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THE SELECTIVE PERMEABILITY OF FLESHFLY MIDGUT TO AN ORALLY TOXIC COBRA VENOM CARDIOTOXIN

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SUMMARY

The permeability of the midgut of the fleshfly, *Sarcophaga falculata*, to the orally toxic polypeptide ($M_r \sim 7000$), a cobra venom-cardiotoxin, was investigated by LM and EM autoradiography, using the radio-iodinated toxin. The histology of the normal and toxin affected midgut was also investigated.

1. The midgut could be classified into segments that were permeable, partially-permeable and non-permeable to the toxin.

2. Histological comparison between the epithelial cells of the permeable and non-permeable segments revealed strong differences in the form of the cells, and in the distribution, form and organization of their microvilli, organelles and basal foldings.

3. The movement of the toxin in the permeable region of the gut is progressive and includes the crossing of the peritrophic membrane, penetration into the apical region of the cell through the microvilli (5 min), movement in the cell's cytoplasm and finally the passage of the midgut as shown by its appearance in extraintestinal tissues (30 min). The data strongly suggest that the crossing of the midgut by cardiotoxin did not follow the common pattern of pinocytotic uptake and vesicular transport.

4. When applied orally in superlethal doses (10 LD_{50} units per fly), cardiotoxin strongly affected the integrity of the epithelial cells in the toxin permeable segment, as expressed in their collapse, rupture and final distintegration. The toxin had no histopathological effect on any other region of the midgut.

These data are interpreted in terms of a postulated specific composition and arrangement of phospholipids in the outer plasma membranes of the epithelial cells in the cardiotoxin-permeable segment of the midgut.

INTRODUCTION

Several scorpion and cobra snake venoms are paralytic and lethal when orally introduced to *Sarcophaga falculata* fleshflies (Primor & Zlotkin, 1978). The oral toxicity of the venom of the South African cobra *Naja mossambica* has been attributed to a specific group of low molecular weight (~7000) basic proteins defined

Key words: Insect gut permeability, cardiotoxin, fleshfly.

as cardiotoxins (Primor & Zlotkin, 1978). With the aid of a radioiodinated cardiotoxin (D_5) and assays of competitive displacement, it has been shown that the oral toxicity of cardiotoxin is a consequence of its ability to cross the flies' digestive system and to bind to target tissues in their body (Primor, Teitelbaum & Zlotkin, 1980).

For a protein to pass through the gut of a fly, it has to overcome several difficulties. Firstly, it has to resist a wide and rich variety of proteolytic enzymes shown to exist in the gut of a fleshfly (Capps *et al.* 1972; House, 1974; Sinha, 1976). Secondly it has to cross through the multilayered and relatively thick peritrophic membrane present in fleshflies (Naponitaya & Misch, 1974), which is supposedly impermeable to large molecules present in the gut lumen (Zhuzhikov, 1964). Lastly it has to pass through the continuous layer of epithelial gut cells (possessing outer and inner plasma membranes) and the basal membrane (Richards, 1975).

The penetration of a toxic protein such as cobra venom cardiotoxin into an insect gut may be attained either by anatomical damage to the gut or by a more specific non-destructive pathway. The 'destructive' pathway hypothesis is supported by the general, well known cytolytic action of cobra venom cardiotoxins, as shown by lysis of blood cells (Condrea, Mammon, Aloof & De Vries, 1964; Condrea, Barzilay & Mager, 1970), tumour cell cultures (Patel, Braganca & Bellare, 1969; Zaheer, Noronha, Hospattankar & Braganca, 1975) and the destruction of tissues such as mammalian heart muscles (Nayler *et al.* 1976). This view has raised the expectation that cardiotoxin may damage the continuity and integrity of the gut tissues, thus enabling its penetration. On the other hand, the fact that paralysis is rapid (15–30 min, Primor & Zlotkin, 1978), and that amounts as low as 0·005 of the oral LD_{50} unit of radioiodinated toxin have been detected in the fly's body tissues (Primor *et al.* 1980), suggest that cardiotoxin may cross the gut not by causing anatomical damage but by following a specific pathway.

The above considerations concerning the possible mode of cardiotoxin's gut penetration have motivated and directed the present study, resulting in some basic information related to the structure and function of the insect's midgut epithelial cells.

MATERIALS AND METHODS

Test insects

Fleshflies of the species *Sarcophaga falculata* (= *argyrostoma*) were bred in the laboratory according to the method presented by Zlotkin, Fraenkel, Miranda & Lissitzky (1971). Female flies, 24–28 h after hatching, deprived of food and water, were employed in the experiments.

Oral application

The test solutions (5 μl per fly) were introduced to the proboscis through a calibrated Hamilton syringe. The responsiveness of the fly was improved when a crystal of sucrose was placed on the tip of the needle. To follow the movement of the orally applied solution in the digestive system by an external observation of the exposed intestine, the substance was dissolved in a 0·2 % solution of the dye erythrosin in distilled water.

Orally toxic protein

The cardiotoxic fraction D_5, isolated and purified from the venom of the cobra snake *N. mossambica* by the method of Primor & Zlotkin (1978), was employed in the experiments on gut penetrability and autoradiography. The lethal potency in LD_{50} values of this toxin is 23·8 μg per 100 mg of body weight for *Sarcophaga* flies. Amino acid analyses have revealed that the cardiotoxin D_5 is identical to the component $V_{II}4$, previously purified by Louw (1974) from the same venom.

Radioiodination

Cardiotoxin D_5 was radioiodinated (^{125}I) using Sepharose (Pharmacia, Sweden) bound lactoperoxidase (Sigma, U.S.A.) by the method of David & Reisfeld (1974) and according to technical details given by Teitelbaum, Lazarocivi & Zlotkin (1979). The product yielded a specific radioactivity of $1{\cdot}4 \times 10^5$ to $2{\cdot}1 \times 10^5$ Ci mol^{-1} (20–30 μCi μg^{-1}). It was chemically stable for a period of several weeks as judged by its column chromatographical (Primor *et al.* 1980) and electrophoretical mobilities (data not shown). In standard experiments the flies were orally applied with the radioiodinated toxin or with the equivalent amount of Na [^{125}I] (Negev Nuclear Center, Israel) corresponding to 3·2 μCi.

Histological techniques

The gut was exposed by a careful longitudinal dorsomedial section of the integument. It was fixed in its intact form, while connected to the body using 2·5 % gluteraldehyde in 0·1 M-cacodylate 5 % sucrose pH 7·4 buffer for 90–120 min at room temperature. For postfixation, 1 % osmium tetroxide was employed. After dehydration in graded ethanol, the whole midgut was removed and embedded in SPURR. For LM, sections of 2 μm were prepared and stained with methylene blue. For LM autoradiography, unstained sections of 3–5 μm were used. For EM (including autoradiography), sections 70–90 nm were stained with 3 % uranyl acetate and lead citrate. Sections were examined in a YOEL 100 CX EM. For LM autoradiography, the sections were treated with Nuclear track emulsion NTB 2 (Kodak, U.S.A.) according to Gude (1968). They were stored for 7–9 days. The emulsion was developed with Kodak D-19 developer. The sections were examined using a phase contrast microscope (Olympus, Japan). In EM autoradiography the sections were placed on grids coated by formvar solution. The grids were coated with Ilford L4 Size A emulsion according to Caro (1969). They were stored for 2·5 months. The emulsion was developed using Kodak D-19 developer.

