Bull. Environ. Contam. Toxicol. (1988) 41:865–871

Environmental Contamination and Toxicology

Zinc Treatment of the Digestive Gland of the Slug *Arion ater* L. 2. Sublethal Effects at the Histological Level

A. Recio, J. A. Marigómez, E. Angulo, and J. Moya

Laboratorio de Citología Histología, Dept. Biología Celular & Ciencias Morfológicas, Fac. Ciencias, Universidad del País Vasco, Apdo. 644-48080 Bilbao, Spain

Several authors have pointed out the necessity when measuring the level of pollution within a biological system on an assessment of the biological effects of pollutants (Bayne 1980; Widdows 1981; Bayne et al 1982). Within this field, histological parameters for the indication of environmentally induced stress response (i.e. destabilization of the lysosomal system, reduction of the mean epithelial thickness of digestive gland cells-MET) could offer an initial approach to determine the impact of a pollutant on the biota (Moore 1986).

The present work is the completion of the histochemical study on cellular distribution of zinc in the digestive gland of the slug Arion ater (part 1 of this investigation). Thus, the histochemical and planimetric study of the effects of zinc-exposure on A. ater is presented as the other aspect in which histology is concerned in pollution research.

MATERIALS AND METHODS

A sublethal toxicity assay was carried out (see part 1 of this investigation for description of experimental conditions). The digestive gland was routinely processed and stained with Best's carmine (plus diastase control) to demostrate glycogen (Martoja and Martoja-Pierson 1970). Arbitrary units in figure 1 were obtained from subjective histochemical results (4 detection levels) converted into percentage values (mean of replicate series) as reported in part 1 of this investigation for metal histochemistry.

Send reprint requests to JA Marigómez at the above address.

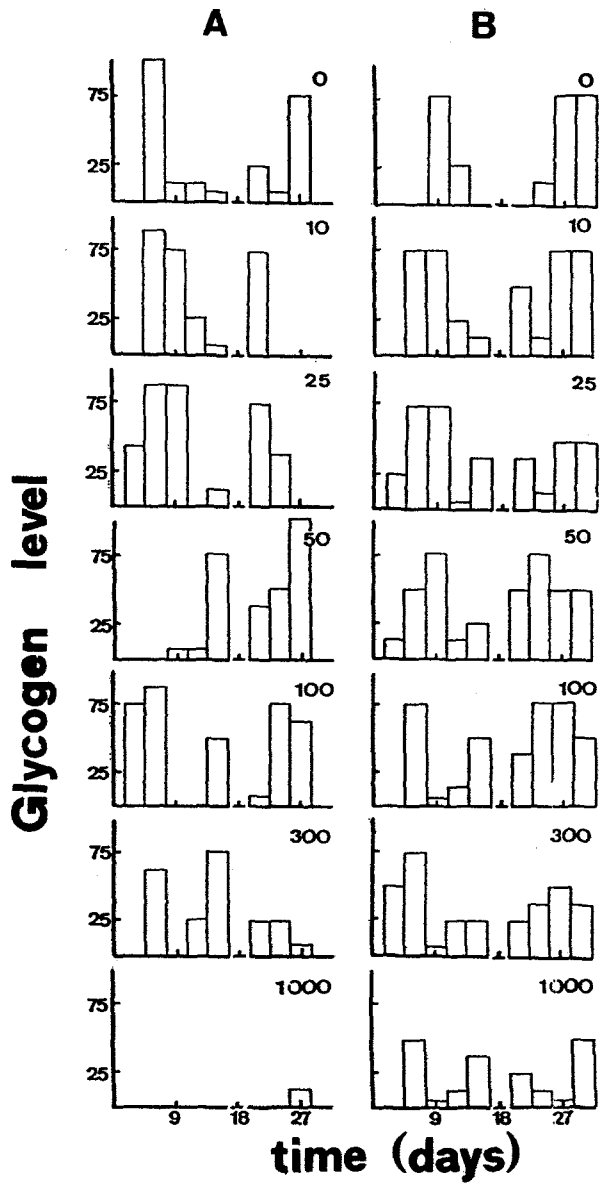

Figure 1. Histochemical levels of glycogen (arbitrary units) in the digestive gland vs. bioassay time for each group of studied slugs (from 0 to 1000 mg Zn/kg food). A, digestive cells; B, Leydig cells.

