MALACOLOGIA, 1989, 31(1): 141–156

ULTRASTRUCTURAL CHANGES IN THE DIGESTIVE TRACT OF *DEROCERAS RETICULATUM* (MÜLLER) INDUCED BY A CARBAMATE MOLLUSCICIDE AND BY METALDEHYDE

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ABSTRACT

Electron microscope investigations reveal different reactions of cells in the digestive tract of *Deroceras reticulatum* to intoxication with carbamate or metaldehyde molluscicides. All enterocytes are more strongly attacked by the carbamate compound Mesurol than by metaldehyde. The better efficiency of Mesurol is primarily attributed to its severe impact on nuclei, leading to other cell damage and finally to an increased macrophage reaction.

Metaldehyde leaves the enterocyte functions more or less intact except for that of mucus cells. It activates mucus extrusion immediately after the onset of intoxication. This mucus serves to dilute the toxin, which passes through the digestive tract and is voided. The severe attack of metaldehyde on the immature mucus cells results in cessation of mucus production, leading to a fatal mucus deficiency in the digestive tract.

Key words: Gastropoda; molluscicides; carbamate; metaldehyde; digestive tract; ultrastructure

INTRODUCTION

To date, the most efficient pesticides against slugs are carbamate compounds, such as Mesurol, which act as nerve toxins by inhibition of the cholinesterase activity (Getzin & Cole, 1964; Pessah & Sokolove, 1983). During the last decade, Mesurol has replaced metaldehyde as the primary commercial molluscicide, because metaldehyde loses most of its efficiency in humid climates (Martin & Forrest, 1969). In the literature (Pappas et al., 1973), however, it is not only Mesurol but also such other carbamate compounds as Carzol, Furadan and Zectran that are mentioned as having an increased efficiency compared to metaldehyde (Getzin, 1965; Prystupa et al., 1987). Whereas in most investigations LD_{50} tests are used (Bakhtawar & Mahendru, 1987), there are only a few publications concerning cellular mechanisms induced by molluscicides (Ishak et al., 1970; Banna, 1977, 1980a, b; Pessah & Sokolove, 1983). Up to the present, little attention has been paid to the fact that both carbamate compounds and metaldehyde are in use as oral toxins (cf. Henderson, 1969), and, as a consequence, the first possible targets for molluscicidal action might be the cells of the intestinal epithelia.

In fact, only one study covers the influence of molluscicidal agents on the cells and tissues of the alimentary tract of slugs after intoxication (Manna & Ghose, 1972). To the best of my knowledge, ultrastructural investigations are completely lacking. Thus, the present electron microscope study was designed to investigate the different cellular responses to molluscicidal intoxication in the digestive tract of the grey garden slug, *Deroceras reticulatum*. A further purpose of the paper is to elucidate the reasons for the superior efficiency of carbamate molluscicides by comparing the ultrastructural damage after oral application of carbamate and metaldehyde.

MATERIALS AND METHODS

Laboratory-reared specimens of *Deroceras reticulatum* were fed pellets containing 4% of the carbamate compound Mercaptodimethur (4-(methylthio)-3,5-xylyl-methyl-carbamate; Mesurol; Bayer) or 4% metaldehyde (Spiess Urania 2000). The pellets were weighed before and after the slugs had fed, and the amount of toxicant effectively ingested was calculated. On an average, the animals took

up 200 µg Mesurol or 9 mg metaldehyde/g wet weight. Animals fed carbamate were dissected after one, five and 16 hours. The metaldehyde group was fixed after five hours. For primary fixation a 2% glutaraldehyde solution in cacodylate buffer (0.01 M, pH 7.4) was injected into the body cavity. Then oesophagus, crop, stomach, intestine and digestive gland were isolated in fixative and fixed for two hours in 2% glutaraldehyde at 4°C. The tissues were rinsed in cacodylate buffer and postfixed in 1% osmium-ferrocyanide (Karnovsky, 1971) for two hours. After rinsing in cacodylate and 0.05 M maleate buffer (pH 5.2), the specimens were stained en bloc in 1% uranylacetate in 0.05 M maleate buffer overnight at 4°C. The samples were dehydrated and embedded either in Araldite or in Spurr's medium (Spurr, 1969).

Ultrathin sections cut on a Reichert ultramicrotome were counter-stained with lead citrate for 30 minutes and finally examined in a Zeiss EM 9.

RESULTS

Macroscopic observations

The macroscopic reactions of the animals after molluscicide application correspond to the reactions described as typical for carbamate or metaldehyde intoxication by Godan (1979). By 30 minutes after ingestion of Mesurol pellets, the animals show violent muscle convulsions. The anterior body begins to swell while the posterior flattens. The tentacles are relaxed, the animals release a lucid mucus and take up liquid from the environment. After three hours they lie almost motionless on one side. Usually they die 20 to 30 hours later, but recovery is also possible.

After the application of metaldehyde, the animals lose much more slime than after carbamate ingestion. In this case, muscle convulsions and relaxation of the tentacles could not be observed.

Electron microscopical investigations

Histology of the epithelia in control animals

Oesophagus: The oesophageal epithelium consists of four cell types, three of which reach the lumen (Fig. 1a):

Type I: Columnar storage cells (Figs. 2a, 5) characterized by high amounts of lipid and storage carbohydrate (glycogen or galactogen) (Fig. 34). In the central cytoplasm, the nucleus, small Golgi complexes, mitochondria and a few peroxisome-like vesicles are located, while smooth and granular endoplasmic reticulum occasionally appear in basal regions of the cells. Under the microvillous border a band of mitochondria can be found (Fig. 10).

Type II: Columnar secretory cells of an eccrine type (Fig. 1a), with basally situated granular endoplasmic reticulum and an elaborate Golgi apparatus (Fig. 29) producing electron-lucent secretory vesicles. Mitochondria and small amounts of lipid and glycogen are dispersed over the cytoplasm. The nucleus is located in the center of the cell.

Type III: Secretory cells of a holocrine type (mucus producing goblet cells, in the following called "mucus cells") (Figs. 1a, 3a, 5, 25), with conspicious granular endoplasmic reticulum characterized by a spacious lumen, large Golgi apparatus and mucus vacuoles that merge in the apical part of the cells. The nuclei of these cells are situated in the basal, dilated regions. Young mucus cells (Fig. 3a) do not contain high amounts of mucus vacuoles and are characterized by a conical cell shape.

Type IV: Small electron-lucent cells (Fig. 5), conical in shape, that are dispersed amongst the other cells. Their apices do not reach the lumen. Containing characteristic lysosomes, dictyosomes and a prominent nucleus, they resemble the haemolymph macrophages.

The basal surfaces of all cell types have no infoldings (Fig. 5). In addition to numerous microvilli, the luminal surface of cell types I and II may bear cilia (Fig. 2a). The microvilli of the mucus cells are smaller than those of the other cell types (Fig. 3a).

A strong muscle layer, connective tissue cells and nerves with different neurosecretory vesicles can be found subtending the epithelium (Fig. 2a, 40, 42). In longitudinal section, the muscle filaments are all roughly parallel, while in transverse section there is a quasi-lattice of thick and thin filaments (Fig. 40). In the haemolymph space some macrophages can be observed. They are characterized by a large nucleus, small Golgi apparatus and a few small vesicles of various electron-density (Fig. 2a).