RESULTS

The alimentary canal of *Sarcophaga faculata* is a long convoluted tube about three times the length of the body (Chaudhry, 1972). The gut is differentiated into three primary regions: the foregut, the midgut and the hindgut. The midgut, where the digestion and absorption of food occur, comprises about 90 % of the length of the alimentary canal. Part of the tubular midgut shows a helix-like coiling in the abdominal cavity (Fig. 5). This coiled region of the midgut was the main object of our study.

A section at this region resulted in 3–6 gut cross sections at different locations of the midgut. Gut segments preceding and following the coiled region were also examined.

Penetrability of [^{125}I] D_5 cardiotoxin

A total number of 37 female flies were orally dosed with 3·2 μCi of [^{125}I] D_5 each. They were subdivided into groups of 4–9 flies each, dissected and fixed at intervals of 5, 15, 30, 60, 120, 180 and 360 min after the oral application. There were two control groups. One contained five flies dosed with water and sucrose in order to exclude possible chemographic artefacts (Rogers, 1973). The second contained 20 flies dosed with 3·2 μCi of Na [^{125}I], fixed at the same time intervals as in the experimental groups. This second control was used to exclude the possibility that autoradiography may represent the free radioactive iodine of the degraded rather than the authentic toxin.

The examination of several hundred histological gut sections resulted in the following information:

(1) Both controls were clearly negative. The Na [^{125}I] was washed out during the process of tissue preparation and only traces of radioactivity were occasionally detected in the lumen of the midgut, but never in the epithelial cells. This indicates that the autoradiographic reaction obtained with [^{125}I] D_5 is due to the presence of the intact toxin, and is strongly supported by the data obtained by Primor *et al.* (1980) in which orally applied [^{125}I] D_5 was identified in the tissues of the fly following its competitive displacement with unlabelled D_5.

(2) Examination of cross sections simultaneously obtained from the coiled region of the midgut indicated strong differences in the permeability to [^{125}I] D_5 of different segments (Fig. 1). These segments were classified as permeable (Fig. 1A), partially permeable (Fig. 1B) and nonpermeable (Fig. 1C). Their location in the midgut is schematically presented in Fig. 5. The following information refers mainly to the permeable segment of the midgut.

(3) In permeable segments, the progression of the [^{125}I] D_5 through the midgut wall occurred as a continuous front during the first 20 min after its oral application. This progression included its appearance in the lumen, the crossing of the peritrophic

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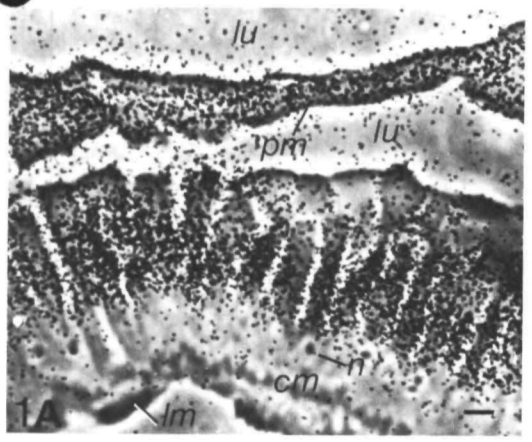
Figs 1–3. Autoradiography of orally applied [^{125}I] cardiotoxin (3·2 μCi) in the midgut of *Sarcophaga falculata* flies. Abbreviations: *cm*, circular muscles; *lm*, longitudinal muscles; *lu*, lumen; *mv*; microvilli; *n*, nucleus; *pm*, peritrophic membrane; *t*, tracheae.

Fig. 1. Sagittal sections from three different segments of the midgut of the same fly 1 h after the oral application of radioiodinated cardiotoxin. (A) Permeable segment – demonstrating the massive presence of the toxin in the lumen beyond the peritrophic membrane and in the cells. (B) Partially-permeable segment – relatively few autoradiographic grains are found in the cells. (C) Non-permeable segment – penetration through the peritrophic membrane may be noticed, but there is practically no entrance of the toxin into the epithelial cells. Scale bars, 10 μm.

Fig. 2 Sagittal sections at the permeable segments of midguts at different time intervals after the application. (A) 5 min – penetration through the peritrophic membrane and the beginning of the entrance into a cell can be noticed. (B) 30 min – advancement of the radioactive substance to the basal parts of the epithelial cells. Large arrows indicate sites of passage through the entire length of the cell. Notice the relatively higher density of the grains around the apical margins of the cells which may correspond to the location of microvilli (see also Fig. 4C, D). Scale bars, 10 μm.

Fig. 3. Thirty minutes after the oral application of [^{125}I] D_5 – the grains are located on extraintestinal tracheae. Scale bar, 10 μm.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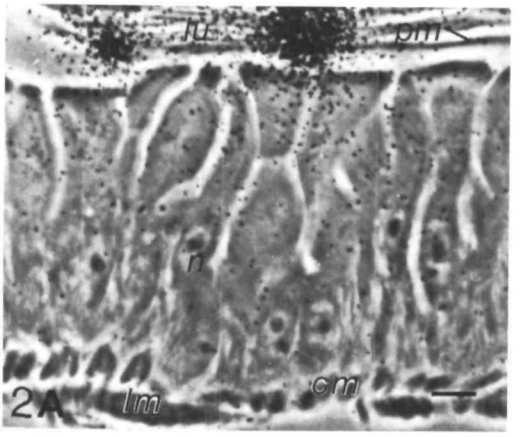
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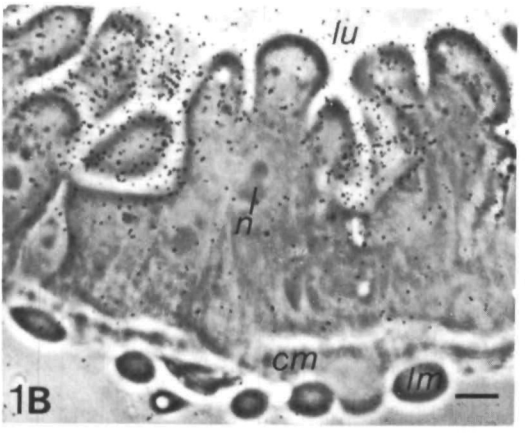
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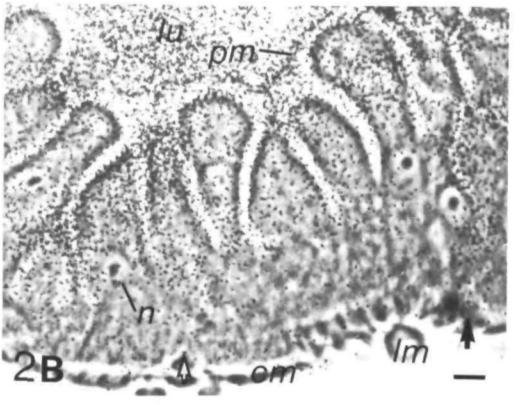
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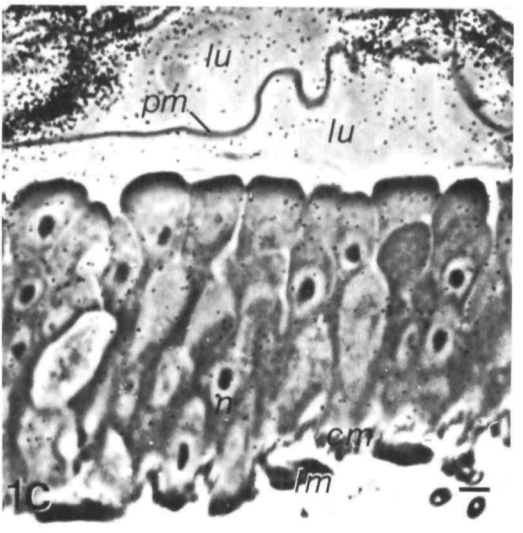
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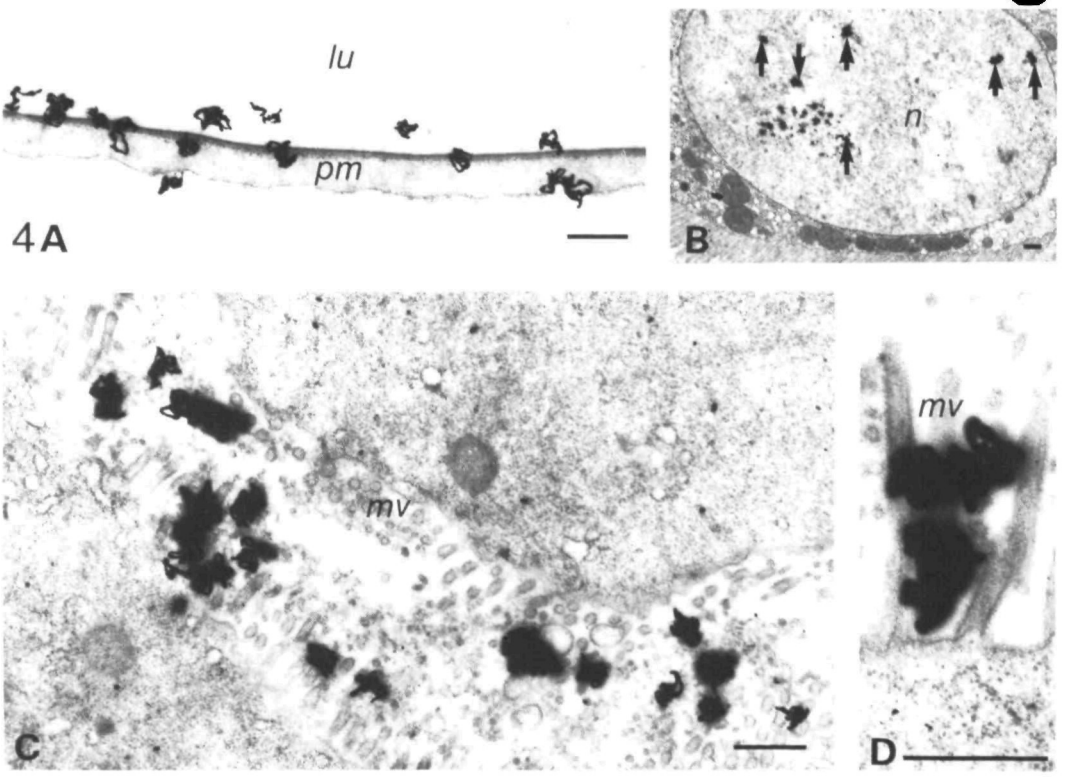


