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Distribution and Speciation of Cadmium in the Terrestrial Snail, *Helix aspersa*

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Cadmium appears to be toxic to all life forms whether ingested, injected or inhaled. It is a cumulative poison, which may result in chronic disease after a number of years. In adults, about one third of the body burden of cadmium lies in the kidneys and the cadmium:zinc ratio in these organs increases from near zero at birth to 2:3 in middle age (SCHROEDER et al. 1967). The best known occurrence of cadmium poisoning in man is the Itai-Itai disease of northern Japan (KOBAYASHI et al. 1970, MURATA et al. 1969). Clinical symptoms included osteomalacia and nephropathy. Sub-acute symptoms include tiredness, loss of appetite, headache and breathlessness. Toxicological effects of cadmium on experimental animals suggest that cadmium is linked to hypertension in man as cadmium is known to induce hypertension in rats, rabbits and dogs whether injected intraperitoneally (SCHROEDER et al. 1966), intra-arterially (PERRY and YUNICE 1965) or orally (PERRY and ERLANGER 1974). Some immunity may be induced by concurrent injections of zinc (PARIZEK 1957, MASON et al. 1964) or selenium (MASON et al. 1964). However, Zn^{2+} as a treatment of chronic cadmium poisoning has no record of success.

Ingested cadmium is poorly absorbed when compared with inhaled cadmium, and the distribution of cadmium throughout the body is altered by the mode of absorption. One of the most interesting aspects of cadmium metabolism is the similarity of its absorptive mechanism to those of zinc (SAHAGIAN et al. 1966, 1967) mercury, copper (VAN CAMPEN 1966), and iron (VALBERG et al. 1976).

Metallothionein found in equine renal cortex contains sulphur, cadmium and zinc in the ratio of approximately 4:3:1. Amino acid analysis shows a high cysteine content and absence of phenylalanine, tyrosine, tryptophan and histidine. Of the 20 cysteinyl residues present all are titratable with Ag^{+} and thus no disulphide bonds are present (BREMNER 1974).

Stability constants for zinc binding proteins are greater than for cadmium binding proteins when the binding ligands contain either nitrogen or oxygen but the converse is true when co-ordination takes place via sulphur atoms. Thus cadmium prefers to bind to 'softer' ligands such as the disulphide linkage of cystine resulting in the formation of the S-Cd-S system. Experiments with zinc containing proteins have demonstrated that the zinc is easily replaced by cadmium (BLUNDELL and JENKINS 1977). Such

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replacement alters the functionality of the biochemical species. For example, replacement of zinc with cadmium in zinc carboxypepsidase alters the enzyme's activity and specificity (COLEMAN and VALLE 1961). The consequence of this interchangeability is a tenacious retention of cadmium by the body.

We have recently reported the isolation of a cadmium bearing metalloprotein complex from marine molluscs (HOWARD and NICKLESS 1977) taken from the heavily polluted Severn Estuary. The origin of the zinc and cadmium pollution is generally associated with local heavy industry and in particular a zinc smelter. We now report the results of our studies on the zinc and cadmium content of the terrestrial mollusc Helix aspersa, taken from polluted land near the industrial complex together with preliminary details of the isolation of metalloprotein compounds from this species.

MATERIALS AND METHODS

All glassware and plastic containers were soaked in 25 % v/v reagent grade HNO_3, rinsed twice with singly distilled water and dried (110°, 12 h) before use. Chemicals were analytical grade and water for preparation of standards and samples was double distilled from an all glass apparatus.

Samples (≃1 g wet weight) were digested with concentrated nitric acid (10 mL) overnight at room temperature and then gently refluxed (5 h). Cooled digests were filtered (Whatman, grade 541) and made up to 50 ml with double distilled water. A blank was prepared with each batch of samples. Standards for cadmium and zinc for the ranges 0-1 ppm and 1-5 ppm were prepared from 1000 ppm stock solutions and were renewed every second week. Analysis was by atomic absorption spectrophotometry employing a Varian Techtron Model A.A.5 with background correction. For both elements response was linear over the range 0-5 ppm.

