majority of cases the epithelial surface may be devoid of extruding cell discharges and, though some extrusions are often observed, their number is too small to suggest any significance in the matter of secretory activity, these occur on top of old worn out cells and appear to represent extrusion of old degenerating cells. In some normally feeding forms, they may be entirely absent. On the other hand, in specimens starved for long periods, cell extrusions in the form of cytoplasmic globules, separated cell tips, bursting and extruding cells may be in abundance. These results show that the various cell extrusions often observed in histological preparations do not represent secretory activity and enzyme production but represent cellular degeneration.

Zusammenfassung. Ein Indizienbeweis wird geliefert, wonach die Zellextrusionen im Mitteldarm mancher Insekten nicht (Verdauungs-) Sekrete darstellen, sondern durch Zelldegeneration bedingt sind.

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Mitochondrial Changes Induced by Diphtheria Toxin in Chicken Embryo Heart Cell Cultures

The effects induced by diphtheria toxin on the morphology and metabolism of various cell strains have been described by several authors[1–6]. Besides the cytopathic effect, Strauss[7,8] and Collier and Pappenheimer[9,10] have recently indicated that the oxidative phosphorylation is not significantly affected by diphtheria toxin in HeLa cells, while Kato et al.[4] consider this effect as manifest.

But no study has yet been made on the action of diphtheria toxin on the mitochondria of living cells, although many uncoupling substances are also well-known mitochondrial swelling agents. A study of this kind, by morphological and biochemical methods, has recently been carried out by Kadis[11] who used the murine plague toxin. This toxin induces a clear swelling in isolated mitochondria and at the same time a clear uncoupling effect on oxidative phosphorylation. The present study was undertaken to observe the mitochondrial behaviour under action of diphtheria toxin in chicken embryo heart cells cultured in vitro.

Materials and methods. Cell cultures: Primary fibroblastic cell cultures were obtained from the hearts of 6-day-old chicken embryos. The hearts were minced finely with scissors and washed twice in 100 ml of Hanks balanced salt solution (BSS) and the cells dispersed by trypsinization. The dispersed cells were centrifuged in a conical graduated tube at 500 rpm for 10 min, suspended in 10 ml of outgrowth medium consisting of 10% inactivated calf serum and 0.5% lactalbumin hydrolysate in Hanks BSS, and centrifuged again as described above. A 1:200 dilution of the cell pack was prepared in the outgrowth medium. This suspension was then planted in Leighton tubes containing a cover glass in a volume of 2 ml and incubated at 37 °C.

Diphtheria toxin: Crude lyophylized diphtheria toxin (Sclavo)[12] containing 60 Lf/ml and 13 DLM/Lf was used. The diphtheria toxin was diluted with Hanks BSS to obtain the following concentrations: 0.6, 1.2, 6.0, and 12.0 Lf/ml. The final pH of toxin dilutions, adjusted with $NaHCO_3$ 1.4%, was 7.4.

Experiments. After 20 h of incubation the outgrowth medium was eliminated from the Leighton tubes, the cell culture was washed twice with Hanks BSS (previously heated to 37 °C) and the medium was substituted with diphtheria toxin dilutions. After 15, 30, 60, and 120 min from toxin inoculum, the cover glasses were extracted from the Leighton tubes, placed in a perfusion chamber containing the same toxin dilution and observed under a phase contrast microscope (Leitz Ortholux with a × 70 fluorite immersion objective). Some specimens were fixed in glutaraldehyde (Fluka) 0.25% in phosphate buffer (pH 7.4), dehydrated with ethyl alcohol and stained with uranil acetate (0.95% in ethyl alcohol) and with phosphotungstic acid (1% in absolute ethyl alcohol)[12]. These permanent specimens were also observed

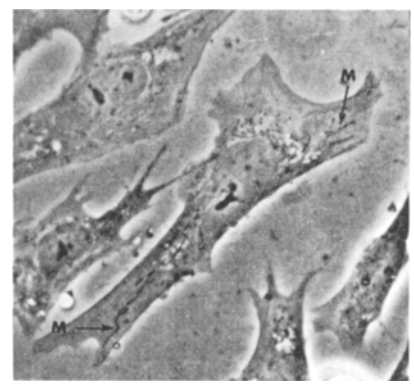

Fig. 1. A normal chicken embryo heart cell. M, mitochondria. Phase contrast microscope. × 1000.

