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Effects of Metals on the Total Lipid Content in the Gypsy Moth (*Lymantria dispar*, Lymantriidae, Lepid.) and Its Hemolymph

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Previous work on the gypsy moth, *Lymantria dispar*, was focused on the influence of Cd, Pb, Cu and Zn on its life cycle (diverse vitality parameters), stage-specific accumulation potential and implications on one of its parasitoids *Glyptapanteles liparidis* (Gintenreiter et al. 1993 a,b; Ortel et al. 1993). Results of these studies suggested that metal exposure of *L. dispar* at NOEC (No-Observed-Effect-concentration) levels may influence its hemolymph composition. We decided, therefore, to analyze the hemolymph composition for the main substance classes protein, lipids and carbohydrates of fourth instar larvae of *L.dispar* exposed to concentrations of Cd, Pb, Cu and Zn in the range of NOECs determined by Gintenreiter et al. (1993a).

This study presents the first results of the determination of lipid concentration in the hemolymph of fourth instar larvae as well as of total lipid content of the corresponding larvae.

MATERIALS AND METHODS

Gypsy moth egg clusters were collected from the field (Neusiedl/Zaya, lower Austria). Before hatching of the larvae, eggcluster surfaces were sterilized in dilute formaldehyde solution (4%). Larvae hatched after being placed in covered Petri dishes at 20°C after 10 days.

From hatching on, larvae were kept at 24°C and 12D:12L light regime and fed on an artificial diet (main components: water, wheat germ, casein, saccharose, yeast) according to Bell et al. (1981). The diet was contaminated seperately with four metals each at two concentrations: 10 and 30 μg/g cadmium (Cd10, Cd30), 20 and 60 μg/g lead (Pb20, Pb60), 30 and 50 μg/g copper (Cu30, Cu50), and 300 and 500 μg/g zinc (Zn300, Zn500), all based on dry weight. Control individuals fed on uncontaminated artificial diet (C) with background metal levels of 0.1 μg/g Cd, 0.5 μg/g Pb, 7 μg/g Cu, and 28 μg/g Zn (Gintenreiter 1994) as well as on oak leaves (natural diet, EF).

For hemolymph collection, the tip of an abdominal proleg was cut with scissors

and blood samples were collected in glass capillaries (10 and 20 µL) as it flowed from the wound by gentle squeezing of the body. Hemolymph was either taken from day-3, fourth-instar larvae of *L. dispar* (hemolymph of 3-8 individuals was pooled to obtain 100 µL samples), or - for a reduced number of test-groups (C, Cd6, Cu6, Cu10, Zn60) - taken from single specimens from day 1 to 5 (L4). All hemolymph samples were stored at -20°C until analysis. Larvae yielding hemolymph samples were dissected from digestive tracts, dried to constant weight at 80°C , homogenized and analyzed for total lipid content.

Twenty µL aliquots of cell-free hemolymph (10-15 samples per test group) of each sample were digested in 300 µL conc. H_2SO_4 at 100°C for 10 minutes. For whole body analyses, 2-3 mg of dried homogenized larvae were digested in 1 mL conc H_2SO_4 at the same conditions as mentioned above.

Lipid concentrations were measured photometrically by the phosphovanillin method (Merckotest 3321) on a Shimadzu UV-VIS recording spectrophotometer (model UV-.160).

Data were treated with the following statistical tests: Kolmogorov-Smirnov Goodness of Fit Test, Anova (Analyses of Variance) and t-test.

RESULTS AND DISCUSSION

Simpson and Raubenheimer (1993) stressed the importance of hemolymph providing a constantly updated indication of an insect's nutritional state. Therefore, chemical analyses of the hemolymph may also reveal sublethal impairment of insect larvae due to metal exposure.

Water and nitrogen content of a diet are the main factors for growth limitation in insects (Slansky and Scriber 1985). The artificial diet used in the present study had a higher protein and water content than the oak leaves (Nußbaumer 1992) and, hence, was of better nutritional quality for *L.dispar* larvae. Results of the present study seem to support this in respect to lipid content of larvae, too, since oak-leaf reared larvae had significantly lower lipid content in both hemolymph (Fig. 1A) and total body (Fig. 1B) than individuals fed on the uncontaminated artificial diet (71.5% and 62.3% of C-group, respectively; $p<0.01$). Consequently, metal-contaminated test groups were only compared within one metal and to the C- group.

