THE IDENTIFICATION OF GRANULES OBSERVED UNDER THE PHASE-CONTRAST MICROSCOPE OF CELLS IN TISSUE CULTURE

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Doku kültüründe Yetiştirilen Hücrelerde Faz-Kontrast Mikroskobu ile Görülen Taneciklerin Teşhisi

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Summary: The presence of some structures in the cytoplasm of normal and abnormal cells of albino rats in tissue culture were found to exist when the cells were examined under phase-contrast microscope. The cells were examined under phase-contrast microscope after they were stained vitally with Janus green. Another group of cells were also examined after being fixed with Regaud iron hematoxylin. It was concluded that some of the structures observed in the cytoplasm of the cells with phase-contrast microscope were mitochondria and the rest of them were thought to be different kinds of structures.

INTRODUCTION

The mitochondria were first observed by ALTMAN (1894) at the end of the last century and described as "Bioblasts". Later, these structures were named as "Mitochondria" by BENDA (1897). In 1900, for the first time MICHAELIS stained these structures supravitally by Janus green see (De ROBERTIS et al, 1965).
The mitochondria are cell organelles stained vitally with Janus green and are found in the cytoplasmas of protozoa of animal and of plant cells. They are also detected under phase-contrast microscope and dark-field illumination. Especially, the mitochondria of the cells in tissue culture can be seen clearly under the phase-contrast microscope and dark-field illumination (BOURNE and TEWARI, 1964; BRACHET, 1957; De ROBERTIS et al, 1965; NOVIKOFF, 1961). These structures, when fixed with specific fixatives and stained, can also be observed easily under normal light-microscope. The mitochondria show great variations in their morphology, size, amount, distribution and in their movements in the cytoplasm from cell to cell, and from tissue to tissue (BOURNE and TEWARI, 1964; BRACHET, 1957; De ROBERTIS et al, 1965; GIESE, 1968; HALL and PALMER 1969, KARLSON, 1968; LEHNINGER, 1964; MAZUR and HARROW, 1968; NOVIKOFF, 1961; ŞENGÜN, 1954, ŞENGÜN, 1967). Different shapes of these structures can be detected even in a single cell. They can exist as granules, rods, granules with clear central zones, vesicular with a central clear zone, filamentous (or thread-like) and as a net-work formed by the filaments (BOURNE and TEWARI, 1964; BRACHET, 1957; De ROBERTIS et al, 1965; GIESE, 1968; HALL and PALMER, 1969; KARLSON, 1968; LEHNINGER, 1964; MAZUR and HARROW, 1968; NOVIKOFF, 1961; ŞENGÜN, 1954; ŞENGÜN, 1967). It has been found that in a fibroblast cell one single mitochondria in an interval of 60 seconds, showed 29 different shapes (LEHNINGER, 1964).

In our laboratories, the structures observed as granules in the cytoplasms of cells, normal and abnormal cells in tissue cultures, under phase-contrast microscope were first thought to be the mitochondria. Since these structures appeared in different shapes and sizes in several experiments and especially in the cases where carcinogenic chemicals were added to the media of the cells, experiments were performed to determine whether or not they were mitochondria.

The research work, mentioned below, was carried out in order to make a decisive morphological identification of these structures with the use of classical mitochondrial stains and of phase-contrast microscope.

MATERIALS AND METHODS

In the experiments the embryonic cells in tissue culture, of albino rats (Rattus norvegicus) were used as normal cell types. The abnormal cell types in tissue culture were derived from tumors obtained by the application of croton oil (J.H. MÜLLER, Hamburg) and 7,12-Dimethylbenz (a) anthracen (Fluka AG. Buchs SG) on the skin of albino rats. The tumors appeared 8 months after the application of the carcinogenic materials. After the tumors developed well in size, they were removed from the rats under sterile conditions. The cells obtained from tumors were cultured and propagated by the use of tissue culture techniques in July 1969. Since then continuous culture of these cells are made in our laboratories. Some of these cultured tumor cells are also kept as stock cells in liquid nitrogen.
The rat embryonic cells were cultured and propagated from pregnant rats in our laboratories whenever needed.

Both the normal and abnormal cells were inoculated on sterile coverglasses in petri-dishes by the use of tissue culture technics. On each cover-glass 0.1 ml of cells, in a suspension containing approximately $5 \times 10^5$ cells/ml, were inoculated. The petri-dishes containing the cover-glasses with cells attached on them, were incubated at 37°C for 48 hours in a continuously circulating atmosphere containing 95% air and 5% CO$_2$. These cells were then employed in the experiments.

