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INCORPORATION OF CADMIUM INTO *DROSOPHILA*

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ABSTRACT

The purpose of this study was to determine the toxicity tolerance of Drosophila *to cadmium in order to obtain flies with a high Cd body burden. $CdCl_2$ was added in various concentrations to the larval rearing medium. Development was normal in media containing less than 10^2 μg/g Cd (wet wt). Adult flies reared on a medium with 31 μg/g Cd (dry wt) contained an average of 73 μg/g Cd (dry wt). Much of the Cd incorporated into the body of the adult fly was located in the soft tissues and the haemolymph; only negligible amounts were found in the cuticle.*

INTRODUCTION

Although the literature on *Drosophila* is not inconsiderable, it has not been possible to find any reference specifically relating to the tolerance, uptake and site of accumulation of cadmium in this insect. Data of this sort were needed because I have been using *Drosophila melanogaster* as a convenient food for the predatory sea-skater *Halobates* (Heteroptera: Gerridae) (Cheng, 1974), which has recently been found to contain exceptionally high levels of Cd in nature (Cheng *et al.*, 1976; Bull *et al.*, 1977) and to which I wished to supply additional Cd in the diet. I accordingly determined the toxicity tolerance of *D. melanogaster* to Cd in the rearing medium, the incorporation of this element in the adult flies and its distribution between the soft and hard parts of the insect.

MATERIALS AND METHODS

Vestigial-winged *D. melanogaster* stocks, obtained from the genetics laboratory of the University of California, San Diego, were reared in 200-ml heavy-glass bottles

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containing 50 ml of a standard cornmeal molasses medium supplemented with brewer's yeast (Demerec, 1950). Adult flies transferred to fresh medium were allowed to lay eggs for three days before being discarded and flies developing from the resulting larvae were used for the experiments described here. Cadmium (as $CdCl_2$) was added and mixed with the hot medium in some bottles; an equal volume of NaCl was added to the control medium in others. After cooling, the media were inoculated with flies and kept at 25 °C. The larvae were allowed to pupate without interference and, upon emergence, adult flies were removed daily and kept frozen in closed polyethylene vials until sufficient numbers were obtained for various treatments and analyses. The usual precautions (use of non-metal instruments and specially cleaned glass or plastic containers) were taken to avoid contamination.

For Cd analyses, samples in individual polyethylene vials covered with a layer of tissue paper (Kimwipes; Kimberly-Clark) were dried in a small drying oven at 60 °C for 48–72 h. All samples (unless otherwise mentioned) were analysed for trace metals by using a special optical emission spectrometer system at the University of California, Los Angeles (Cheng *et al.*, 1976). For our sample size (< 10 mg) the limit of detection of this system for Cd is 3 ppm.

For tests on Cd toxicity tolerances, I reared flies on media to which was added 5 ml of $CdCl_2$ of the following concentrations: 0·001 %, 0·01 %, 0·1 %, 1·0 % and 10 %, giving final Cd concentrations in the media from 1 to 10^4 μg/g. The series was set up in duplicate. Thirty adult flies were introduced into each vial.

For investigating the incorporation of Cd into *Drosophila*, flies were again reared on control medium and medium enriched with 50 μg/g Cd (wet wt) and the Cd concentrations of the media and the flies respectively were determined.

In order to determine whether the Cd taken up by the larvae was incorporated in the soft tissues or the hard parts of the adult flies, further batches of flies were reared on control and Cd-enriched media and about 1500 adult F_1-generation flies were collected from each medium. Known aliquot weights (≃ 300 mg wet wt) of the flies were squashed between layers of filter paper (Whatman No. 40, ashless) protected on each side by a sheet of waxed paper (Schleicher & Schuell, No. B-2); the chitinous exoskeletons were carefully rinsed into a polyethylene funnel lined with filter paper and then washed, drained and dried. Samples of disrupted flies (homogenised in a Potter tube with a Teflon pestle) were also digested with a protease (Sigma No. P-5130) in tris-EDTA buffer at pH 7·5 and at 28 °C for 24 h, Na_3N being added to prevent bacterial action. Samples (≃ 100 mg wet wt) of control flies (in duplicate) and Cd-enriched flies (in triplicate) were used for the digestion experiments. The homogenised flies were put into 15-ml Corning polycarbonate centrifuge tubes with 8 ml of a buffer solution comprising 87 ml of 0·15M NaCl, 10 ml of 0·25M Na_3N, 1 ml of 1M tris and 2 ml of 0·5M EDTA, the final pH being adjusted to 7·5 with HCl. Five milligrammes of the enzyme were added to each of the fly samples; a buffer–enzyme blank was kept for reference. All the tubes were then incubated in a water-bath at 28 °C for 24 h. The fly remains were then collected by centrifugation in a Clinical

