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Variations of Some Elements in Cadmium-Induced, Malformed Fish

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There have been many reports of experiments on the development of vertebral malformations due to agrochemicals, as follows: MEYER (1966); KIMURA & MATSUSHIMA(1969); McCANN & JASPER(1972); MEHRLE & MEYER(1975). However, little is known about morphological anomalies caused by heavy metals. Reports of malformation induced by cadmium such as the appearance of vertebral anomalies in carp(*Cyprinus carpio* L.) have been described by the present author (1972, 1979, 1980a,b). PIKERING & GAST(1972) reported a case of malformation in fathead minnow due to cadmium. EATON(1974) described a similar effect in bluegill and NAKAMURA(1975) reported on dace(*Tribolondon nakonensis*). MURAMOTO(1979, 1981a,b) suggested malformed fish showed a slight decrease in the ash weight/dry weight ratio in bone tissues, and significantly deficiences of calcium and phophrus compared with normal fish.

In this paper, the appearance of such malformed fish by exposure to cadmium was confirmed in a repeat experiment. Decalcification of the fish was studied from spinal X-ray photographs and the results of some elements analysis.

MATERIALS AND METHODS

Experimental conditions

Ten carp(*Cyprinus carpio* L.) 8-10g in weight and 6-7 cm long in the first experiment and 9-11g in weight and 6-8 cm long in the second experiment, were kept singly in each 60-L glass aquaria at 17.0-19.0 °C throughout the experiment. The fish were exposed to cadmium ($CdCl_2.2.5H_2O$) at nominal concentrations of 0.01, 0.05, and 0.1 ppm; one control group was prepared. Test water was prepared with tap water and changed twice a week. Water characteristics(mg/L) were : Ca 4.7-4.9; Mg 1.4-1.5; Na 3.6-3.9; K 0.77-0.88; SO_4 2.8-3.0; Alkalinity as $CaCO_3$ 13.3-14.8; Cl 2.6-3.3; Dissolved solid 41.0-42.2; Cd 0.001-0.002; Cu 0.004-0.005; Zn 0.07-0.09; and Pb 0.04-0.05. The pH was 6.8-7.1.

Analysis

Three parts of the fish, viscera, gills and vertebrae, were removed from the bodies. Each sample was dried at 60 °C for 24-h in a hot-air drier and tured into ash at 400°C(viscera, gills) or 450°C (bone), for 24-h in an electric muffle furnace. The ash sample was dissolved in HNO_3-$HClO_4$(2:1), and made up to a fixed valume by

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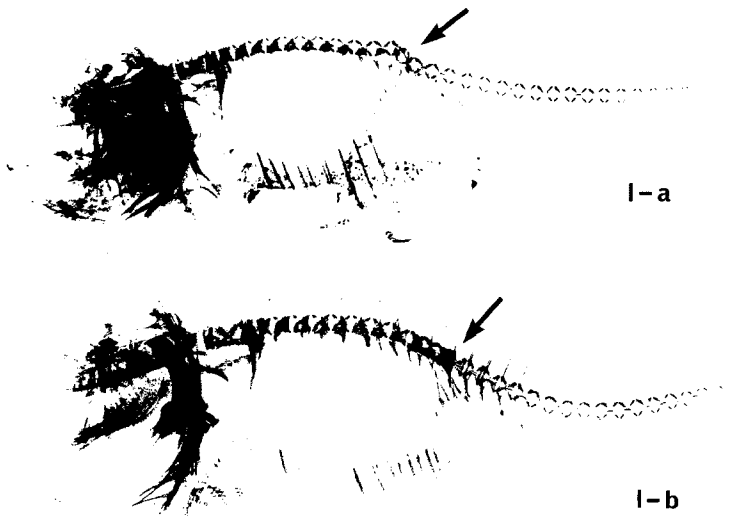
addition of 1N-HCl. This solution was used for the determination of metals of Cd after application of the APDC-MIBK extraction method. Phophorus was analysed by the method of BOLTZ(1958). Calcium and magnesium were determined by atomic absorption spectrophotometry in the presence of $LaCl_3$. Fish exposed to Cd conveniently were divided into two groups by observation with the naked eye: (1) malformed fish, and (2) normal fosh. X-ray photographs were taken using Softex (Japan Softex Co.) to show skeletal deformities.