Hemolymph lipid concentrations of day-3 L4 *L.dispar* were significantly reduced in all but the Pb20, Pb60 and Cu50-groups (Fig. 1A), but a correlation of lipid levels in the hemolymph and of the total body content (Fig. 1B) was only observed for these three groups (Corr.coef. + 0.739, + 0.651, + 0.741; $p< 0.01$). The hemolymph cholesterol level in the bug *Chrysocoris stolli* decreased continually after an injection of 5 µg $CdCl_2$/individual (Islam and Roy 1983). Alterations in hemolymph lipid concentrations proved to be dose-dependent for the two essential

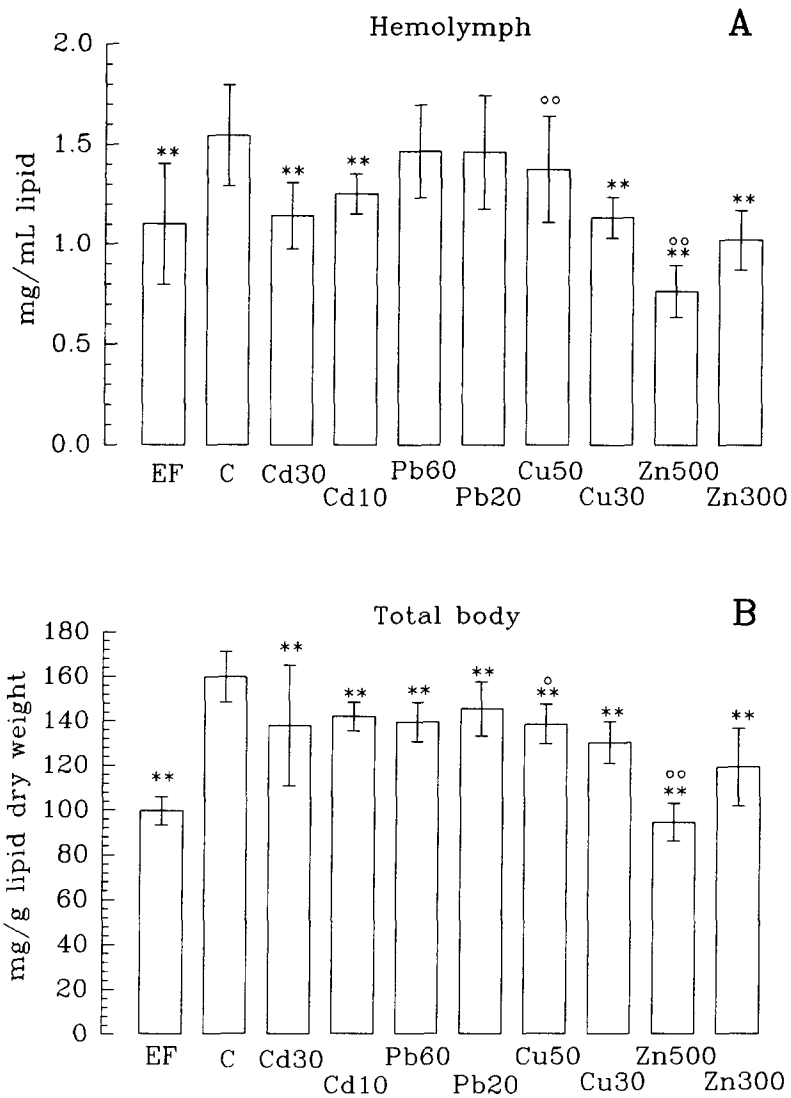

Figure 1. A) Lipid concentrations (mg/mL) in the hemolymph of day-3 *L.dispar* larvae (L4) exposed separately to four metals each at two concentrations.
B) Lipid content (mg/g dw) of corresponding day-3 *L.dispar* larvae (digestive tract dissected) exposed separately to four metals each at two concentrations.
Averages ± s.d. are given (N=10-15/ group); not different scaling.
EF= natural diet (oak leaves), C= uncontaminated artificial diet, Cd30, Cd10 = 30µg/g and 10 ug/g cadmium, Pb60, Pb20= 60 µg/g and 20 µg/g lead, Cu50, Cu30= 50 µg/g and 30 µg/g copper, Zn500, Zn300= 500 µg/g and 300 µg/g zinc all based on dry weight of the artificial diet
** ($p<<0.01$) and * ($p<0.05$) significantly different to C, °° ($p<<0.01$) and ° ($p<0.05$) dose-dependent significant difference within one metal.

