Effect of insecticides (Dimiline WP 25, Torak EC 24 and Gamacide 20) on hydra (*Hydra vulgaris* Pallas)

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ABSTRACT Investigations showed that the three insecticides used had the most damaging effect upon hydra immediately after treatment. The tentacles and the hypostome are the parts most often damaged. Inse the affected cells, lesions appear in the intracellular membranes, the nucleus shell and the membranes of the mitochondria, Golgi complex and the endoplasmic reticulum, while the cell membrane is preserved. The damaged parts of the body regenerate within three days. Zymogen cells play a significant role in the course of regeneration. They dedifferentiate into gastrodermal interstitial cells and later into other types of cells of the ectoderm and the gastroderm. Apart from their intense participation in regeneration, these totipotent cells also invariably participate in the formation of new hydra buds. It was observed that Dimiline WP 25 and Torak EC 24 in the concentrations used stimulate asexual reproduction of this animal.

KEY WORDS: *differentiation, dedifferentiation, regeneration, zymogen cells, gastrodermal interstitial cells*

Introduction

Many chemical compounds, and consequently insecticides in stronger doses are lethal for hydra and in weaker doses cause various morphological and cytological-histological changes (Znidaric and Lui, 1983; Znidaric *et al.*, 1987). Some insecticides, like Dimiline, are known to act upon the pentose cycle resulting in the accumulation of glucose in the hemolymph of insects (Denneulin and Lamy, 1982). In hydra, this insecticide causes swelling of endodermal cells which are ejected into the gastral cavity from where they accumulate in the budding region. This influences the increase in the number of buds in the treated hydra (Znidaric *et al.*, 1987). Lindan affects biomembranes so that it acts upon the double link of unsaturated fatty acids in phospholipids, making lysosomal membranes labile, which causes the release of proteolytic enzymes (Carevic, 1979). Gamacide 20 has a strong effect upon hydra so that some of its parts become completely destroyed (Kalafatic *et al.*, 1991). Organophosphorous insecticide dialifos has lipophilic properties and damages membranes of blood cells (Potas and D'Angelo, 1987). In hydra, some organophosphorous insecticides (fosalon) not only damage membranes but also entire hydras and during regeneration certain deformities occur (Znidaric *et al.*, 1990). In addition, pesticides as well as many other compounds in small doses can have a stimulating effect upon reproduction (Stebbing, 1982).

The purpose of our work was to establish the effect of insecticides used upon *Hydra vulgaris* Pallas species, including morphological effects, cytological-histological changes in the structure and regeneration, dedifferentiation and differentiation of cells, and possible changes in hydra morphogenesis and budding.

Results

Morphological changes

On the first day after treatment, the damage of the tentacles in all the hydras treated was noted. In thinner solutions the tentacles were shortened and shrunk and their tips were spherically thickened. In higher insecticide concentrations the tentacles were damaged to their base. Damage of the hypostome (approx. 25%) can be seen in hydras treated with Torak and Gamacide, while Dimiline did not cause greater damage on the hypostomes of the treated hydras. All the treated animals were shrunk within 24 hours. Only hydras treated with Dimiline were budding. The buds of the treated hydras did not differ from the buds of the control. On the second day after treatment, hydras treated with Dimiline regenerated their tentacles completely and did not differ from the control. Hydras treated with Gamacide and Torak have closed wounds on the hypostome. The recovery of the tentacles progressed but they were still shorter than the control. Hydras treated with Gamacide and Torak also formed new buds, whose appearance did not differ from the control. On the