The normal embryonic cells were always used for experimental purposes 48 hours after their secondary passages. In our experiments, the tissue culture medium was Medium 199 which contained 10 % calf serum and a mixture of antibiotics.

In order to investigate both cell types vitally, Janus green (E. Merck AG, Darmstadt) was prepared at concentrations of 1/50,000 and 1/500,000 in tissue culture medium containing serum. A well type slide and the cover-glass containing the cells were mounted on "a special microscope slide chamber" (A. ÖZALPAN, unpublished data) containing the mixture of medium and Janus green. The cells were in contact with stain. They were investigated under the phase-contrast microscope vitally and their microphotographies were taken.

The cells which were fixed by Regaud fixative were stained with Regaud iron hematoxylin (GALIGHER and KOZLOFF, 1964). The coverglasses containing these cells were then mounted on microscope slides, examined under normal light microscope and their microphotographies were taken.

RESULTS

When the normal embryonic cells which were not stained vitally, were investigated under the phase-contrast microscope, the presence of large amounts of granular structures, which were mostly small granules, were observed. These granular structures were either accumulated at a specific area (such as the periphery of the nuclear membrane) or distributed over the whole cell. Especially in the cell periphery and also in a smaller amount at the central part of the cell, mostly around the nucleus the presence of rod-like or filamentous structures were also seen (Fig. 1).

When the normal embryonic cells were vitally stained with Janus green (at a concentration of 1/50,000 and 1/500,000), pale green structures identical with the classically defined mitochondria were observed. Their shape is differing from granules to long thin filaments (Fig. 2-3).

The observation obtained from abnormal cells, non-stained and stained by Janus green were similar to those of normal cells (Fig. 4-6).
In both types of cells the mitochondria appeared as small rods or granules when examined under 40 x 10 magnification. At 100 x 10 magnification, structures like thin long filaments and small granules exactly identical with the classical shapes of mitochondria were observed. Any movement of the structures were not seen at 40 x 10 magnification. At 100 x 10 magnification, the typical movements of mitochondria such as agitation and wriggling were seen. The structures were also found to be collected as a net-work around the nucleus.

In the cells which were fixed by Regaud fixative and stained with Regaud iron hematoxylin blue-black mitochondria in different shapes (identical with the classical definitions) and dispersed in the cytoplasm were observed (Fig. 7a,b).

DISCUSSION

The appearance of structures in both cell types, when stained with Janus green or with Regaud iron hematoxylin after being fixed with Regaud fixative, fits in with those of the classically defined mitochondria (BOURNE and TEWARI, 1964; BRACHET, 1957; De ROBERTIS et al, 1965; GIESE, 1968; HALL and PALMER, 1969; KARLSON, 1968; LEHNINGER, 1964; MAZUR and HARROW, 1968; NOVIKOFF, 1961; ŞENGÜN, 1954; ŞENGÜN, 1967). For this reason, we can conclude that the structures identified by both methods are mitochondria. Since the amount and the size of granules observed under the phase-contrast microscope differed from the ones stained with the above mentioned methods, we can assume that all the granular structures observed under the phase-contrast microscope are not mitochondria.

The cytological text-books indicate that in the cytoplasm besides the mitochondria, there are other round structures such as the lysosomes (COHN and FEDORKO, 1969; NOVIKOFF, 1961). Further information about these structures will be given by E. TUNCEL in another paper.

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REFERENCES


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Fig. 1: The appearance of mitochondria in vitally unstained normal embryonic cell under the phase-contrast microscope. Mag. 100 × 10.

Fig. 2: The appearance of mitochondria in the normal embryonic cell which is stained with Janus green (at a concentration of 1/50,000). Mag. 100 × 10.

Fig. 3: The appearance of mitochondria in the normal embryonic cells which is stained with Janus green (at a concentration of 1/500,000). Mag. 100 × 10.
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Fig. 4: The appearance of mitochondria in vitally unstained abnormal cell under the phase-contrast microscope. Mag. 100 x 10.

Fig. 5: The appearance of mitochondria in the abnormal cells which are stained with Janus green (at a concentration of 1:50,000). Mag. 100 x 10.

Fig. 6: The appearance of mitochondria in the abnormal cells which are stained with Janus green (at a concentration of 1:500,000). Mag. 100 x 10.
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Fig. 7: The appearance of mitochondria, after the cells were first fixed with Regaud fixative and then stained with Regaud iron hematoxylin. Mag. 100 × 10.

a — Normal embryonic cell.

b — Abnormal cell.