Fig. 4. Autoradiography of orally applied [^{125}I]cardiotoxin (3·2 μCi) in the midgut of *Sarcophaga falculata*: electron micrographs. (A) 5 min after the application. The evident presence of the grains on the peritrophic membranes indicates permeability to the toxin. (B) 30 min after the application. The presence of the photographic grains (arrows) is evident in the nuclei of the epithelial cells. (C) and (D) demonstrate the association of the toxin with the microvilli (see also Fig. 2B), supposedly the site of penetration into the cell. Scale bars, 0·7 μm. Abbreviations as for Fig. 1.

membrane (Figs 1A, 2A, B and 4A) and its arrival at and penetration into the microvilli (Figs 2A, 4C, D) 5 min after application. After 15 min the [^{125}I] D_5 could be found in the cytoplasm of the apical part of the epithelial cells and in the intercellular spaces. Thirty minutes after the application, the front of the autoradiographic grains in the epithelial cells reached the level of the nuclei (Fig. 2B). Beyond this level the movement of [^{125}I] D_5 became more randomly dispersed, as reflected in the distribution of grains over the entire length of the epithelial cells (Figs 1A, 2B).

(4) At time intervals above 30 min after the oral application, no significant changes in the distribution of the grains in the epithelial cells were observed except that cardiotoxin was observed in the nuclei of the epithelial cells (Fig. 4B). The presence of [^{125}I] D_5 in the cytoplasm was not associated with any cell organelles. The final evidence for the crossing of the gut is given by the presence of grains around extra intestinal tissues such as tracheae (Fig. 3).

The cytology of the cardiotoxin permeable and non-permeable segments of the midgut of Sarcophaga faculata

Histology of the permeable and non-permeable segments was examined in 10 flies

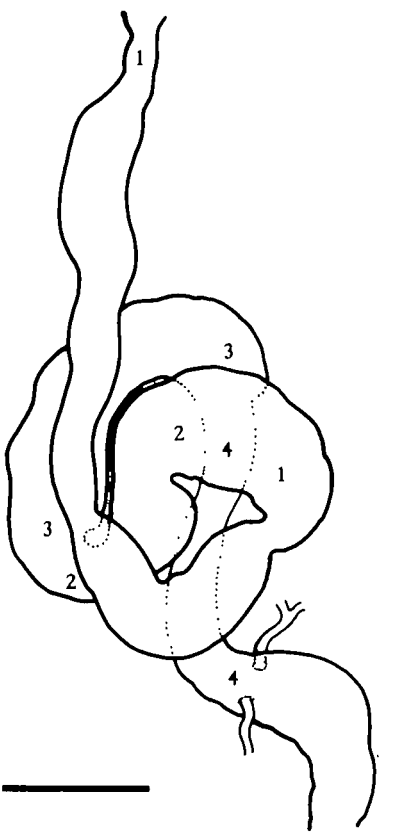
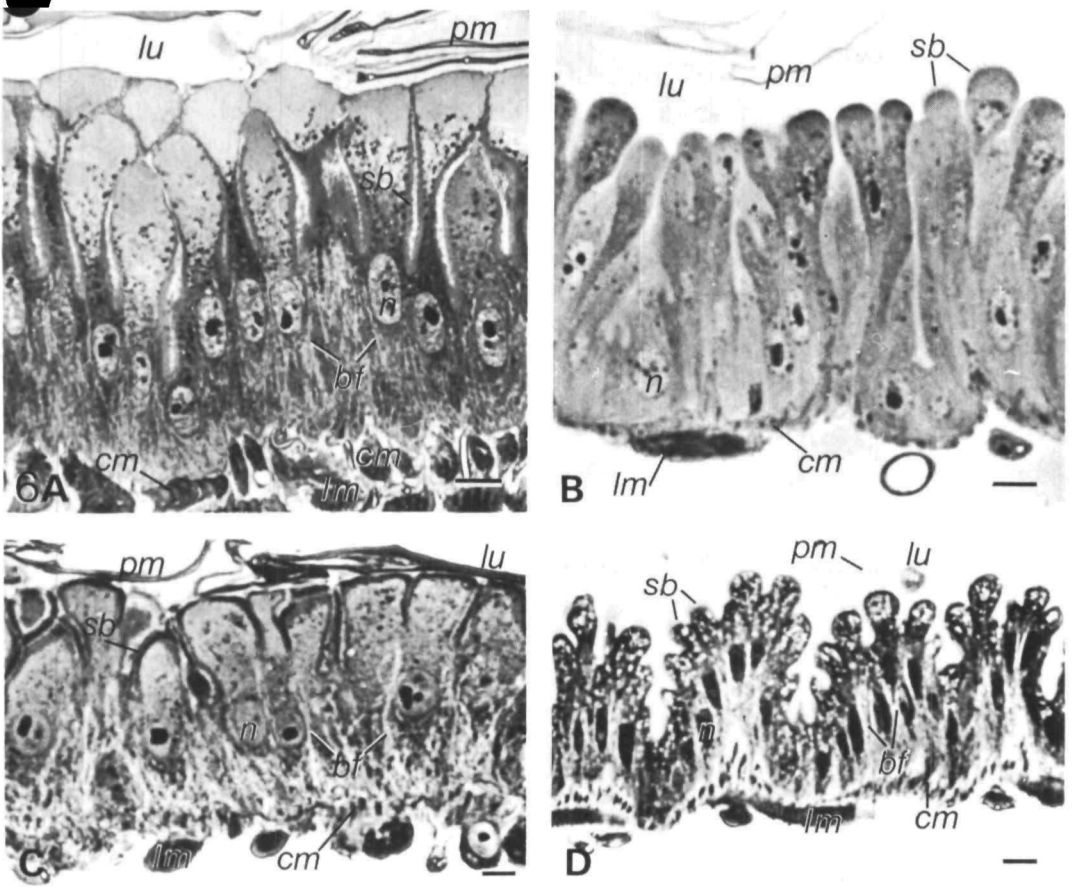


