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Changes in the fine structure of the gut epithelium of Pieris brassicae induced by the δ-endotoxin of Bacillus thuringiensis

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The morphological changes induced by the δ-endotoxin of *Bacillus thuringiensis* BERL. in the midgut epithelium of *Pieris brassicae* L. were investigated by electron microscopy. Micrographs taken a few minutes after administration of the δ-endotoxin showed the beginning of a vigorous disrupture of the fine structure of the epithelial cells. The subcellular components such as microvilli and mitochondria undergo rapid swelling. The content of the mitochondria seems to be dissolved during the continuous enlargement leaving finally spherical cavities. The fine and regular network of the endoplasmatic reticulum disintegrates by forming vacuoles of various sizes in the cytoplasm. The connections between the epithelial cells are loosened by the separation of the cell membranes.

Preparations based on *Bacillus thuringiensis* BERL. are considered as ideal insecticides for the control of several important pest insects belonging to the order *Lepidoptera*. The crystalline δ-endotoxin which is produced during the sporulation process of the *Bacillus* is responsible for the insecticidal activity whereas the spore plays only a secondary role by invading the hemocoel of the insect after destruction of the gut epithelium by the δ-endotoxin.

The mode of action of the δ-endotoxin which is a pure protein is still unknown although a number of different hypotheses have been presented. The first symptoms of intoxication appear a few minutes following ingestion of the protein crystals which are also called parasporal bodies. The larvae stop feeding and their movements are greatly reduced. There is general agreement that the gut epithelium is the primary site of action since this tissue undergoes destruction usually in less than an hour if a lethal dose of δ-endotoxin has been consumed by the insect. In highly susceptible larvae the first morphological changes occur even within 10–20 min as shown by ANGUS (1970) in a light microscope study using larvae of *Bombyx mori* L. The epithelial cells of the intestine are rapidly enlarged and simultaneously they form bubble-like protrusions which enventually burst. Despite a heavily damaged gut epithelium and general paralysis the larvae can remain alive for a prolonged period of time before death occurs.

This study was performed to reveal morphological changes of subcellular components in the presence of the δ-endotoxin. Investigations by electron microscopy alone will probably not result in an ultimate answer of the mode of action but any findings based on physiological and biochemical studies must be supported by the corresponding morphological events during intoxication.

MATERIALS AND METHODS

Organisms

The δ-endotoxin was derived from a strain of *B. thuringiensis* var. *thuringiensis* (LBG B 4412). The bacteria were grown in a semisynthetic medium (YOUSTEN & ROGOFF, 1969) on a rotary shaker at 30 °C. 500-ml Erlenmeyer flasks with 100 ml of substrate were used as culture vessels. After completion of the sporulation process, spores and crystals were harvested by centrifugation and washed three times with distilled water. Subsequently, the crystals were separated from the spores by a method described by DELAFIELD *et al.* (1968).

The investigations were carried out with midgut epithelia of fifth instar larvae of *Pieris brassicae* L. This insect is very susceptible to the above mentioned strain of *B. thuringiensis*. Each larva received a purified crystal suspension of 0.01 ml containing 1.10^6 parasporal bodies. Controls were conducted by force-feeding 0.01 ml of distilled water to the larvae.

Fixation and embedding of the midgut epithelium

Fixation was carried out with 3% glutaraldehyde in 0.05 M cacodylate buffer with a final osmolarity of 400 mMos. The fixative was administrated directly into the gut as well as into the hemocoel of the insects. Before dissection, the larvae were placed for 1 h into a solution of fixative. Disection was carried out in the fixative and the midgut epithelium was cut into small pieces after careful removal of the tube-like peritrophic membrane containing the food particles. The specimens were then transferred for 12 h into fresh fixative and postfixed with 1% osmium tetroxide for 4 h at 4 °C. Dehydration was carried out according to a method proposed by MULLER & JACKS (1975) using 2,2-dimethoxypropane. The specimens were embedded in araldite/epon and polymerized for 3 days at 60 °C. Thin sections of 500–800 Å were cut with a LKB Ultratom III and placed on Formvar-coated 200 mesh copper grids. The sections were stained with uranyl acetate and lead citrate (REYNOLDS, 1963) and examined with a Philips electron microscope at 100 kV.