Crop: Apart from a few mucus (Type III) and small electron-lucent cells (Type IV), the cylindrical epithelium of the crop is dominated by a single cell type, resembling the storage

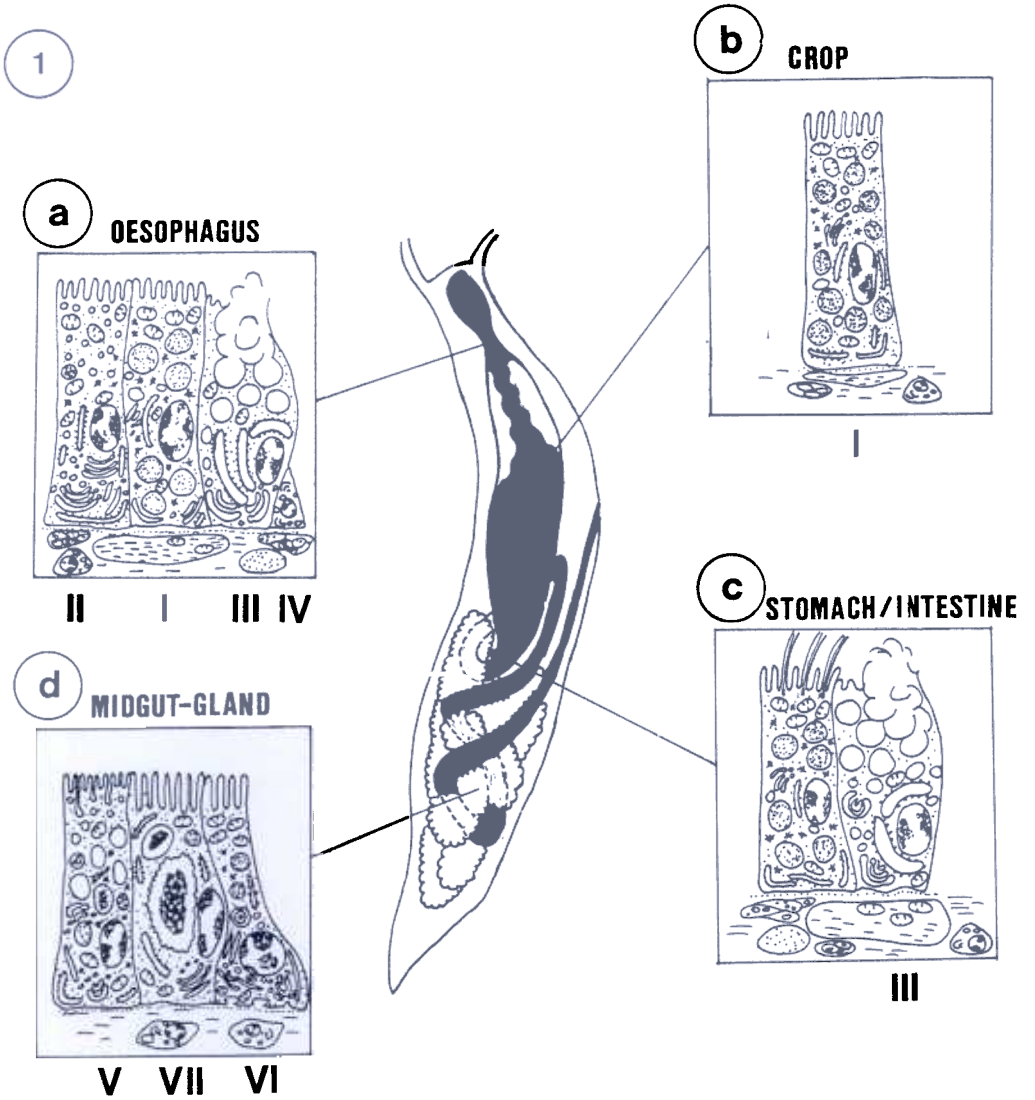

FIG. 1. Diagram of the digestive tract of *Deroceras reticulatum* illustrating the cells investigated in the present study.

1a. Oesophagus: Storage cell (I), secretory cell of an eccrine type (II), secretory cell of a holocrine type, called mucus cell (III), and small electron-lucent cell (IV)

1b. Crop: Storage cell

1c. Stomach and adjacent intestine: Storage (I) and mucus cell (III)

1d. Mid-gut gland: Digestive cell (V), crypt cell (VI), and excretory cell (VII)

cell (Type I) of the oesophagus (Fig. 1b). Only in regions of the crop adjacent to the stomach do these cells bear cilia.

A small muscle layer with associated connective tissue and nerves underlies the epithelium.

Stomach and adjacent intestine: Half of the stomach epithelium is made up by cells resembling the storage cells of the oesophagus with respect to their ultrastructural organisation and storage products (Fig. 1c). The cells always bear microvilli and cilia (cf.

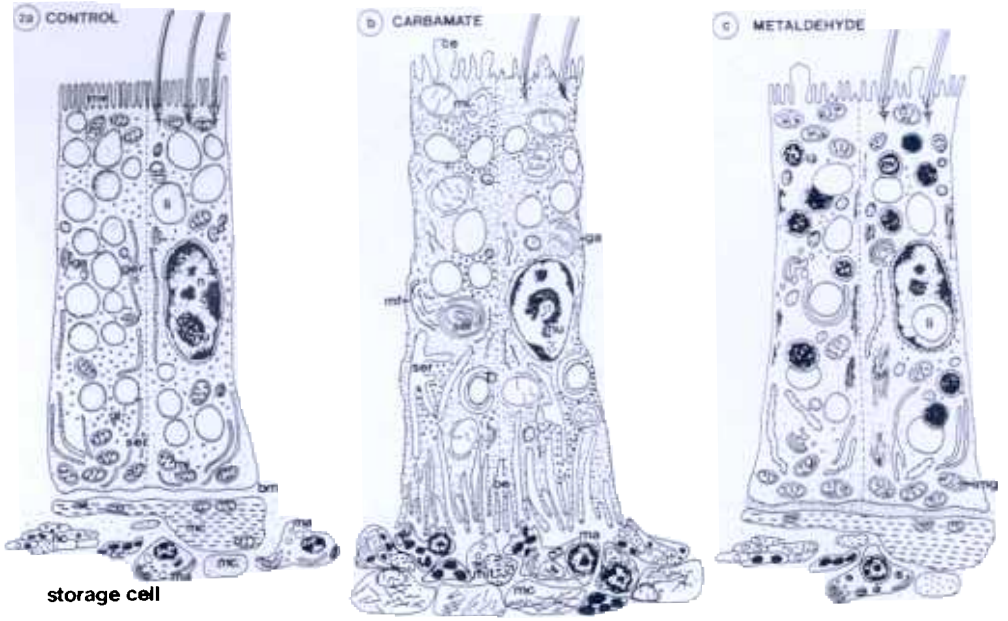

FIG. 2. Reconstruction of an unciliated or ciliated storage cell (2a) and its habit after carbamate (2b) and metaldehyde (2c) intoxication

Walker, 1972). In the stomach crypts, the cilia are longer and more numerous (cf. Häffner, 1924). The rest of the epithelium is made up by mucus cells (Type III). In the adjacent intestine, half of the cells are mucus cells (Type III), and only a quarter are storage cells (Type I). The other quarter of the cells are secretory (Type II). An underlying muscle layer is well developed. It can be compared with that of the oesophagus (Fig. 40). Many nerve fibres can be detected.