Fig. 5. The approximate subdivision of the midgut of *Sarcophaga falculata* at the abdominal region into segments according to their permeability to the cardiotoxin. 1, permeable segment; 2, non-permeable segment; 3 and 4, partially-permeable segments. Scale bar, 1 mm.

Table 1. *Cytological comparison between the cardiotoxin permeable (Fig. 5, segment 1) and non-permeable (Fig. 5, segment 2) segments of the midgut of* Sarcophaga falculata *flies*

	Permeable segment	Reference of figures	Non-permeable segment	Reference of figures
General description of the epithelial cell	Elongated cells – Dimensions: 90 (±8·1) × 13·5 (±0·7) μm*	6A	Relatively shorter cells Dimensions: 66 (±6) × 6·3 (±1·0) μm*	6B
	The apical ends are uneven and touch each other	6A	Apical ends are rounded and separated	6B
	Cytoplasm of the apical parts of the cells stains lighter than the rest of the cell	6A	Cytoplasm stains homogeneously all over the cell	6B
	Organelles absent in apical part	7A	Organelles present all over the cell	7B
	Nuclei are arranged in one plane (centre of the cell)	6A	Nuclei are not in same plane	6B
	Outer plasma membrane is bumpy	6A	Outer plasma membrane is rounded without folds	6B
	Striated border is evident	7A		7B
Microvilli	Present only at the lateral margins of the apical part	7A	Present all over the apical part of the cell	7B
	Long: 4·3 (±0·11) μm	7A	Short: 0·84 (±0·13) μm	7B
	Orientated parallel, straight and uniform in size and thickness – 0·11 (±0·03) μm. Surrounded by thick layer of glycocalyx	8A	Disorientated and variable in their thickness – 0·12 (±0·1) μm. Surrounded by very thin layer of glycocalyx	8B
Rough endoplasmic reticulum	Circular in their arrangement	7A 9A	Elongated in their arrangement	7B 9B
Basal folding of plasma membrane	Thicker in diameter 0·36 (±0·1) μm	10A	Thinner in diameter 0·1 (±0·036) μm	10B

* The data in brackets represent standard deviations of the mean determined in 10 different measurements.



Figs 6–10. Cytological comparison between segments of different permeabilities to cardiotoxin (in the absence of toxin) in the midgut of the *Sarcophaga falculata* flies. Abbreviations: *bm*, basal membrane; *bf*, basal foldings; *cm*, circular muscles; *lm*, longitudinal muscles, *lu*, lumen; *m*, mitochondria; *mv*, microvilli; *n*, nucleus, *pm*, peritrophic membrane; *rer*, rough endoplasmic reticulum; *sb*, striated border.

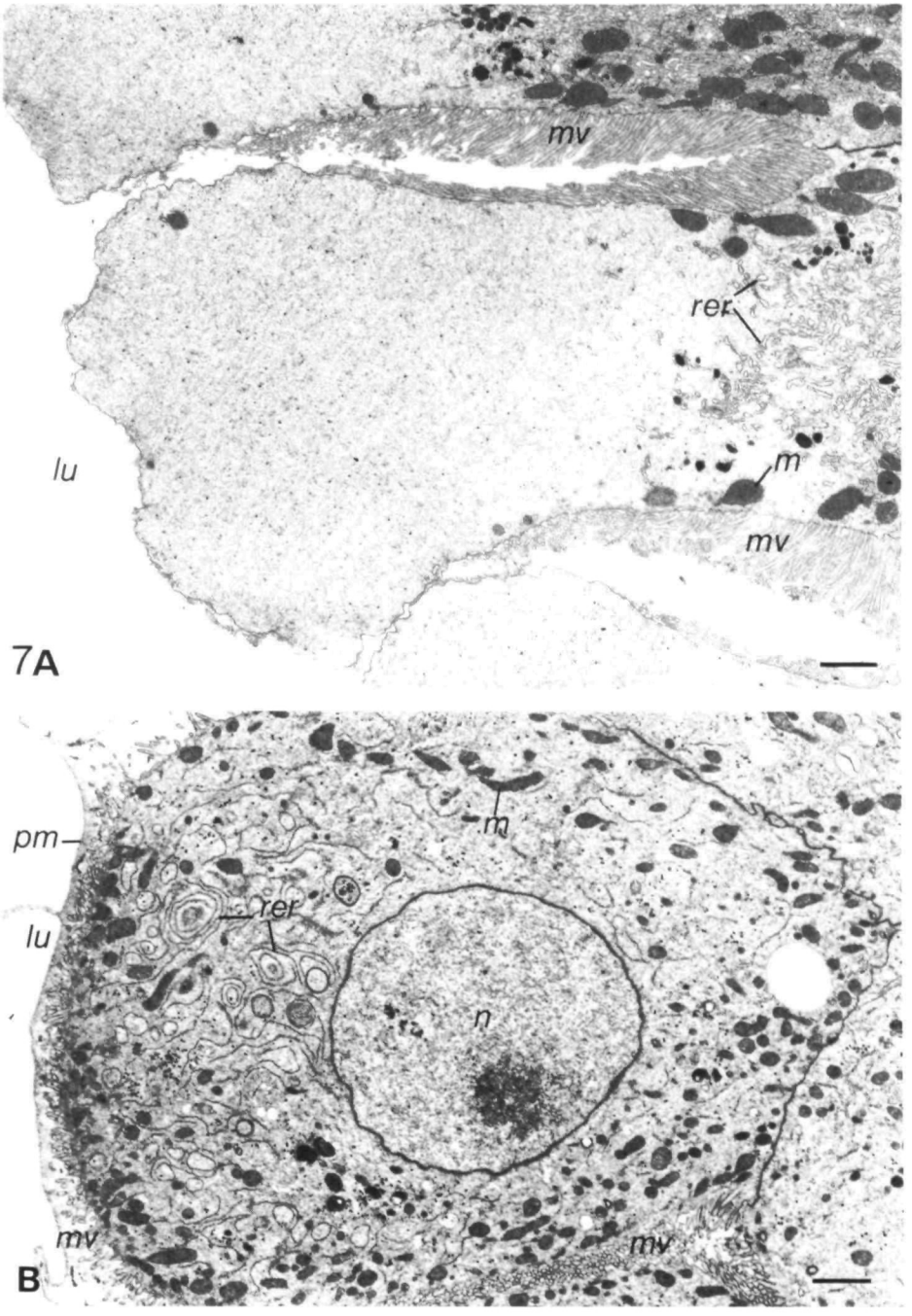
Fig. 6. Sagittal sections of the cardiotoxoin permeable (A) Fig. 5, segment 1; non-permeable, (B) Fig. 5, segment 2; and partially-permeable (C and D) Fig. 5, segments 3 and 4 respectively, segments of the midgut. (A) and (B) for details, see Table 1. Scale bars, 10 μm. (C) Relatively thick cells [dimensions: 65 ($\pm$4·8) 16·3 ($\pm$2·4) μm with homogeneously stained cytoplasm, regularly arranged nuclei, clearly visible striated border and emphasized and expanded regions of the basal foldings. (D) Densely stained thin cells [dimensions: 56 ($\pm$2·9) 6·9($\pm$1·17 μm arranged in *villi* and characterized by rounded and vacuolated apical ends, large intensively stained nuclei and enlarged regions of the basal foldings. Scale bars, 10 μm.

