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Histopathological Effects of Phenol on the Digestive Gland of *Amphimelania holandri* Fér. (Gastropoda, Prosobranchia)

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Phenolic wastes are common water pollutants generated from a variety of industrial processes used in oil refineries, gas operations, coke ovens, coal gasification and by natural processes such as the decomposition of plant matter (Buikema et al. 1979). Relatively high concentrations of phenol are found in rivers near the outlets of channels into which industrial waste waters have been discharged (Buikema et al. 1979).

There are data about the toxic effects of phenol on fish (Holcombe et al. 1982; Krajnovic-Ozretic and Ozretic 1988; Smith et al. 1991), and on some invertebrates, including snails (Erben 1982; Gupta et al. 1984; Mc Cahon et al. 1990). However, little is known about histopathological changes induced by phenol's toxic effects, and these changes might be a basic indicator in assessing the condition of a particular water ecosystem. The existing data are mostly relevant for fish (Mitrovic et al. 1968; Kumar and Mukherjee 1988; Kirk and Lewis 1993), and we know very little about the snail's histopathology; however, the snail is a good research model due to its effectiveness as a pollution indicator species.

There are some reasons why the digestive gland was chosen for research. In the first place, this gland has a central position in digestion of food in molluscs (Hyman 1967). Secondly, it is well known that digestive organs are one of the routes by which phenol enters the organism (Buikema et al. 1979). Furthermore, the digestive gland plays an important role in the metabolism of endogenous and xenobiotic compounds in snails (Wilbrink et al. 1990). And finally, histopathological changes in the hepatopancreas are important indicators of the condition of water ecosystems, and they are used frequently in monitoring research (Yevich and Barszcz 1983; Sunila 1987).

In the present study an attempt has been made to establish the structure of the normal digestive gland and histopathological changes as a result of exposure to phenol. Phenol concentrations used in the test were close upon to high

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concentrations of phenol (more than 300 mg/L) which were measured in the Sava River downstream of the city of Zagreb as a result of its accidental discharge (Munjko 1974). At the same time, these concentrations were near its 48 and 96 hr median lethal concentration (LC 50) for snails (Buikema et al. 1979).

Amphimelania holandri Fér. is a freshwater snail distributed in streams, rivers and lakes of Balkan Peninsula, and areas along the Danube River (Illies 1978). In karst rivers (NW Croatia) this species has many relatively stable local populations and has an important role in food webs.

MATERIAL AND METHODS

Adult specimens of the snail *Amphimelania holandri* Fér. were collected from the Mreznica River, near the city of Karlovac, and acclimatized to laboratory conditions in dechlorinated tap water for 48 hr. The specimens used for the study averaged 1.75 cm (range: 1.5 to 2.0 cm) in height. The tap water was left in open tanks for at least 1 wk and than dechlorinated by filtration through activated carbon filters. Sodium thiosulfate was added to remove the residual chloramines.

Pure phenol (99%) was obtained from the INA-OKI, Industry of Crude Oil and Organic Chemistry in Zagreb, Croatia. Stock solutions were prepared in distilled water.

The experiment was carried out in six aerated, rectangular glass dishes (containers), each containing 5 L of dechlorinated tap water. The snails were exposed to phenol concentrations of 100, 150, 200, 250 and 300 mg/L. One dish was used as the control receiving dechlorinated tap water only. There were 30 snails per dish. The test solutions and control water were renewed daily (semi-static test). The snails were not fed during the exposure period. In order to avoid effects of starving on the digestive gland the snails were exposed to phenol for 7 d. The physico-chemical properties of the test solutions and control water were determined every 24 hr by routine procedures (APHA 1985). Water characteristics were as follows: temperature 20° to 21°C; pH 7.2 to 7.6; dissolved oxygen 7.1 to 8.2 mg/L; hardness 279 to 288 mg/L as $CaCO_3$.

After exposure to phenol, survived snails were removed to clean dechlorinated tap water for 3 wk recovery period. During the recovery period control and exposed animals were fed with algae.