Samples for gel-permeation chromatography (GPC) were homogenised (20 min) with a pH7 phosphate buffer (0.025 M in Na_2HPO_4, 0.025M in KH_2PO_4). The homogenate was centrifuged (16,000 G, 5 h, 4°), after which time the supernatant was gently decanted from the solid pellet. The supernatant and pellet were sampled for metal analysis. Freeze drying of the supernatant gave a suitable sample for gel permeation chromatography.

Gel-permeation chromatography was performed on a column (90 x 2.6 cm) packed with Sephadex G-50 (M.W. range 1,500-30,000), using a pH7 phosphate buffer (0.025 M in Na_2HPO_4, 0.025 M in KH_2PO_4) as eluant. The molecular weight of proteins present was determined as described elsewhere (WHITAKER 1963). The column effluent was monitored automatically using an ultra-violet spectrophotometer (SP 1700, Pye Unicam, Cambridge, U.K.) at 230, 250, and 280 nm using a silica flow cell. The collected fractions (5 ml each) were subsequently analysed by atomic absorption spectrophotometry for cadmium and zinc which could thus be correlated with protein molecular weight.

The homogeneity of metallo-proteins thus isolated was examined by disc gel electrophoresis (ORNSTEIN, 1964) on laboratory constructed equipment. Gels were of polyacrylamide (7½ % and 15 %) buffered to pH 8.6 with a tris/glycine buffer, [glycine 28.8 g; tris{2-amino-2(hydroxymethyl)-propane-1,3-diol}, 6 g; double distilled water, 1 ℓ .; sodium hydroxide solution to pH 8.6]. Samples were prepared for electrophoresis as follows. The cadmium containing fractions from the Sephadex G-50 column were collected, lyophilised, and applied to a second Sephadex G-50 column (90 x 2.6 cm) and eluted with 0.1 M tris/HCl of pH 8.6. The cadmium containing fractions were again collected, lyophilised and analysed electrophoretically.

RESULTS AND DISCUSSION

Zinc/cadmium relationships in snails from unpolluted (Pembrokeshire, Wales) and polluted areas (Avonmouth, England) are given in Table 1. Table 2 illustrates the distribution of cadmium and zinc within Helix aspersa taken from a contaminated area.

The total metal levels of the terrestrial snails conform to the pattern suggested by their respective origins. Thus snails from the relatively clean environment (Pembrokeshire) carried relatively low body burdens of both zinc and cadmium when compared with those from the polluted (Avonmouth) area. The difference in speciation of cadmium and zinc in vivo is suggested by the Cd/Zn ratio of 'soluble' metal and the 'insoluble' metal. The distribution of cadmium and zinc throughout the body of the snail (Table 2) parallels that observed by Coughtrey and Martin (1976) for the same species. Both investigations imply that the digestive gland is an important store of cadmium and zinc and that about 95 % of the total cadmium and zinc is contained therein.

The gel-permeation chromatography experiments demonstrate the presence of metal binding proteins in Helix aspersa. In the common limpet, Patella vulgata, cadmium appears to bind mainly to a protein (M1) of molecular weight 10,000 daltons although some cadmium binds to a protein (M2) of molecular weight 22,000 daltons. Zinc, in the same species, binds almost exclusively to M2. In the terrestrial snail however, the majority of cadmium, and that zinc which is extractable, are bound to M2. There is some evidence for the existence of M1 but at a much reduced level to that found in the limpet. The existence of a metal binding protein M2 in both species is thus indicated. A simple comparison of molecular weights of M1 and M2 suggests that M2 is the dimer of M1. It is possible, (though unlikely in view of the relative amounts present), that M2 is generated by the partial removal of metal atoms from M1 during sample preparation. The molecular weight of the protein M1 is close to the reported values of the apparent molecular weights of mammalian metallothioneins. It has been suggested (WEBB and STODDART 1976) that the discrepancy between the apparent molecular weight of mammalian metallothionein obtained by gel-permeation (10,000 daltons) and the values derived from amino acid analysis (6,600 daltons) is due to the presence of a carbohydrate moiety in

TABLE 1

Levels of cadmium and zinc found in Helix aspersa from clean (Pembrokeshire) and contaminated (Avonmouth) sites. (Mean values from several samples.)