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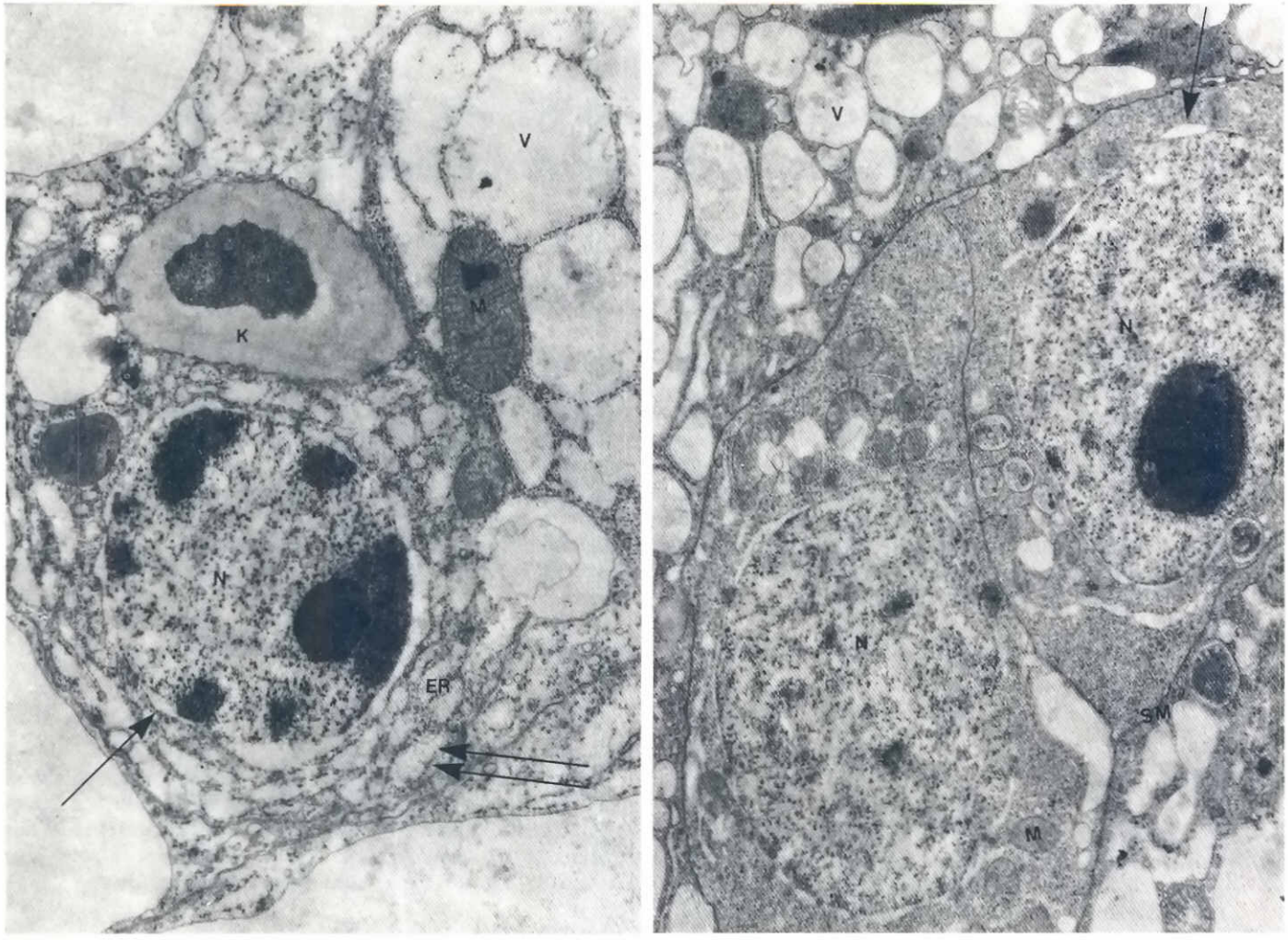


Fig. 1. Hydra vulgaris Pallas treated with 10 mg/l Gamacide 20 for 3 hours, and fixed immediately afterwards. *The membranes of the nucleus in interstitial cells are damaged and dilated (arrow). Membranes of endoplasmic reticulum (two arrows). Differentiation of cnidoblasts proceeds normally. K, cnidoblast; M, mitochondria; V, vacuole; ER, endoplasmic reticulum; N, nucleus. x11500.*

Fig. 2. Hydra vulgaris Pallas treated with 10 mg/1 Torak EC 24 for 24 hours, and fixed immediately afterwards. *Cellular membranes are normal. The membranes of the nucleus are dilated in places (arrow). Membranes of mitochondria and endoplasmic reticulum are damaged. Vacuoles are small but numerous. M, mitochondria; N, nucleus; V, vacuole; SM, cellular membranes. x8000.*

third day after treatment all the hydras treated had completely regenerated their damaged body parts and did not differ in appearance from the control, except those treated with Gamacide.

