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Biochemical changes in selected body tissues of the scallop *Patinopecten yessoensis* under long-term exposure to low Cd concentrations

Z. S. Evtushenko[1], O. N. Lukyanova[2] and N. K. Khristoforova[3]

[1] Laboratory of Comparative Physiology and Chemical Ecology, [2] Institute of Marine Biology, Far East Science Center, Academy of Sciences of the USSR, Vladivostok 690022, USSR
[3] Laboratory of Geochemistry, Pacific Institute of Geography, Far East Science Center, Academy of Sciences of the USSR, Vladivostok 690022, USSR

ABSTRACT: Effects of low cadmium concentrations (0.5 µg Cd l^{-1}) on uptake, alkaline phosphatase activity and zinc content of scallops *Patinopecten yessoensis* were investigated during 25 d exposure. Cadmium accumulations in digestive gland were bound to cytoplasmic intermediate molecular weight proteins. No adverse damage was observed.

INTRODUCTION

Spreading via water and air, heavy metals have become pollutants of great local, regional and even global importance; they add to the concentrations of toxic substances in environments and organisms, sometimes very distant from man's activities. High concentrations of metals (and other pollutants) in the environment is a new ecological factor to which organisms must adapt in order to survive. In recent years, effects of metals on various plant and animal species and communities as a whole have been studied intensively. Largely because of the growing interest in mariculture, it is important to understand the mechanisms by which some trace elements influence organisms and the potential adaptations to these effects in commercially important invertebrates (Carpene and George, 1981; Viarengo et al., 1981). In our region, the scallop *Patinopecten (Mizuhopecten) yessoensis* (Jay) has been, for some time, important for fishery and cultivation. It is a low-boreal species inhabiting Pacific coasts of Asia.

This scallop (180 × 170 mm shell) has been recorded in the Sea of Japan at Primorye, North Korea, western Sakhalin, northern Honshu and Hokkaido; in the Okhotsk Sea, at Aniva Bay and Bousse Lagoon; and in the South Kuriles (Scarlato, 1976, 1981). The ability of various species of Pectinidae to accumulate large amounts of heavy metals in nature and under laboratory conditions is well-documented (Brooks and Rumsby, 1965; Bryan, 1973; Nelson et al., 1976; Saenko et al., 1976; Pesch and Stewart, 1980; Carmichael and Fowler, 1981); however, there is little information on biochemical mechanisms of detoxication of trace elements in molluscs.

This paper presents results of a model experiment dealing with the effect of low Cd concentrations on the scallop *Patinopecten yessoensis* under long-term exposure. The nature of Cd accumulation in molluscan tissues and the binding of Cd with cytoplasmic proteins of the digestive gland were studied. The functional state of test animals was estimated by the activity of alkaline phosphatase in selected organs of the scallop.

MATERIALS AND METHODS

The molluscs were collected in Vityaz Bay (Peter the Great Bay, Sea of Japan; 32 ‰ salinity) in January 1982. We selected 100 to 120 mm long scallops, with shells free of fouling organisms. The scallops were kept in tanks with sea water, to which cadmium chloride was added at a final concentration of 0.5 µg l^{-1}. This concentration is one-tenth of the admissible concentration of Cd in rivers and marine fishery areas. Duration of exposure was 25 d. The medium was replaced every second day. Scallops were fed on water extracts of *Laminaria* and *Ulva* homoge-

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nates, and twice on yeast extracts. On Days 3, 7, 15, 20 and 25 of the experiment, 3 individuals were removed from every test and control tank, and Cd and Zn contents in gonad, mantle, digestive gland and muscle were determined. Digestive glands of test scallops were removed and examined for cytoplasmic proteins. Digestive glands were homogenized in 3 volumes of 0.05 M Tris-HCl buffer at pH 7.5. Centrifugation of homogenates was carried out at 16,000 g for 20 min. Calcium and magnesium salts were added to the supernatant to precipitate mitochondria and microsomes (Kamath and Narayan, 1972). Clear supernatant was used to separate proteins. Cytosol (5 ml) was loaded into an 80 × 2.5 cm column with Sephadex G-75. Proteins were eluted with 0.05 M Tris-HCl buffer (pH 7.5) at a rate of 18 ml h^{-1}. The column was first calibrated with Cytochrom C (molecular weight 13,400), used as a marker protein for determining elution volumes of intermediate molecular weight proteins (10,000 to 15,000). Proteins were quantified in preparations as described by Lowry et al., 1951.