RESULTS

The midgut epithelium is composed of two different cell types, namely columnar and goblet cells which are shown as thin sections in fig. 1 and fig. 2. The whole epithelium is covered by a dense brush border of microvilli originating from the columnar cells. Microvilli also occur in the cavities of the goblet cells. The longitudinal as well as the cross sections (insets fig. 1 and fig. 2) reveal distinct differences in the morphology of the microvilli of the two cell types. In addition, microvilli of goblet cells frequently contain mitochondria.

The mitochondria and the endoplasmatic reticulum are the other organelles which have been investigated (fig. 1 and fig. 2). The mitochondria are easily recognized as deeply stained bodies which are as a rule of elongated shape. Inside the mitochondria the fine structure of the cristae are visible. The endoplasmatic reticulum forms a fine and dense network within the cytoplasm. It has to be added

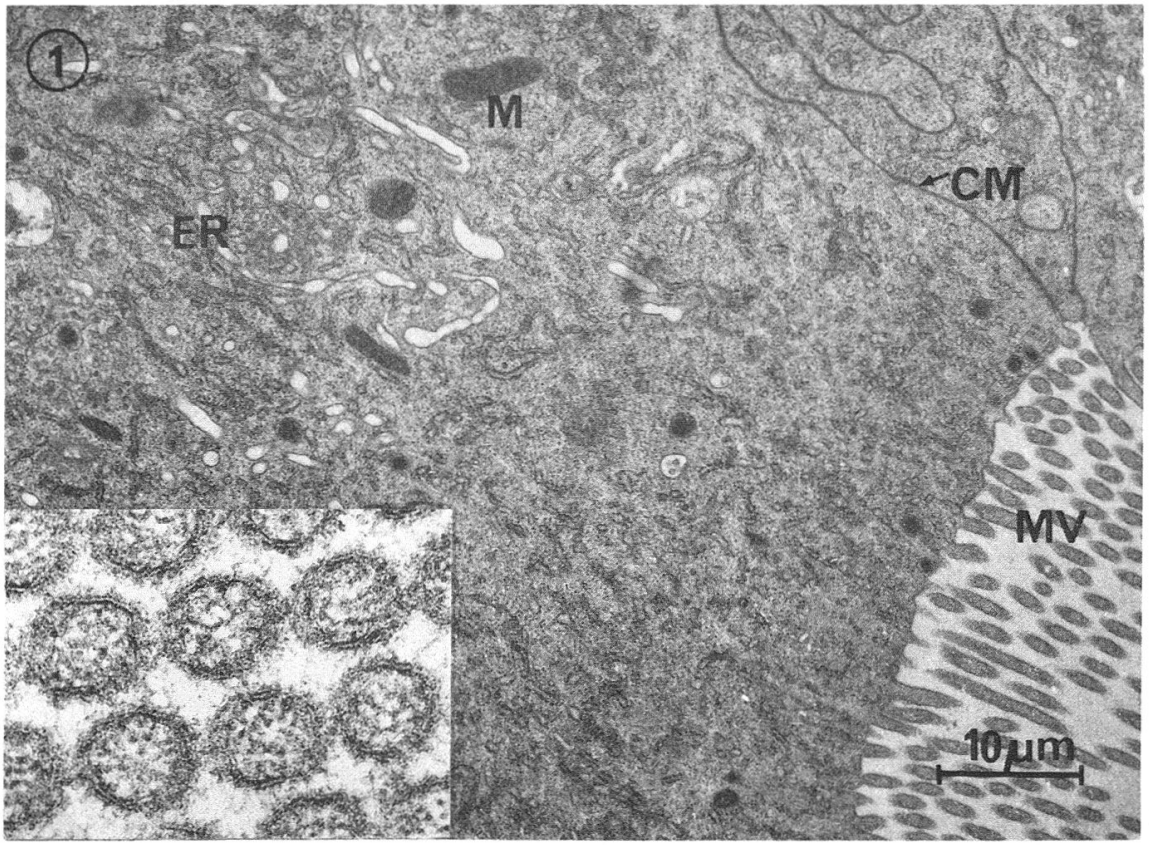

Fig. 1: Section out of an untreated columnar cell of the gut epithelium of *Pieris brassicae*. Visible as organelles are microvilli (MV), mitochondria (M), endoplasmatic reticulum (ER) and the cell membrane (CM). Inset: cross section of microvilli.