TABLE 1

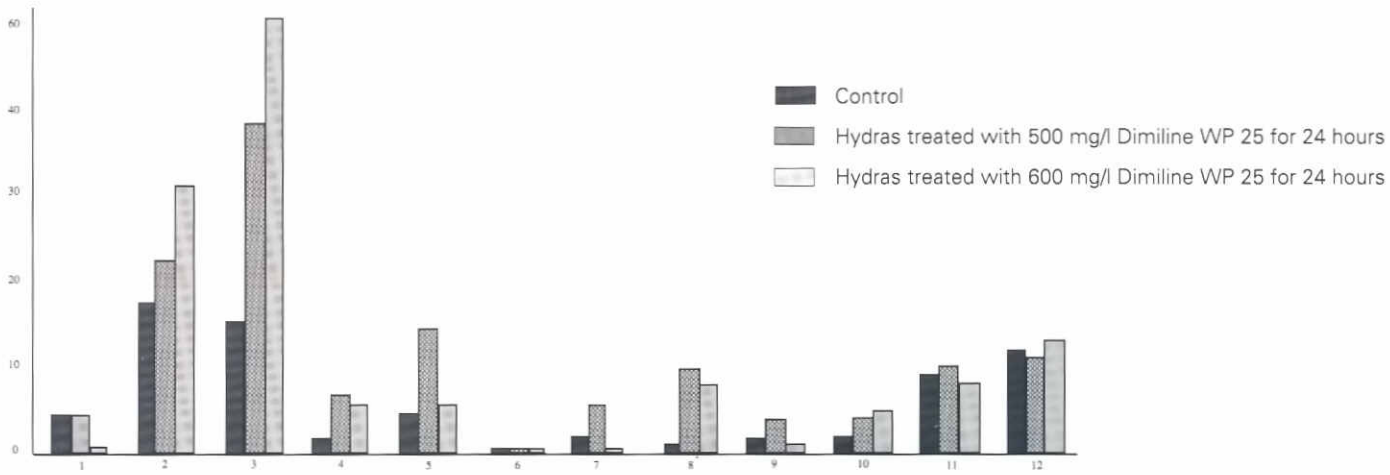
NEWLY FORMED BUDS OF HYDRA TREATED WITH DIMILINE WP 25 FOR 24 HOURS, AND THE CONTROL HYDRAS WITHIN THE PERIOD OF 20 DAYS

Days	1-3	3-6	6-18
Controls	36	5	63
500 mg/l	65	20	103
600 mg/l	82	10	62

Hydras treated with Gamacide after their regeneration had 5% deformed forms. Some had an increased number of tentacles, others had buds that did not separate from their mother individuals and a certain number had forked tentacles.

During the experiment, hydras treated with Dimiline budded more intensely than the controls (Table 1, Graph 1). Statistical analysis was made on the basis of a numerical matrix which contained frequencies of buds with regard to two factors. Thefour degrees of freedom yielded a result of 17.87, which is significant at the level of P=0.0013.

Hydras treated with Torak also budded more intensely than the controls (Table 2, Graph 2), X^2 was 65.75, which is significant at the level of P=0.001. Hydras treated with Gamacide also budded more intensely (Table 3, Graph 3), but the X^2 test was 3.82 and p=0.43, which is not at the level of significance.



Graph 1. Budding of Hydra vulgaris Pallas treated with Dimidine WP 25 for 24 hours.

