THE IMPORTANCE OF MUCUS PRODUCTION IN SLUGS' REACTION TO MOLLUSCICIDES AND THE IMPACT OF MOLLUSCICIDES ON THE MUCUS PRODUCING SYSTEM

R. TRIEBSKORN, D. EBERT
Zoologisches Institut I, Ökologie/Morphologie,
Im Neuenheimer Feld 230, D-6900 Heidelberg, F.R.G.

ABSTRACT

In *Deroceras reticulatum*, mucus cell ultrastructure and the histochemistry of the epidermis and the digestive tract epithelia were shown to be influenced by carbamate and metaldehyde, two molluscicides used as both contact and oral toxins. After carbamate poisoning mucus production is increased and the chemical composition of the slime is modified. Ultrastructural damage is less intense than after metaldehyde poisoning. After metaldehyde application mucus secretion is activated immediately and the production of mucus is prevented by the severe effect of this chemical, especially on undifferentiated mucus cells.

INTRODUCTION

Increased mucus secretion is one of the first reactions of slugs to mechanical or chemical irritation, as e.g. caused by molluscicidal poisoning (Godan 1979). The production of mucus enables the animals to form a protective barrier preventing direct contact between the toxin and the surfaces of the epithelial cells. At the same time, it may serve to dilute the toxin. Although the target epithelia for molluscicides are either the skin or the digestive tract, depending on whether contact or oral toxins are applied, there is only limited information on cellular effects in skin and digestive tract epithelia induced by molluscicides (Ishak *et al.* 1970, Banna 1977, 1980, Pessah & Sokolove 1983). The present study was designed to elucidate the reactions of mucus-producing cells in skin and digestive tract to carbamate or metaldehyde poisoning. Particular emphasis was put on the cellular basis for the well-known effect of metaldehyde on the water regulation system (Godan 1979, Getzin &.Cole 1964) and on finding an explanation for its reduced efficiency compared with carbamate molluscicides under humid conditions.

MATERIALS AND METHODS

In order to study the efficiency of molluscicides as oral toxins, laboratory-reared *Deroceras reticulatum* were fed pellets containing either 2% of the carbamate Phenol-2-(2-chloro-1-methoxy-ethoxy)-methylcarbamate or 4% metaldehyde. The contact poison effect was studied by exposing animals to a 1% suspension of the carbamate or a 0,02% solution of metaldehyde, both applied in a known volume on a filter paper. Control animals were fed lettuce or held on tap water-soaked filter paper.

Animals were dissected one or five hours after ingestion or three hours after external application of the molluscicides. For electron-microscope studies, the tissues were routinely fixed and examined in a Zeiss EM 9.

To compare the quantity of mucus, before and after poisoning, by light-microscope, we took advantage of the fact that mucus shows a high affinity to FR-amide antibody and is thus easily to be detected in a fluorescence microscope.

For identification of neutral, acid, sulphated and carboxylated acid mucopolysaccharides, PAS-alcian blue and alcian blue-alcian yellow staining was applied.

Unspecific esterases were stained using the method of Davis & Ornstein (1959).

RESULTS

ECTRON-MIICROSCOPE STUDIES

Digestive tract

In the digestive tract of *D. reticulatum*, one type of mucus cell is predominant. It can be found in the oesophagus, the stomach and the intestine. Fully differentiated, this pear-shaped cell is characterized by a wide-luminar granular endoplasmic reticulum (gER), the tubules of which reach a width of up to 280 nm and contain typical microtubular structures. Furthermore, a basally located nucleus, large Golgi complexes and mucus vacuoles merging in the apical parts of the cell can be found. Eventually. these vacuoles are released into the lumen of the digestive tract. Immature cells are of conical outline and contain only moderate amounts of mucus vacuoles.

Carbamate poisoning

After carbamate poisoning, the number of large mucus vacuoles in both mature and immature cells increases. The cis-face cisternae of the Golgi complexes are compressed and the gER cisternae are slightly enlarged. In a few cases, the membranes are ruptured and devoid of ribosomes. Mitochondria are swollen and the number of cristae is reduced. The nucleus appears less electron-dense and the amount of heterochromatin decreases.