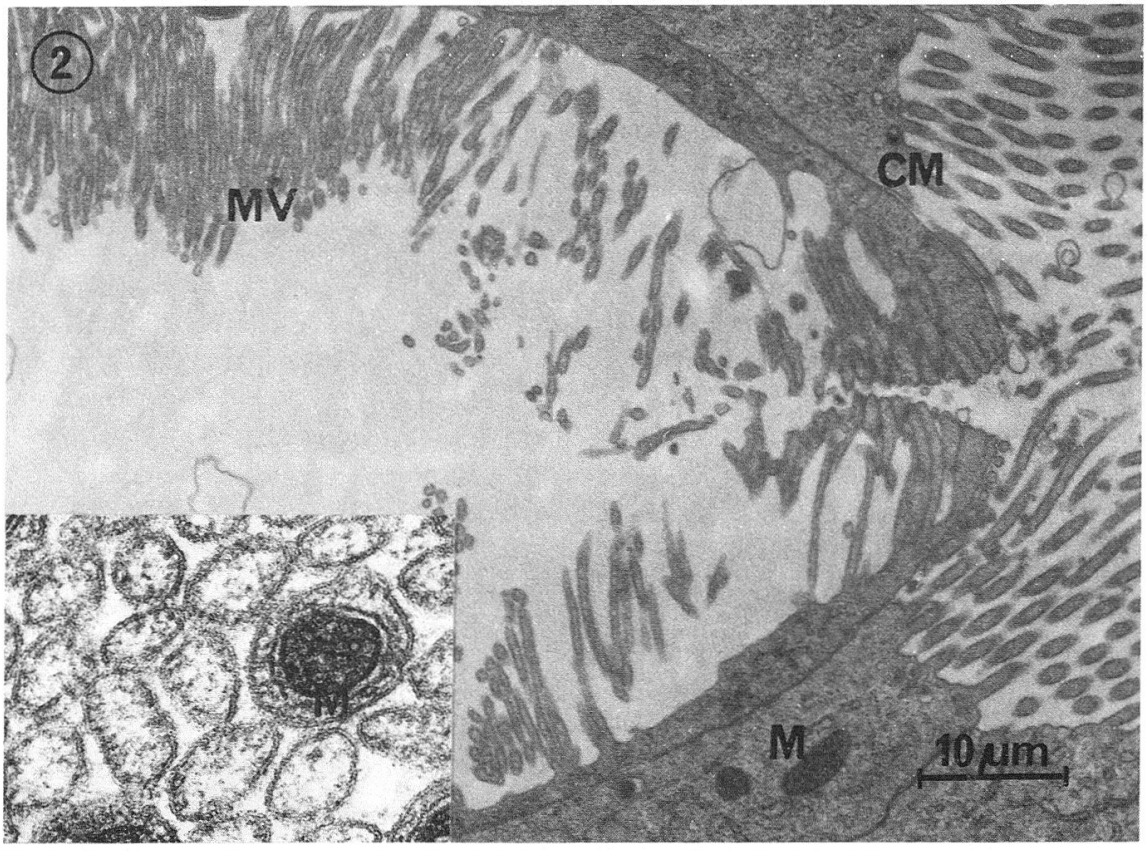

Fig. 2: Section out of an untreated goblet cell. Inset: cross section of microvilli.