Cytological-histological changes

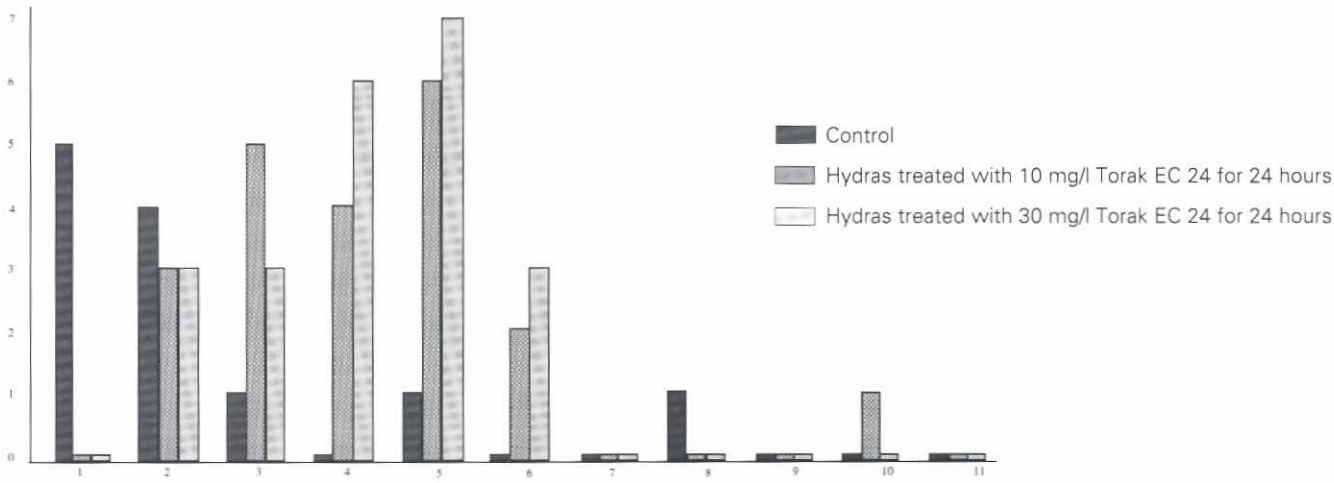
On the first day after treatment, in all the treated hydras damage of the outer mucous layer covering the ectoderm was visible. In some of these places myoepithelial cells had also been destroyed. Interstitial cells were rare. Damage of intracellular membranes in the cells of both layers could be seen. Mitochondria, Golgi complex and endoplasmic reticulum were damaged. Membranes were damaged or dilated. In places, membranes of the nucleus shell in myoepithelial cells were damaged. Vacuoles were small but numerous. Differentiation of cnidoblasts proceeded normally (Figs. 1 and 2).

The hypostome and hypostomal mucous wrinkles in hydras treated with Dimiline were fairly well preserved, while in some of the hydras treated with Torak and Gamacide they were badly damaged. Wounds were open and at the place of the hypostome damaged cells and mucous, instead of the ectodermal and gastrodermal layer, were present. There were no interstitial cells in the wounded part of the ectoderm as well (Fig. 3). Gastrodermal interstitial cells were present in a greater number than in the controls. Nearly all the zymogen cells had changed in shape and position and most of them were at the stage of dedifferentiation into gastrodermal interstitial cells and at the stage of differentiation into mucous cells. Buds showed minor changes in relation to mother individuals. Damage

