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.

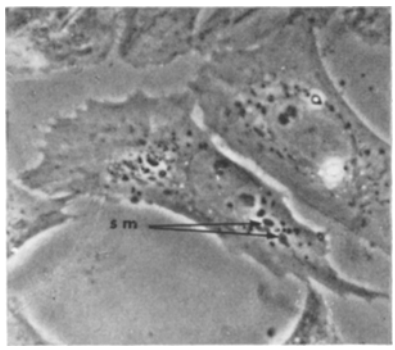

Fig. 2. A chicken embryo heart cell 15 min after the inoculum with diphtheria toxin (1.2 Lf/ml). Note the 'thickened' mitochondria (sm). Phase contrast microscope. × 1000.

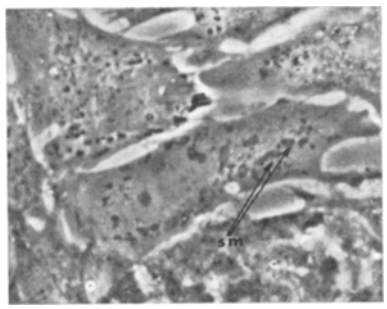

Fig. 3. A chicken embryo heart cell 30 min after inoculum with diphtheria toxin (6.0 Lf/ml). The swollen mitochondria (with clear differences in their optical density) are evident (sm, swollen mitochondria). Phase contrast microscope. × 1000.

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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plays an important part in protein synthesis, the nucleolus and the nucleolar emissions have gained considerable importance. The present study covers the histochemical studies of nucleolus and nucleolar extrusions in the egg cells of *Periplaneta americana*, *Acheta domesticus*, *Locusta migratoria*, *Chrotogonus* sp. and *Dysdercus cingulatus*. The ovary of the first four species is of the panoistic type, while that of the last is of the telotrophic type. Methyl green/pyronin G technique[8] was employed for RNA detection; and Sudan black acetone[9] and bromphenol blue[10] techniques were employed for bound lipids and proteins respectively.

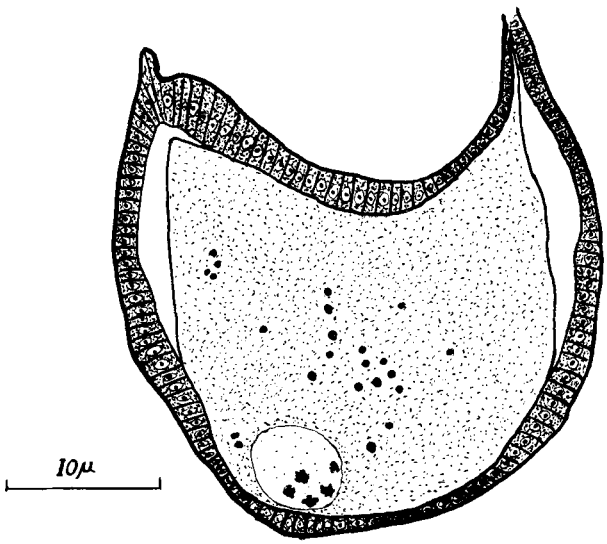

Fig. 1. L.S. of oocyte of *Dysdercus cingulatus*, showing nucleoli in the nucleus and nucleolar extrusions in ooplasm by Methyl green pyronin G technique.