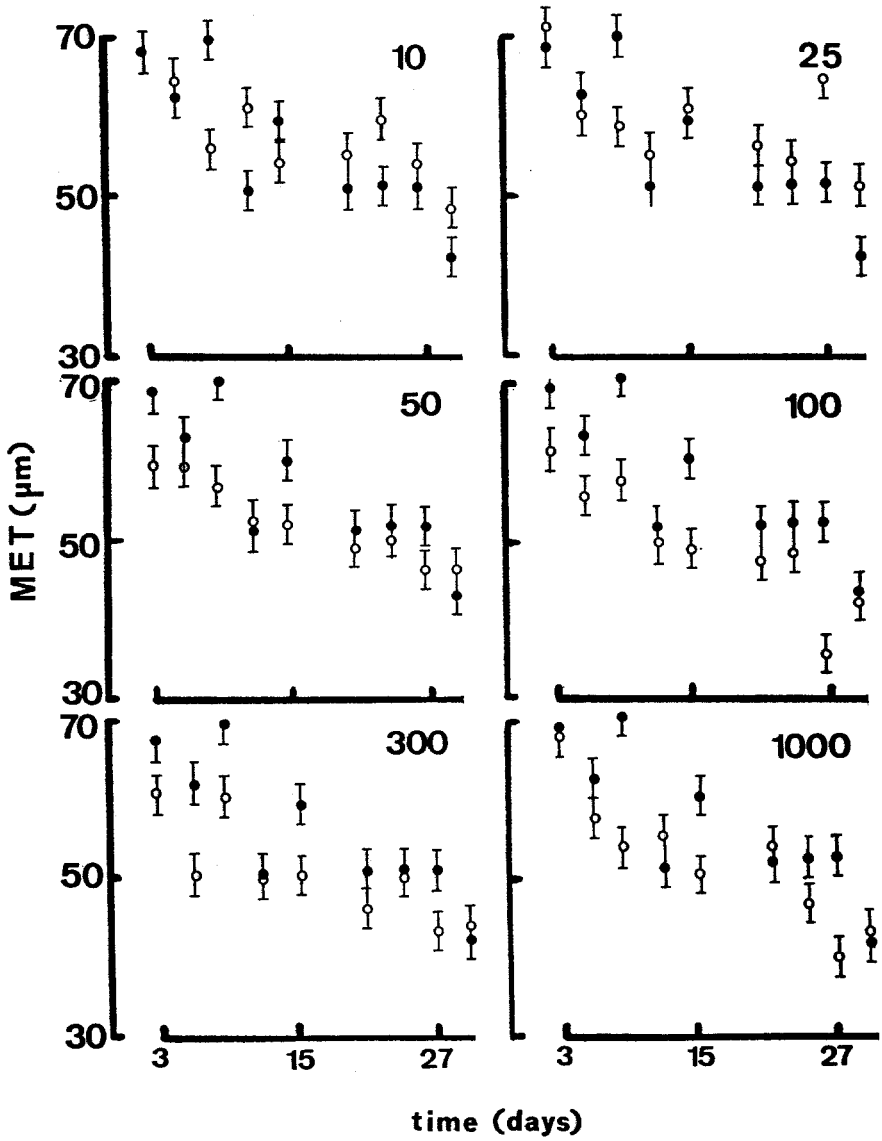

Figure 2. Results of the morphometric analysis. Dots indicate the mean epithelial thickness (MET) of the digestive gland for control (●) and treated (O) slugs. Intervals indicate the standard 99% confidence limits. Numbers at the top of each graphic indicate the zinc dosage.

MET was calculated using a planimetric procedure. Drawings of the digestive tubule sections were realised with the aid of a camera lucida attachment on a Nikon "Optiphot" microscope. Six sections randomly distributed throughout 250 µm of tissue, and 5 acini per section in each treatment group were measured with the aid of a digitizer connected to a Olivetti "M-24" computer. MET was calculated on 6 µm paraffin sections stained with haematoxylin and eosin following the method of Marigomez et al (in prep.). This method is based on the geometrical transformation of the acinar section shape into a parallelogram. Two-way ANOVA was calculated to determine the influence of both exposure time and zinc dosage on the MET.

RESULTS AND DISCUSSION

The used concentrations of zinc exposure cause no death in A. ater (male phase) as reported by Marigómez et al (1986b). Thus, it may be said that the present study only includes the range of sublethal response, which is more related to the supervivence of biological populations that to the individual tolerance.

It is thought that one of the most general sublethal effects of environmental stressors is the decline of polysaccharide reserves. Stress sources such as temperature can inhibit the synthesis of glycogen in mussels (Cracium 1980). Richards and Ireland (1978) observed an inverse relationship between glycogen absorption in the earthworm Dendrobaena rubida and the environmental concentration of lead. Lowe-Jinde and Niimi (1984) described the loss of glycogen in cadmium-exposed salmon Salmo gardnieri. Marigómez et al (1987) reported a decrease in the glycogen content of the winkle Littorina littorea exposed to cadmium. As a matter of fact, not only membrane alterations or inhibition of enzymatic activity, but also a physiological situation similar to starvation (brought on by environmental stress) could be the cause of glycogen decrease. This would not cause the death of individuals, but the weakening of the population.

Besides, the question is more complex because most of the physiological events in molluscs are of a cyclic pattern. In the present work it has been observed that a shortening in the glycogen variation pattern (Fig.1) is related to an increase in glandular activity, and to the shortening of other glandular cyclic activities (unpublished data). Such results could cause indiscernible interferences when we try to analise the

Table 1. 2-way ANOVA table: T, main effect of time; D, main effect of zinc dosage; TxD time dosage interaction; R, replication (within animals).