Fig. 7. Electron micrographs of the apical portions of midgut cells from permeable (A) and non-permeable (B) segments. See Table 1. Scale bars, 1,9 μm.

Fig. 8. Electron micrographs of cross sections of the microvilli of cells from the cardiotoxin permeable (A) and non-permeable (B) segments of the midgut. For details, see Table 1. Arrows indicate the glycocalyx layer. Scale bars, 0·2 μm.

Fig. 9. Electron micrographs of the rough endoplasmic reticulum in epithelial cells from permeable (A), and non-permeable (B) segments of the midgut. Notice the circular 'vesicular' arrangement of the reticulum in (A) as compared to the elongated forms in (B). Scale bars, 0·2 μm.

Fig. 10. Electron micrographs of the basal portions of midgut cells from permeable (A) and non-permeable (B) segments. Notice the differences in the thickness and coiling of the basal folding of the plasma membrane. See Table 1. Scale bars, 0·2 μm.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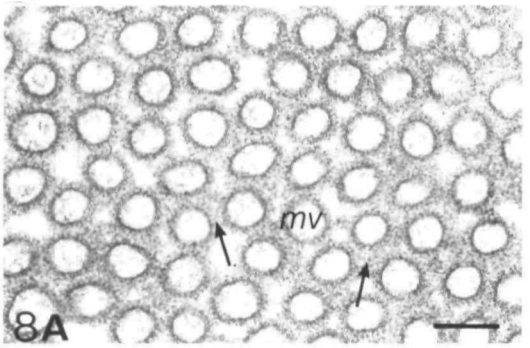

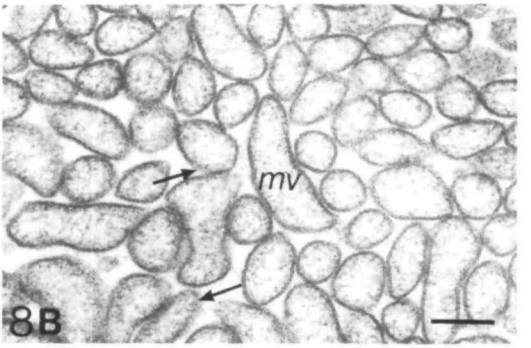

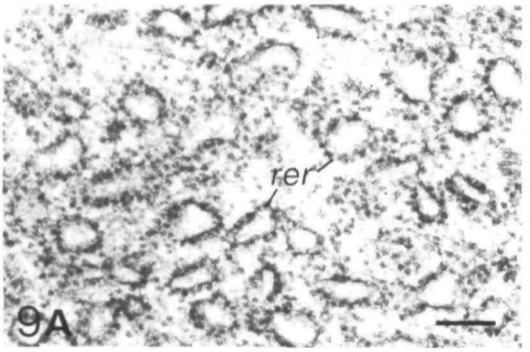

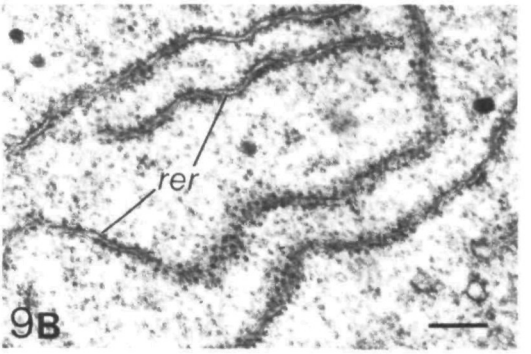

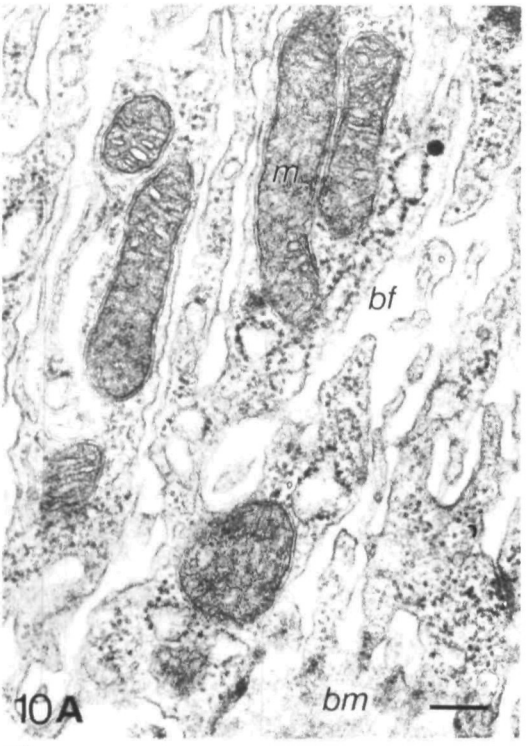

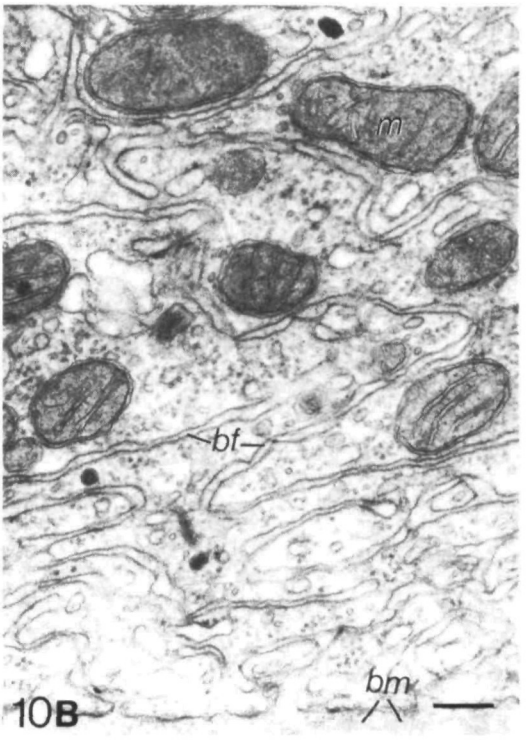

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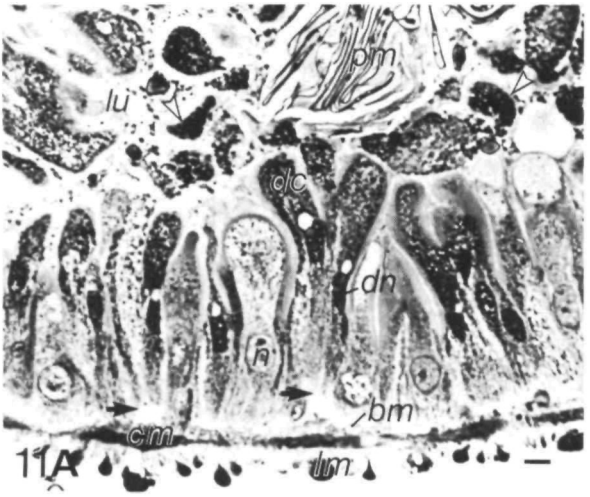
(*For legend see Fig. 6*)



