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Fate of Dietary Cadmium at Two Intake Levels in the Odonate Nymph, *Aeshna canadensis*

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While it is known that Cadmium (Cd) is concentrated from the water to the tissues of aquatic biota through respiration and surface adsorption (Wong 1987), the role of food in the uptake of Cd is not well understood, and current evidence is contradictory. In studying the flux of dietary Cd through aquatic invertebrates, it has been repeatedly noted that the Cd concentration of faecal pellets is much greater than that of the food source. Enrichment factors from food to faecal pellets of 2- to 25-fold have been recorded in the opossum shrimp, Mysis relicta, in several species of gastropod and mollusc, and in the euphausid zooplankter, Meganyctiphanes norvegica (VanDuyn-Henderson and Lasenby 1986; Brown 1986; Benayoun et al. 1974). This seems to indicate that the majority of dietary Cd is subsequently egested, and that food is therefore not an important source of Cd accumulation. Nevertheless, mass balance studies indicate that only 84% of dietary Cd in the euphausid (Benayoun et al. 1974), and 16% of dietary Cd in the crab, Pugettia producta (Boothe and Knauer 1972) was egested in the faecal pellets. The remaining Cd must then have been accumulated into the tissues of the organism, or excreted by some other means. However, studies comparing the difference in accumulation of animals exposed to soluble Cd alone, versus those exposed to both soluble Cd and a Cd-enriched food source, have indicated that food does not contribute significantly to the metal body burden (Jennings and Rainbow 1979; Sick and Baptiste 1979; Carney et al. 1986). Both of these preceeding methods of determining Cd uptake from a dietary source are subject to problems. Often the exact quantity and Cd concentration of the food consumed is unknown, with the food items being either sediment or a mixture of microplankton, from which the organism may be selecting particles of higher metal concentration (Brown 1986). Surface adsorption of Cd onto the exoskeleton and external organs of organisms exposed to its soluble form may serve to exaggerate the actual assimilation efficiency of uptake from solution (Luoma 1983), and cause an underestimate of the importance of the dietary source of the metal when organisms are exposed to both sources of Cd.

In the present study we monitored the flux of dietary Cd using the mass balance technique with the dragonfly nymph (Aeshna canadensis). The use of a predatory test organism eliminates the problem of the predator selecting food of high Cd concentration, as the animals are fed discrete, quantifiable prey items of known metal concentration. Faeces of predatory invertebrates are generally excreted in the form of compact pellets facilitating chemical analysis and determination of metal egestion. Nymphs were first fed rations of a Cd concentration typical of prey items found in relatively unpolluted waters, and were then exposed to a Cd-enriched diet to determine if a change in metal flux and body accumulation occurred at elevated levels of dietary intake.

MATERIALS AND METHODS

Odonate (Aeshna canadensis) nymphs were collected from a brownwater pond in the

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Muskoka-Haliburton region of eastern Ontario in November 1986, and kept unfed for approximately 4 wk, in aerated pondwater at 4 ° C. The prey species, blackflies (Simulium sp.) were collected from Thompson's Creek near Peterborough, Ontario and also maintained at this temperature. Two feeding experiments were conducted in an identical manner. Eight previously-starved odonate nymphs of the same instar (X = 0.059 g dry weight) were placed in individual acid-cleaned 1-L beakers containing 750 mL of filtered riverwater and a square of nylon mesh as a perch. In the first experiment, each nymph was handfed a recorded number of 8-mm long blackflies daily until two or three faecal pellets were produced (usually 4 to 5 days). Eight samples of 20 clumped 8-mm blackflies were taken for dry weight determination and Cd analysis. Faecal pellets produced by each nymph were removed from the beakers, individually dried, weighed and analysed for Cd. Cd concentration of food and faecal pellets were compared and total Cd ingested and egested by each nymph was calculated.

In the second experiment, blackflies were placed in a solution of 500 ug Cd/L for 2 d, then put in clean water for 3 d to remove loosely bound Cd from their external surfaces. A feeding experiment identical to the first was then undertaken and a Cd mass balance was calculated. Blackfly samples were analysed for Cd concentration regularly throughout this experiment to account for temporal changes in Cd concentration which might occur. Blackflies for this experiment were found to contain from 49 to 75 ug Cd/g dry weight.

Nymph Cd body burdens were also monitored. Two nymphs were analysed prior to any feeding experiments, 4 and 5 nymphs were analysed after the first and second feeding experiments, respectively.