RESULTS

Appearance of malformed fish

Fish with deformed vertebra were observed on the 47th, 85th and 73rd day after the beginning of the experiment in 0.01, 0.05, and 0.1 ppm Cd in the first experiment, and on the 58th and 95th day after the beginning of experiment in 0.01 and 0.05 ppm Cd in the second experiment; these deformed fish survived throughout the experimental period. The frequency of development of malformed fish was not constant. The vertebral column of some fish developed the abnormalities shown in Figs. 1-a, 1-b.



Figs. 1-a, 1-b. Magnified X-ray photographs of malformed fish exposed to (1-a) 0.01 ppm Cd in the first experiment, (1-b) 0.01 ppm Cd in the second experiment.

The concentrations of Cd, Ca, Mg ans P in the fish

The concentrations of Cd, Ca, Mg and P in each sample of fish (μg/g in ash) are shown in Table 1. The cadmium levels in the fish given cadmium tended to rise in their viscera, gills and vertebrae with the increase in the concentration of cadmium of the breeding water (MURAMOTO 1980b, 1981), and was higher than in the control fish (Fig. 2-a). However, there were marked differnces in the viscera and gills of the malformed and normal fish. The levels of calcium in the viscera, gills and vertebrae of fish given cadmium tended to be lower than in the control fish.

TABLE 1

The concentrations(μg/g in ash) of Cd, Ca, P and Mg in malformed fish and normal fish exposed experimentaly to different concentrations of Cd, and in control fish in the first and second experiment

The concentrations(μg/g in ash) of Cd, Ca, P and Mg in malformed fish and normal fish exposed experimentaly to different concentrations of Cd, and in control fish in the first and second experiment

	Metal	Part	Cd 0.01ppm	Cd 0.05ppm	Cd 0.1ppm	Control
First experiment	Cd	Viscera	16.2(40.5)	44.6(34.3)	61.3(65.7)	0.221
		Gills	13.0(14.5)	11.8(25.7)	15.8(51.4)	0.286
		Vertebrae	4.18(10.8)	5.74(7.51)	5.93(11.5)	0.11
	Ca	Viscera	74.6(65.9)	56.2(58.2)	65.8(65.6)	84.4
		Gills	505(488)	435(409)	519(398)	669
		Vertebrae	187000(131000)	176000(138000)	178000(125000)	213000
	P	Viscera	45.4(52.5)	48.0(38.8)	43.6(32.5)	51.6
		Gills	348(359)	319(303)	366(349)	468
		Vertebrae	93100(73100)	86500(75900)	85300(79000)	104100
	Mg	Viscera	10.9(10.7)	10.3(9.4)	11.8(13.9)	10.5
		Gills	25.8(27.0)	25.3(26.1)	27.3(27.2)	23.3
		Vertebrae	3960(3970)	3930(3880)	3860(3810)	3780
Second experiment	Cd	Viscera	17.8(35.1)	57.2(59.9)	63.6	0.227
		Gills	11.2(21.1)	12.4(56.8)	16.5	0.311
		Vertebrae	5.76(12.8)	6.39(8.33)	6.05	0.14
	Ca	Viscera	73.0(64.7)	60.6(71.8)	66.1	83.3
		Gills	566(439)	449(432)	507	681
		Vertebrae	188000(133000)	179000(144000)	181000	223000
	P	Viscera	47.4(43.4)	47.5(40.8)	41.7	59.1
		Gills	378(357)	361(338)	348	414
		Vertebrae	93100(74100)	95300(79700)	84600	106000
	Mg	Viscera	10.6(11.0)	9.2(10.5)	12.4	10.2
		Gills	25.1(25.8)	25.6(27.3)	26.9	23.6
		Vertebrae	3980(3880)	3950(4090)	4010	3790