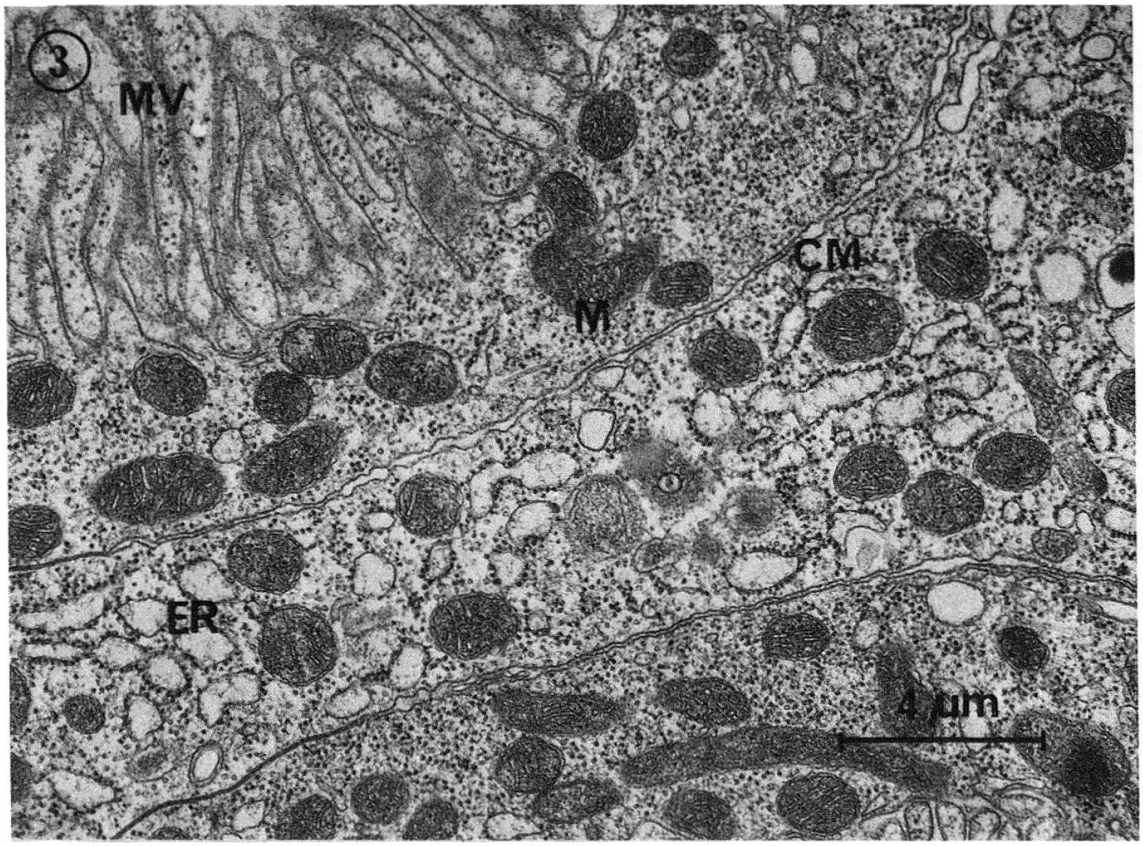

Fig. 3: General view of the morphological changes induced by the δ-endotoxin after an exposure time of 12 min.