Mid-gut gland: The epithelium of the mid-gut gland is arranged in tubules that are bound together by a meshwork of connective tissue. An underlying muscle layer is lacking. Three cell types can be distinguished (Fig. 1d, 4a):

Type V: The columnar digestive cells, highly vacuolized absorptive cells, that dominate the epithelium. The vacuoles vary in size and are generally largest towards the basal regions of the cells. Pinocytotic vesicles develop along the apical plasma membrane, where endocytotic channels can also be found. The absorptive area is increased by numerous microvilli. The digestive cell cytoplasm contains a little granular endoplasmic reticulum, a few mitochondria and an occasional small Golgi apparatus. Lipid and glycogen storage can be found. The nuclei of these cells are basally located.

Type VI: The crypt cells are conical in shape with broad bases abutting on to the haemolymph space. Serving secretory functions, they are characterized by a large amount of granular endoplasmic reticulum (Fig. 22), a great number of Golgi stacks and secretory vesicles in the perinuclear cytoplasm. The nuclei are basally situated, possess a large nucleolus and have scattered patches of heterochromatin. Mitochondria are located near the apical and the basal surfaces of the cells. Lipid and carbohydrate storage, as well as membrane-bound spherites are present (Fig. 4a). The microvilli are longer than those of the digestive cells, and the basal labyrinth is well developed.

Type VII: The goblet-like excretory cells are characterized by large and small vacuoles containing electron-dense material (Fig. 27). The membrane of the large vacuole shows numerous infoldings. In the cytoplasm a small Golgi apparatus, a small amount of smooth and granular endoplasmic reticulum and lipid, and a few mitochondria can be found. The

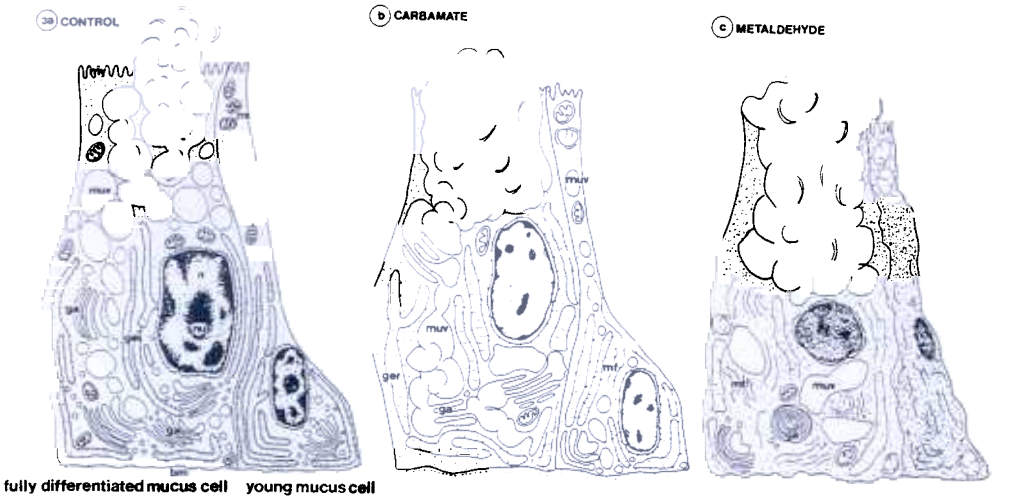

FIG. 3. Reconstruction of a young and a fully differentiated mucus cell in control animals (3a), after carbamate (3b), and after metaldehyde intoxication.

microvilli of these cells are as long as those of the crypt cells.

To compare the different theories concerning the genealogy of these cells and the different nomenclatures, see David & Götze (1963) and Walker (1970).

Histopathological alterations (Figs. 2, 3, 4)

Generally speaking, the cytological reactions in the digestive tract originate in isolated cells and spread over the epithelium during the following hours. Sixteen hours after ingestion of the molluscicides, a high percentage of the cells are significantly damaged.

The reactions observed after five and 16 hours generally resemble those after one hour, but they are more intense.

Reactions that appear in the anterior part of the digestive tract immediately after the molluscicide treatment became apparent in cells of the posterior part with a time lapse corresponding to the transport rate of toxic foodstuff.

Sixteen hours after carbamate ingestion, a lot of cells have been extruded from the epithelium.

Reactions of the basal and apical cell surfaces

MESUROL: Immediately after the application of Mesurol, the basal surfaces of storage and secretory cells (Type I and II) are slightly stretched (Comp. Figs. 5 and 6). After five and 16 hours, the cells exhibit considerably extended basal cell extensions (Fig. 7) that sometimes contact nerve or muscle cells (Fig. 8). In the mid-gut gland the basal cell extensions are less distinct than in the alimentary tract. However, the basal labyrinth of crypt cells is distended, and the intercellular spaces are enlarged (Fig. 35).

Comparable to the reactions of the cell bases, the apical surfaces of the cells react very quickly with a reduction of microvilli and cytoplasmic protrusions in the anterior, and after five or 16 hours in the posterior parts of the digestive tract (Figs. 10, 11, 12).

An intensified vacuolization in the digestive cells often leads to a breakdown of the apical membrane.

METALDEHYDE: After intoxication with metaldehyde, basal cell extensions are lacking; the basement membrane is thickened and becomes more electron-dense (Fig. 9).

Protrusions of the apical cytoplasm and reduction of microvilli can occasionally be found.

After intoxication with carbamate and metaldehyde, the shape of all cell types becomes more irregular (Figs. 2, 3, 4).

Reactions of the cytoplasm

MESUROL: After carbamate intoxication, the cytoplasm of storage and secretory cells appears slightly condensed (Fig. 7) or elec-

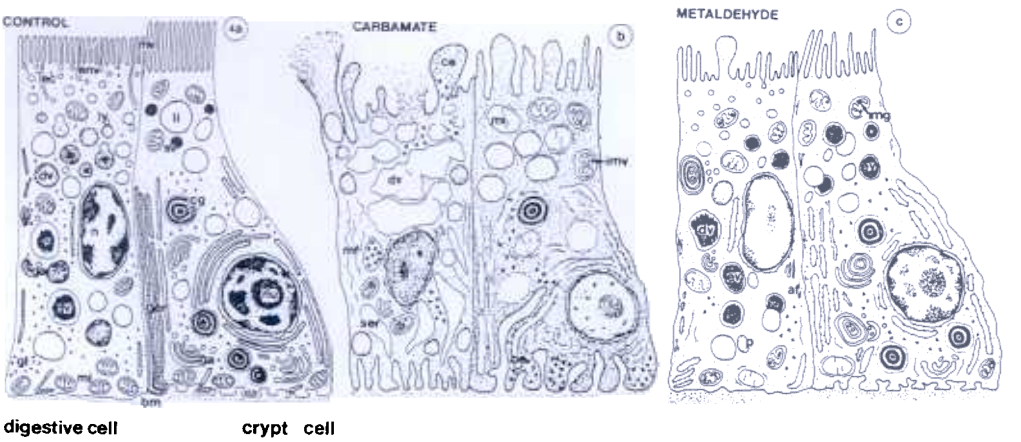

FIG. 4. Reconstruction of a digestive and a crypt cell of the mid-gut gland (4a) and its variable habit after carbamate (4b) and metaldehyde (4c) ingestion.

tron-lucent (Fig. 13). In the digestive cells of the mid-gut gland, it is either extremely electron-lucent, or totally electron-dense, depending on the degree of vacuolization. Electron-dense cytoplasmic areas often surround the nuclei.

METALDEHYDE: In mucus cells the cytoplasm is displaced by the enlarged mucus vacuoles (Fig. 33). In all cell types it appears less electron-dense.