Metaldehyde poisoning

After metaldehyde poisoning, mature and immature mucus cells of the digestive tract are severely damaged. The membranes of the gER and the the Golgi cisternae as well as those of the mucus vacuoles are destroyed. The lumen of the gER is enlarged and the mernbranes are often devoid of ribosomes. In mature cells, most of mucus has been extruded from the cells. Mitochondria are partly inflated.

Epidermis

In the skin of *D. reticulatum* three types of mucus cells have been investigated:

The pedal gland cell is located in the footsole and its ultrastructure can be compared to that of the mucus cells in the digestive tract. The secretory product looks either spotted and electron-dense or fluffy and less electron-dense, with all transitional stages possible.

The mantle gland cell is the most common mucus cell in the skin of *D. reticulatum*, occuring in the mantle, the dorsal skin and the foot. It is characterized by a prominent Golgi apparatus consisting of 10-20 stacked lamellae. The secretory vacuoles, filled up with a slightly electron-dense material, are produced distally. In mature cells, the nucleus and the cytoplasm are displaced to the cell periphery by enlarged mucus vacuoles.

The club-shaped peripodial gland cells can be found in the foot margins. Their irregularly formed nuclei are basally located, and the gER is characterized by long cisternae arranged in parallel reaching 35 nm in diameter. As in mature pedal gland cells, secretory vacuoles, arising from the trans-face cisternae of the large Golgi complexes, fill the cell and displace the nucleus and the cytoplasm to the periphery of the cell.

Carbamate poisoning

In general, mucus cell effects in the skin are comparable to those in the digestive tract. However, in all cell types, the Golgi complexes reveal carbamate-specific alterations: In the pedal and mantle gland cells the cisternae are curved and often surround small vesicles. Large mucus vacuoles can rarely be detected. In the peripodial gland cell, the Golgi cisternae appear almost circular, enclosing vesicles at the center. In all cell types, the gER is more dilated than in the mucus cells of the digestive tract. In the peripodial gland cell, it shows an irregular shape, and the typical parallel arrangement of the cisternae is lost. In the mantle gland cell, the amount of glycogen increases.

Metaldehyde poisoning

As already described for the mucus cells in the digestive tract, the gER and the Golgi apparatus are severely damaged in all mucus cells of the skin. In the peripodial gland cell, the cis-face cisternae of the Golgi complexes are conspicuously inflated. In the mantle gland cell, typical lysosomes can be detected containing an electron-dense, membrane-like, fluffy material, glycogen-like crystals and small lipid droplets.

HISTOCHEMICAL STUDIES

Carbamate poisoning

Carbamate application results in an increased mucus production in the stomach and the intestine of *D. reticulatum*. The PAS-alcian blue staining provided evidence for an immediate increase in acid mucopolysaccharide production. Five hours after carbamate application, a lot of acid mucus is extruded from the cells. As a result, a large amount of neutral mucopolysaccharides can be detected in the epithelia. An intense reaction of acid mucopolysaccharides can be demonstrated in the lumen of the digestive tract. Frequentely, acid mucus surrounds the intestinal contents.

An alcian blue-alcian yellow staining allowed further separation of the acid mucopolysaccharides. Immediately after carbamate intoxication, large amounts of sulphated secretions were extruded from the mucus cells of the digestive tract and the skin, and subsequentely, carboxylated and mixed mucopolysaccharides were produced.

Metaldehyde poisoning

After metaldehyde poisoning, we failed to stain mucopolysaccharides in the mucus cells, since most of the secretory product had been secreted. In immature mucus cells of the digestive tract, high activity of unspecifc esterases randomly dispersed in the cytoplasm could be detected.

DISCUSSION

This present study describes the impact of two molluscicidal agents on the ultrastructure of the mucus cells and the chemical composition of the slime in the digestive tract and the skin of *D. reticulatum*. After carbamate application, the cellular damage is less severe than after metaldehyde. Carbamate causes a deformation of the mucus-producing system while metaldehyde induces a total destruction even at low concentrations.