Figures in parentheses : Malformed fish

The levels in the viscera and gills of malformed and normal fish were much the same, but there were significant defects (approximately 59-88%) in the vertebral column of the malformed fish (Fig. 2-b). The levels of magnesium in the gills and viscera of the fish given cadmium showed little difference between the malformed and normal fish, but were slightly different in the gills (approximately 11-18%), in the vertebrae (approximately 1-5%).

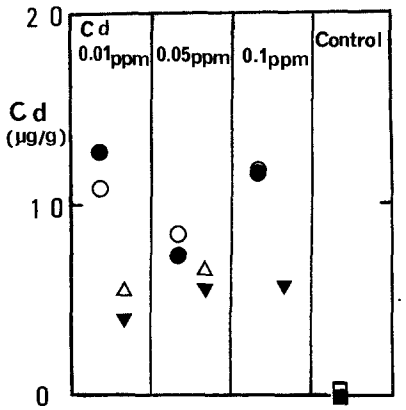


Fig. 2-a

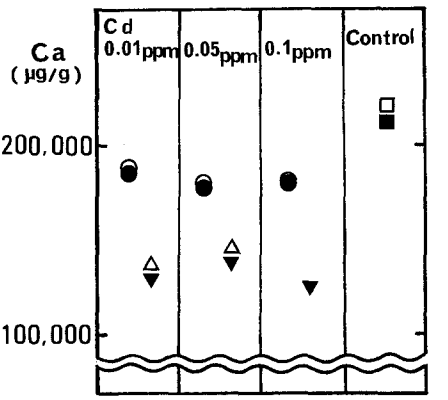


Fig. 2-b

Figs. 2-a, 2-b Metal concentrations in the vertebral column (μg/g in ash)