Cadmium and zinc contents were measured by atomic-absorption spectrophotometry in protein fractions and in pooled samples of molluscan tissues after acid mineralization in an autoclave.

Preparations for determining alkaline phosphatase activity were obtained by centrifugation of tissue homogenates at 16,000 g for 20 min. Enzyme activity was determined with P-nitrophenylphosphate as a substrate in 0.1 M carbonate buffer, pH 9.6 (Echeteby, 1980).

RESULTS

Concentrations of cadmium and zinc from selected body tissues of the scallop are given in Table 1. Digestive glands had a high background level of Cd, although the scallops were collected in an uncontaminated area. Cadmium contents of digestive glands ranged up to 53.6 µg g^{-1} dry matter; this is 20 to 30 times more than in other organs. The gills, for example, contained 8 µg Cd g^{-1}, the gonads 3.4 µg Cd g^{-1} dry matter. Zinc content varied: 62 µg g^{-1} in the digestive gland and 160 µg g^{-1} dry matter in the mantle. Initially, Cd content of the digestive gland increased and was maximal on the 15th day (89 µg g^{-1}); it then fell sharply to 21 µg g^{-1}. Cadmium content either increased consistently (gonads and mantle) or did not change measurably (gills and muscles).

During the experiment, Zn content in gills increased constantly. In digestive gland and gonads, zinc content varied with no apparent trend. It is emphasized that the highest Cd content in the digestive gland on Day 15 was accompanied by the lowest Zn concentration, but by Day 25, when the Cd content of the digestive gland fell, the Zn content increased.

Examination of cytoplasmic proteins for metal contents showed that cytosol contained measurable quantities of Cd and Zn. Cd concentration in cytoplasmic proteins of experimentals was 0.23 µg mg^{-1} of protein on Day 25. Zn content of cytosol did not change significantly during the experiment.

The elution profile of cytoplasmic proteins extracted from the digestive gland of control scallops and metal contents of protein fractions are given in Fig. 1. Separation of cytosol aliquot on Sephadex G-75 gave 2 main peaks: one corresponded to high-molecular weight proteins, the other to the region of elution of Cytochrom C (molecular weight 13,400). All protein fractions had a significant Zn content; the highest concentration (2 µg mg^{-1} protein) was found in proteins of molecular weight of about 13,000. However, the amount of these proteins was small (optical density 0.2 at 254 nm). Cadmium distribution was different: proteins of high molecular weights were practically Cd-free, and intermediate molecular weight proteins contained the main portion of the Cd. The highest concentrations of Cd and Zn were coincident and associated with proteins of a molecular weight of 13,000.

Fig. 2 and 3 give elution profiles of cytoplasmic proteins extracted from scallop digestive glands on the

Table 1. *Patinopecten yessoensis*. Mean cadmium and zinc concentrations (µg g^{-1} dry matter) in selected organs after exposure for 25 d to 0.5 µg Cd l^{-1}

	Start (Day zero)		Day 7		15		20		25	
Organs	Cd	Zn	Cd	Zn	Cd	Zn	Cd	Zn	Cd	Zn
Gonad	3.4	137.5	4.8	111.8	16.5	139.5	9.6	108.0	14.0	81.2
Mantle	2.6	161.8	6.9	88.7	6.8	113.0	10.9	133.8	9.4	112.5
Digestive gland	53.6	62.3	60.2	130.9	89.9	95.9	–•	–•	21.6	130.6
Gills	8.0	118.6	13.2	138.5	8.2	148.6	10.2	183.6	–•	–•
Muscle	1.5	69.4	1.0	68.3	1.1	79.3	1.9	76.8	2.0	75.1

• No data

15th and 25th days of exposure, and metal concentrations of protein fractions. By Day 15, the amount of proteins containing high concentrations of Cd increased (Fig. 2, optical density 1.4 at 254 nm). Zn was found in all protein fractions, Cd only in 2 fractions (Fig. 2). By Day 25, redistribution of metals was observed: Cd concentration in low-molecular proteins increased almost 2-fold to 0.7 μg mg^{-1} proteins (Fig. 3); moreover, as in the case of the 15 d exposure results, a peak persisted which corresponded to a protein with significant content of this metal (in the region of elution of proteins with a molecular weight above 13,000). High molecular weight proteins contained insignificant amounts of both Cd and Zn.