After carbamate poisoning, the mucus cells of the digestive tract activate mucus production and modify the chemical composition of the slime. As the carbamate tested is known to be unstable at low pH (Künast, personal communication) the increased secretion of acid mucopolysaccharides may be regarded as an incidental attempt to reduce the efficiency of specifically this chemical in the digestive tract. Moreover, the activation of SH-groups and of γ-glutamyltransferase (Triebskorn 1988), as well as the extrusion of sulphated mucopolysaccharides, confirm the assumption that the secretory product of mucus cells in the alimentary tract contributes to detoxification of carbamates, possibly by glutathion- or sulphate conjugation. Metaldehyde causes an increased secretion of mucus immediately after application of the chemical. Subsequently, the severe impact of metaldehyde on the mucus-producing system, e.g. Golgi apparatus and gER, results in a cessation of mucus production. The ultrastructural damage and the increased activity of unspecific esterases in immature cells after metaldehyde application might be interpreted as symptoms of autolysis. Therefore, the replacement of mature mucus cells by immature ones is inhibited.

With regard to the skin epithelia, the animals are no longer able to produce the necessary surface mucus. In addition, the intensified mucus secretion leads to an increased loss of liquid, that can only be compensated for in a humid habitat.

Concerning the alimentary system, the extruded mucus passes through the digestive tract and is excreted. As a result, an internal desiccation takes place if the loss in the mucus cannot be replaced by taking up liquid from the environment. Applied either as oral toxin or contact poison, the effects of metaldehyde are therefore less intense under humid conditions. With regard to the

attack on mucus cells, metaldehyde is superior to the tested carbamate, but with respect to the general impact on the epithelia of skin and digestive tract carbamates exhibit an equal or even greater effect. These results are in line with the investigations of Riemschneider & Heckel (1979) and Prystupa *et al.* (1987), who studied the advantages of carbamates using LD_{50} tests.
This study revealed the cellular basis for the different reactions of slugs to carbamate and metaldehyde intoxication, especially in humid conditions. Furthermore, it provides evidence for the importance of the mucus-producing system in slugs' reaction to molluscicidal attack. Mucus cells should be considered as sensitive targets for toxins, especially with regard to the development of new specific molluscicidal agents.