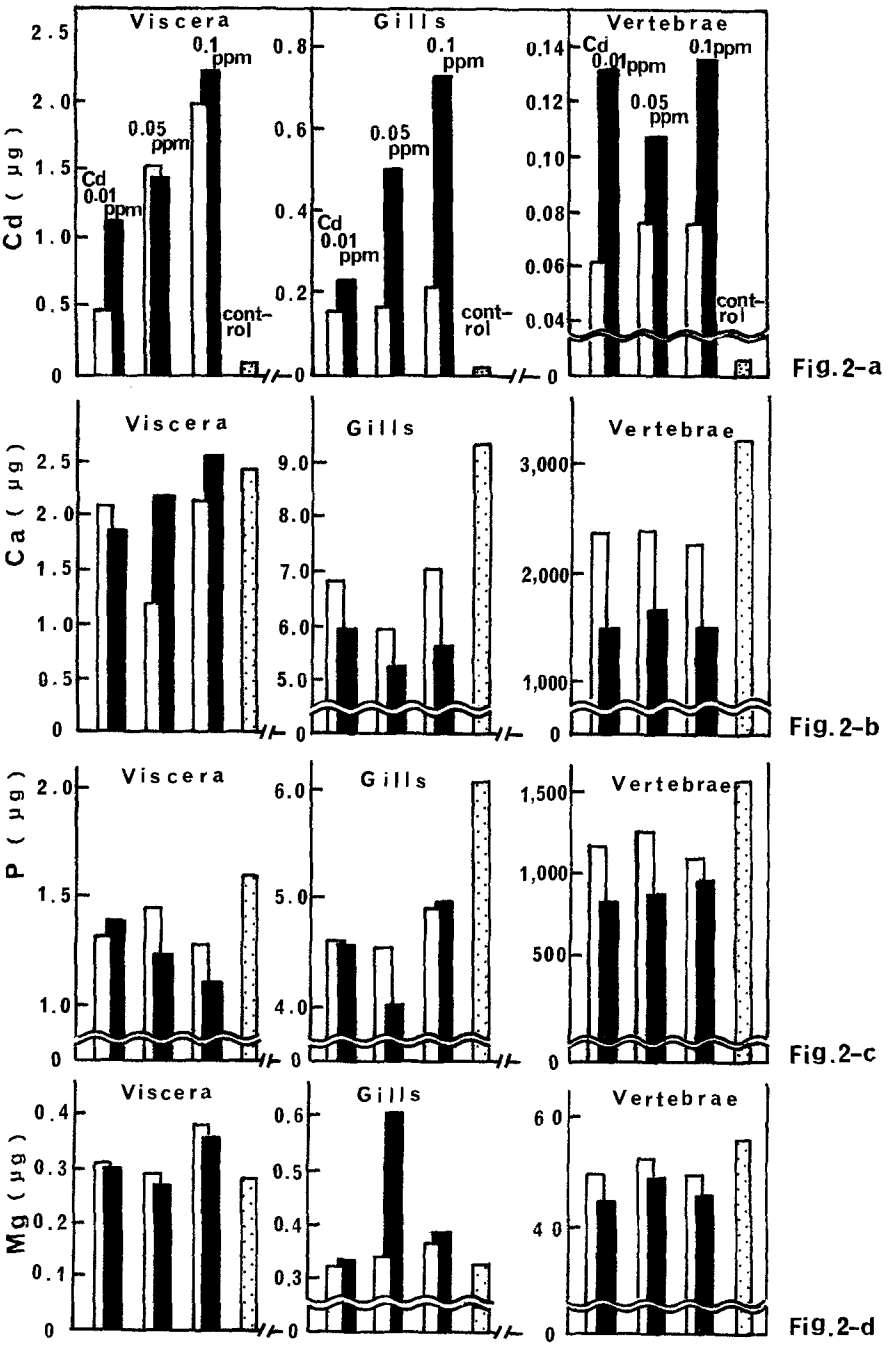
1st experiment
●:normal fish
▼:malformed fish
■:control fish

2nd experiment
○:normal fish
△:malformed fish
□:control fish

The contents(μg) of Cd, Ca, Mg and P in the viscera, gills and vertebrae

The content(μg/g in dry matter) of the elements in viscera, gills and vertebral columns of the fish are shown in Fig. 3. Table 2 indicates the differences between deformed and normal fish. The Cd content of viscera of malformed fish was higher (approximately 1.4 - 2.2 times) than that of normal fish. The Mg and Ca content was nearly the same (approximately 0.93-0.99 times and 0.76-1.1 times). By contrast, the P content was lower (approximately 0.68-0.85 times) in the gills, and the Cd content was higher in the malformed fish than in normal fish; Mg and P were nearly the same (approximaely 0.78-1.04 times, and 0.89-1.0 times) in both, and Ca was lower (approximately 0.81-0.89 times). In no case was any significant difference observed between the deformed and the normal fish.

In the vertebrae, however, the contents of these elements were markedly different: the Cd content in the malformed fish was significantly higher (approximately 1.4 -2.1 times; $P < 0.01$) and those of Ca and P were significantly less (approximately 0.62-0.69 times



Figs. 2-a - 2-d
Average of metal content(μg in dry matter)of vertebral column in malformed and normal fish exposed to Cd, and in control fish
□ :normal fish; ■ :malformed fish; ⬚ :control fish

and 0.70-0.89 times; $P < 0.01$ and $P < 0.05$) than in the control fish. When the content of each of these metals was compared with the content in the control fish, Cd was higher in every part of the fish body (approximately 57-67 times) whilst Ca, P and Mg showed a tendency to decrease (approximately 0.46-0.51 times, 0.53-0.60 times and 0.89-0.92 times) in the malformed fish.