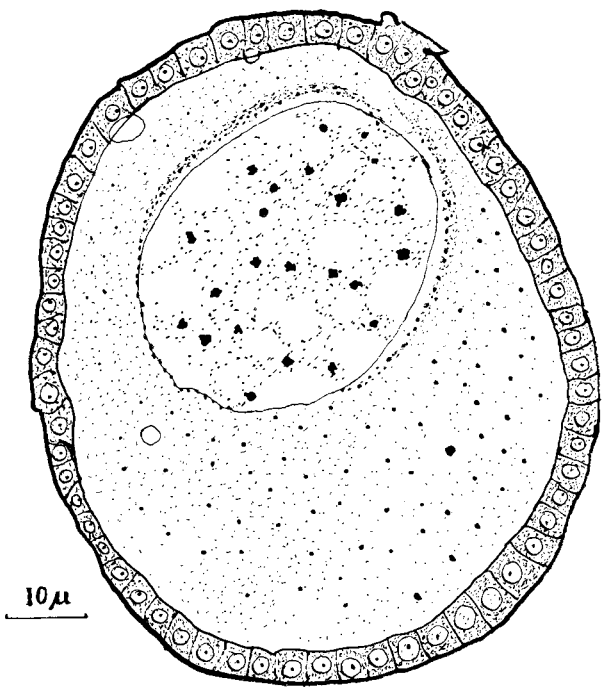

Fig. 2. T.S. of oocyte of *Locusta migratoria*, showing perinuclear ring of nucleolar extrusions by acetone Sudan black technique.

In *Periplaneta americana*[11,12] and *Dysdercus cingulatus*[13] the early oocyte contains a big rounded nucleolus in the nucleus with very little cytoplasm. During prophase the single nucleolus divides into secondary nucleoli and then tertiary nucleoli, the latter come out in the ooplasm through the nuclear membrane. The nucleolar extrusions are present throughout the nucleoplasm and ooplasm in early oocytes (Figure 1), later they move to the periphery of the ooplasm. The nucleolus and nucleolar extrusions in early oocytes are strongly positive for proteins and RNA, but do not contain bound lipids. Later on in the growth phase, bound lipids also develop in the nucleolus and nucleolar extrusions. In *P. americana* and *L. migratoria* the nucleolus in early stages is homogeneous but later becomes vacuolated, while in *D. cingulatus* and *Chrotogonus* sp. it stains uniformly.

In the other three species, viz. *Acheta domesticus*, *Chrotogonus* sp. and *Locusta migratoria*, a perinuclear ring of fine nucleolar extrusions is formed (Figure 2). The nucleolus and the fine granules of the perinuclear ring are positive for RNA, proteins, and bound lipids.

The nucleoplasm in the animals studied is moderately positive for proteins. In *P. americana* and *D. cingulatus* (Figure 1), the nucleoplasm reacts negatively for RNA and bound lipids while in the remaining three species, viz. *A. domesticus*, *L. migratoria* (Figure 2), and *Chrotogonus*, the nucleoplasm is positive for RNA and bound lipids. The ooplasm reacts positively for proteins in all the five species, but negatively for bound lipids except in *Chrotogonus* sp. The contents of the ooplasm in early stages of all the five species are rich in RNA, except in *P. americana* in which RNA develops later. During vitellogenesis the RNA content of the nucleolar extrusions decreases.

There is clear evidence that messenger RNA comes out from the nucleus to the cytoplasm in the form of nucleolar extrusions[14]. The extrusions may be in the form of big granules as in *Periplaneta* and *Dysdercus* (cf. *Lithobius*[2] and *Saccocirrus*[15]), or very fine granules as in *Acheta* and *Chrotogonus* (cf. Crayfish[3], *Palaemon* and *Paratelphusa*[16]). In *A. domesticus* and *Chrotogonus* sp. the nucleolar extrusions of the perinuclear ring do not migrate into the general ooplasm but just disappear in situ; nevertheless, the general ooplasm is strongly pyroninophil.

Zusammenfassung. Kernausstossungen in der Oozyte von *Periplaneta americana*, *Dysdercus cingulatus* und *Locusta migratoria* sind grobkörnig, während sie in *Acheta domesticus* und *Chrotogonus* sp. besonders fein sind. Kern und Kernausstossungen erweisen sich für RNS, Proteine und Lipoproteine positiv.

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Department of Zoology, Panjab University, Chandigarh (India), December 10, 1965.

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[17] Acknowledgment: I am grateful to Prof. Vishwa Nath, Panjab University, for his direction and supervision of this work.