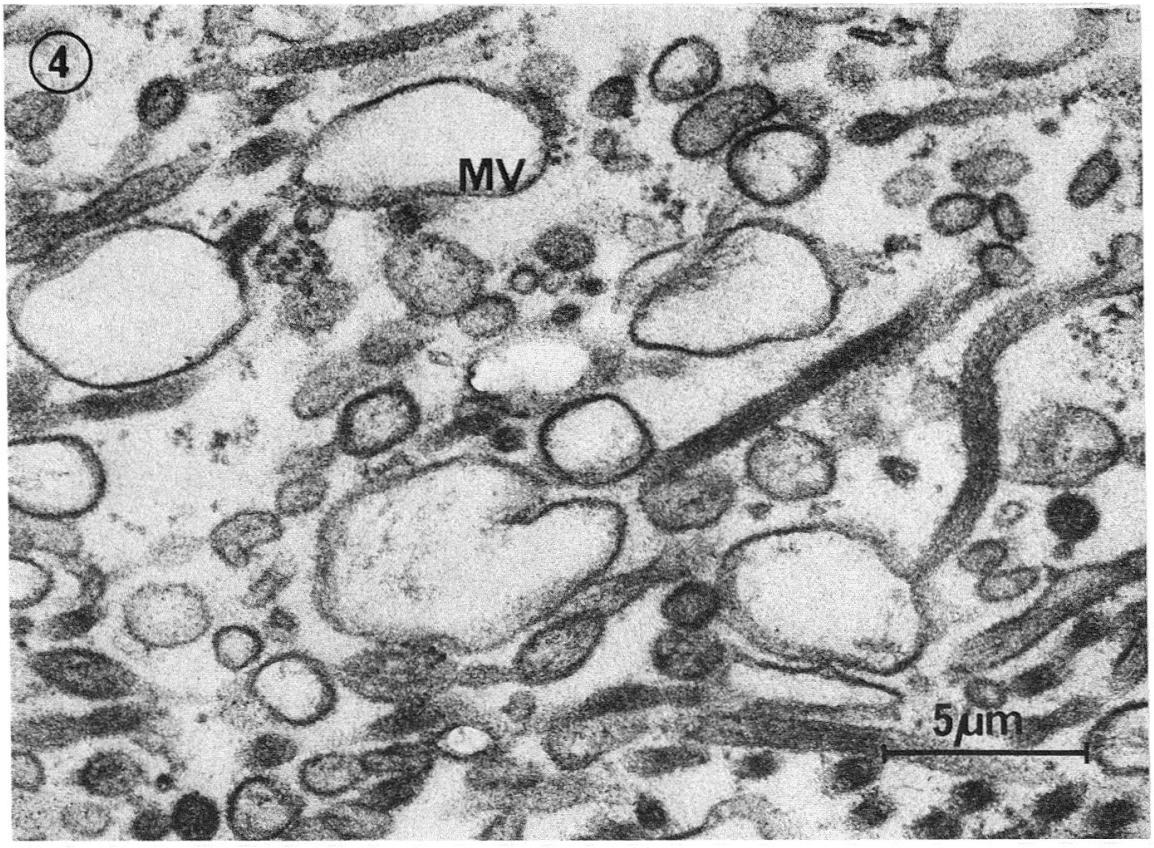

Fig. 4: Microvilli (MV) of a columnar cell after 12 min exposure to the δ-endotoxin.

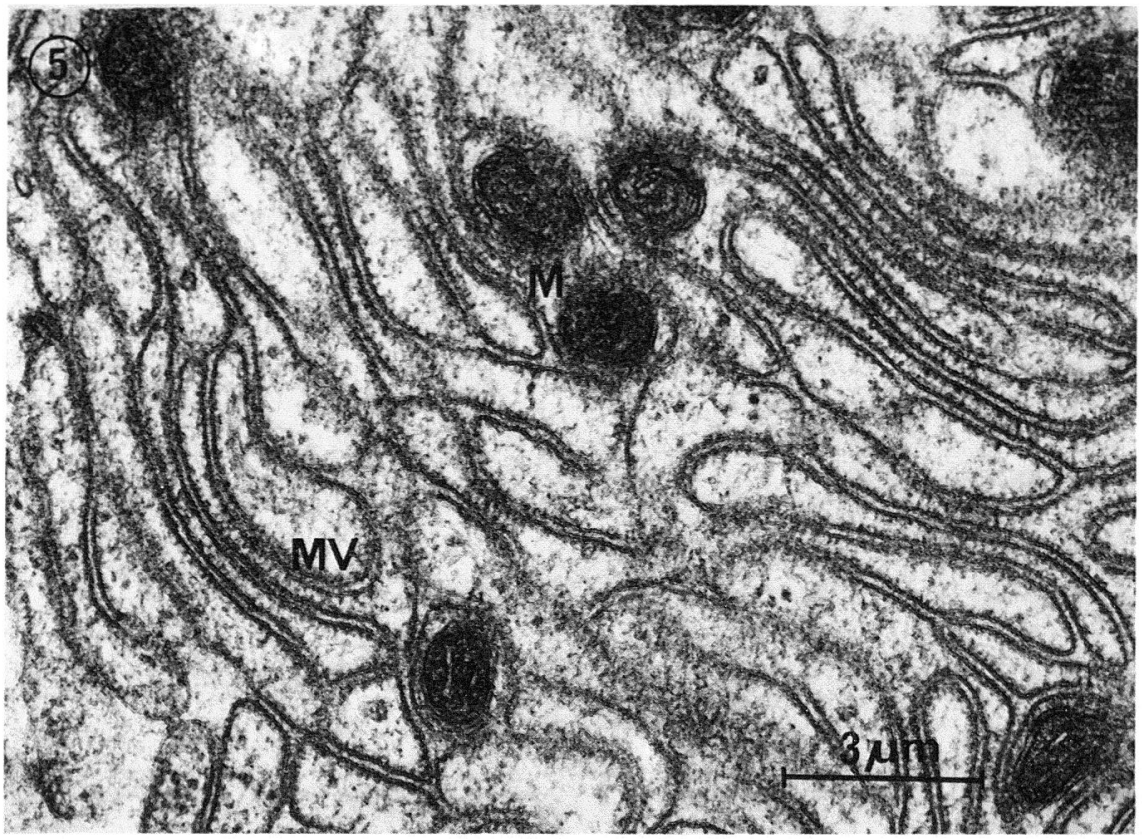

Fig. 5: Microvilli (MV) of a goblet cell, exposed for 8 min to the δ-endotoxin.