Source	d.f.	SS	MS	F ratio
T	8	561269.27	70158.65	138.64 (P<0.01)
D	6	28106.26	4686.37	9.25 (P>0.05)
TxD	48	125475.94	2614.08	5.16 (P>0.05)
R	1827	928545.05	506.04	
TOTAL	1889	1639396.54		

damage caused by environmental irritants. However, glycogen depression is actually evident (Fig. 1) at the highest zinc-exposure (long times with 1000 mg Zn/Kg food). The overall effect is in agreement with a decrease in feeding activity and growth described by Marigomez et al (1986b) under the same experimental conditions. These results are also in agreement with the data obtained on MET variations (Fig. 2).

MET has been proposed to be indicator of the degree of non-specific stress induced by environmental irritants. Thus, Lowe et al (1981) related the stress induced by the WAF of a crude oil to a reduction in the MET of the digestive gland of the mussel M. edulis.

Marigómez et al (1986a) determine a significant reduction of the MET in copper-exposed A. ater. Axiak et al (1988) determine a significant reduction in the mean height of the digestive cells of WAF-exposed Venus verrucosa. The present statistical data treatment has demostrated non-significant reduction of the MET in zinc exposed A. ater (Table 1). However, a trend to such reduction can be observed during the last days of treatment for the highest concentration of zinc (Fig. 2). It is not surprising that glycogen reserves (Fig. 1), feeding activity and growth (Marigómez et al 1986b) seem to be affected only at this level of exposure.

In summary, in the view of both parts of this investigation as a whole, it must be underlined that A. ater (male phase) is resistant to high dosages of zinc, though this metal is accumulated in the digestive gland, being located mainly in excretory and calcium cells. Zinc elimination occurs directly from lipofuscin material of excretory cells, and from spherules of calcium cells, where it is accumulated at calcium sites. Excretion of lipofuscin material, and

apo/holocrine extrusion from all cell types has been the mechanism observed for zinc release into the acinar lumen, and subsequently elimination via faeces. Pathological effects such as a reduction in polysaccharide reserves are only evident at the highest metal exposure. A trend in MET reduction can also be observed at this level of metal exposure.

Acknowledgments. The authors are deeply grateful to Dr MP Ireland for providing many helpful comments to improve the manuscript, and to Mr A Mateo for his excellent technical assistance.

REFERENCES

Axiak V, George JJ, Moore MN (1988) Petroleum hydrocarbons in the marine bivalve Venus verrucosa: accumulation and cellular response. Mar Biol 97:225-230

Bayne BL (1980) Physiological measurements of stress. Rapp P-v Reun Cons int Explor Mer 179:56-61

Bayne BL, Widdows J, Moore MN, Salkeld P, Worrall CM, Donkin P (1982) Some ecological consequences of the physiological and biochemical effects of petroleum compounds on marine molluscs. Phil Trans R Soc Lond B 297:219-239

Cracium C (1980) Effect of high temperatures on the ultrastructure of leydig cells in Mytilus galloprovincialis. Mar Biol 60:73-79

Lowe DM, Moore MN, Clrake KR (1981) Effects of oil on digestive cells in mussels: quantitative alterations in cellular and lysosomal structure. Aquat Toxicol 1:213-226

Lowe-Jindi L, Niimi AJ (1984) Short-term and long-term effects of cadmium on glycogen reserves and liver size in rainbow (Salmo gardnieri Richarson). Arch Environ Contam Toxicol 13:759-764

Marigómez JA, Angulo E, Moya J (1986a) Copper treatment of the digestive gland of the slug Arion ater L. 2. Morphometrics and Histophisiology. Bull Environ Contam Toxicol 36:608-615

Marigómez JA, Angulo E, Sáez V (1986b) Feeding and growth responses to copper, zinc, mercury and lead in the terrestial gastropod Arion ater (Linne). J Moll Stud 52:68-78

Marigómez JA, Cajaraville M, Sáez V, Moya J (1987) Histochemical study of the effects of experimental Cd-exposure on the glycogen contents in the digestive gland of Littorina littorea (Gastropoda: Prosobranchia). 22nd European Mar Biol Symposium. Barcelona 17-22 August

Martoja M, Martoja-Pierson M (1970) Técnicas de histología animal. Toray-Mason. Barcelona
Moore MN (1986) Molecular and cellular indices of pollutant effects. NATO ASI Series 69.Strategies and advanced techniques for marine pollution studies: Mediterranean Sea (Giam CS, Dow HJ-M eds) Berlin
Richards KS, Ireland MP (1978) Glycogen-lead relationship in the earthworm Dendrobaena rubida from a heavy metal site. Histochemistry 56:55-64
Widdows J, Bayne BL, Donkin P, LivingstoneDR, Lowe DM, Moore MN, Salkeld PN (1981) Measurement of the responses of mussels to environmental stress and pollution Sullom Voe: a based-line study. Proceedings R Soc Edinburgh 80B:323-338

Received April 21, 1988; Accepted June 1, 1988.