For histological analyses live specimens were removed from each exposure concentration and control on days 1, 2, 3, 4, 7 and at the end of the recovery period. The shells were removed by cutting the columella muscle. Animals were placed in Bouin's fixative for 24 hr. After fixation, the snails were embedded in paraffin and cut with a microtome into 6 to 8 µm thick slices. The sections were then stained with haematoxylin and eosin.

RESULTS AND DISCUSSION

Analysis of histological preparations of the control animal group shows that the digestive gland (hepatopancreas) consists of numerous, compressed small channels (tubules) (Figure 1). Between the tubules lie loose connective tissues and hemolymphatic spaces. The tubule epithelium consists of two cell types: digestive cells and basophil cells. The digestive cells are long, and their shape varies from cubical to cylindrical. The basophil cells are short, triangular in shape and with cilia on the apical surface. In transverse sections the tubules are seen as circular structures. The cytoplasm of digestive cells is filled with secretory granules of different sizes. Many cells contain vacuoles with phagocytotic content and the nucleus is placed toward the basal membrane. The basophil cells are characterized by the presence of cell granules of calcium carbonate. In comparison with the structure of digestive gland of other species of snails, the digestive gland of *A. holandri* is simpler, because this gland may have two to four cell types (Hyman 1967). No pathological changes in the digestive gland of control snails could be observed.

Changes in the digestive gland were observed in snails exposed to 250 and 300 mg/L phenol by the first day of the experiment. Vacuolation of digestive cells became apparent, and the vacuoles of various sizes were observed (Figure 2). Also, the gathering of amoebocytes in areas between the tubules increased (Figure 2), which is characteristic in acute inflammatory processes. The first necrotic changes also occurred in 250 and 300 mg/L phenol and were observed on the second and third day of the experiment. These changes manifested as the erosion of cytoplasm of the digestive cells. The lumen of tubules contained exudate and cell remains as well as granules from basophil cells (Figure 3). On the fourth day of the experiment, the processes of atrophy went even further and in the spaces between the tubules necrosis of connective tissue took place. In some parts of the gland, the tubules lost cell organization simultaneous with expansion of hemolymphatic areas (Figure 4). In places, total breakdown of tubules occurred, and only aggregation of cell particles remained.

At 200 mg/L phenol, cell vacuolation was observed after 3 d of exposure to phenol, but the process of necrosis was slower, so that not until the seventh day did the necrotic changes become apparent.

At a lower concentration of phenol (100 and 150 mg/L) vacuolation of cells, corresponding to the first degenerative change, was observed in digestive gland of some animals on the fourth day. Necrotic changes were also established in subchronical conditions of poisoning on the seventh day of the experiment, but the digestive glands of the greatest number of the snails showed only inflammatory reaction.

Similar histopathological changes in the digestive gland of molluscs, as a result of the influence of phenol, have been reported by Fries and Tripp (1976) in the