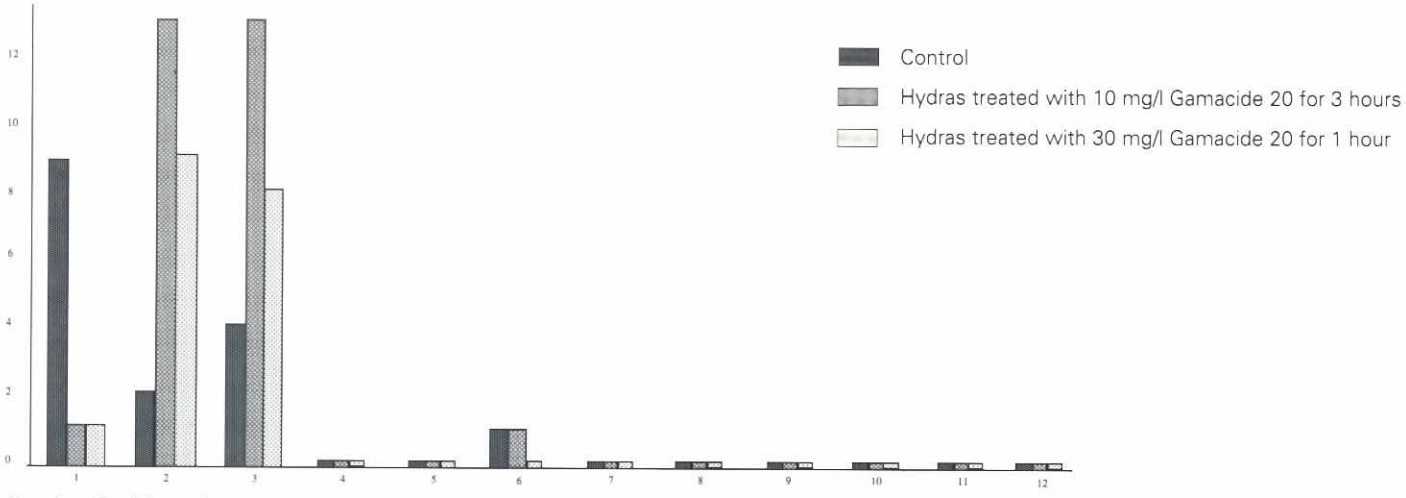
TABLE 2

NEWLY FORMED BUDS OF HYDRA TREATED WITH TORAK EC 24 FOR 24 HOURS, AND THE CONTROL HYDRAS WITHIN THE PERIOD OF 20 DAYS

Days	1-3	3-6	6-18
Controls	10	1	1
10 mg/l	8	12	1
30 mg/l	6	16	1



Graph 2. Budding of Hydra vulgaris Pallas treated with Torak EC 24 for 24 hours.



Graph 1. Budding of Hydra vulgaris Pallas treated with Gamacide 20 for 1 and 3 hours.

could be seen in their outer mucous layer. In the gastroderm of the buds some of the zymogen cells had also changed. *On the second day* after treatment the wounds were closed. In the hydras treated with Gamacide for 3 hours and 20 minutes, the wounds were closed only by gastroderm, while in hydras treated with Torak for 24 hours, the wounds were closed by both the ectoderm and the gastroderm. The outer mucous layer was still partly damaged in all the treated hydras. A smaller number of myoepithelial ectodermal cells was also damaged. There were more interstitial cells in the ectoderm than the day before. Mucous wrinkles of the hypostome were a bit larger. Zymogen cells kept their changed shape and position. The gastral cavity was filled with numerous free cells. Most of these single cells and cells in groups had a spherical shape. The buds of hydras appeared so far also had their mucous covering layer damaged in places. Some of the zymogen cells of the bud were changed. This was also characteristic of the control buds which were at the stage of intense growth. In both the control and the treated hydras zymogen cells could be found in the stage of dedifferentiation into gastrodermal interstitial cells (Fig. 4). *On the third day* after treatment, the wounds were completely closed in all the treated hydras, and the hypostome with tentacles had also regenerated as well. Hypostomal wrinkles were more scarce than those in the control. Interstitial cells were missing only in some places of the ectoderm. In the gastroderm there were still altered zymogen cells. They were generally sphere-shaped and the zymogen granules in them were not numerous. The buds of the treated hydras did not differ from the control. *On the sixth day* after treatment, hydras were equal to the control in their cytological-histological structure.