Reactions of the nuclei

MESUROL: One hour after intoxication the nuclei are severely damaged.

The karyoplasm becomes less electron-dense (Fig. 13, 14, 15), lipid droplets can be detected in it (Fig. 15), the nucleoli are irregularly deformed (Fig. 16), and the amount of heterochromatin is reduced. In some cases, the karyoplasm appears totally condensed (Fig. 17), or, especially in crop cells, small vesicles can be found in it (Fig. 16). Mitotic processes are evident. However, even after 16 hours, there are still some unafflicted nuclei next to totally damaged ones (Fig. 17), emphasizing the heterogeneity in cellular reaction.

METALDEHYDE: After metaldehyde ingestion, damage to the nuclei is less intense than after carbamate application. The karyoplasm becomes less electron-dense, and in a few cases it bears lipid droplets (comp. Fig. 14). Especially in the crypt cells of the mid-gut gland, the amount of heterochromatin is reduced.

Reactions of the mitochondria

MESUROL: After carbamate intoxication, mitochondrial effects originate in the oesophagus and crop cells from the cell apex, while in the posterior parts of the digestive tract mitochondria located in the basal cytoplasm are afflicted earlier.

Especially in the stomach and the digestive gland, electron-dense granules different from the common intramitochondrial granules can be found in mitochondria, located in membrane-bound compartiments (Fig. 18). Furthermore, the organelles are heavily inflated and their cristae are reduced (Fig. 19).

METALDEHYDE: After metaldehyde intoxication, the mitochondria are less afflicted than after carbamate ingestion. The common intra-mitochondrial granules are often enlarged (Fig. 20), and only in a few cases the organelles are swollen.

Reactions of the endoplasmic reticulum

MESUROL: After Mesurol application, the smooth and granular endoplasmic reticulum proliferates in basal regions of storage, digestive and crypt cells. In most cases, the smooth endoplasmic reticulum is heavily distended (Fig. 21). Degranulation of granular endoplasmic reticulum can be observed in basal re-

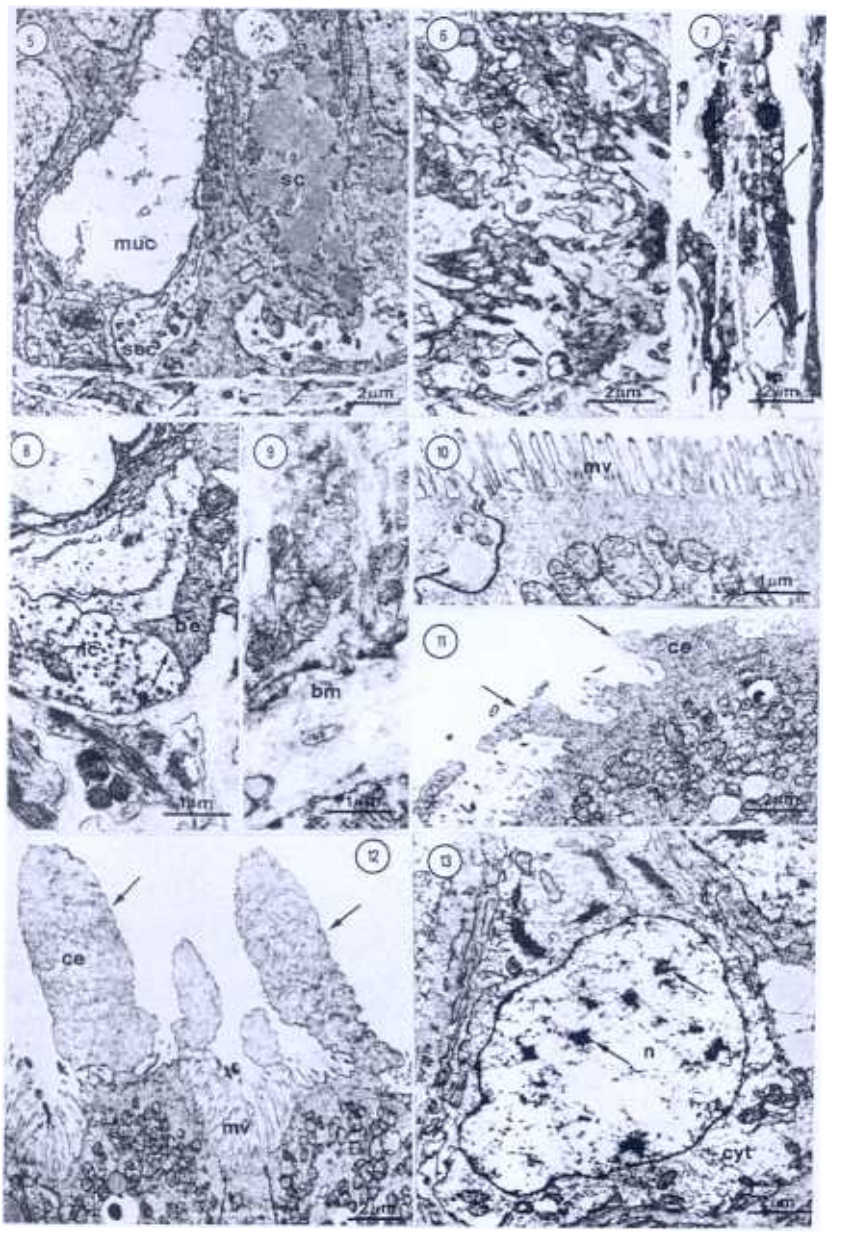

FIG. 5. Oesophagus, control: Section through the basal part of the oesophagus epithelium showing a mucus (muc), storage (sc) and small light cell (slc). There are no infoldings of the cell basis (arrows).
FIG. 6. Oesophagus, Mesurol, 1 h: Slight basal extensions (arrows).
FIG. 7. Oesophagus, Mesurol, 5 hs: Strong basal extensions (arrows).
FIG. 8. Oesophagus, Mesurol, 1 h: Contact of a basal cell extension (be) to a nerve cell (nc,arrow).
FIG. 9. Crop, metaldehyde, 5 hs: Thickening of the basal membrane (bm).
FIG. 10. Crop, control: Apex of a storage cell, showing regularly orientated microvilli (mv).
FIG. 11. Oesophagus, Mesurol, 1 h, storage cell: Slight cytoplasm extrusions (ce, arrows).
FIG. 12. Oesophagus, Mesurol, 1 h, storage cell: Intensified cytoplasm extrusions (ce, arrows).
FIG. 13. Oesophagus, Mesurol, 16 hs, secretory cell: Electron-lucent cytoplasm (cyt) and nucleus (n) with the heterochromatin reduced (arrows).

