Flux of cadmium through the marine nematode *Enoplus brevis* Bastian, 1865

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SUMMARY

Pulse-labelling with ^{109}Cd was used to study the movement of the metal through the marine nematode *Enoplus brevis*. When exposed to filtered seawater containing ^{109}Cd as cadmium chloride, the metal first appeared in association with the cuticle.

EXPOSURE TO ^{109}CD-LABELLED SEAWATER

Approximately 100 nematodes were exposed for 12 hours to seawater containing 3 mg l^{-1} cadmium chloride and 100 µCi 50 ml^{-1} ^{109}Cd giving the solution a high level of activity (1.64 × 10^5 cpm µM Cd^{-1}). ^{109}Cd was obtained from Amersham International plc, England. After this period the nematodes were rinsed in clean seawater and placed into unlabelled seawater containing 3 mg l^{-1} cadmium chloride for a further period of 11.5 days. Animals were exposed in covered Petri dishes, containing a sterile, artificial substrate of Ballatini glass beads (Anderson & Coleman, 1977), in a constant temperature room at 10°. Solutions were not aerated but were changed every 24 hours.

Groups of twenty animals were removed at intervals of 0, 1, 3, 6 and 12 days from the start of the experiment and the tissues analysed for total cadmium and ^{109}Cd as described later.

EXPOSURE TO ^{109}CD-LABELLED SEDIMENT

Sediment from the Blyth estuary, Northumberland, known from previous work to be significantly contaminated by cadmium (R. Howell, unpubl. data) was collected and sieved to remove nematodes and other members of the meiofauna. The filtrate and washings were collected and after mixing with the sediment retained by the sieve, the mixture was evaporated down at room temperature until a residue with the approximate consistency of the original sediment was obtained. A sample of this reconstitued sediment was placed into ^{109}Cd-labelled seawater containing 100 µCi 100 ml^{-1} for 1 hour and then the mixture was again evaporated down at room temperature until the sediment resembled natural Blyth estuary sediment.

Approximately 100 nematodes were exposed to this labelled sediment in Petri dishes maintained in a constant temperature room at 10° as before. After 12 hours exposure the animals were collected, rinsed in clean seawater and placed into unlabelled Blyth sediment from which the meiofauna had been removed by sieving as described earlier. The animals were exposed to this sediment for a further 11.5 days.

Samples of twenty animals were removed at time intervals of 1, 3, 6 and 12 days from the start of the experiment and treated as follows.

DISSECTION AND ANALYSIS OF TISSUES

Groups of nematodes taken from the exposure media (seawater or sediment) were rinsed in clean seawater and narcotised to the death point using a 2 % solution of propylene phenoxitol in seawater. They were then subjected to mild ultrasonication to remove gut contents after which individuals were dissected into six regions as follows. The pharynx, up to the oesophago-intestinal junction, and the tail, up to the anal aperture, were removed first. The remainder was carefully manipulated to produce a gut tube, a cuticular tube, the eggs, and « other tissues ». The latter included the hypodermis and muscle layers and the reproductive organs which could not be successfully separated from one another.

Tissues from the twenty animals in each group were pooled into two groups of ten, dried on ashless filter paper and weighed. The pooled tissues were then homogenised in 0.1 ml of 0.3 M sucrose at 0° and the homogenate subjected to ultrasonication for 10 minutes at 0° as described by Howell and Smith (1983). 5 µl aliquots of the tissue extracts were used to determine the total cadmium concentration by flameless atomic absorption spectrophotometry using a Varian Techtron atomic absorption spectrophotometer fitted with a carbon rod atomiser. A 50 µl aliquot was taken from each tissue extract and added to 10 ml of NE 262 scintillant and then analysed for ^{109}Cd activity using a Beckman LS 100 C liquid scintillation counter, set to a counting efficiency of 2 %.

DETERMINATION OF THE MOLECULAR WEIGHTS OF THE OBSERVED CADMIUM-BINDING PROTEINS

Analysis of total cadmium and ^{109}Cd activity in the different tissues indicated that the eggs, tail and pharynx were of minor importance to the flux of cadmium through the nematodes during the experiment. It was also noted that peaks of ^{109}Cd activity in the cuticle, gut and " other tissues " occured at days 1 and 3 after the start of the experiment. It was therefore decided to confine the final part of the experiment to those tissues at those times to maximise the use of the available animals.