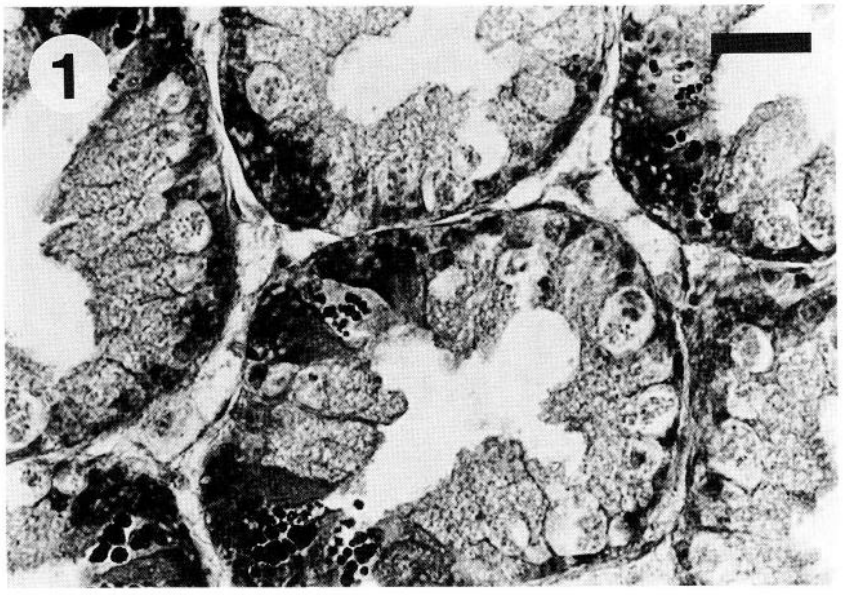

Figure 1. Normal digestive gland. Control. The tubules are built of digestive cells (light color) and basophil cells (dark color). Between the tubules there is connective tissue with hemolymphatic areas. H&E; Scale bar =20 µm.

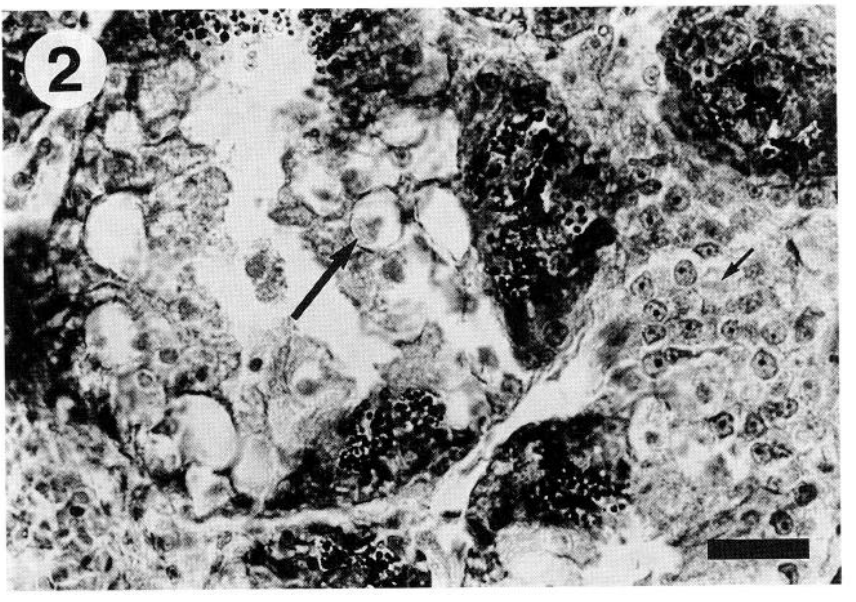

Figure 2. Digestive gland of a snail treated with 300 mg/L of phenol for 1 d. The increased vacuolation of the cells (large arrow) is followed by accumulation of amoebocytes (small arrow) in the hemolymphatic areas. H&E; Scale bar =20 µm.