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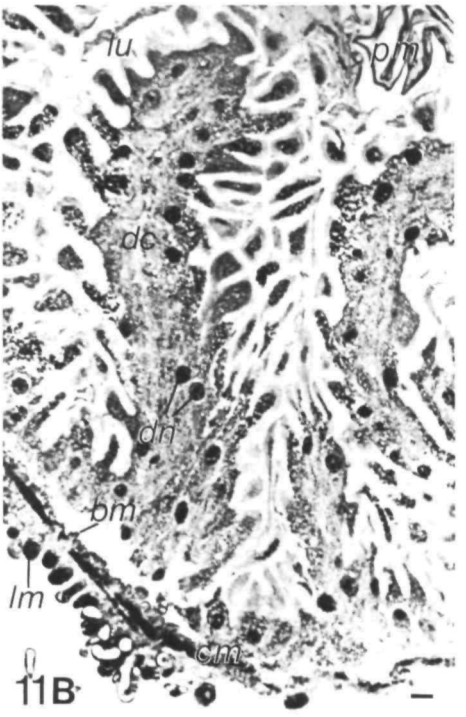

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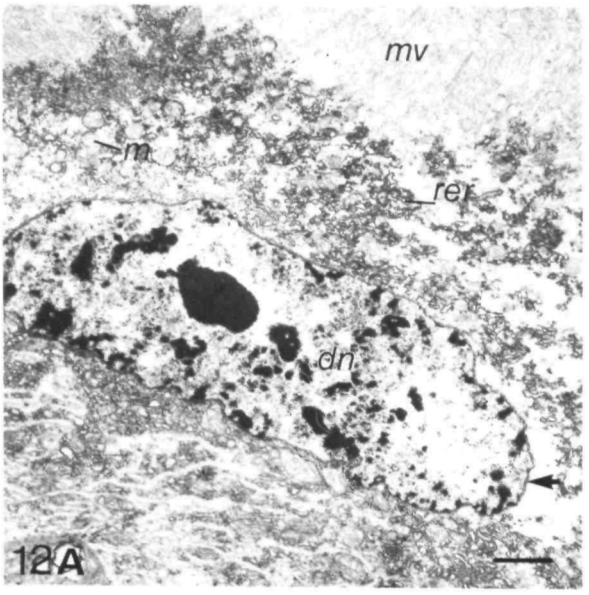

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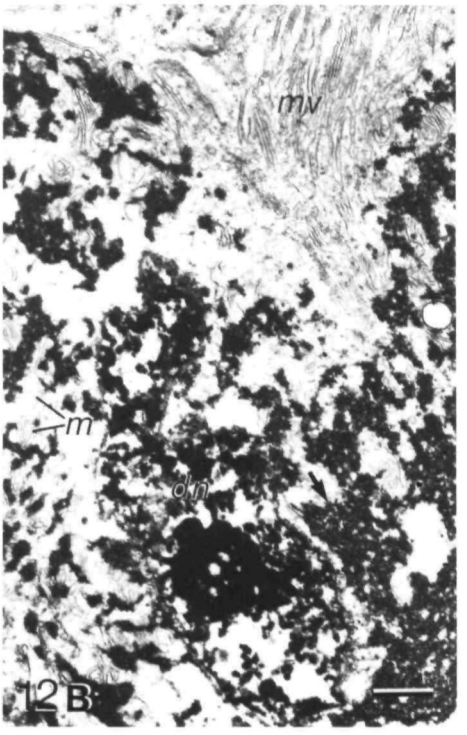

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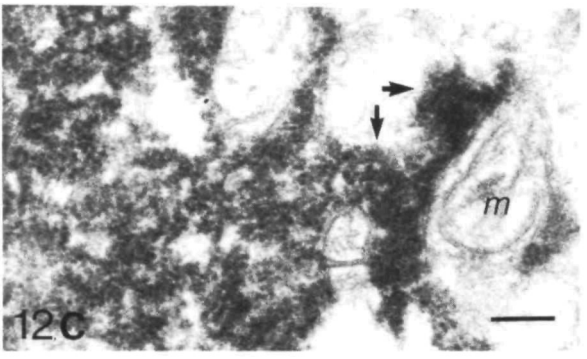

12C

of the same sex, age and pretreatment as those used in the autoradiography experiments. The digestive systems were fixed 60 min after the oral application of the erythrosin solution (see Materials and Methods). The results are given in Table 1 and Figs 6 to 10. For comparative purposes, sagittal sections of the partially permeable segments (Fig. 5, segments 3 and 4) are also given in Fig. 6.

Histopathology

The amount of cardiotoxin orally introduced per fly in the autoradiography studies corresponded to about 0·005 LD_{50} units. At this dosage no histopathological changes whatsoever were observed in the midgut, so the effects of superlethal doses were examined. Fifteen flies of the same sex, age and pretreatment as those used in the previous experiment were orally dosed with 125 μg (~10 LD_{50} units) of toxin each. Upon the occurrence of the paralysis symptoms, which appeared within 20–40 min after the oral application, the midguts were dissected and fixed.

Epithelial cells of the permeable segment underwent extreme structural changes, expressed in the aggregation and shrinkage of cytoplasm and nuclei, dissociation of the cells from the basal membrane and finally disruption and disintegration of the cells (Fig. 11). Electron-microscopy revealed that the affected cells contained disrupted cytoplasmic membranes and deformed and destroyed organelles (Fig. 12). Some of the histopathological effects resemble those induced by the cardiotoxic component of the Indian cobra in a mammalian heart preparation (Nayler *et al.* 1976).

No histopathological changes were detected in the non-permeable and the partially-permeable parts of the midgut. The resistance of the partially-permeable segments to the histopathic effect of cardiotoxin is probably due to their limited permeability which may prevent the accumulation of a critical local concentration necessary for anatomical damage.

DISCUSSION

Superlethal doses (10 LD_{50}) of orally applied cobra venom cardiotoxin were able

Figs 11, 12. Histopathology of the cardiotoxin permeable segment (Fig. 5 segment 1) of the midgut introduced by the oral application of 125 μg of cardiotoxin per fly. Abbreviations: *bm*, basal membrane; *cm*, circular muscles; *dc*, damaged cell; *dn*, damaged nuclei; *lm*, longitudinal muscles; *lu*, lumen; *m*, mitochondria; *n*, nucleus; *mv*, microvilli; *pm*, peritrophic membrane; *rer*, rough endoplasmic reticulum.

Fig. 11. (A) Sagittal section showing strongly damaged cells adjacent to normal cells or partially damaged cells. Notice dense aggregates in the cytoplasm and the nuclei of the damaged cells; dissociation of the damaged cells (arrows) from the basal membrane and surrounding muscular layer; the presence of cellular fragments (arrowheads) in the lumen of the gut. (B) Final stage of pathology. Notice the clusters of damaged cells, separated from the basal membrane collapsing into the lumen. Scale bars, 10 μm.