Tissue and faecal samples were dried to a constant weight on acid-washed polyethylene strips at 60 ° C, then digested in nitric acid and hydrogen peroxide and analysed for Cd concentration using a Scintrex flameless atomic adsorption spectrophotometer with a tungsten filament. Three analytical blanks were always digested simultaneously and were found to contain consistently less than 10% of sample metal concentrations. Analytical accuracy was checked against NBS standard reference No. 1755a (bovine liver) and were within the range of the certified values ($X \pm SM$ = 0.44 ± 0.08, n = 5).

RESULTS AND DISCUSSION

The Cd concentration of faecal pellets was significantly higher than that of the food source concentration (Figure 1, t-tests, $P < 0.05$) in both experiments, resulting in metal enrichment factors of 7.95 and 5.3 for experiments 1 and 2, respectively. Mass balance calculations indicate that total Cd egested in experiment 1 was significantly greater than the total Cd ingested (paired t-test, $P < 0.02$). Although a similar trend occurred in experiment 2, the difference was not significant (Figure 2). Aeshnids have a feeding assimilation efficiency of 90%, which appears to vary only minimally among prey types (Folsom and Collins 1982). Thus, only 10% of the food intake is excreted in the faecal pellets, whereas all the Cd was excreted, resulting in the high faecal concentrations. Assimilation efficiencies of 90 to 98% were also recorded for mysids fed zooplankton, resulting in similar enrichment factors (VanDuyn-Henderson 1985).

The mass balance of experiment 1 indicated that not only was all the ingested Cd being excreted, but that faecal pellets actually accumulated Cd from some other source because total Cd egested was greater than that ingested. Although this additional egested Cd may be a result of the excretion of a previously accumulated body burden, this is unlikely as there was no change in metal concentration between nymphs analysed before and after the feeding experiment. Another explanation for the excess Cd in the odonate faecal pellets is their probable ability to scavenge metal ions out of the water column. Odonate faecal pellets are wrapped in a peritrophic membrane, which is a semipermeable structure lining the midgut region of invertebrates consuming solid food. This membrane is continually being replaced, because it passes with the excretia into the hindgut and is egested with the faeces. It is composed of chitin and proteins very similar to the cuticle, or outermost layer

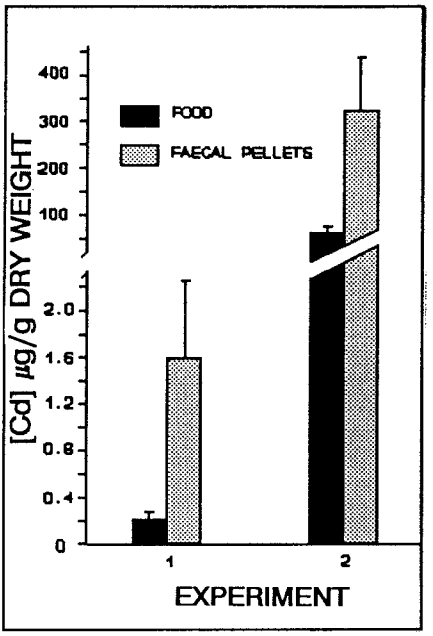

Figure 1. Mean Cd concentration of food source and faecal pellets of nymphs during the two experiments. Bars indicate 1 standard deviation.

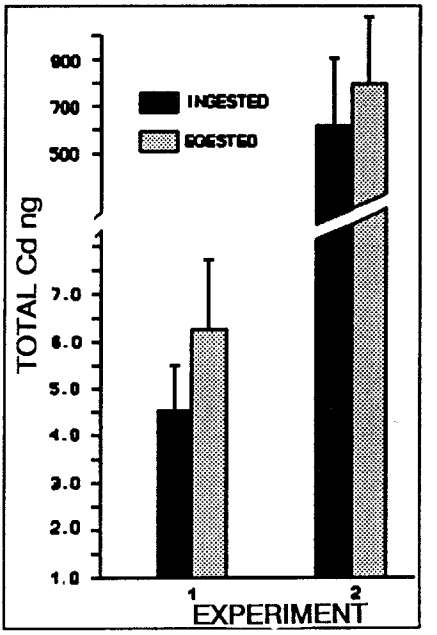

Figure 2. Mean total Cd ingested and egested by nymphs in the two experiments. Bars indicate 1 standard deviation.

of the arthropod exoskeleton (Pfadt 1985), and like the exoskeleton undoubtedly has a strong affinity for metals (Clubb et al. 1975b; VanDuyn-Henderson 1985). The filtered river water in which the dragonflies were kept contained a concentration of 0.08 ug Cd/L, thusthe 750-mL volume would have provided 60 ng of soluble Cd. The mean Cd mass by which the faecal pellets exceeded the food intake was 1.7 ng, therefore the water provided ample Cd to account for this difference. The binding capacity of the peritrophic membrane may cause an overestimation of the mass of Cd actually egested by the odonate nymphs and other invertebrates producing similar faecal pellets. Nevertheless, it did not appear that dietary Cd at low intake levels contributed to the body burden of the nymphs in this study, as their tissue concentrations remained unchanged, implying that all Cd was excreted.