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under phase contrast microscope. In the control cultures the medium was substituted with 2 ml of Hanks BSS at the same temperature and pH but without diphtheria toxin.

Results. The lower diphtheria toxin concentrations (0.6 Lf/ml) were unable to produce any effect on the cells.

The cells treated for 15 min with 1.2 Lf/ml toxin concentration show a mitochondrial 'thickening' (Figure 2). The mitochondria, on the other hand, do not show any difference of optical density in their structure. With this toxin concentration, the first generalized cellular injury develops only after 120 min after toxin inoculum as a vacuolar degeneration. The higher concentrations of toxin (6.0 and 12.0) have a similar action but their swelling effect is more evident, with clear differences in optical density of swollen mitochondria. Owing to these dilutions, the 'thickening' and swelling effect is already completed 15 min after toxin inoculum. Further observations (30, 60, and 120 min) do not show an increasing swelling effect.

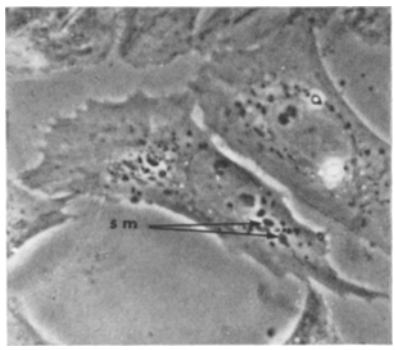

Fig. 2. A chicken embryo heart cell 15 min after the inoculum with diphtheria toxin (1.2 Lf/ml). Note the 'thickened' mitochondria (sm). Phase contrast microscope. × 1000.

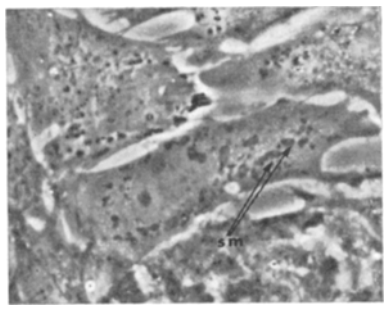

Fig. 3. A chicken embryo heart cell 30 min after inoculum with diphtheria toxin (6.0 Lf/ml). The swollen mitochondria (with clear differences in their optical density) are evident (sm, swollen mitochondria). Phase contrast microscope. × 1000.

Discussion. The earlier effect of diphtheria toxin on chicken embryo heart cells cultured in vitro is a mitochondrial swelling. It is complete 15 min after toxin inoculum: this effect is never common either to all cells or to all mitochondria of the same cell. The swelling, once evident, does not develop any further with time: it is the same, for each toxin concentration, at 15 min as at 2 h. The mitochondrial swelling is, within certain limits, sensitive to toxin concentrations: it is highest for 6.0 and 12.0 Lf/ml toxin concentrations, without differences between these conditions. The cytopathic effect, which appears after 1 h with higher toxin concentrations, is comparable to that obtained by other authors[3] in the HeLa cells strain.

The mitochondrial swelling can be related to a metabolic lesion. This phenomenon is now under study from the morphological and biochemical viewpoint.

Riassunto. L'autore ha preso in considerazione l'effetto della tossina difterica in varie concentrazioni sulle cellule di cuore di embrione di pollo coltivate in vitro. L'effetto saliente consiste in un effetto rigonfiante, assai precoce, sui mitocondri. Ogni altro effetto citopatico è più tardivo. L'autore prospetta l'ipotesi che il rigonfiamento mitocondriale sia correlato a lesione biochimica del metabolismo energetico.

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Clinica delle Malattie Infettive dell'Università, Napoli (Italy), January 17, 1966.

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Histochemical Studies of Nucleolus and Nucleolar Extrusions in Insect Oogenesis

Nucleolar extrusions in the cytoplasm have now been observed in different types of cells of widely different groups of animals, particularly in oogenesis, and mostly with light microscopy[1,2]. Recent electron microscope studies in oogenesis, combined with cytochemical studies of oocyte organelles with light microscopy, have also made valuable contributions to our understanding of the fine structure and the chemical composition of the emissions, and their role in yolk formation[3,4].

Indeed, students of electron microscopy have shown the actual pathways in the nuclear membrane through which mutual exchange of material takes place between the nucleus and the cytoplasm (see review by WISCHNITZER[5]).

With the discoveries of CASPERSSON[6] and BRACHET[7], by two entirely different techniques, that the nucleolus is the site of RNA in different types of cells and that RNA

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