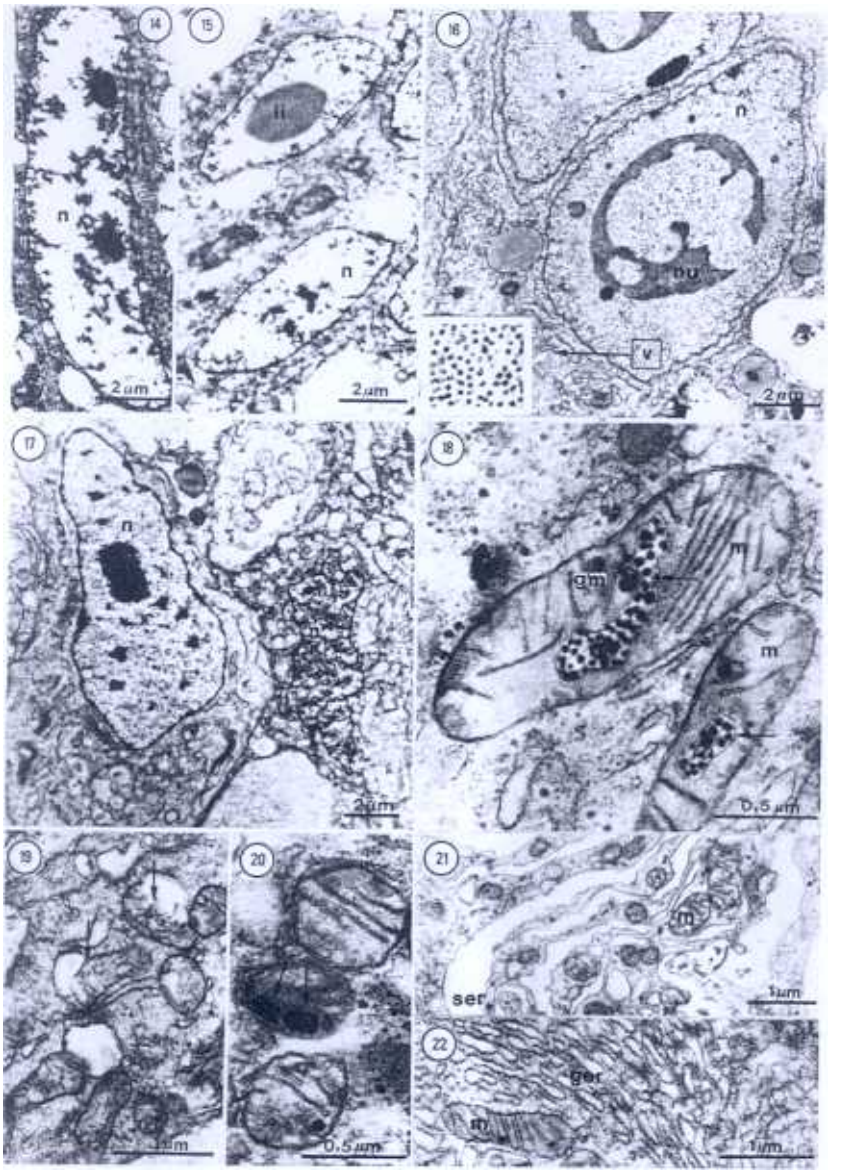

FIG. 14. Oesophagus, Mesurol, 5 hs, storage cell: Nucleus (n) with a totally dissolved karyoplasm.
FIG. 15. Stomach, Mesurol, 5 hs: Nuclei with lipid inclusions (li) in an electron-light karyoplasm.
FIG. 16. Crop, Mesurol, 1 h: Nucleus with an irregularly formed nucleolus (nu) and small vesicles (v) in the karyoplasm (inset, ×6).
FIG. 17. Crop, Mesurol, 16 hs: Totally damaged nucleus next to an unafflicted one in the adjacent crop cell.
FIG. 18. Mid-gut gland, Mesurol, 16 hs, crypt cell: Mitochondrion (m) with inclusions in membrane-bound areas (arrows); common intramitochondrial granules (gm) are visible.
FIG. 19. Oesophagus, Mesurol, 1 h, secretory cell: Destruction of cristae in mitochondria (arrows).
FIG. 20. Stomach, metaldehyde, 5 hs, storage cell: Mitochondrion with enlarged intramitochondrial granules (arrows).
FIG. 21. Intestine, Mesurol, 5 hs, storage cell: Dilation of smooth endoplasmic reticulum (ser) in the basal parts of the cell.
FIG. 22. Mid-gut gland, control, crypt cell: Cisternae of the granular endoplasmic reticulum (ger).

gions of the crypt cells (comp. Figs. 22 and 23).

In mucus cells the granular endoplasmic reticulum is dilated and disorientated. After several hours, the membranes become fragmented.

METALDEHYDE: The damage to the granular endoplasmic reticulum in mucus cells is more intense after metaldehyde than after carbamate intoxication. The cisterna are heavily dilated, and the membranes are disarranged, ruptured and sometimes coiled to form myeline figures (comp. Figs. 25 and 26).

In the crypt and excretory cells of the mid-gut gland, the granular endoplasmic reticulum disintegrates into short cisternae with fragmented membranes, frequently devoid of ribosomes (Fig. 28). The cisternae of the endoplasmic reticulum often form fingerprint-like structures (Fig. 24). In many instances, the membranes are ruptured.

Reactions of the Golgi apparatus

MESUROL: In the oesophagus, the Golgi complexes of the secretory cells (Type II) are heavily damaged. Especially the trans-face cisternae are either compressed or highly inflated (comp. Figs. 29 and 30). Associated with this is a reduction in the number of secretory vesicles.

In the mucus cells, the cisternae of the Golgi apparatus are strongly dilated (Fig. 31), and the regular arrangement of the Golgi stacks is often lost.

METALDEHYDE: Except for the mucus cells, the reaction of the Golgi apparatus is less intense after metaldehyde than after carbamate ingestion. The trans-faces of the cisternae are slightly dilated. In the mucus cells, the Golgi cisternae are swollen, often the membranes are arranged as concentric whorls (Fig. 32), and the mucus containing vacuoles are enlarged (Fig. 33).

Alteration of storage products

MESUROL: After carbamate ingestion, compact areas containing glycogen or galactogen can be found between lipid droplets, especially in central and basal parts of digestive cells and in crypt cells of the mid-gut gland (Fig. 35).

METALDEHYDE: In storage, secretory, digestive, crypt and excretory cells, the amounts of lipid and glycogen are reduced after metaldehyde poisoning (comp. Figs. 34 and 36). This reduction is related to the presence of vesicles containing electron-dense material with a typical lamellar fine-structure (Fig. 37). Furthermore, an increased number of peroxisome-like structures appears.

Reaction of the cytoskeleton

MESUROL: After Mesurol intoxication, no reaction of the cytoskeleton is visible.

METALDEHYDE: In the center of storage and secretory cells (Type I and II), condensed actin-like microfilaments appear (Figs. 38, 39).

Reactions of the underlying muscle connective and nerve tissues

MESUROL: After the application of the carbamate molluscicide, the muscle tissue is fragmented, and the regular arrangement of the muscle filaments is disturbed (comp. Figs. 40 and 41). Granules similar to peroxisomes (with regard to their size and their electron-density) appear in connective tissue cells showing intensive contact to smooth muscle and nerve cells, as well as in nerve cells themselves (Figs. 42, 43).

METALDEHYDE: After application of metaldehyde, no reactions of muscle, nerve and connective tissue could be found.

Reactions of the macrophages

MESUROL: After ingestion of Mesurol, the number of macrophages in the haemolymph space increases. In many cases, mitotic processes can be observed (Fig. 44). Particularly 16 hours after intoxication, many of these cells penetrate the epithelium (Fig. 45). Other macrophages contain membrane fragments incorporated into vacuoles (Fig. 46).

METALDEHYDE: Reactions of the macrophages are lacking.