Approximately 100 nematodes were exposed to ^{109}Cd-labelled seawater for 12 hours and then to unlabelled seawater containing 3 mg l^{-1} cadmium as cadmium chloride for a further period of 2.5 days. Similarly 100 nematodes were exposed to labelled, then unlabelled Blyth sediment. Groups of 50 animals were taken from the exposure media 1 and 3 days after ths start of the experiment.

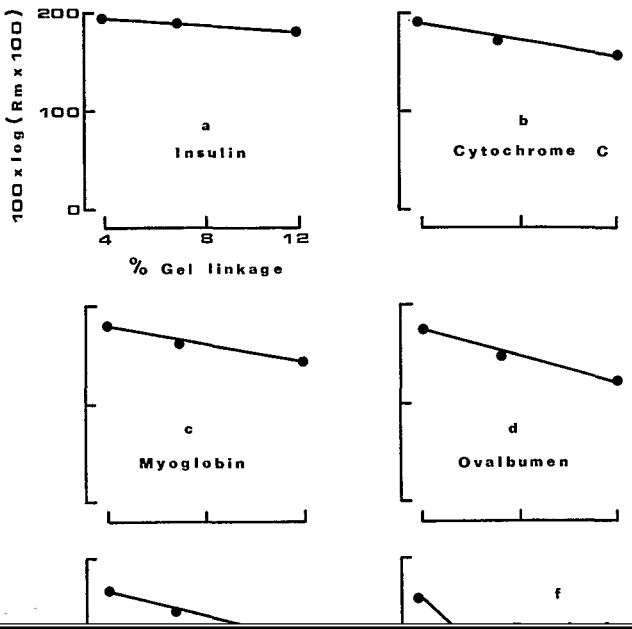
The gut contents were removed as described previously, the nematodes were dissected into the six regions and protein extracts were made of the different tissues as described by Howell and Smith (1983).

Polyacrylamide gel electrophoresis was carried out according to the method of Hendrick and Smith (1968). Replicate 25 µl aliquots of the protein extracts from the tissues were mixed with 5 µl of Bromophenol Blue tracking dye and run at the same time in gels with 4 %, 7.5 % and 12 % cross-linking, using a basic buffer. There were two gels at each level of cross-linking, one of which was stained for protein and the other used to detect ^{109}Cd activity. Five standard protein markers were run under the same conditions and all gels were stained for protein using the following procedure. Gels were first fixed in

an isopropanol/acetic acid/deionised water mixture (Anon., 1974). Proteins were then stained using Coomassie Blue (Swank & Munkres, 1970). Destaining was by a Shandon Southern transverse destainer. Gels were quantified using a Joyce-Lobel densitometer.

^{109}Cd activity was estimated by liquid scintillation counting after preparation according to Fillingame (1975).

A mixture of proteins run in basic buffer does not separate according to molecular weight as occurs in sodium dodecyl sulphate (S.D.S.) electrophoresis. However if the same protein mixture is run in several gels of different percentage cross-linking the relative mobility (Rm) of a particular protein in the different gels correlates with its molecular weight (Hendrick & Smith, 1968). The relative mobility of each standard protein in each gel was expressed as $100 \times \log (Rm \times 100)$. These values were plotted against the gel percentage cross-linkage as shown in Figure 1. The slopes of the lines which were calculated are proportional to the molecular weights of the proteins. Thus a plot of M.W. against slope produces a straight line (Fig. 2) allowing the