In all organs of the scallop, no sharp change of alkaline phosphatase activity was observed during the experiment (Table 2). Only in gills was there an appreciable decrease in alkaline phosphatase activity during Cd exposure. By the end of the experiment, the activity of this enzyme in all organs of test scallops was somewhat higher than in the controls.

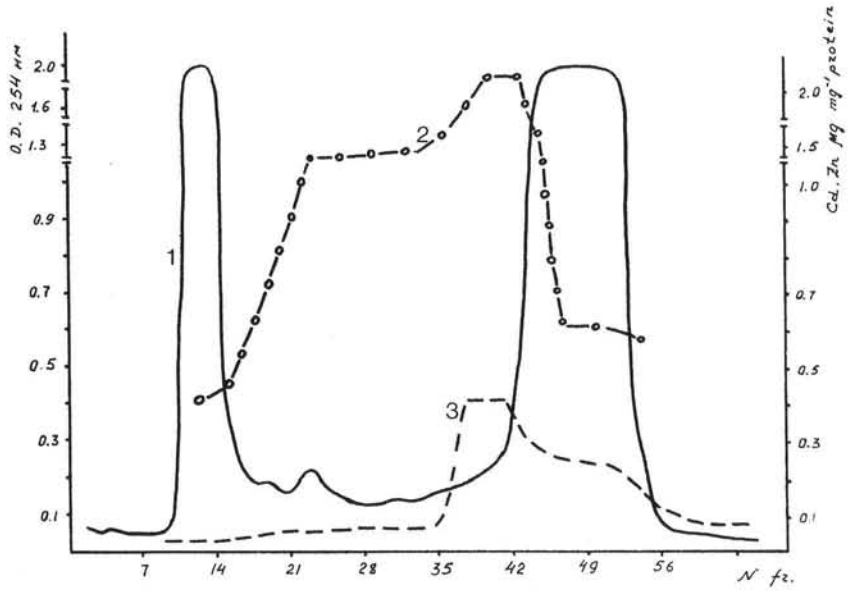

Fig. 1. *Patinopecten yessoensis*. Gel chromatography of cytoplasmic proteins of digestive gland (control) on Sephadex G-75. 1: elution profile of cytoplasmic proteins at O.D. 254 nm; 2: Zn content in protein fractions; 3: Cd content in protein fractions

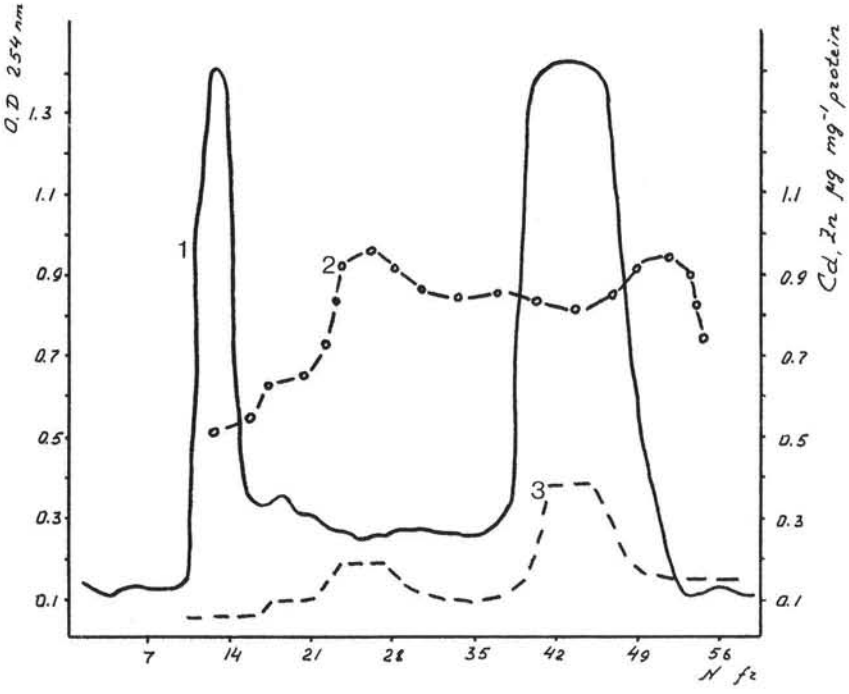

Fig. 2. *Patinopecten yessoensis*. Gel chromatography of cytoplasmic proteins of digestive gland (Day 15) on Sephadex G-75. 1: elution profile of cytoplasmic proteins at O.D. 254 nm; 2: Zn content in protein fractions; 3: Cd content in protein fractions