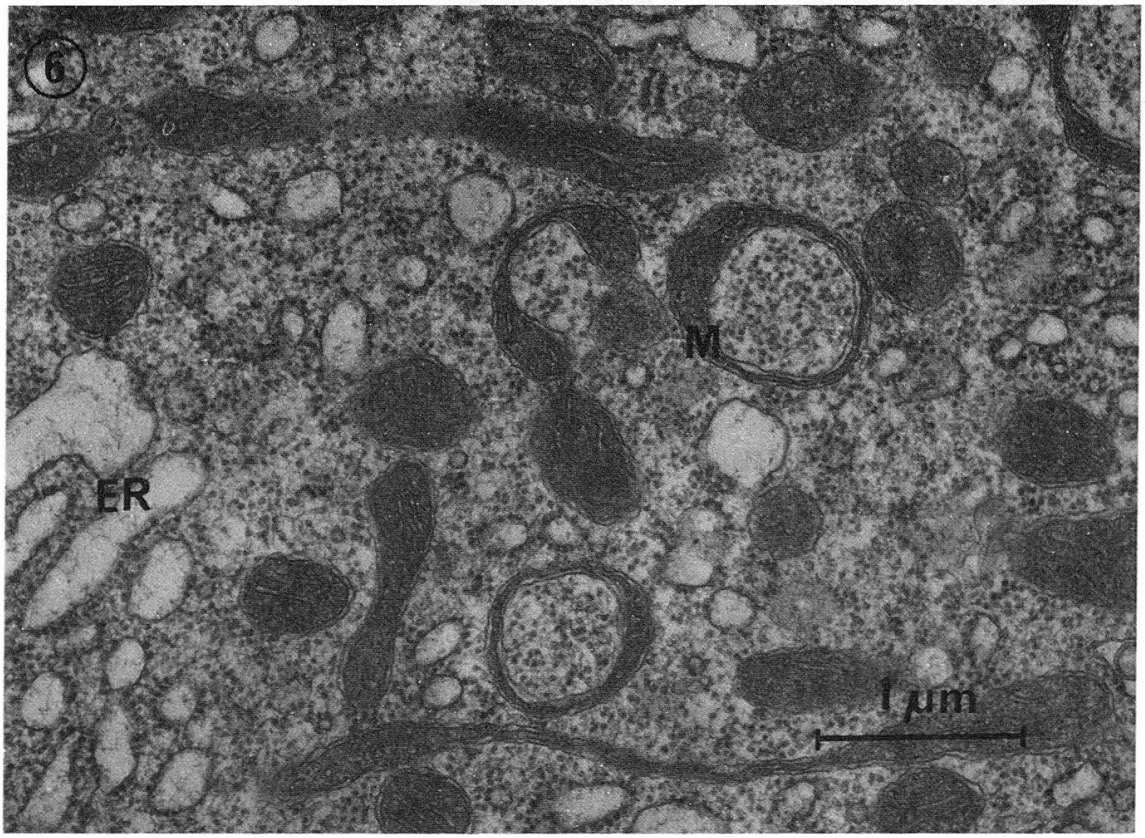

Fig. 6: Mitochondria exposed to the δ-endotoxin for 10 min.

