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Environmental Contamination and Toxicology

# Copper Treatment of the Digestive Gland of the Slug, *Arion ater* L. 1. Bioassay Conduction and Histochemical Analysis

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Effects of experimental treatment with copper on the digestive gland of the terrestrial slug, Arion ater, have been studied from a histochemical perspective.

Studies involving the use of molluscs as monitors of metallic pollution have increased over recent years. Three main reasons justify these kind of works: (a) demonstration of bioaccumulation of metal ions in molluscs, mainly in the digestive gland; (b) the low mobility which characterizes a variety of molluscs makes them proper representatives of concrete ecosystems; and (c) a great number of molluscan species are widely distributed in the world, so that criteria for environmental pollution monitoring program can be standardized (Simkiss et al. 1982).

Simkiss and Mason (1984) report that the purposes of histotoxicological studies must be: (a) to explain metal-metal interaction; and (b) to describe regulating mechanisms with regard to the use of molluscs in environmental analysis of metals.

## MATERIALS AND METHODS

We have conducted a chronic toxicity bioassay. Two simultaneous sets of seven groups of animals were treated with different copper concentrations in diet (0, 10, 25, 50, 100, 300 and 1000 ppm Cu) for a month (Russell et al. 1981). 198 individuals of the species Arion ater were collected from a field near the University Campus the last week of July, being selected in relation to size (4-5 cm length) and weight (5-6 g). The slugs were taken to the laboratory and distributed in 14 plastic boxes (20x10x8 $cm^3$). Animals were starved for 6 days prior to copper treatment in order to minimize physiological differences and provide acclimation to laboratory conditions (Akerlund 1969). Copper treatment was started with 12 animals per box. Mean environmental temperature during the assay was 20°C