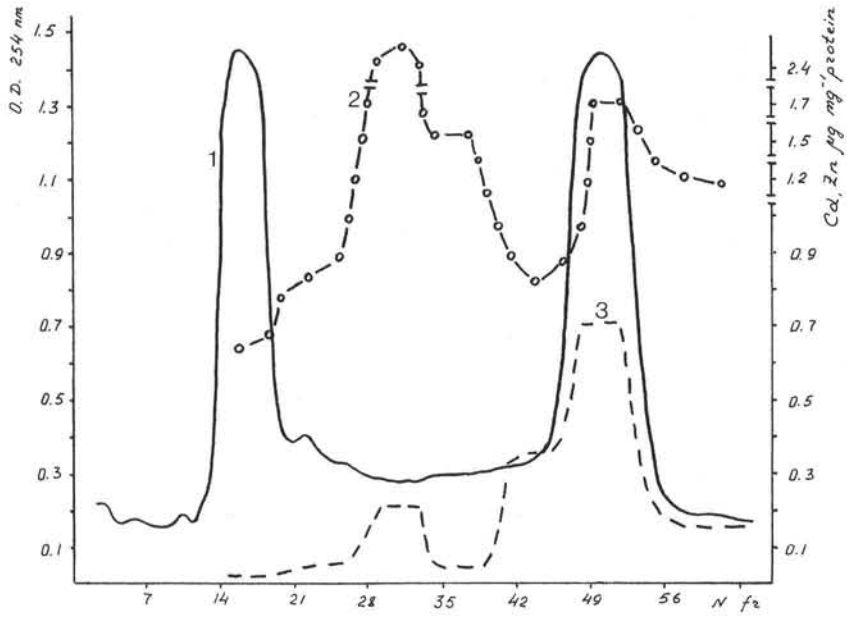

Fig. 3. *Patinopecten yessoensis.* Gel chromatography of cytoplasmic proteins of digestive gland (Day 25) on Sephadex G-75. 1: elution profile of cytoplasmic proteins at O.D. 254 nm; 2: Zn content in protein fractions; 3: Cd content in protein fractions

Table 2. *Patinopecten yessoensis.* Alkaline phosphatase activity in selected tissues, during 20 d exposure to 0.5 µg Cd l^{-1}. Mean values in µmol min^{-1} mg^{-1} protein ± 1 standard deviation

Day	Mantle		Gills		Muscle	
	Controls	Experimentals	Controls	Experimentals	Controls	Experimentals
0	0.46 ± 0.17	0.46 ± 0.17	0.65 ± 0.18	0.65 ± 0.18	0.20	0.20
3	0.29 ± 0.03	0.16 ± 0.05	0.81 ± 0.04	0.79 ± 0.13	0.23 ± 0.07	0.21 ± 0.05
8	0.37 ± 0.04	0.38 ± 0.04	0.43 ± 0.09	0.71 ± 0.17	0.37 ± 0.08	0.23 ± 0.06
15	– *	0.26 ± 0.03	– *	0.42 ± 0.08	– *	0.26 ± 0.05
20	0.32 ± 0.06	0.53 ± 0.09	0.28 ± 0.1	0.38 ± 0.07	0.21 ± 0.03	0.27 ± 0.1

Day	Gonads		Digestive gland	
	Controls	Experimentals	Controls	Experimentals
0	0.27 ± 0.03	0.27 ± 0.03	0.25 ± 0.04	0.25 ± 0.04
3	0.17 ± 0.07	0.21 ± 0.07	0.24 ± 0.03	0.41 ± 0.03
8	0.18 ± 0.04	0.16 ± 0.03	0.34 ± 0.02	0.35 ± 0.04
15	– *	0.20 ± 0.05	– *	0.26
20	0.16 ± 0.03	0.26 ± 0.01	0.40 ± 0.07	0.43 ± 0.03

* No data

DISCUSSION

Toxicological studies on mechanisms of heavy metal influences upon organisms usually test high concentrations of heavy elements, not likely to be encountered under field conditions. In animals exposed to high heavy metal concentrations, significant changes may occur in organs and body tissues as indicated by biochemical, cytological, histological and other methods. After long-term exposure to comparatively low metal concentrations, organisms usually adapt to the new conditions, but the mechanisms by which this is accomplished are imperfectly understood. In our experiment, cadmium concentrations were only 5 times above background in the waters of Peter the Great Bay (Patin et al., 1980).