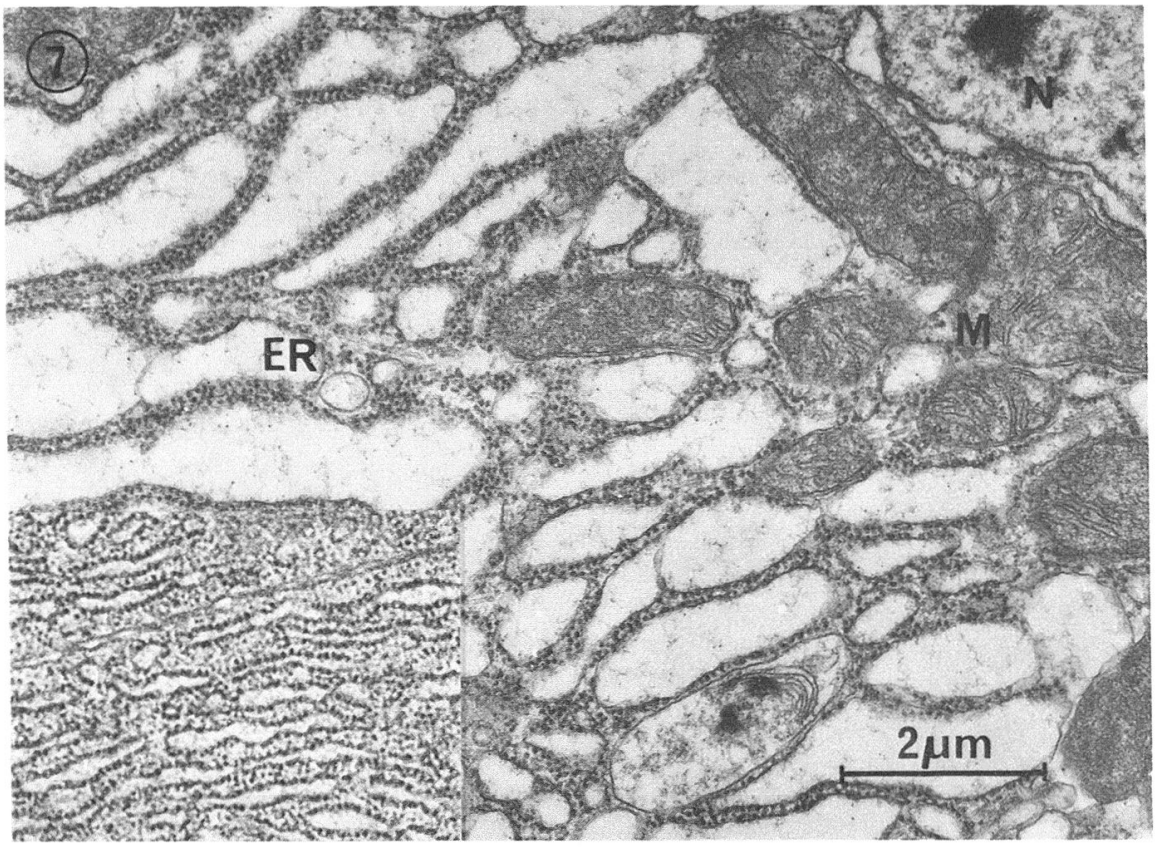

Fig. 7: Endoplasmatic reticulum (ER) following a 12 min exposure to the δ-endotoxin. Inset: endoplasmatic reticulum of a healthy cell. Ribosomes are visible as deeply stained points.