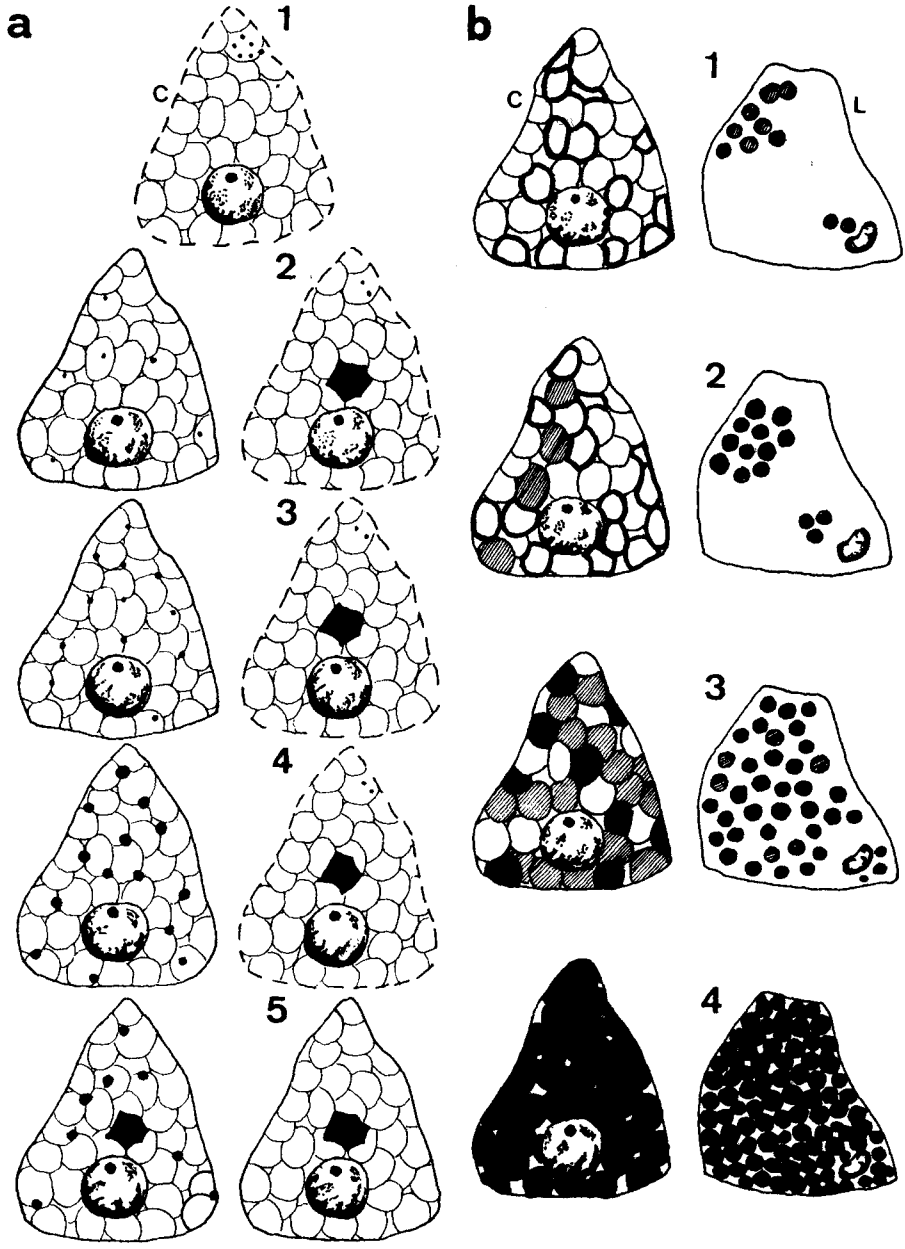


Figure 1. Estimated levels of metals in tissues. Calcium cells (C) and Leydig cells (L) schematical reconstruction is shown for copper (a) and calcium (b) estimated levels. Discontinuous shape means scarce occurrence of the represented morphological appearance at the reported level.

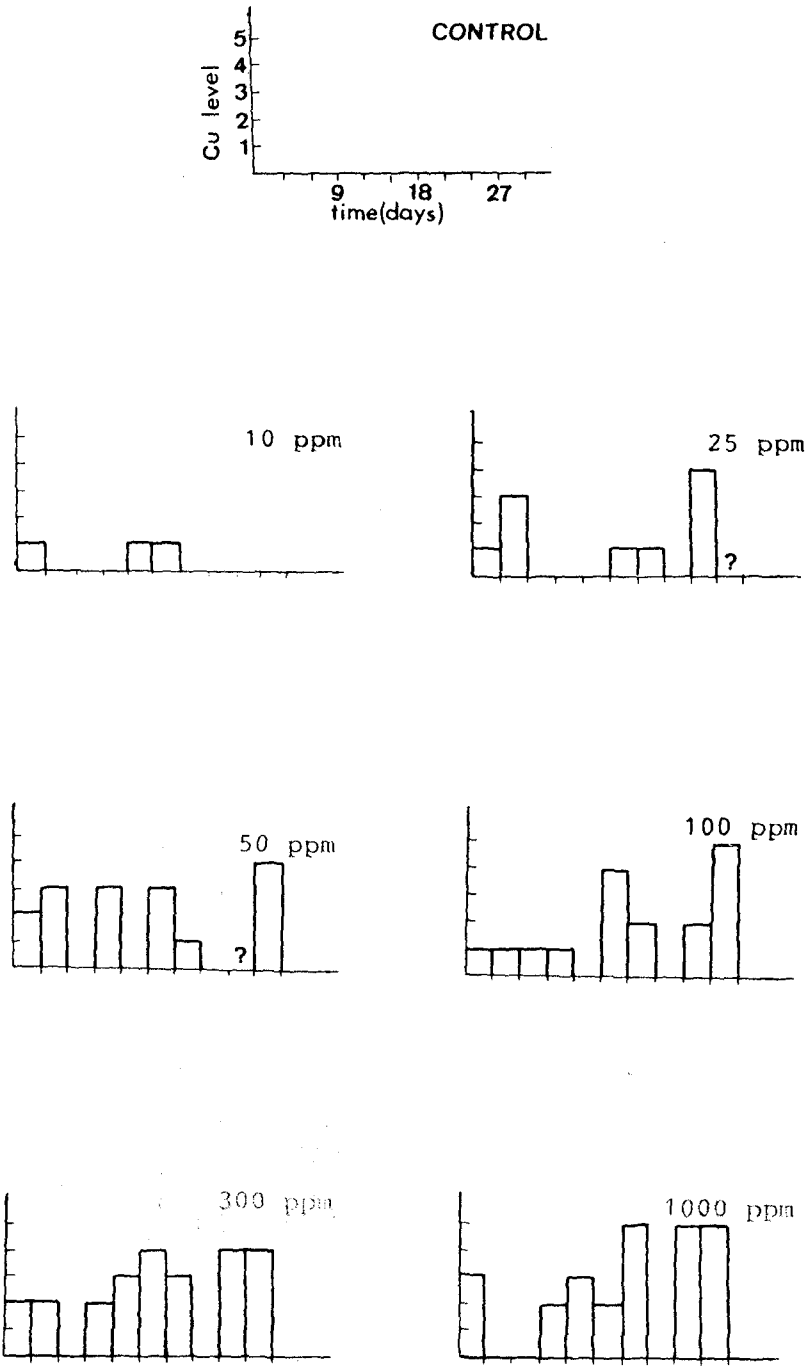


Figure 2. Copper levels in the digestive gland vs. bioassay time for each group of studied slugs. Levels were histochemically determined.