In contrast to other commercially important bivalves (oysters and mussels), the scallop *Patinopecten yessoensis* can accumulate in its digestive system considerable amounts of Cd, even in a contamination-free area (Lukyanova and Evtushenko, 1982; Khristoforova, 1983). Many authors (Mullin and Riley, 1956; Brooks and Rumsby, 1965; Segar et al., 1971; Bryan, 1973; Nielsen and Nathan, 1975; Vattuone et al., 1976) find this accumulation to be typical of all molluscs of the family Pectinidae.

We observed that scallops held at 0.5 μg Cd l^{-1} for 25 d did not exhibit any pathological changes; this may be due to the comparatively high resistance of scallops to Cd. For example, the LC-50 for juvenile *Argopecten irradians* is 1.48 mg Cd l^{-1} and 0.033 mg Ag l^{-1} (Nelson et al., 1976). Pesch and Stewart (1980) found at chronic exposures of scallops to high Cd concentration that scallops accumulate 2,000 to 3,000 mg Cd kg^{-1} dry weight, when compared to 7 mg kg^{-1} for controls. Further, accumulation is greater in summer, and occurs at more rapid rates; this is typical both of scallops (Bryan, 1973) and of oysters (Zaroogian and Cheer, 1976). In the oyster, Cd accumulation rates in summer were twice those in winter. Our experiment was conducted in January, when metabolic processes in scallops were significantly retarded, and this may account for the comparatively moderate rates of Cd accumulation by various scallop tissues. Nevertheless, even this small variation in Cd concentration in scallop tissues caused significant alterations in the chemical binding of this element by cytoplasmic proteins. Cadmium occurred in 2 protein peaks by the 15th day of exposure, and by the 25th day Cd concentration in the protein fraction with molecular weight of 12 to 15,000 was twice that of controls (Fig. 1 to 3).

It is known that Cd is primarily accumulated by cytosol cellular fractions, where it is bound to specific proteins, metallothioneins (Webb and Cain, 1982). Moreover, some experiments showed that a significant portion of the Cd content may be bound in membranes or accumulated in intracellular granules (Carmichael and Fowler, 1981; Metal-binding . . ., 1981). Profiles of Cd levels in protein cytoplasmic fractions indicate strongly that proteins of low and intermediate molecular weights participate in Cd binding. Cadmium concentrations of 0.5 μg l^{-1} are quite likely to occur in natural habitats of molluscan populations; however, this concentration did not appear harmful to scallops. The capacity of cytoplasmic Cd-binding proteins apparently enables the organism to adapt readily to cadmium stress.

Our study considered not only chronic Cd effects but also variations of Zn content in the same experimental molluscs. Zn is a chemical analogue of Cd. Due to the similarity of their chemical characteristics, these elements may compete for Cd binding sites.

Estimation of the functional state of molluscs by the activity of alkaline phosphatase showed that experimental scallops differed only slightly from controls. Moreover, during the 25 d experiment, all scallops showed a decrease in activity of the enzyme in gill tissues; this may be associated with restraint of movement in scallops maintained in tanks during the experiment.

Alkaline phosphatase contains 1 Zn atom in its active center; substituting cadmium, copper or mercury for Zn decreases enzyme activity levels (Spiro, 1978) in experiments *in vitro*. Exposure to 0.5 μg Cd l^{-1} caused a slight rise in alkaline phosphatase activity, suggesting a stimulation effect at low doses of Cd upon alkaline phosphatase activity of scallops. Patin (1979) pointed to the stimulatory effect of low doses of heavy metals on the sea organisms.

Thus, Cd concentrations of 0.5 μg l^{-1} did not cause pathological effects in scallops during exposure for 25 d. We conclude that additional amounts of cadmium accumulated by the molluscan digestive gland are bound by cytoplasmic proteins of intermediate molecular weight. Small doses of the element did not inhibit alkaline phosphatase activity, on the contrary: 0.5 μg Cd l^{-1} elevated alkaline phosphatase levels, particularly in mantle and gill tissues.

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