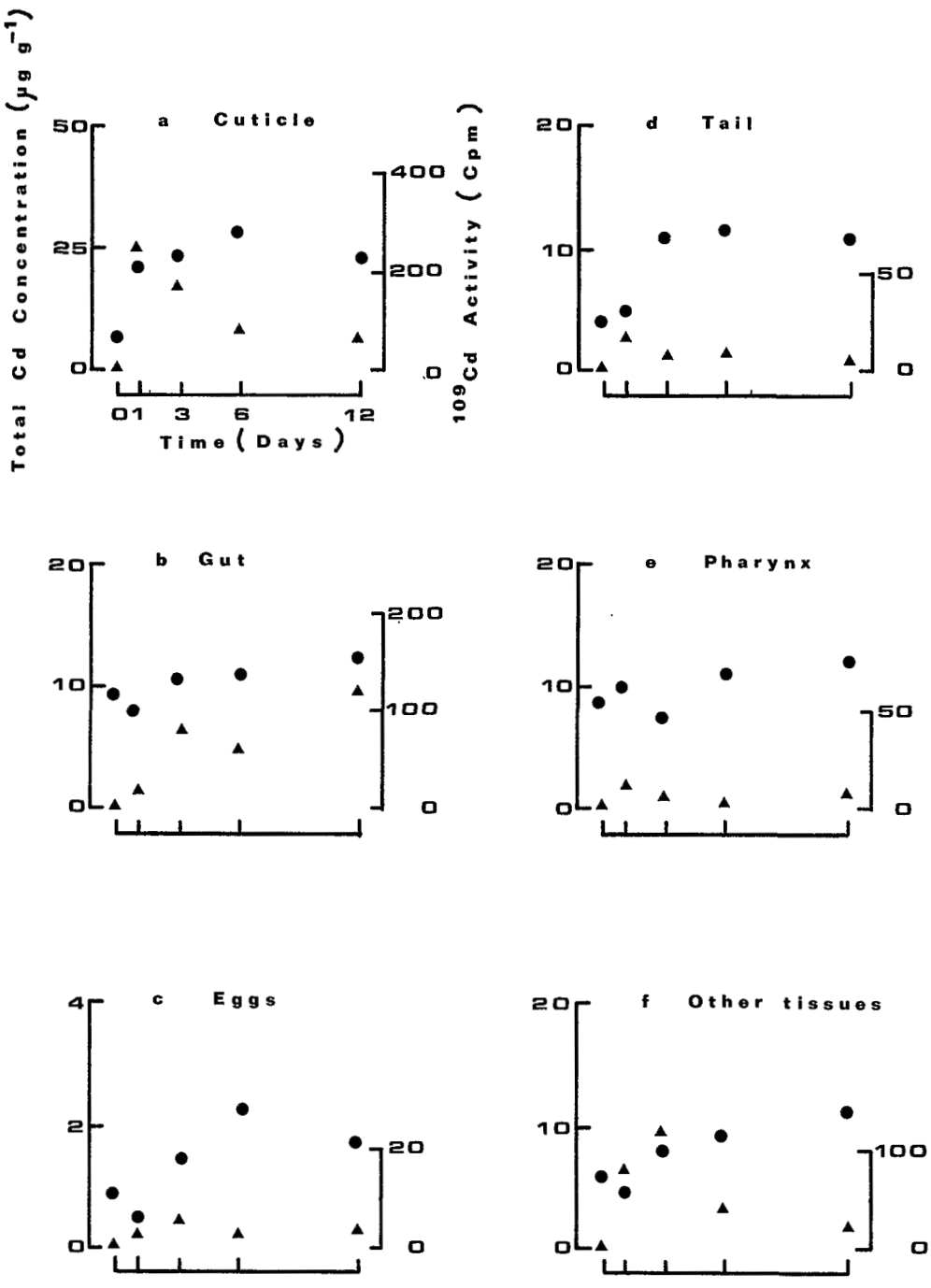


Fig. 3. Graphs of total cadmium concentration (black circle) and ^{109}Cd activity (black triangle) in tissues of *E. brevis* following exposure to cadmium-labelled seawater. Each point represents the mean of two pooled groups of ten animals. Axes labelled as for the cuticle in each case.