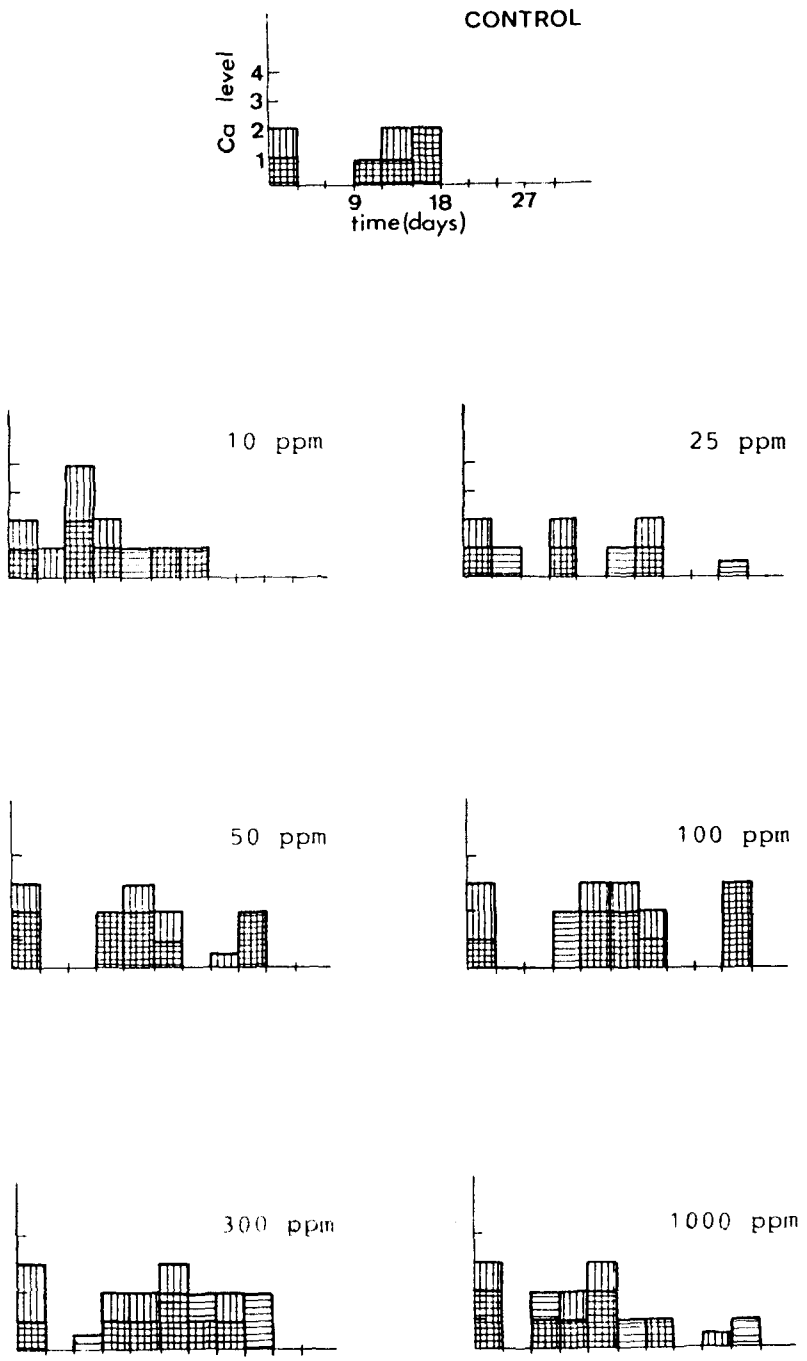


Figure 3. Calcium levels in the digestive gland vs. bioassay time for each group of studied slugs. Levels were histochemically determined ( |||||| in Leydig cells; ≡ in calcium cells).

and relative humidity was maintained at saturation levels. "Natural diet" (equiproportional triturate of lettuce, apple, carrot and pumkin with an 1.5% agar aq solution) (Zubiaga 1982) was supplied to slugs mixed with copper sulphate in the proper concentration for each group of treatment.

Histochemical tests were made every three days. The biggest portion of the posterior lobe of the digestive gland was studied. Tissues were always removed between 5:00 and 7:00 p.m. and fixed 4% formol overnight. Paraffin-sections were stained with the method of Okamomoto and Utamura (Lillie 1965) and the Stoeltzner technic (Martoja and Martoja-Pierson 1970) to detect copper and calcium respectively.

After careful observation of the histochemical results a semiquantitative valuation of metal levels in the digestive gland was considered. Five levels on copper detection and four on calcium were estimated (Figure 1).

RESULTS AND DISCUSSION

No effect of copper treatment on mortality was observed in A. ater (male phase) during assay, as only three of the 168 slugs died: one each in 1000 ppm, 10 ppm and 0 ppm exposure.