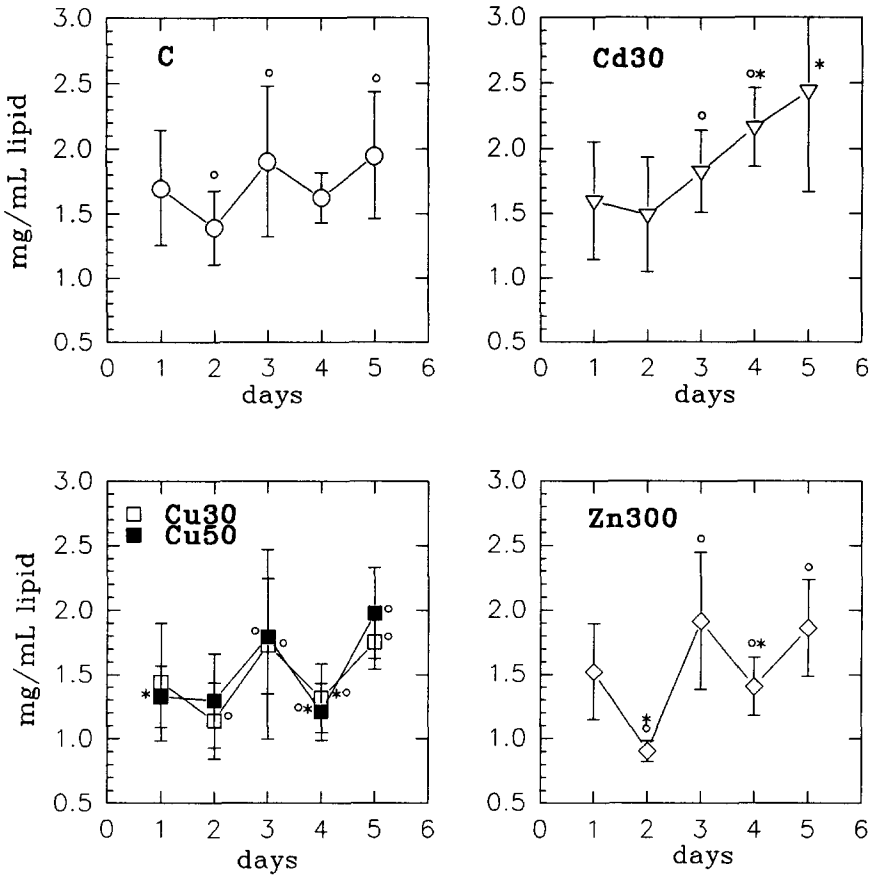

Figure 2. Lipid concentrations (mg/mL) in the hemolymph of *L. dispar* from first to fifth day of the 4th instar exposed separately to different metals. Each point represents the average value ± s.d. (N=15 (single specimens)/test group); group code see Fig. 1. * significant difference to C-group within the same day, ° sign. difference between the day assigned and the day before within one group.

metals applied (Zn, Cu). Modifications in the fatty acid composition of lepidopteran larvae can be subjected to rearing conditions, the diet consumed (Barnett and Berger 1970; Grau and Terriere 1971) and to the developmental stage of the species (Chang and Friedman 1971). Similar changes in the quantitative composition of mono-, di- and triacylglycerols in the hemolymph may also be produced by sublethal metal stress. Therefore, a detailed analysis of lipid classes in the hemolymph of *L. dispar* larvae should be further conducted, perhaps leading to a better interpretation of the present results.

Whole body (larvae with digestive tract removed) (Fig. 1B) results paralleled, in general, those of the hemolymph, but lipid content decreased in both Pb- and Cu-contaminated groups in comparison to the C-group (Fig. 1B). Again, Zn and Cu affected lipid levels depending on the concentration applied. Studies of lipid content in metal-exposed insects are scarce, but Ortel (1991) noted a significant

decrease in total lipid content in adult *Pimpla turionellae* after seven days of cadmium contamination via food.

Dry weights of the L4-larvae were lower in all contaminated groups ($p << 0.01$) except the Pb20 and Cu50 groups, which contrasts with earlier observations of Gintenreiter et al.(1993a) who reported detrimental effects on larval weights for these two concentrations.