DISCUSSION

The present study reveals the impact of the carbamate compound Mesurol and of metal-

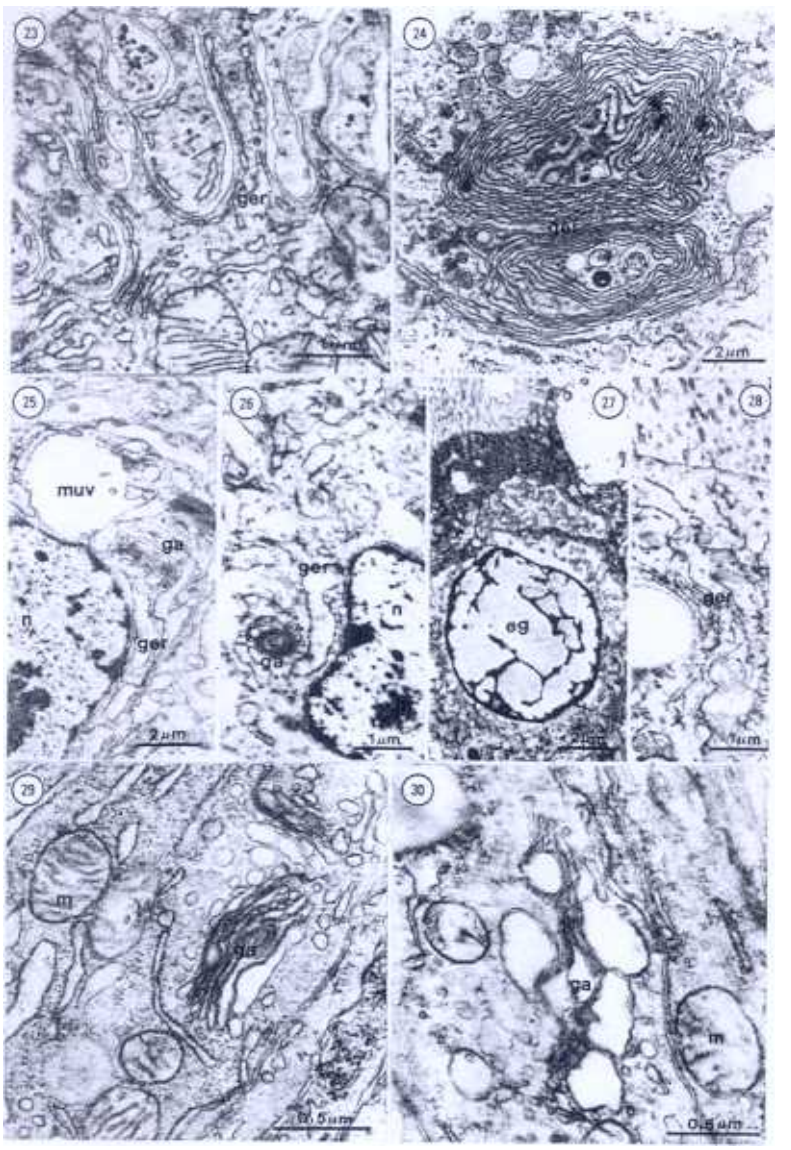

FIG. 23. Mid-gut gland, Mesurol, 1 h, crypt cell: Degranulation of granular ER in the basal part of the cell (arrows).
FIG. 24. Mid-gut gland, metaldehyde, 5 hs, crypt cell: Granular ER (ger) forming fingerprint-like structures.
FIG. 25. Oesophagus, control, mucus cell: Elaborate Golgi system (ga), granular ER (ger) and mucus vacuoles (muv) in a regular arrangement.
FIG. 26. Oesophagus, metaldehyde, 5 hs, mucus cell: Dilated, electron-lucent cisterae of the granular ER and Golgi membranes (ga) forming concentric circles in a cellular disarrangement; nucleus (n) with an electron-lucent caryoplasm.
FIG. 27. Mid-gut gland, control, Excretory cell: excretory cell with excretory granule (eg).
FIG. 28. Mid-gut gland, Mesurol, 5 hs, crypt cell: Short, fragmentary cisternae of the granular ER (ger) frequently devoid of ribosomes.
FIG. 29. Oesophagus, control, secretory cell: Trans- and cis-face of the Golgi apparatus (ga) can clearly be distinguished.
FIG. 30. Oesophagus, Mesurol, 1 h, secretory cell: Heavily inflated cisternae of a Golgi apparatus (ga).

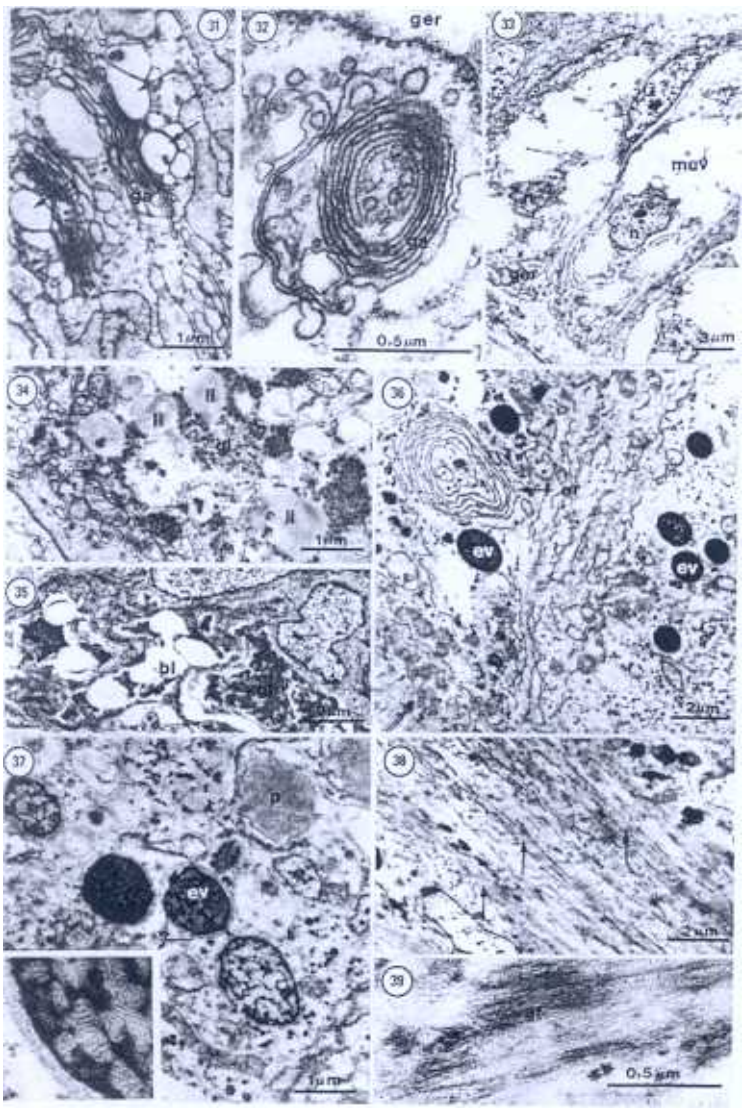

FIG. 31. Stomach, Mesurol, 5 hs, mucus cell: Golgi apparatus (ga) with dilated cisternae (arrows).
FIG. 32. Oesophagus, metaldehyde, 5 hs, mucus cell: Membranes of the Golgi apparatus (ga) are arranged as concentric whorls surrounding Golgi vesicles.
FIG. 33. Stomach, metaldehyde, 5 hs: General view of the mucus cells after metaldehyde application; dilated cisternae of the Golgi apparatus and the granular ER, large mucus vacuoles (muv) and nuclei (n) with an electron-lucent karyoplasm.
FIG. 34. Oesophagus, control, storage cell: High amount of stored lipid (li) and glycogen or galactogen (gl).
FIG. 35. Mid-gut gland, Mesurol, 5 hs, digestive cell: Condensation of glycogen (gl) and dilation of the sER and the basal labyrinth (bl).
FIG. 36. Oesophagus, metaldehyde, 5 hs, storage cell: Lipid and carbohydrate reduction; electron-dense vesicles (ev) and ER membranes forming a fingerprint-like structure (arrow).
FIG. 37. Oesophagus, metaldehyde, 5 hs, storage cell: Electron-dense vesicles (ev) characterized by a typical lamellar fine-structure (inset, ×4) and by peroxisome-like organelles (p) in the center of the cell.
FIG. 38. Crop, metaldehyde, 5 hs: Reduction of storage products and aggregation of actin-like filaments (arrows).
FIG. 39. Crop, metaldehyde, 5 hs: Actin-like filaments (af) in the center of the enterocytes.