Copper was exclusively accumulated inside spherules of glandular calcium cells. Copper rubeanate was not present in tissues of control animals. Besides, copper levels found in epithelia were higher in animals exposed to high copper concentrations in diet than in those exposed to low concentrations. No linear but cyclic behaviour was observed in glandular content in relation to copper concentration in diet. We have established a copper concentration cycle common to all groups of studied slugs and characterized by total absence of copper rubeanate on the 6th and 24th bioassay days (Figure 2).

Coughtrey and Martin (1976) report that copper accumulation occurs in the calcium granules of the digestive gland in Helix aspersa, and Schoettli and Seiler (1970) observed that zinc accumulation in Arion rufus has the same location. In the present work, copper has also been detected inside these same membrane structures. However, we must mention that other authors have demonstrated copper accumulation in digestive cells lysosomes of marine molluscs (Martoja and Tue 1980; Harrison and Berger 1982).

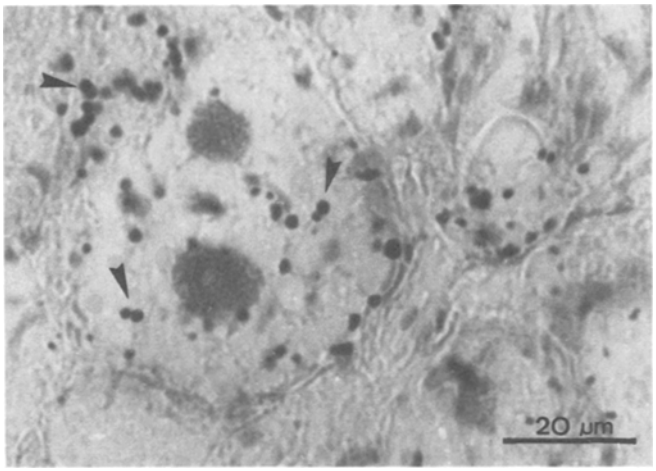


Figure 4. Calcium cells with granules of copper rubeanate (►). Okamoto-Utamura method with rubeanic acid.

Calcium was mainly located inside calcium and Leydig cells. Chemical nature of calcium deposits in both cells types is different: mainly as calcium carbonates and to a lesser extent as phosphates in the first type, and exclusively as phosphates in the second. This difference can be determined using the Stoeltzner technique (McGee-Russell 1958). Differences in the nature of calcium deposits have also been reported by McGee-Russell (1958) in Helix aspersa and Sen Gupta (1977) in Bensonia monticola.

Considering the estimated calcium levels in the digestive gland vs. bioassay time duration, the following observations were made: (a) a close parallelism between calcium cycle in both cell types is observed; (b) there is no apparent relationship between copper concentration in diet and calcium level in tissues; and (c) a calcium cycle common to all groups of slugs characterized by no calcium detection in epithelia on the 6-9th and the 24-27th bioassay days (Figure 3).

No continuous accumulation but cyclic detoxication can be described in experimentally copper treated A. ater. A close parallelism between copper and calcium variation cycles is observed in the digestive gland: both cycles - the one natural of calcium, and the "artificial" of copper- have superposable minima and similar behaviour. We propose that copper detoxication is a calcium cycle dependent mechanism. In fact, secretion of calcium cells containing copper granules was frequently observed in animals treated with high copper concentrations over long periods.

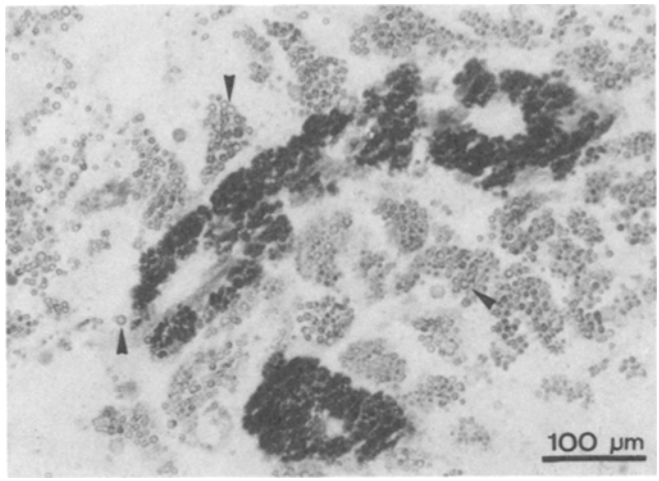


Figure 5. Capillar vessels and calcium cells with calcium deposits. Dark rings of calcium spherules indicate the presence of carbonate complexes (►). Homogeneous black or deep brown colour demonstrates the existence of calcium phosphates. Stoeltzner technic with cobaltous nitrate.

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