There was no significant difference in the Cd concentration of nymphs analysed prior to

Table 1. Cadmium concentration and total cadmium body burden of aeshnid nymphs prior to experiments, and after experiments 1 (low Cd food) and 2 (high Cd food). Means followed by the same letter were not significantly different ($P < 0.01$).

	n	Cd concentration ug/g, mean (SE)		Cd body burden ug, mean (SE)	
Prior to experiments	2	0.219 (0.081)	A	0.013 (0.001)	A
After experiment 1	4	0.214 (0.032)	A	0.014 (0.002)	A
After experiment 2	5	1.748 (0.152)	B	0.152 (0.034)	B

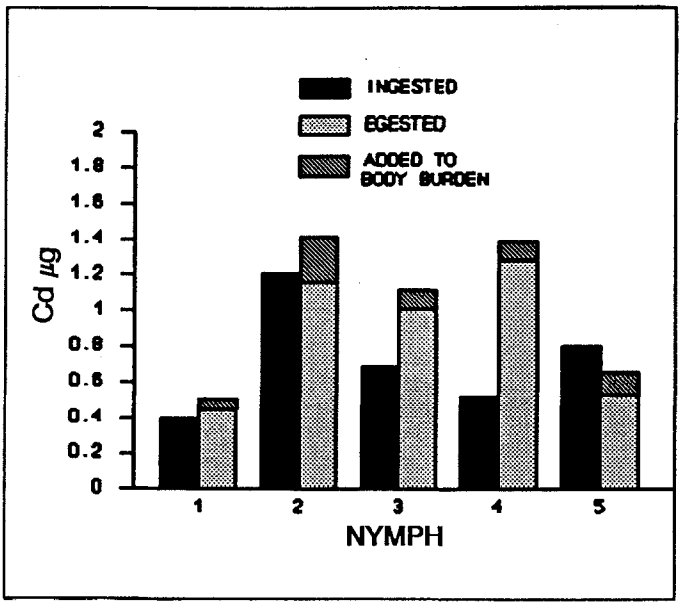

Figure 3. Individual Cd mass balances for 5 nymphs from experiment 2 (high Cd food source).

experiment 1 and those analysed after being fed the low Cd diet of experiment 1 (Table 1). However, nymphs fed the high Cd diet of experiment 2 had significantly higher Cd than did the previous two groups (ANOVA, $P < 0.01$).

Because of the tenfold increase in body Cd concentrations in experiment 2, a more complete mass balance was calculated for each of the five sacrificed nymphs. We assumed nymphs had initial mean Cd concentrations equal to those nymphs analysed from experiment 1 and prior to the experiments. Increases in Cd body burdens were calculated by subtracting estimated initial burdens from measured final burdens. In four of the five nymphs, the quantity of Cd egested or accumulated into the body, was greater than the quantity ingested, resulting in an overall net increase of the metal (Figure 3). This anomaly is potentially a function of the variation in Cd concentration of the food source. Although an effort was made to account for temporal differences in Cd concentration, it is possible that at these high levels of contamination, misrepresentations of the actual Cd burden of individual prey items may well have resulted in an underestimate of Cd intake. As well, the scavenging of metal ions from the water by the peritrophic membrane of the faecal pellets as described earlier may have contributed to an overestimate of the egested Cd. Our experiment was not designed to test this possibility.

The mass balance technique appears to be a potentially useful method of monitoring metal fluxes in aquatic invertebrate predators. At low levels of contamination, where the concentration of the prey species is stable and reliably measured, the problem of metal scavenging by the peritrophic membrane of the faecal pellets, could be overcome by maintaining the test organisms in a Cd-free, man-made water solution, rather than natural water. When spiked food organisms are used, they should be exposed to the metal both in their food source and in the soluble form, to better represent natural conditions, and to ensure that the metal is more stably bound within their tissues. The consistency of the metal concentration, both between prey organisms and over time, should be ascertained to provide for an accurate assessment of the metal intake of the predator.

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