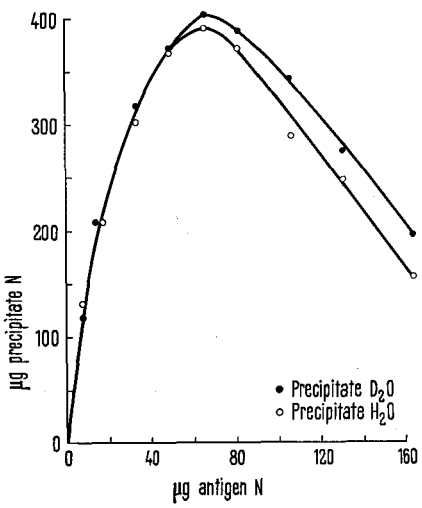
ordinary water[3]. Non-specific aggregation of antibody globulin in heavy water did not appear to contribute to this increased antigen excess zone precipitation. It was previously demonstrated by MORTON[4] that 7S globulin aggregated by X-irradiation, and likewise native macroglobulin, manifested increased UV absorption and an increased 250/280 optical density ratio when compared to equal weights of native 7S material. In the present experiment, comparable alterations in UV spectra were not observed, suggesting minimal, if any, aggregation.



Formation of immunological precipitates in buffered systems containing H_2O or 50% D_2O.

The partial replacement of amide, plus side chain N–H, O–H, and S–H hydrogens of normal antibody by deuterium as described here would be analogous only in part to the molecule formed in the deuterated animal, since in the latter a substantial proportion of non-exchangeable hydrogens would also be replaced as a result of biosynthetic processes[2]. Thus the possibility remains that the antibody molecule as synthesized in the deuterated animal may possess altered chemical and biological properties[5].

Zusammenfassung. Wenn die Lösung zu 50% aus schwerem Wasser bestand, blieb die Bildung von Antigen-Antikörperpräcipitat im Bereich des Antikörperüberschusses unverändert. Dies zeigt, dass die Substitution mit Deuterium die spezifischen Bindungseigenschaften nicht verändert. Dagegen fand sich eine erhöhte Niederschlagsmenge im Bereich des Antigenüberschusses, was auf eine Herabsetzung der Löslichkeit des normalerweise löslichen Antigen-Antikörperkomplexes hinweist.

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Department of Pathology, University of Oregon Medical School, Portland (Oregon, USA), January 4, 1966.

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On the Digestive Enzymes and the Cytology of Midgut of *Leogryllus bimaculatus* Sauss.

The various cell extrusions, often observed along midgut epithelia of insects as cytoplasmic globules, separated cell tips, bursting and extruding cells, some or other of which have been regarded by a large number of previous workers, such as VAN GEHUCHTEN[1], GRESSON[2], HODGE[3] and SAKSENA[4], to be evidence of secretory activity, have been considered by some other workers, such as HENSON[5], WOODRUFF[6], DAY and POWNING[7] and KHAN and FORD[8], to represent cellular degeneration. The present author[9–11], on the basis of his investigation on structure and activity of the epithelial cells in midgut and hepatic caeca of certain insects under normal and induced pathogenic conditions, had also earlier asserted that these cell extrusions appear to represent cellular degeneration.

The present investigations have been carried out to see if there is any relationship between the production of digestive enzymes and extrusion of the so-called secretory vesicles etc. in midgut of *Leogryllus bimaculatus* Sauss.

Normally feeding insects and specimens starved for long periods, from a stock of *Leogryllus bimaculatus* Sauss. maintained in the laboratory on a diet of bread, liver and sugar, were dissected, part of their midgut was fixed by Yao-Nan and Mann-Kopsch techniques and 4–5 μ thick sections were obtained, while the rest of the parts of midgut were employed for qualitative enzyme estimations.

Experiments conducted to determine the enzymes present in normal feeding specimens reveal the presence of amylase (Potassium iodide-iodine test), invertase (Barfoed test), maltase (Barfoed test), lipase (bromo-thymol blue and milk test), lactase (Barfoed test) and protease (egg albumin test). These enzymes show gradually weaker reactions in starved specimens and are entirely absent in specimens starved for long periods. Histological preparations from normally feeding specimens show that in the

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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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Zoology Department, Lucknow University (India), December 31, 1965.

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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

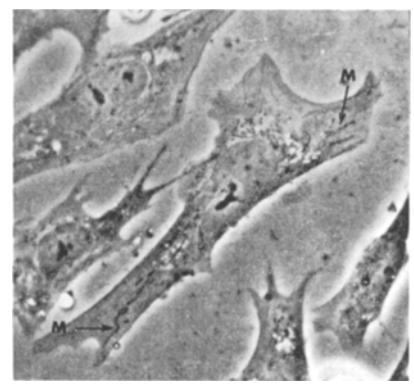

Fig. 1. A normal chicken embryo heart cell. M, mitochondria. Phase contrast microscope. × 1000.

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