The Ca/P ratio contained in malformed and normal fish exposed to cadmium and in control fish

Figure 3 shows that the Ca/P ratios of the vertebrae, viscera and gills of malformed and normal cadmium-exposed fish. This ratio was higher in the viscera (approximately 110%) and lower in the gills (approximately 84%) in control fish, and in the vertebrae(approximately 85%) than in control fish. The normal fish showed a significantly lower ratio in the gills and viscera (approximately 7.8%, 29%) and vertebrae (approximately 14%): 1.8 as compared with 2.1 in the control.

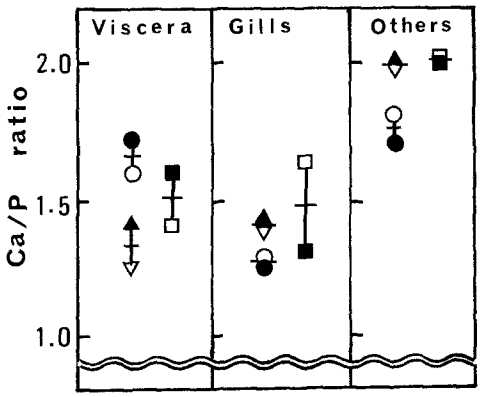


Fig. 3 The Ca/P ratio in malformed and normal fish exposed to Cd, and in control fish
●:normal fish; ▲ :malformed fish; ■ :control fish (1st experiment)
○:normal fish; △ :malformed fish; □ :control fish (2nd experiment)

The ratio of ash weight/dry weight in malformed, normal fish and in control fish

The ratio of ash weight/dry weight(%) of the areas analysed was significantly($P < 0.01$) decreased in the vertebrae of the malformed fish, but was not significantly decreased in the viscera and gills (Fig. 4). It is supposed that the decrease of the ratio of ash weight/dry weight in the vertebrae was due to a slight reduction of the content of some minerals, such as Ca, P, in the bone tissues.

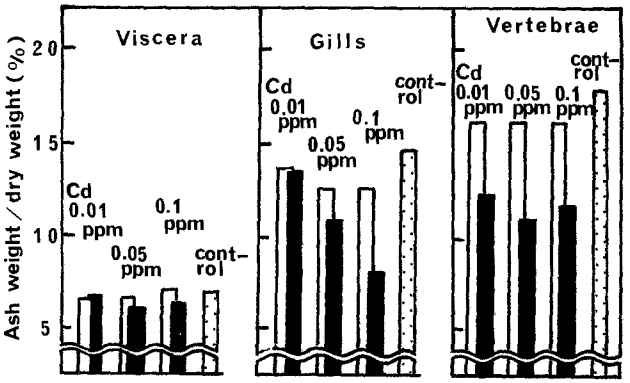


Fig. 4 Average ratio of ash weight/dry weight(%) in malformed and normal fish exposed to Cd, and control fish
□:normal fish; ■ : malformed fish; ⊡:control fish

Development of vertebral malformation due to decalcification of bones of fish

The vertebral abnormalities of fish described in this study suggest that the most marked variations in these metals occur in the spinal column. The cadmium content in the malformed fish was significantly less than in the normal fish. The ratio of ash weight/dry weight in bone decreased the vertebral curvature was concentrated between the 7th and 19th joint, and compared with the control fish, cadmium was increased in all areas of the fish body. The results were confirmed in a repeat experiment. This leads to cavitation, shortening and assimilation of cartilage. Moreover, the muscular action of swimming may lead to vertebral curvature, since the spinal column is used as a fulcrum. The replacement of calcium, phosphorus and zinc by cadmium has been postulated in rats (ABE et al. 1972). KOBAYASHI(1969) bred rats using food containing one part of Cd per 10,000, and observed a decrease of bone ash as a result of Ca deficiency. In this study, a similar phenomenon was observed. The marked escape of calcium and phosphorus from bone might have caused symptoms reminiscent of Osteomalacia similar to the results for Cd-administered rats (ISHIZAKI & FUKUSHIMA 1968).

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