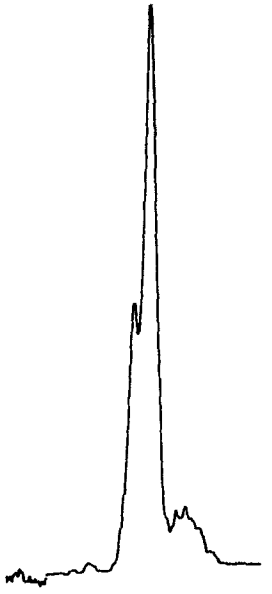
Sample	Pembrokeshire			Avonmouth		
	Cadmium ppm	Zinc ppm	Ratio Cd/Zn	Cadmium ppm	Zinc ppm	Ratio Cd/Zn
Digestive gland (whole sample)	2.8	40.2	1:14	22.9	166	1:7
Muscle (whole sample)	0.6	6.5	1:11	1.7	8.6	1:5
Insoluble fraction of digestive gland	1.1	19.5	1:20	6.4	183	1:28
Soluble fraction of digestive gland	8.0	57.8	1:7	122	83.8	3:2

TABLE 2

Typical distribution of cadmium and zinc throughout a single sample of Helix aspersa taken from the contaminated site (Avonmouth)

Sample	Cadmium ppm	Zinc ppm	Ratio Cd/Zn
Digestive gland	14.4	234	1:16
Muscle	<1	4.4	-
Rest of Tissue	0.94	5.8	1:6
Shell	<1	1.1	-
Digestive gland insoluble fraction	6.1	426	1:70
Digestive gland soluble fraction	136	161	4:5
Muscle insoluble fraction	<1	4.3	-
Muscle soluble fraction	<1	14.3	-

Figure 1



Disc gel electrophoresis of the metalloprotein M2

the protein. In this context it is perhaps significant that a microanalysis of M2 from the terrestrial snail indicated 40 sulphur atoms, (6.0 % S), were present which may be compared with the 20 found in metallothionein from equine renal cortex (BREMNER 1974).

Disc gel electrophoresis of the protein M2 implied (Figure 1) that this fraction was heterogeneous when examined at 280 and 560 nm.

By considering the Cd/Zn ratios of the soluble extracts and pellets a definite trend is observed. The proportion of cadmium relative to zinc in the soluble extract is much greater than the proportion of cadmium relative to zinc in the solid residue. Comparison of the Pembrokeshire (relatively uncontaminated) and Avonmouth (relatively contaminated) snails reveals a Cd/Zn ratio for both sets of snails in the insoluble fraction of about 1:20, whilst the ratios for Cd/Zn in the soluble extract are 1:7 and 3:2 respectively. Thus the soluble extract displays a large increase in cadmium level and a roughly constant zinc level when changing from an uncontaminated to a contaminated sample. Conversely, the solid residue shows a large increase in the zinc level relative to the cadmium level for the same two sites. These considerations point to the conclusion that zinc is complexed in such a manner that it is either insoluble in, or not extractable by water. However, cadmium is complexed such that it may be extracted.