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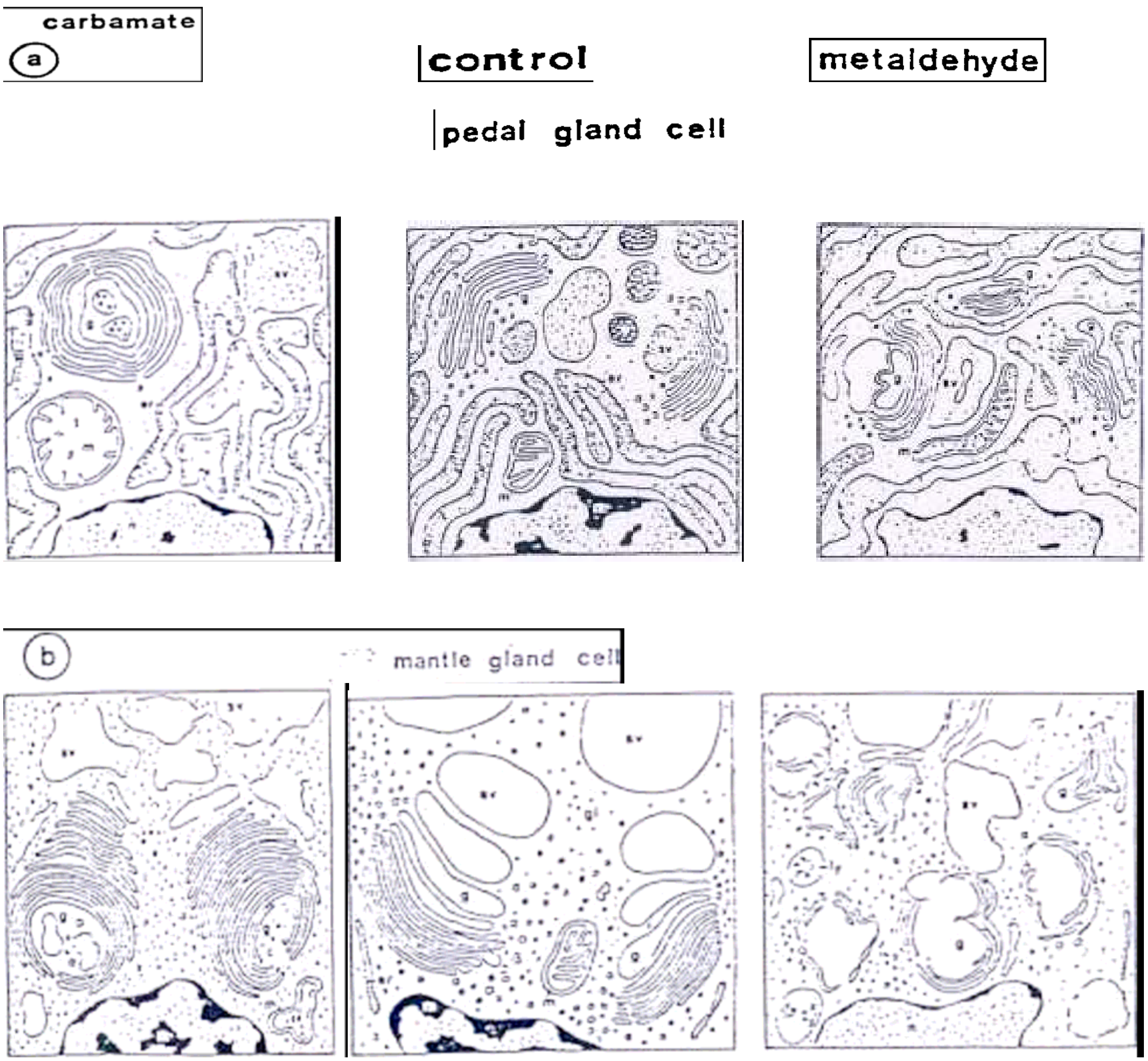
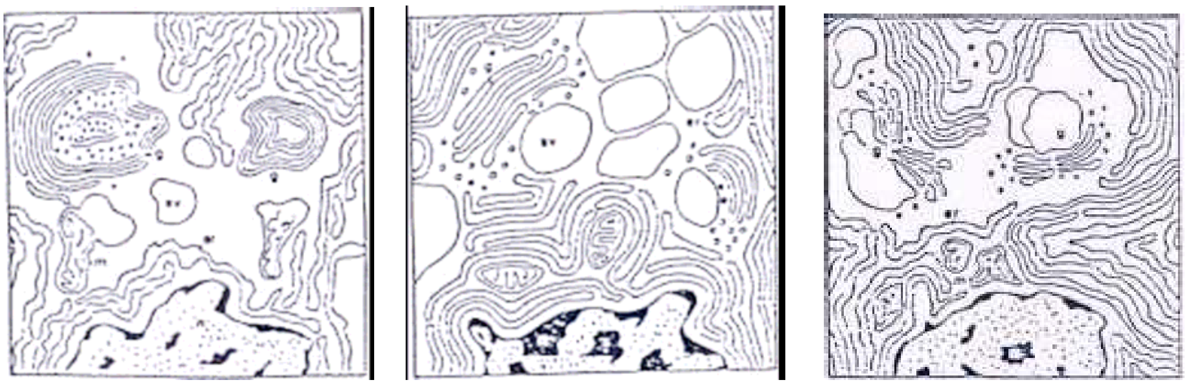


(c)

periopodial gland cell



Ultrastructural changes after metaldehyde and carbarnate poisoning in a) the pedal gland cell, b) the mantle gland cell c) the peripodial gland cell.

Abbreviations: granular endoplasmic reticulum (er) Golgi apparatus (g), lysosome (l), mitochondria (m), nucleus (n), secretory product (s), secretory vacuole (sv).