centrifuge for 15 min, rinsed twice with buffer, drained, rinsed with distilled water, drained and then dried as before. One sample of the control and one sample of the Cd-enriched flies were analysed by OES; duplicate samples of each were also measured with a conventional flameless atomic-absorption spectrophotometer (Perkin Elmer) after digestion with acids (a mixture of four parts HNO_3:one part H_2SO_4).

RESULTS AND DISCUSSIONS

From the first series of experiments on the toxicity tolerance of *Drosophila* to Cd in the rearing medium, it was found that no larvae developed in media containing more than 10^3 μg/g Cd; either the adult flies died without laying eggs or the eggs failed to hatch. In media with 0–10^2 μg/g Cd, apparently normal larvae were found, but normal pupation and adults were obtained only from media containing less than 10^2 μg/g Cd; those in media containing 10^2 μg/g Cd failed to pupate. F_1 adults from larvae raised on Cd-enriched media were re-inoculated into fresh media with Cd concentrations of 50 μg/g Cd (wet wt). In such media F_2 and F_3 adults completed their development successfully and in the usual time ($\simeq$ 12 days). There was no significant difference in the weights of flies reared on control and Cd-enriched media (Table 1). There was also no apparent effect of Cd, at the concentration used, on the development of the flies. No attempts were made to follow this aspect further since the objective of this study was simply to obtain flies with a high body burden of Cd to be used as food for *Halobates* (see Cheng *et al.*, 1979).

As expected, no Cd was detected in analysed samples of the control medium or the flies reared from it. Cadmium was detected only in the experimental medium. (The concentrations of other trace elements in the two media were very similar; e.g. Cu and Zn concentrations were, respectively, 18·9, 19·8 and 12·1, 9·4 μg/g in the control

TABLE 1

CADMIUM CONTENT IN *Drosophila* FLIES REARED ON A MEDIUM WITH OR WITHOUT ADDED CADMIUM (300–500 FLIES WERE USED IN EACH SAMPLE)

Fly treatment	*No. of samples*	*Mean wet wt (mg/fly)*	*Mean dry wt (mg/fly)*	*Cadmium (μg/g dry wt)*	*Cadmium (ng/fly dry wt)*
Control					
Whole	6	0·995 ±0·019	0·292 ±0·023	<3	—
Enzyme-digested	1	—	—	<3, 0·8[a]	—
Reared on Cd medium					
Whole	2	0·994	0·264	98·1, 80·9	25·9, 21·4
Squashed	2	0·127	0·064	32·2, 36·6	2·1, 2·3
Enzyme-digested	1	—	—	2·9,[a] 3·4[a]	—

[a] Cd values obtained by AAS; the others were determined by OES.

and Cd-enriched media.) Two 8 mg (dry wt) samples of flies reared on a medium containing 31 μg/g Cd (dry wt) contained an average of 73 μg/g Cd (61 and 85 μg/g, respectively). It is evident that Cd was incorporated by the larvae from their food and accumulated in the adults.

Results from the squashing and enzyme digestion experiments are presented in Table 1. It can be seen that almost all of the Cd incorporated by the larvae of *Drosophila* was found in the soft parts of the adult flies. I could detect only about a tenth of the Cd in the squashed and washed flies (exoskeletons) and only negligible amounts in the washed remains of whole flies that had been digested with protease. It is of interest to compare this result with the data obtained by Cheng *et al.* (1977) on the accumulation of other heavy metals by beach flies (*Fucillia* sp.), where it was found that polonium was concentrated almost exclusively in the insoluble cuticular fraction. Evidently, in *Drosophila*, cadmium is held in combination with soft tissues, which are largely proteinaceous, since most of it can be expressed from the flies' bodies, and almost all can be removed by protein digestion. (It may possibly be combined with a metallothionein; Webb, 1975; Clubb *et al.*, 1975.) Studies such as these are of significance to biological studies of other liquid-feeding arthropods—in particular spiders—which, by consuming a large proportion of the insects in the world, could effect an ecological fractionation of their elemental components.

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