Reference to studies of intracellular calcium granules by Walker (1975) and Simkiss (1976) suggests a possible mechanistic pathway for the uptake of cadmium and zinc into terrestrial snails. Cadmium and zinc are first ingested. Both elements form strong metal-sulphur bonds but the Cd-S bond is more stable than the Zn-S bond. Zinc binding protein (M2) appears to be naturally present in the body and the zinc is probably partially replaced by cadmium. In addition cadmium and zinc uptake induces the synthesis of extra M2 which preferentially binds cadmium. The majority of the zinc, which has little opportunity to bind to protein because of cadmium competition, probably binds to an alternative group, for example phosphate, eventually being laid down as an insoluble zinc salt in the granules (WALKER 1975). Recently zinc containing granules have indeed been found in the digestive glands of snails taken from the Avonmouth site. This hypothesis implies that synthesis of the binding protein M2 is induced by the rise in metal concentration and it thus acts as a detoxifying agent for cadmium, albeit accidentally.

CONCLUSIONS

Cadmium is taken up by terrestrial snails living in an environment with a relatively high cadmium concentration. The metal becomes bound to protein with a molecular weight of approximately 22,000 daltons. This cadmium-protein complex concentrates in the digestive gland and is present in a form which is soluble in water, (though difficult to extract efficiently). That no efficient excretion mechanism exists for this soluble complex implies that it cannot traverse the cell wall. The failure to

observe a metal-free M2 strongly suggests that formation of M2 is induced by metal insult rather than M2 being naturally present to guard against an increase in zinc and cadmium uptake.

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REFERENCES

BLUNDELL, T.L. and J.A. JENKINS: Chem.Soc.Rev. 6(2), 139 (1977).
BREMNER, I.: Quart.Rev.Biophys. 7, 75 (1974).
COLEMAN, J.E. and B.L. VALLEE: J.Biol.Chem. 236, 2244 (1961).
COUGHTREY, P.J. and M.H. MARTIN: Oecologia (Berl.) 23, 315 (1976).
HOWARD, A.G. and G. NICKLESS: Chem.Biol.Interact. 16, 107 (1977).
KOBAYASHI, J., F. MORII, S. MURAMOTO and S. NAKASHIMA: Jpn.J.Hyg. 25, 364 (1970).
MASON, K.E., J.O. YOUNG and J.E. BROWN: Anat.Rec. 148, 309 (1964).
MURATA, I., T. HIRONO, Y. SAEKI and S. NAKAGAWA: Bull.Soc.Int.Chir. 29, 34 (1969).
ORNSTEIN, L.: Anals.of New York Academy of Sciences 121, 321 (1964).
PARIZEK, J.: J.Endocrinol. 15, 56 (1957).
PERRY, H.M. (Jnr) and A. YUNICE: Proc.Soc.Exp.Biol.Med. 120, 805 (1965).
PERRY, H.M. (Jnr) and M.W. ERLANGER: J.Lab.Clin.Med. 83, 541 (1974).
SAHAGIAN, B.M., I. HARDING-BARLOW and H.M. PERRY Jnr.: J.Nutr. 90, 259 (1966).
SAHAGIAN, B.M., I. HARDING-BARLOW and H.M. PERRY Jnr.: J.Nutr. 93, 291 (1967).
SCHROEDER, H.A., S.S. KROLL, J.W. LITTLE, P.O. LIVINGSTONE and M.A.G. MYERS: Arch.Environ.Health 13, 788 (1966).
SCHROEDER, H.A., A.P. NASON, I.H. TIPTON and J.J. BALASSA: J.Chronic.Dis. 20, 179 (1967).
SIMPKISS, K.: Symposia of the Society for Experimental Biology No. 30, 423 (1976).
VALBERG, L.S., J. SORBIE and D.L. HAMILTON: Amer.J.Physiol. 231(2), 462 (1976).
VAN CAMPEN, D.R.: J.Nutr. 88, 125 (1966).
WALKER, G., P.S. RAINBOW, P. FOSTER and D.L. HOLLAND: Marine Biol. 33, 161 (1975).
WEBB, M. and R.W. STODDART: Biochem.Soc.Trans. 2, 1246 (1976).
WHITAKER, J.R.: Anal.Chem. 35, 1950 (1963).