Fig. 12. Electron micrographs of cardiotoxin-affected cells. (A). A sagittal section of a cell in an advanced stage of damage. Notice the deformed and ruptured microvilli, empty and ruptured mitochondria, partially shrunken and deformed nucleus and cytoplasm. Rough endoplasmic reticulum and the nuclear membrane (arrow) still exist. Scale bar, 1·2 μm. (B) A section from a dissociated epithelial cell in the lumen of the gut in the final stage of damage. The following details deserve attention: nucleus is shrunken and deformed; microvilli are dissociated from the cell and, like the mitochondria, are represented by dispersed threads of their membranes (compare to Fig. 7A). Cytoplasm has converted to dense aggregates (arrow). Scale bar, 1·2 μm. (C) High magnification micrograph of the affected mitochondria and of the dense aggregates of the cytoplasm which seem to be clusters of ribosomes (arrows). Scale bar, 0·2 μm.

to cause a strong structural damage in the epithelial cells of the toxin-permeable segment of the fleshfly midgut, expressed in their dissociation, collapse and final disintegration. However, the toxin could cross the midgut at doses of about three orders of magnitude lower, as shown previously (Primor *et al.* 1980), with no structural changes in the gut, as shown in the present study by light and electron microscopy. Proteins have previously been observed to cross the intestinal wall of an insect in chemical and immunochemical studies (Schlein, Spira & Jacobson, 1976; Nogge, 1971; Nogge & Giannotti, 1980*a*,*b*; Primor *et al.* 1980; Primor & Zlotkin, 1980).

The progression of the cardiotoxin was shown to occur as a continuous front and included the following stages:

(1) The crossing of the peritrophic membrane. This layer, which is a special structure of chitin and protein (Richards & Richards, 1977), was originally thought to be impermeable to polypeptides from the lumen direction (Zhuzhikov, 1964). However, it is permeable to nuclear polyhedrosis virions in lepidopterous larvae (Paschke & Summers, 1975) and to molecules of a magnitude of about 45 000 Da in the tsetse fly (Nogge & Giannotti, 1980*a*).

(2) The penetration into the epithelial gut cells was probably achieved though the microvilli of the apical plasma membrane. It is noteworthy that midgut microvilli absorb the nuclear polyhedrosis virions in lepidopterous larvae (Harrap, 1970). In contrast to the pinocytotic uptake and vesicular transport of various proteins by different insect epithelial tissues (Smith *et al.* 1969; Locke & Collins, 1968; Anderson, 1969), we did not obtain any morphological evidence indicating such a transport for the cardiotoxin in the present study. We also did not observe broadening-extension of intercellular spaces as shown in pathological conditions of mammalian capillaries. Nor did we observe high permeability regions such as the forms of 'fenestrae' characteristic to certain mammalian brain capillaries (Brightman & Broadwell, 1976). The uniqueness of cardiotoxin's penetrability may be attributed to the chemical nature of this compound and its specific interaction with membrane constituents (as mentioned below).

(3) The progressive movement of the [^{125}I] D_5 occurs in the cytoplasm and the nucleus of the epithelial cells reaching their basal region. Beyond this level the advancement of the cardiotoxin becomes more randomly dispersed ('diffusional') throughout the length of the cell. In spite of its being unclear in what way cardiotoxin passes the final part of the pathway, there is no doubt that the crossing of the insect gut does occur. This crossing was previously shown by Primor *et al.* (1980) and was indicated in the present study by the appearance of [^{125}I] D_5 in extraintestinal tissues such as tracheae.

The pattern of penetrability and cytotoxicity of the cardiotoxin in the fleshfly's midgut probably reflects the phospholipid composition of the outer-plasma membrane of the midgut epithelial cells. It is noteworthy that δ-endotoxin from *Bacillus thuringiensis*, which affects insect epithelial gut cells, has been shown to act primarily at the cell surface (Fast, Murphy & Sohi, 1978). Cardiotoxins are known to interact with biological membranes by association with their phospholipid components as expressed in their extremely high binding capacity (Vincent *et al.* 1976), competitive displacement by phospholipids (Patel *et al.* 1969; Zaheer *et al.* 1975), and their binding to artificial liposomes (Dufourcq & Faucon, 1976). In spite of the fact th phospholipids serve as integral and major components of all biological membranes,

cardiotoxins possess a curious pharmacological selectivity distinguishing between closely related cell types such as different erythrocytes (Condrea *et al.* 1964; Lee, Lin & Wei, 1971), strains of Yoshima sarcoma cells (Patel *et al.* 1969), or even different sites located on the same membrane (Vincent *et al.* 1976). The presence and distribution of negatively charged phospholipids such as phosphatidylserine, phosphatidylinositol and phosphatidic acid appears to be an essential prerequisite for the interaction with cardiotoxins. These substances have been shown to serve as receptors of cardiotoxin in liposomal systems (Dufourcq & Faucon, 1976). Their presence in erythrocyte membranes has been correlated with cardiotoxin susceptibility (Condrea *et al.* 1964, 1970) and they are able to protect cells from the lytic action of cardiotoxin (Patel *et al.* 1969) or even to reverse its enzyme blocking activity (Zaheer *et al.* 1975).

The vast majority of studies dealing with the histology of insect digestive systems, including that of the closely related species of *Sacrophaga bullata* (Naponitaya & Misch, 1974), point to a basic homogeneity in the distribution of cellular elements throughout the midgut (i.e. Waterhouse & Wright, 1960; Davies & King, 1977). However, morphologically distinct regions in the insect midgut have been found: in the tsetse fly (Wigglesworth, 1929), in the posterior region of the midgut of Culicidae mosquitoes (Hecker, 1977), in the length of microvilli in the midgut of female *Phlobotamus* (Gemetchu, 1974) and there are differences in the distribution and abundance of rough endoplasmic reticulum and Golgi complexes in the different regions of the plasmid *Carausius* midgut (Beadle, 1972). The functional significance of these morphological diversities is still obscure. We assume that the close accordance, in *Sacrophaga* midgut, between the permeability to cardiotoxin and the well-defined morphological characteristics, shown in the present study, is not coincidental. This may also indicate that differences in the chemical composition and arrangement of the cytoplasmic membranes of the midgut cells are associated with their specific function. In other words, cardiotoxin may serve as a pharmacological tool for the differentiation and identification of functional units in the insect's midgut epithelium.

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REFERENCES

ANDERSON, E. (1969). Oogenesis in the cockroach *Periplaneta americana*, with special reference to the specialization of the oolemma and the fate of coated vesicles. *J. Microscopie* **8**, 721–738.

BEADLE, D. J. (1972). Structural differentiation in the midgut epithelium of the plasmid *Carausius morosus* Brunner. *J. Ent.* **47**, 71–83.

BRIGHTMAN, M. W. & BROADWELL, R. D. (1976). The morphological approach to the study of normal and abnormal brain permeability. In *Transport Phenomena in the Nervous System*, (eds G. Levi, L. Battistin & A. Lajta), pp. 41–54. New York: Plenum Press, Inc.

CAPPS, K. C., FREIER, J. E., FRIEDMAN, S., NIGG, H. S., LARSEN, J. R. & RATNASIRI, N. B. (1972). Diet dependent cycling of protease activity in the midgut of the fleshfly *Sarcophaga bullata*. *Isr. J. Ent.* **7**, 99–108.

CARO, L. G. (1969). A common source of difficulty in high-resolution radioautography. *J. Cell Biol.* **41**, 918.

CHAUDHRY, K. (1972). Morphology of the alimentary canal of *Sarcophaga ruficornis* (Fabricius) (Diptera: Sarcophagidae). *Zool. Beitr.* **18**, 361–369.

CONDREA, E., BARZILAY, M. & MAGER, J. (1970). Role of cobra venom direct lytic factor and Ca^{2+} in promoting the activity of snake venom phospholipase A. *Biochim. biophys. Acta* **210**, 65–73.

CONDREA, E., MAMMON, Z., ALOOF, S. & DE VRIES, A. (1964). Susceptibility of erythrocytes of various animal species to the hemolytic and phospholipid splitting action of snake venom. *Biochim. biophys. Acta* **84**, 365–375.