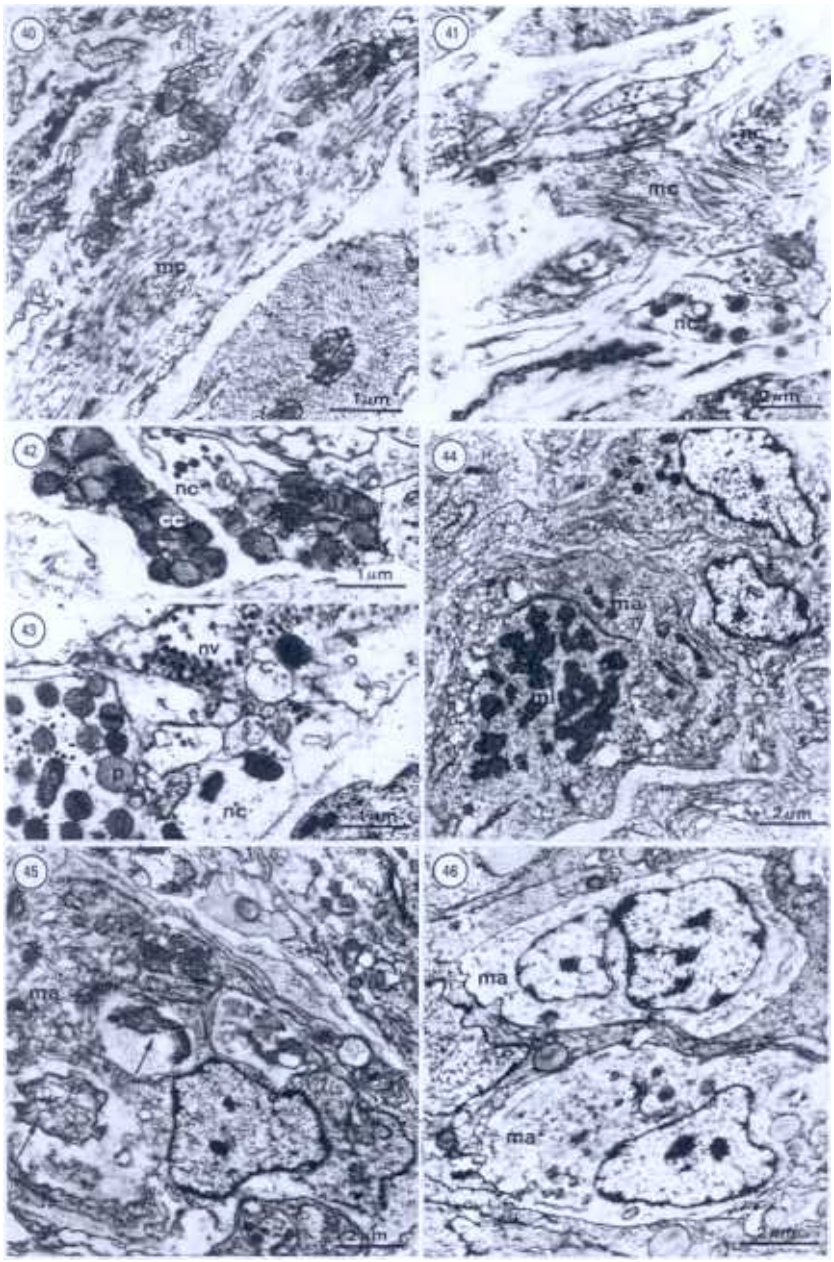

FIG. 40. Oesophagus, control: Muscle cell (mc) with regularly orientated muscle fibres.
FIG. 41. Oesophagus, Mesurol, 5 hs: Fragmentation of muscle tissue in isolated portions and irregular orientation of muscle fibres. Intense contact of nerve (nc) and muscle cell (mc).
FIG. 42. Oesophagus, Mesurol, 1 h: Nerve (nc) and connective tissue cells (cc) containing electron-dense, peroxisome-like structures.
FIG. 43. Oesophagus, Mesurol, 5 hs: Nerve with peroxisome-like structures (p).
FIG. 44. Oesophagus, Mesurol, 5 hs: Mitosis taking place in a macrophage (ma).
FIG. 45. Oesophagus, Mesurol, 16 hs: Macrophage bearing membrane fragments in vacuoles (arrows).
FIG. 46. Oesophagus, Mesurol, 1 h: Two macrophages that penetrate the epithelium.

dehyde on the ultrastructure of the digestive tract of *Deroceras reticulatum.* In addition to the results of Tegelsstrom & Wahren (1972), Godan (1979) and Pessah & Sokolove (1983), who attributed the molluscicidal effects to influences on cholinesterase activity and water regulation, it can clearly be demonstrated that both chemicals interact with several different types of enterocytes.

As evident by the present cytological findings, the carbamate compound Mesurol is absorbed immediately after ingestion in the anterior parts of the digestive tract, i.e. in the oesophagus and the crop. The rapid reactions in these two regions and the quick and intense reaction of the whole animal one hour after feeding support the findings of Fretter (1952), Walker (1972) and Horst et al. (1986), who describe the high absorptive activity of the crop using radioactive labelling and biochemical methods. With regard to the time lapse resulting from the passage of food through the intestinal tract, the cellular responses after one hour in the anterior parts can be compared with those in the posterior parts of the digestive system after five hours. Analysis of cellular responses to metaldehyde and carbamate poisoning after five hours allows three types of reaction to be distinguished: carbamate-specific reactions, metaldehyde-specific reactions, and cell responses that appear in both experiments but with different intensity.

Reactions such as cytoplasm condensation, cytoplasmic protrusions also called "surface blebs" (Whyllie, 1981; Réz, 1986), reduction of microvilli, mitochondrial swelling (Goyer & Rhyne, 1975; Triebskorn, 1988) and dilation of Golgi cisterna (Triebskorn, 1988), endoplasmic reticulum and intercellular spaces (Smuckler & Arcasoy, 1969), as well as ER-membrane proliferation or destruction (Moore, 1979, 1985; Nott & Moore, 1987) resemble those described in bivalves and vertebrates as cellular stress symptoms after intoxication with different xenobiotics. Likewise, the degranulation of the granular ER and the formation of membrane whorls or myelin-like membranes by ER are discussed as general changes of the cell in response to toxicants (Réz, 1986). Most of these reactions are attributed to membrane destabilization and increased membrane permeability to ions under the influence of toxicants, followed by osmotic effects and finally cell death (Sparks, 1972).

Swelling of mitochondria is suggested to be the result of an increased Ca^{2+} influx (Packer et al., 1967; Smuckler & Arcasoy, 1969). Bayne et al. (1985), Moore (1985) and Nott & Moore (1987) relate the sER proliferation to an increase of sER-bound detoxification enzymes, such as the NADPH-neotetrazolium-reductase and many others.