Insect hemolymph composition is known to change with developmental stage and within one stage (Janda 1987; Chang and Friedman 1971). Moreover, *L.dispar* responded to metal-contaminated diets with the prolongation of developmental stages (Gintenreiter et al. 1993a). Hence, the sole interpretation of day-3 hemolymph lipid concentrations may be misleading, since larvae could have been in different developmental phases (within L4) at the time of hemolymph sampling (day 3 of L4). Therefore, in addition to day-3 samples hemolymph lipid concentrations were examined from day 1 to 5 (single specimens) for a reduced number of test groups. In agreement with Janda (1987), lipid concentrations fluctuated from day 1 to 5 (in all test groups but the Cd30 group in a similar way). Significant changes in lipid concentrations with time and within one group, as well as differences between groups, are shown in Fig. 2. These results suggest that the hemolymph lipid concentrations (day 3) are affected directly by metal exposure rather than indirectly by developmental retardation.

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REFERENCES

Bell RA, Owens CD, Shapiro M, Tardif JR (1981) Mass rearing and virus production. In: Doane CC, McManus ML (eds) The Gypsy Moth: Research Toward Integrated Pest Managment. Forest Service, Science and Education Agency, Technical Bulletin 1584 US Department of Agriculture, Washington, DC

Barnett JW, Berger RS (1970) Growth and fatty acid composition of bollworms *Heliothis zea* (Lepidoptera: Noctuidae), as affected by dietary fats. Ann Ent Soc Am 63: 917-924

Chang F, Friedman S (1971) A developmental analysis of the uptake and release of lipids by the fat-body of the tobacco hornworm, *Manduca sexta*. Insect Biochem 1: 63 - 80

Gintenreiter (1994) Gewebsspezifische Akkumulation der Metalle Cadmium, Blei, Kupfer und Zink in *Lymantria dispar* (Lymantriidae, Lepid.) Larven - bei Dauerbelastung und in einer metallkinetischen Studie. Dissertation Inst für Zoologie Univ Wien

Gintenreiter S, Ortel J, Nopp H (1993a) Effects of different dietary levels of cadmium, lead, copper, and zinc on the vitality of the forest pest insect *Lymantria dispar* L. (Lymantriidae, Lepid). Arch Environ Contam Toxicol 25: 62 - 66

Gintenreiter S, Ortel J, Nopp H (1993b) Bioaccumulation of cadmium, lead, copper, and zinc in successive developmental stages of *Lymantria dispar* L. (Lymantriidae, Lepid.) - a life-cycle study. Arch Environ Contam Toxicol 25: 55 - 61

Grau PA, Terriere LC (1971) Fatty acid profile of the cabbage looper, *Trichoplusia ni*, and the effect of diet and rearing conditions. J Insect Physiol 17: 1637 - 1649

Islam A, Roy S (1983) Effects of $CdCl_2$ on the quantitative variation of carbohydrate, protein, aminoacids and cholesterol in *Chrysocoris stolli* Wolf (Insecta: Hemiptera). Curr Sci 52: 215 - 217

Janda V (1987) Beitrag zur Stoffwechselphysiologie der Insektenmetamorphose. Scripta Fac Sci Nat Univ Purk Brun 17 (9-10 Biologia): 515 - 530

Nußbaumer Ch (1992) Der Einfluß von natürlichem Futter und künstlicher Diät auf die Stoff- und Energiebilanz von *Lymantria dispar* L. (Lymantriidae, Lep.). Diplomarbeit Inst für Zoologie Univ Wien

Ortel J (1991) Effects of lead and cadmium on chemical composition and total water content of the pupal parasitoid, *Pimpla turionellae*. Entomol Exp Appl 59: 93 -100

Ortel J, Gintenreiter S, Nopp H (1993) The effects of host metal stress on a parasitoid in an insect/insect relationship (*Lymantria dispar* L., Lymantriidae Lepid.-*Glyptapanteles liparidis* Bouchè, Braconidae). Arch Environ Contam Toxicol 24: 421 - 426

Simpson SJ, Raubenheimer D (1993) The central role of the haemolymph in the regulation of nutrient intake in insects. Physiol Ent 18: 395 -403.

Slansky FJr, Scriber JM (1985) Food consumption and utilization. In: Kerkut GA, Gilbert LI (eds) Comprehensive Insect Physiology, Biochemistry and Pharmacology (vol. 4). Pergamon Press, Oxford, UK