DAVID, G. S. & REISFELD, R. A. (1974). Protein iodination with solid state lactoperoxidase. *Biochemistry, N.Y.* **13**, 1014–1021.

DAVIES, I. & KING, P. E. (1977). The ultrastructure of the midgut cells of *Nasonia vitripennis* (Walker) (Hymenoptera, Pteromalidae). *Cell Tiss Res.* **177**, 227–238.

DUFOURCQ, J. & FAUCON, J. F. (1976). Specific binding of a cardiotoxin from *Naja mossambica* to charged phospholipids detected by intrinsic fluorescence. *Biochemistry, N.Y.* **17**, 1171–1176.

FAST, P. G., MURPHY, D. W. & SOHI, S. S. (1978). *Bacillus thuringiensis* δ-endotoxin: Evidence that toxin acts at the surface of susceptible cells. *Experientia* **34**, 762–763.

GEMETCHU, T. (1974). The morphology and fine structure of the midgut and peritrophic membrane of the adult female *Phlebotomus longipes* Parrot and Martin (Diptera: Psychodidae). *Ann. trop. Med. Parasit.* **68**, 111–138.

GUDE, W. D. (1968). *Autoradiographic Techniques: Localization of Radioisotopes in biological Material*. Englewood Cliffs, N. J.: Prentice Hall, Inc. pp. 27–33.

HARRAP, K. A. (1970). Cell infection by a nuclear Polyhedrosis virus. *Virology* **42**, 311–318.

HECKER, H. (1977). Structure and function of midgut epithelial cells in Culicidae mosquitoes (Insecta, Diptera). *Cell Tiss. Res.* **184**, 321–341.

HOUSE, H. L. (1974). Digestion. In *The Physiology of Insecta*, Vol. 5, (ed. M. Rockstein), pp. 63–118. New York & London: Academic Press.

LEE, C. Y., LIN, J. S. & WEI, J. W. (1971). Identification of cardiotoxin with cobramine B, D. L. F., toxin γ and cobra venom cytotoxin. In *Toxins of Animal and Plant Origin*, Vol. 1, (eds A. Vries & E. Kochva), pp. 307–318. New York, London, Paris: Gordon & Breach Science Pub.

LOCKE, M. & COLLINS, J. V. (1968). Protein uptake into multivesicular bodies and storage granules in the fat body of an insect. *J. Cell Biol.* **36**, 453–483.

LOUW, A. I. (1974). Snake venom toxins. The purification and properties of five non-neurotoxic polypeptides from *Naja mossambica mossambica*. *Biochim. biophys. Acta* **336**, 470–480.

NAPONITAYA, W. & MISCH, D. W. (1974). Developmental cytology of the midgut in the flesh-fly, *Sarcophaga bullata* (Parker). *Tissue and Cell* **6**, 487–502.

NAYLER, W. G., SULLIVAN, A. T., DUNNETT, J., SLADE, A. M. & TRETHEWIE, E. R. (1976). The effect of a cardiotoxic component of the venom of the Indian cobra (*Naja nigricollis*) on the subcellular structure and function of heart muscle. *J. molec. Cell Cardiol.* **8**, 341–360.

NOGGE, G. (1971). Resorption von serumproteinen in die haemolymphe bei larven von *Hypoderma bovis* (De Geer) (Diptera, Hypodermatidae). *Experientia* **27**, 524–525.

NOGGE, G. & GIANNOTTI, M. (1980*a*). Midgut absorption of proteins by tsetse flies, *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). In *Isotope and Radiation Research on Animal Diseases and their Vectors*, pp. 313–317. Wein: IAEA-SM 240/12.

NOGGE, G. & GIANNOTTI, M. (1980*b*). Specific antibodies: a potential insecticide. *Science, N.Y.* **209**, 1028–1029.

PASHKE, J. D. & SUMMERS, M. D. (1975). Early events in the infection of the arthropod gut by pathogenic insect viruses. In *Invertebrate Immunity*, pp. 75–112. New York, London: Academic Press.

PATEL, T. N., BRAGANCA, B. M. & BELLARE, R. A. (1969). Changes produced by cobra venom cytotoxin on the morphology of Yoshida sarcoma cells. *Expl Cell Res.* **57**, 289–287.

PRIMOR, N., TEITELBAUM, Z. & ZLOTKIN, E. (1980). Penetrability of oral toxic protein from cobra venom through the gut of a blowfly. *Biochim biophys. Acta* **627**, 71–81.

PRIMOR, N. & ZLOTKIN, E. (1978). Oral toxicity of venoms and toxins to blowflies. In *Toxins: Animal, Plant and Microbial*, (ed. P. Rosenberg), pp. 1087–1095. Oxford & New York: Pergamon Press.

PRIMOR, N. & ZLOTKIN, E. (1980). Penetrability of proteins through the digestive system of *Sarcophaga faculata* blowfly. *Biochim. biophys. Acta* **627**, 82–90.

RICHARDS, A. G. (1975). The ultrastructure of the midgut of hematophagous insects. *Acta Trop.* **32**, 83–95.

RICHARDS, A. G. & RICHARDS, P. A. (1977). The peritrophic membranes of insects. *Ann. Rev. Ent.* **22**, 219–240.

ROGERS, A. W. (1973). *Techniques of Autoradiography*. Amsterdam, London, New York: Elsevier.

SCHLEIN, Y., SPIRA, D. T. & JACOBSON, R. L. (1976). The passage of serum immunoglobulins through the gut of *Sacrophaga faculata*. Pand. *Ann. trop. Med. parasitol.* **70**, 227–230.

SINHA, M. (1976). Digestive enzymes in the gut and salivary glands of *Sarcophaga ruficornis* Fab. and *Musca domestica* L. (Diptera: Insecta). *Appl. ent. Zool.* **11**, 260–262.

SMITH, D. S., COMPHER, K., JANNERS, M., LIPTON, C. & WITTLE L. W. (1969). Cellular organisation and ferritin uptake in the mid-gut epithelium of a moth, *Ephestia kuhniella*. *J. Morph.* **127**, 41–72.

TEITELBAUM, Z., LAZAROCIVI, P. & ZLOTKIN, E. (1979). Selective binding of the scorpion venom insect toxin to insect nervous tissue. *Insect Biochem.* **9**, 343–346.

VINCENT, D., SHWEITZ, H., CHICHEPORTICHE, R., FOSSET, M., BALERNA, M., LENOIR, M. & LAZDUNSKI, M. (1976). Molecular mechanism of cardiotoxin action on axonal membranes. *Biochemistry, N.Y.* **15**, 3171–3175.

WATERHOUSE, D. F. & WRIGHT, M. (1960). The fine structure of the mosaic midgut epithelium of blowfly larvae. *J. Insect Physiol.* **5**, 230–239.

WIGGLESWORTH, V. B. (1929). Digestion in the tsetse-fly: a study of structure and function. *Parasitology* **21**, 288–321.

ZAHEER, A., NORONHA, S. H., HOSPATTANKAR, A. V. & BRAGANCA, B. M. (1975). Inactivation of (Na^+-K^+) stimulated ATPase by a cytotoxic protein from cobra venom in relation to its lytic effects on cells. *Biochim. biophys. Acta* **394**, 293–303.

ZHUZHIKOV, D. P. (1964). Function of the peritrophic membrane in *Musca domestica* L. and *Calliphora erythrocephala* Meig. *J. Insect Physiol.* **10**, 273–278.

ZLOTKIN, E., FRAENKEL, G., MIRANDA, F. & LISSITZKY, S. (1971). The effect of scorpion venom on blowfly larvae – a new method for the evaluation of scorpion venom's potency. *Toxicon* **9**, 1–8.