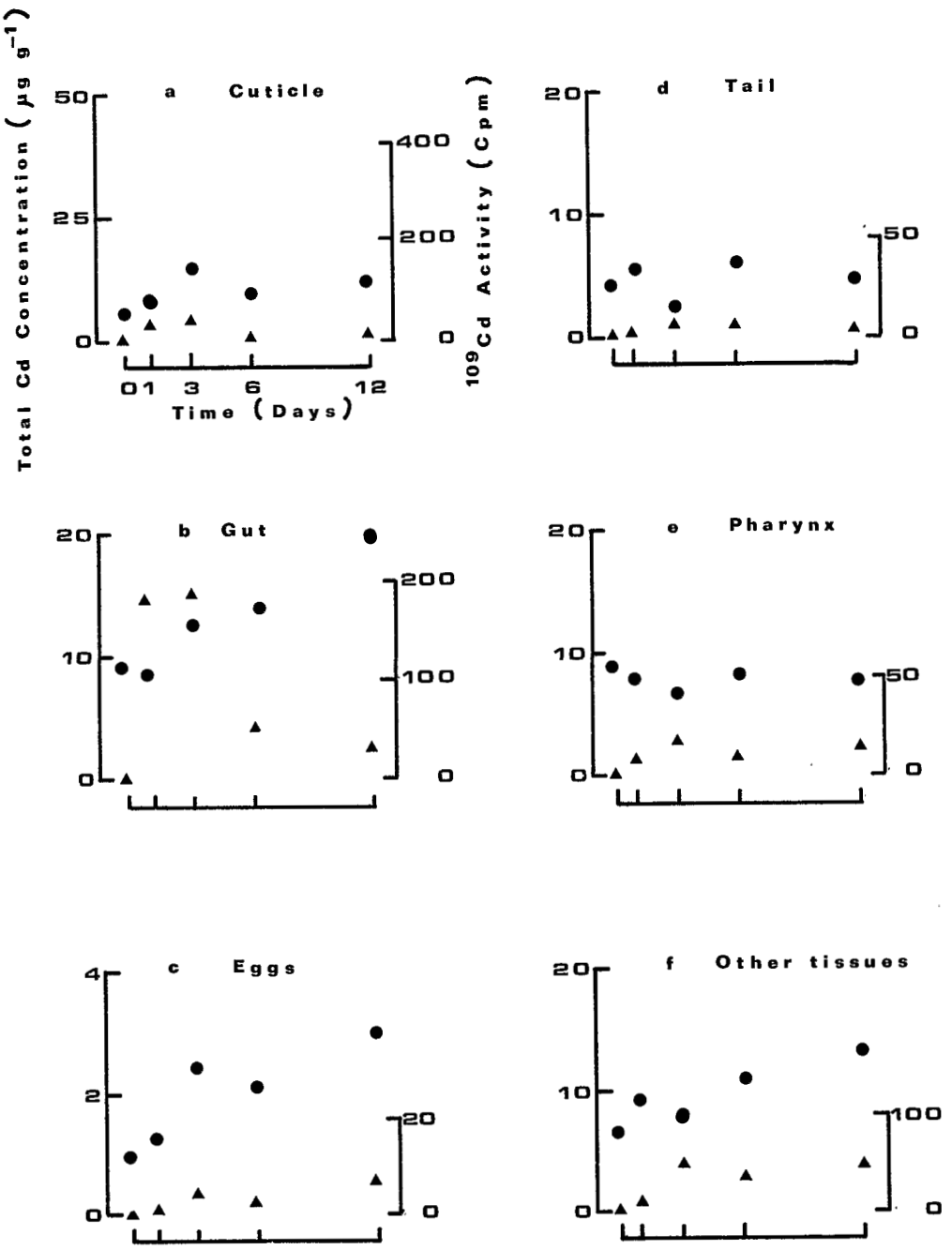


Fig. 4. Graphs of total cadmium concentration (black circle) and ^{109}Cd activity (black triangle) in tissues of *E. brevis* following exposure to cadmium-labelled sediment. Each point represents the mean of two pooled groups of ten animals. Axes labelled as for the cuticle in each case.

animals exposed to cadmium in sediment. In both cases it appears that the eggs, tail and pharynx do not play an important role in the movement of cadmium through the nematodes.

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observed in the " other tissues " is then explained since these tissues lie between, and contiguous with, the cuticle and the gut. Movement of the cadmium, probably in bound form, from the cuticle through the

was present in the pharynx and the " other tissues " and its incidence at these sites may offer a clue as to its identity. The pharynx contains a structure known as the ventral gland which secretes an Alcian Blue positive mucus (Howell, 1982 a). This has been demonstrated to be highly effective at binding heavy metals and may play a role in heavy metal metabolism by the nematodes (Howell, 1982 a). It is also known that buccal glands and digestive glands in the pharynx and foregut secrete mucus and that this passes through the gut tube, to form a lining to the gut as described by T. King (unpubl. observ.). This lining could function to protect the gut wall from mechanical abrasion by imbibed sediment or as suggested by T. King (pers. comm.) it could function as an ion exchange mechanism resulting in the binding of cadmium ions by the gut tissues. Alternatively the gut wall could be binding cadmium directly, possibly utilising protein C which may be acting as a storage system for detoxifying the metal. Cadmium, bound by protein C, could be retained in a detoxified state until it was removed from the body by exocytosis into the gut lumen. This proposed mechanism resembles the system described in the hepatopancreas of crustaceans and in the kidney of molluscs (Brown, 1978; George *et al.*, 1978).

The isolation of protein C from the cuticle provides further evidence to support the suggestion of Howell &

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