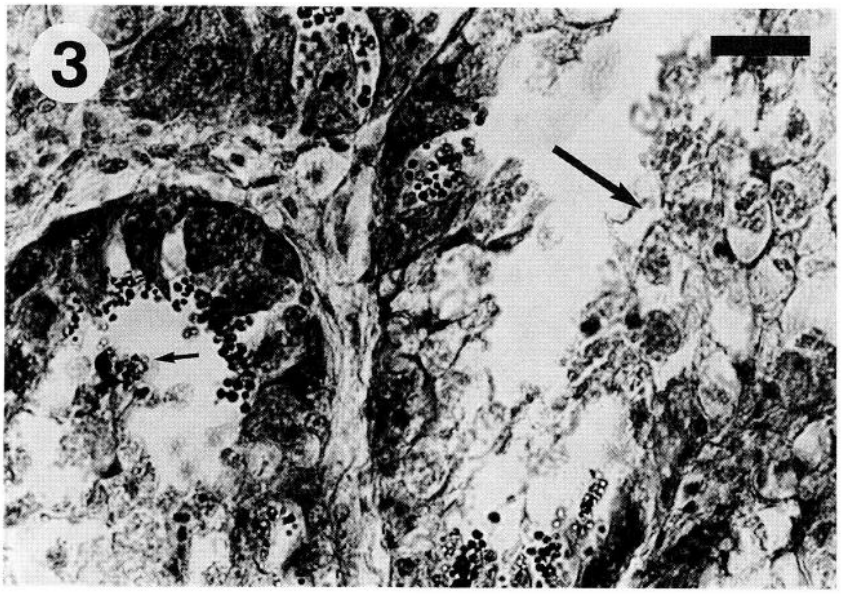

Figure 3. Digestive gland of a snail treated with 250 mg/L of phenol for 2 d. The lumen of the tubules contains exudate and pinchings from the digestive cells (large) arrow) and granules composed of calcium carbonate (small arrow) from the basophil cells. H&E; Scale bar=20 µm.

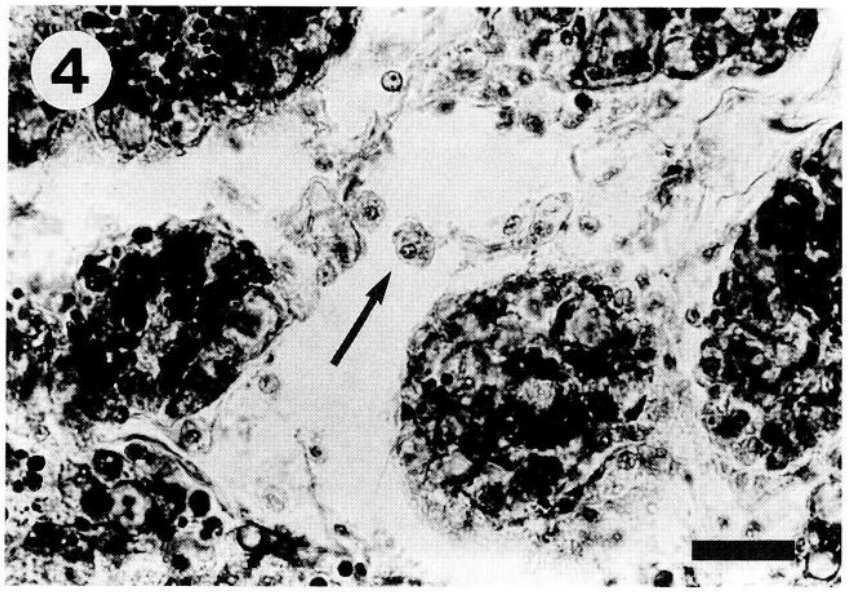

Figure 4. Digestive gland of a snail treated with 250 mg/L of phenol for 4 d. Complete disorganization of a digestive gland is evident. Large amoebocytes (arrow) are present in the expanded hemolymphatic areas. H&E; Scale bar=20 µm.

clam *Mercenaria mercenaria* and by Tutiš (1990) in the snail *Planorbarius corneus.* Similar disturbances of the normal structure in the molluscan digestive gland are caused by other chemical compounds, especially heavy metals (Calabrese et al. 1984; Sunila 1984; Minniti 1987).

In the present study no snails survived for 7 d in 250 and 300 mg/L of phenol. At phenol concentration of 200 mg/L only 10% of snails survived the exposure, but they died in the recovery period. However, in the two lowest phenol concentration of 100 and 150 mg/L survived 60% and 30% of snails. The recovery of these animals was good (mortality was lower than 5%).

Necrotic changes which have been described earlier were irreversible and they might be a possible cause of death of snails in acute and subchronic conditions of poisoning. Inflammatory reaction and degenerative changes such as vacuolation were reversible. This is confirmed in our study by histological examination of the snails which recovered for 3 wk. The digestive glands of these snails were in the same condition as in the control animals.

As we said in introduction, it is known that digestive gland is a major organ for digestion and resorption of food in snails (Hyman 1967). The histopathological changes seen in the present study confirmed that phenol affected digestive cell function. We presume that this disfunction of digestive gland, along with other effects of phenol exposure, reflected on the growth and reproduction of snails which survived. Reason for that was the spawning observed in the control snails, whereas the snails exposed to 100 and 150 mg/L of phenol did not spawn during the recovery period. Future studies should focus on these problems.

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