Discussion

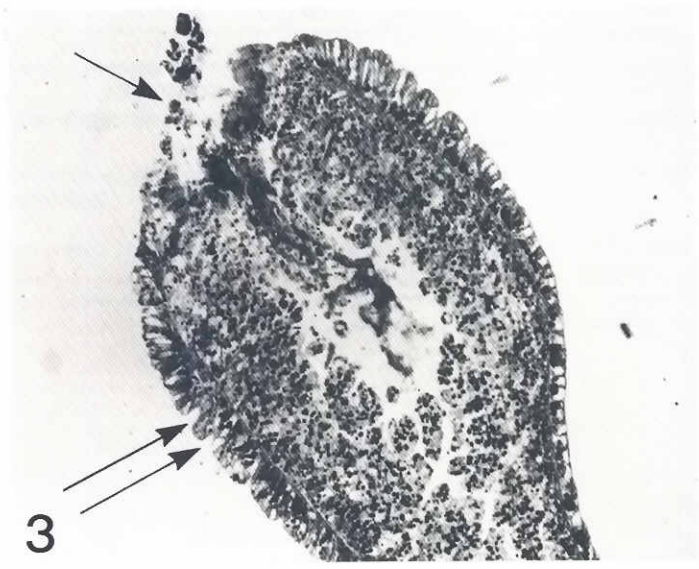
All three insecticides used caused considerable morphological and cytological-histological changes during the treatment and immediately afterwards. Dimiline was the most benign and damaged only the tentacles. Gamicide and Torak, apart from damaging the tentacles, also caused damage to the hypostome. The state of contraction of the body, which is characteristic of all the animals treated with a higher concentration, gradually disappeared on the first day after treatment. In hydras treated with Gamacide, the exchange of diffusion potential which is also characteristic of plant cells (Schefczik and Simonis, 1980), as well as the inhibition of the activity of cholinesterase of organophosphoric insecticides, seem to cause changes in innervation of hydra, which influenced a prolonged contraction of their body. Damaged parts of the body regenerated gradually so that the hydras treated with Dimiline had an almost normal appearance. More seriously damaged hydras treated with Gamacide and Torak did not resume their normal appearance until the third day. Hydras treated with Gamacide showed certain anomalies in the regeneration and morphogenesis of ruined parts of the body. Some of them had an increased number of tentacles and others had a Y-shaped body and newly-formed buds remained coalesced permanently with their mother individual. In addition, a certain number of these hydras had several forked tentacles.

Although morphological and cytological-histological damage was considerable and small changes in young newly-formed buds could be seen immediately after treatment, this did not have any influence upon the intensity of budding. It is well known that many compounds, and pesticides among them, have a stimulating effect upon reproduction (Stebbing, 1982). Thus, the hydras treated either with Dimiline or Torak budded more intensely than the control (Tables 1 and 2, Graphs 1 and 2), as determined by mathematical analysis. The process of budding developed normally as in the control, although their number was much greater. In earlier work with Dimiline, the capacity for stimulating budding was also noticed (Znidaric *et al.*, 1987). This is explained by the fact that Dimiline

TABLE 3

NEWLY FORMED BUDS OF HYDRA TREATED WITH GAMACIDE 20 FOR 1 AND 3 HOURS

Days	1-3	3-6	6-18
Controls	15	1	1
10 mg/l	27	1	0
20 mg/l	18	0	0



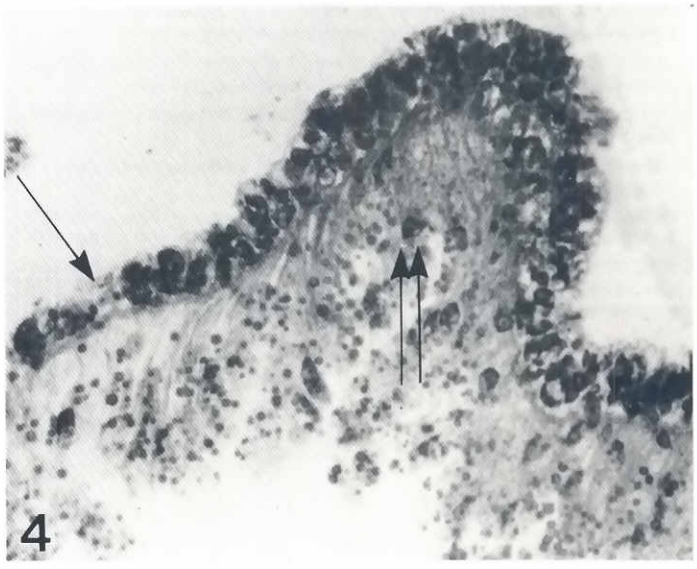


Fig. 3. Hydra vulgaris Pallas treated with 30 mg/1 Torak EC 24 for 24 hours, and fixed immediately afterwards. *The tentacles of the hydras are shortened to their base. Open wounds can be seen on the hypostome (arrow). The number of interstitial cells is reduced (two arrows). Toluidine blue, x233.*