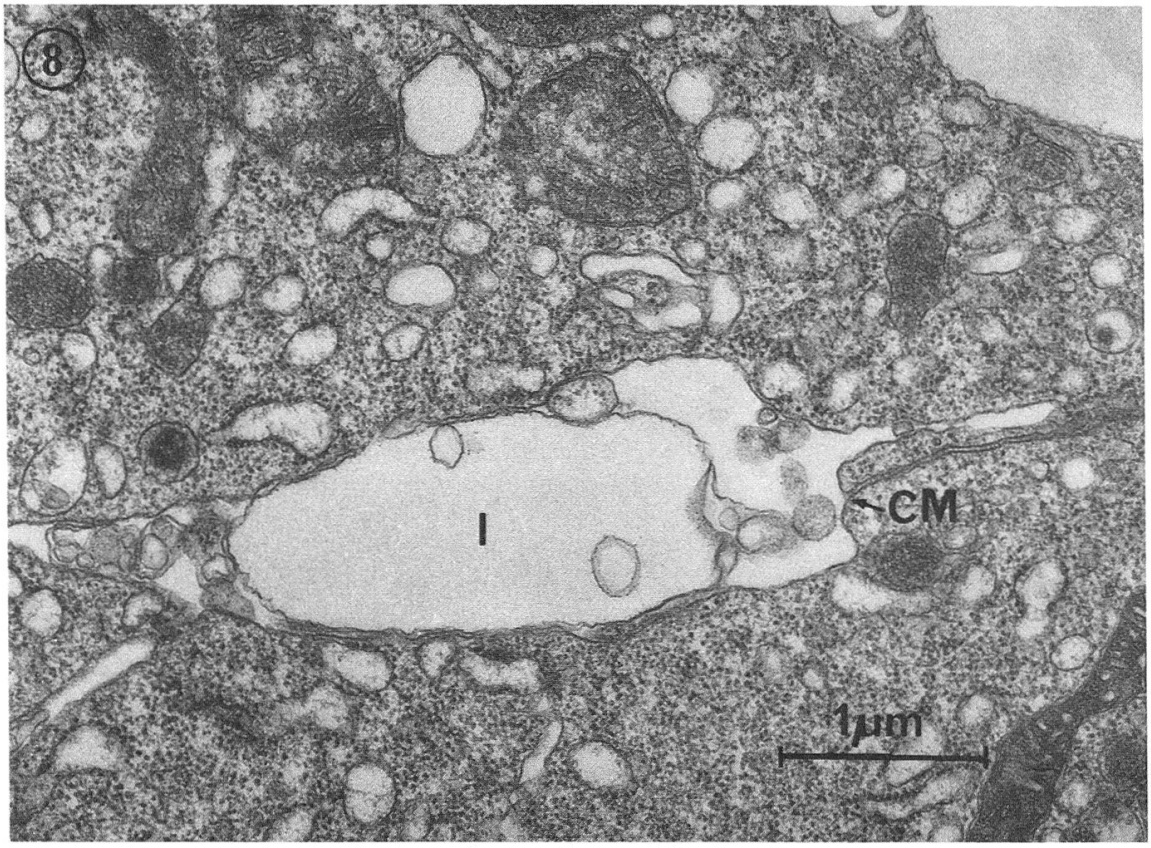

Fig. 8: Separation of cell membranes (CM) and formation of interspace (I) 12 min after administration of δ-endotoxin to the insect.

that the distribution of the cell organelles is not uniform. The number of mitochondria and the amount of endoplasmatic reticulum is decreasing towards the lumen of the gut.

A general view of the destruction caused by the δ-endotoxin is given in fig. 3. This gut tissue was in contact with parasporal protein for a period of 12 min. Under the influence of the δ-endotoxin a rapid swelling of the epithelial cells occurred which was accompanied by the development of protrusions growing out towards the lumen of the gut. Columnar and goblet cells are likewise affected by the δ-endotoxin.

Changes in the structure of the microvilli can be observed as early as 5 min following administration of protein crystals to the larvae. They show irregular swelling and distortion that leads to rupture in a later stage. Toxin treated microvilli of a columnar and of a goblet cell are shown in fig. 4 and fig. 5.

Mitochondria do not exhibit a uniform morphological alteration in the presence of δ-endotoxin. They increase in size either by adopting a spherical shape or by elongation of the whole structure (fig. 6). As a rule the cristae are disintegrated and then dissolved which leaves an empty shell that continues to grow up to the point of rupture.

The changes which are caused at the endoplasmatic reticulum are presented in fig. 7. The development of vacuole-like bodies can be observed which steadily increase in volume. It may be assumed that the formation of these structures is a result of the disrupture of the connecting sites of the membranes. The same reaction seems to take place between the cell membranes which separate from each by forming empty interspaces (fig. 8).

The nucleus is the only structure of the epithelial cells which is not dramatically altered by the δ-endotoxin. Only prolonged exposure to parasporal protein seems to induce some contraction of the nuclear material.

DISCUSSION

The obtained results clearly show the rapid and vigorous destruction of the midgut epithelium of a susceptible insect species. With the exception of the nucleus, all subcellular components which were under investigation such as microvilli, mitochondria and endoplasmatic reticulum undergo destruction. It is of interest to notice that the morphological changes of the different organelles resemble each other. A general increase in size is followed by the loss of fine structure leading ultimately to complete dissolution of the contents and leaving only the membraneous shells.

It has been shown that the protein crystals are dissolved by the gut juice proteases yielding an active peptide with a molecular weight of around 100000 (TRUEMPI, 1976). The first symptom of intoxication is the blockage of the uptake of food as early as 2 min following administration of parasporal bodies. According to our results it may be assumed that the feeding inhibition is caused as soon as the swelling of the epithelium is initiated bringing about a feeling of satiety for the insect larva. The increase in volume of the gut epithelium must very likely be induced as soon as the first peptides have been cleaved from the parasporal bodies by the proteases.

The rapid action and the total disrupture of the cellular fine structure favor a hypothesis based on the interaction of the δ-endotoxin with the membrane of the epithelial cells. The breakdown of the permeability control could easily lead to the general destruction of the epithelial cells caused by the high digestive power and by the high concentration of cations of the gut juice (LUETHY, 1973). Some evidence that the δ-endotoxin acts on the cell membrane has recently been presented by FAST *et al.* (1976). δ-Endotoxin bound to Sephadex beads which are too big to be engolfed by the cells was able to damage tissue culture cells of *Choristoneura fumiferana.* The authors measured the damage by reduction of the ATP content of the whole cultures. In contrast to this hypothesis, TRAVERS *et al.* (1976) showed a direct effect of the δ-endotoxin on mitochondria where the δ-endotoxin stimulated the oxygen uptake and inhibited at the same time the ATP production.

The dose of 1.10^6 protein crystals which was administrated to each larva is rather high since the LD_{50} for fifth instar larvae of *P. brassicae* lays in the range of 5.10^4 crystals. In future experiments a reduction of the dose and shorter time intervals between administration of the δ-endotoxin and fixation of the epithelium are planned in order to obtain a clear sequence in the whole process of destruction.

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