K. GONATAS, J. Cell Biol. 21, 429 (1964).

⁹ This study was supported by the Ministry of Health and Welfare (Cancer Research Subsidy, 1970/1). We thank Prof. T. NAGAYO, Dr. K. KOJIMA and Dr. M. HOSHINO for their advice, and Mr. T. SATO for his technical assistance.

In Vitro Fertilization of the Mongolian Gerbil Egg

Heated bovine follicular fluid (BFF) is reported¹⁻³ to endow epididymal spermatozoa from mice and hamsters with the capacity to penetrate homologous eggs in vitro. This success prompted an attempt to achieve in vitro capacitation and fertilization for Mongolian gerbil gametes in the presence of BFF; unusually high irradiation resistance, exhibited even by the early zygote, makes this rodent a subject of particular experimental interest^{4,5}.

Mature female Mongolian Gerbils were superovulated by injection of 20-40 IU PMS (Equinex, Ayerst) and 48-52 h later 10 IU HCG (APL, Ayerst). Eggs were recovered from the oviduct 13-15 h after HCG injection in the manner described by YANAGIMACHI and CHANG⁶. A sperm suspension was prepared by cutting the cauda epididymis and pressing out the spermatozoa in a watch-

glass, containing 0.5 ml of Tyrode's solution; there were $15-20 \times 10^6$ sperm cells/ml in this suspension. BFF was collected by means of a sterile syringe from large follicles in ovaries from freshly slaughtered cows. The fluid was centrifuged at 2,500 rpm ($800 \times g$) for 15 min and stored at -4°C . Before incubation BFF was heated as described

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⁶ R. YANAGIMACHI and M. C. CHANG, J. exp. Zool. 156, 361 (1964).