In the digestive tract of *Deroceras reticulatum,* such unspecific reactions are more intense after carbamate than after metaldehyde treatment except for the mucus cells of oesophagus, stomach and intestine. This might arise from the fact that the amount of metaldehyde taken up by the animals is supposed to be closer to the sublethal dose than that of carbamate. Therefore, after Mesurol intoxication, many more cellular reactions associated with cell death are involved. However, the comparison of reactions to different molluscicides in several regions of the digestive tract, as well as in several cell types at three times allows reactions of general nature to be distinguished from specific ones. Thus, for example, in mucus cells the destruction of Golgi cisternae, rER and mitochondria is more prominent after metaldehyde than after carbamate ingestion. This severe impact of metaldehyde on the mucus producing cells correlates well with the known influence of this molluscicide on water regulation. Metaldehyde enhances the extrusion of available mucus immediately after intoxication. This mucus might serve to dilute the toxin but may also have the capacity to detoxify it (Triebskorn, 1988). It passes the digestive tract and is voided quickly due to the intensified mucus extrusion of the whole animal. Furthermore, the replacement of the necessary mucus is blocked by destruction of the cellular secretory apparatus, especially in immature cells. If the resulting loss of liquid cannot be compensated, the animal will desiccate. Therefore, the effects of metaldehyde are reversible in a humid climate.

These considerations are in line with other investigations studying the advantages of carbamates compared with metaldehyde using LD_{50} tests (Riemschneider & Heckel, 1979; Prystupa et al., 1987). They support the findings of Getzin & Cole (1964), who postulate the effect of metaldehyde to be the result of water loss by stimulation of mucus secretion.

Furthermore, metaldehyde poisoning reduces cellular lipids, increases the number of electron-dense vesicles and peroxisome-like particles, and leads to a thickening of the basement membrane and the condensation

of actin-like filaments in the cytoplasm. Until now, there are no targets known for the attack of toxins in the cytoskeletal system. Nor is there any intelligible explanation for the thickening of the basement membrane.

With regard to lipid reduction, my own enzyme-histochemical studies have shown that catalase activity can be found in the periphery of lipid droplets after molluscicide intoxication (Triebskorn, in prep.). One might speculate that the observed lipid reduction is correlated with lipid peroxidation (cf. Tappel, 1975). To reinforce this idea, the presence of detoxification products resulting from such reactions as well as the nature of the electron-dense vesicles and the peroxisome-like structures should be investigated. Beyond this, the reaction of macrophages and connective tissue cells after intoxication with Mesurol could be of interest. While the present study demonstrates an increased number of peroxisome-like structures in connective tissue cells after Mesurol intoxication, Sminia (1972) was able to demonstrate peroxidase activity in haemolymph cells, localized in similar peroxisome-like vesicles. In addition, my own light-microscope investigations reveal an intensified catalase activity in the connective tissue underlining the epithelium of the digestive tract and in the haemolymph, where macrophages can be found (Triebskorn, in prep.). Because peroxidative reactions are known to be involved in detoxification (Belding et al., 1970; Recknagel, 1967), I assume that similar processes are of importance in the digestive tract of slugs after molluscicide intoxication. Whereas they might be found after metaldehyde treatment in the enterocytes themselves, carbamate poisoning leads to a disturbance of essential functions of these cells so quickly that detoxification processes cannot be established in them in time. This deficiency might be compensated by macrophage activity. The function of these cells in detoxification is also verified by the results of Moore (1979). He could demonstrate the activation of MFO-enzymes in the haemolymph cells of *Mytilus edulis* after polycyclic hydrocarbon poisoning. Furthermore, the haemocytes are known to be able to penetrate the gut epithelium and to phagocytise decaying cells (Sminia, 1972).

The macrophage reaction, nucleic damage and a changed glycogen metabolism appear one hour after the ingestion of the carbamate. The Mesurol attack on the nucleus seems to be the most important effect of this chemical and accounts for its better molluscicidal activity compared with metaldehyde. While karyolysis is often described as a late reaction to intoxication in vertebrates and invertebrates (Bayne et al., 1985), the reaction of nuclei in the present study does not seem to be an ultimate one revealing cell death, but a primary cell response that leads to cell death. Since, in most cells with damaged nuclei, other cell death symptoms are lacking, the heterochromatin disorganization and increase in mitotic activity seem to reflect a central process in intoxication. The damage to the nuclei could explain the reinforcement of other cellular responses, such as the disturbances in glycogen metabolism or the destruction of Golgi apparatus, already described by Flickinger (1971).

In contrast to these reactions, the formation of basal cell extensions requires several hours. This is probably due to the inhibition of cholinesterase activity by carbamates. The blockade of the esterase center of the enzyme (Wegler, 1970) interrupts nerve stimuli conduction, leading to uncontrolled muscle contraction and finally to muscle atony. Uncontrolled contractions in the muscle layer that underlies the gut epithelium are responsible for the basal cell deformation.

In conclusion, the present results suggest that the cells might respond to environmental stress in different ways. Lipid mobilization in storage cells, for example, only takes place after intoxication with less effective molluscicides, such as metaldehyde. Carbamate ingestion stimulates the activity of the macrophages. Furthermore, peroxidative reactions have been suggested to be the biochemical pathway of detoxification of carbamate and metaldehyde. According to this opinion, the detoxification processes in molluscs resemble those described for insects by Wegler (1970).

The present electron microscope study was able to extend former observations on whole animal behavior following intoxication. Suggestions about cellular mechanisms induced by different molluscicides are developed. However, other techniques, such as enzyme histochemistry, biochemistry and autoradiography, are necessary to further specify these results and to give more detailed information about the function of the structures described.

ACNOWLEDGEMENTS

This work was partly supported by the German Research Council (DFG Sto 75/9; Ja 407/

1–1). Personal thanks go to Günter Vogt, Thomas Braunbeck and Ulrich Bielefeld for the revision of the paper and for help with the English version. Thanks are also due to Dr. Janssen, to Prof. Storch and to Dr. Künast (BASF), who encouraged the present investigation and provided laboratory facilities and chemicals.

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Revised Ms. accepted 20 June 1989

ABBREVIATIONS

af	:	actin-like filament
be	:	basal extension
bl	:	basal labyrinth
bm	:	basal membrane
c	:	cilia
cc	:	connective tissue cell
ce	:	cytoplasmic extrusion
cg	:	calcium granule
cyt	:	cytoplasm
dv	:	digestive vacuole
ec	:	endocytotic channel
eg	:	excretory granule
env	:	endocytotic vesicle
ev	:	electron-dense vesicle
ga	:	golgi apparatus
ger	:	granular endoplasmic reticulum
gl	:	glycogen
gm	:	intramitochondrial granule
imv	:	intramitochondrial vesicle
li	:	lipid
ly	:	lysosome
ma	:	macrophage
mc	:	muscle cell
mf	:	membrane fragments
mi	:	mitochondria
mit	:	mitosis
muc	:	mucus cell
muv	:	mucus vacuole
mv	:	microvilli
n	:	nucleus
nc	:	nerve cell
nu	:	nucleolus
nv	:	neurosecretory vesicle
p	:	peroxisome-like vesicle
sc	:	storage cell
sec	:	small electron-lucent cell
ser	:	smooth endoplasmic reticulum
sv	:	secretory vesicle