Fig. 4. Hydra vulgaris Pallas treated with 10 mg/1 Gamacide 20 for 3 hours, and fixed on the second day after treatment. *Outer mucus layer is destroyed (arrow). In the bud most of the zymogen cells can be found in the stage of differentiation into gastrodermal interstitial cells (two arrows). Toluidine blue, x512.*

increases the concentration of monosaccharids in gastrodermal cells of hydra and because of this, these cells became turgescent and are ejected into the gastral cavity. The effect of Torak is very similar. Released cells accumulate in the budding region and here they differentiate into the cells of the body of new buds and so their number greatly increases.

Cytological-histological findings show that the insecticides damaged the hydra. The cells of the tentacles and of the hypostomal part of the body were the most sensitive, while in other cells only their inner structures were hurt. Zymogen cells, as in earlier findings (Lui and Znidaric, 1968; Znidaric, 1970) showed totipotence as they can dedifferentiate into gastrodermal interstitial cells and differentiate into mucous cells of the mouth region (Lui and Znidaric, 1973; Znidaric *et al.*, 1980; Znidaric and Lui, 1983; Znidaric *et al.*, 1987). The dedifferentiation of these cells was noticed not only in the wound region, but also in a greater area of the gastral region. Namely, it has been found that these cells are much more active in the presence of the insecticides used than in the condition of normal growth, regeneration and budding. Moreover, growth and regeneration went on as normal, with only Gamacide causing an insignificant number of anomalies. However, anomalies do not happen as a consequence of changes in the genome, as in the experiment clonic genetically identical individuals were used and reproduced themselves by budding. Differences appeared due to the changed demonstration of genome of injured cells so they changed the differentiation and morphogenesis of surrounding healthy cells by their activity, which had also happened in the treatment with other insecticides (Znidaric and Lui, 1983).

The damage observed by means of an electronic microscope in the structures of cells also healed very quickly so that the recovered cells were almost equally active as the control undamaged cells.

Materials and Methods

Cloned individuals of the *Hydra vulgaris* Pallas species were kept in aquarium water at room temperature. They were fed with *Artemia salina* larvae everyday. Twenty-four hours after feeding, hydras of equal size and similar development were selected for the experiment. They were treated for 24 hours with 500 mg/l and 600 mg/l Dimiline Wp 25 (diflubenzurone C NHCONHCO-⬡, «Galenika» Belgrade), and with 30 mg/l and 10 mg/l Torak EC 24 (dialifos, S-/2-chloro-phtalimidoethyl/00-diethyl phosphorodithyoate, «Pliva», Zagreb), and with 20 mg/l and 10 mg/l Gamacide 20 (gamma isomer hexachlorcycloxexane, «Pliva» Zagreb), for 1 and 3 hours, respectively.

During the treatment, hydras were kept with the thermostat at 22°C and were transferred into clean aquarium water afterwards. Morphological changes were observed daily by means of a stereomicroscope. For the cytological-histological analysis, hydras were fixed in Bouine on the first, second, third and sixth days after treatment. Paraffin blocks were cut with a microtome. The cuts were 7 μm thick. The preparations were dyed with 0.1% toluidine blue.

For electronic microscopy, the animals were fixed in 1% glutaraldehyde pH 6.9 buffered with 0.01 M cycodylate buffer and postfixed in 1% osmium tetroxide buffered with 0.01 M cycodylate buffer immediately after treatment. Afterwards, the animals were dehydrated and immersed in araldite. The preparations were cut with a glass knife on the ultramicrotome. They were put up on the net and dyed with Pb citrate.

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