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BIOLOGICAL EFFECTS OF CADMIUM DETOXIFICATION

The biochemical separation techniques used at SCCWRP enable us to make precise measurements of the distibution of metals between sites of detoxification (metallothionein) and toxic action (enzymes) in the liquid fraction (cytosol) of cells. Although the effects of acute cadmium exposure appear to occur when the binding capacity of metallothionein is exceeded and excess metals "spill over" into an enzyme-containing fraction (Brown et al. 1984; Pruell and Engelhardt 1980), it is not known if a similar relationship exists between metallothionein binding and the sublethal effects resulting from chronic exposure. This paper presents the results of biochemical measurements on scorpionfish that were chronically exposed to cadmium for 16 weeks. The objectives of these measurements were to determine the enzymatic effects, physiological consequences, and energetic cost of chronic cadmium exposure and to relate these changes to detoxification processes (i.e., the binding of cadmium by metallothionein).

The activities of three enzymes were measured in order to examine effects at the molecular level. Two of these enzymes, catalase and selenium-dependent glutathione peroxidase (Se-GSH-Px), are indicators of the cell's ability to protect itself against endogeneous oxidant stress (Fridovich 1976). Both of these enzymes catalyze the decomposition of hydrogen peroxide generated by normal metabolism, thereby reducing the production of highly reactive hydroxyl radicals which can cause toxicity by initiating destructive peroxidation reactions in the cell (McCord 1979). In addition, catalase has been reported to be a site sensitive to cadmium toxicity (Jackim et al. 1970). The third enzyme measured, alkaline phosphatase (ALP), served to indicate other types of molecular effects. This enzyme contains zinc which is essential for its proper function of removing phosphate from various compounds. Antagonistic effects on zinc metabolism by cadmium which reduce the amount of zinc available to enzymes may therefore cause a reduction in enzyme activity. ALP is associated with the cell membrane, where it is

thought to play a role in phosphorous transport processes. An alteration in ALP function resulting from damage to this membrane may be expressed as a change in enzyme activity.

Measurements of changes in blood plasma composition and energy storage compounds were also made to examine potential effects on physiology and energy reserves, respectively.

Two types of biological responses by the scorpionfish to cadmium were observed. At the low exposure level (0.1 mg Cd/L), a compensatory physiological stimulation was evidenced by increased levels of calcium in the blood. The high exposure level produced significant increases in the amount of cadmium bound to the enzyme-containing (ENZ) pool of all tissues examined and caused changes in enzyme activity in the kidney and intestine.

MATERIALS AND METHODS

All measurements of biological effects were carried out on the same fish used for the metals analyses described in the preceding paper (Bay et al., this volume). Samples of blood, whole tissue, or tissue extracts were analysed in seven preexposure fish and in either six or seven fish exposed to 0 (control), 0.1, or 1.0 mg Cd/L for 4, 8, or 16 weeks. Details of the experimental methods and tissue preparation are described in the preceding paper.

Enzyme Analyses

The activities of three enzymes were determined in liver, kidney, intestine, and gill tissue from each of the cadmium exposure groups for 4 and 8 weeks. Se-GSH-Px activity was measured in samples of the ENZ pool obtained from Sephadex G-75 chromatography of tissue cytosols. A coupled assay procedure was used in which the enzyme-catalyzed oxidation of glutathione to glutathione disulfide by hydrogen peroxide was linked to the more easily measured oxidation of NADPH to NADP (Paglia and Valentine 1967; Flohe and Brand 1970; Gunzler et al. 1974). The rate of NADPH oxidation was measured in a spectrophotometer at 340 nm and was equal to the GSH oxidation rate (SE-GSH-Px activity). Measurements were made at 37°C using reagent concentrations which yielded the most accurate data. One unit of Se-GSH-Px activity represented the quantity of enzyme catalyzing the oxidation of 1.0 µmole of GSH/min.

Catalase activity was measured in tissue cytosol samples at 25°C using hydrogen peroxide as a substrate (Aebi 1974; Beers and Sizer 1952). Because the initial activity of this enzyme follows first-order reaction

kinetics, catalase activity was expressed in terms of the first-order rate constant, k . The specific assay conditions used minimized secondary interfering reactions (Maehly and Chance 1954).

ALP activity measurements were made on homogenate samples which were centrifuged at either 1000 x g (4 weeks) or 2500 x g (8 weeks) to remove tissue debris. Enzyme activity was determined by measuring the rate of conversion of p-nitrophenyl phosphate to p-nitrophenol (Walter and Schutt 1974). Measurements were made at a wavelength of 405 nm. One unit of ALP activity was defined as the amount of enzyme needed to hydrolyze 1.0 µmole of substrate/min at 25°C.

All enzyme measurements were expressed as specific activities by dividing the activity by the cytosol or homogenate protein content. Protein concentrations were measured using Coomassie Blue (Bradford 1976).

Blood Chemistry

Approximately 2-4 ml of blood was taken from each fish and centrifuged at 500 x g to sediment the red blood cells. The supernatant (plasma) was removed and diluted 2-5 times to obtain the minimum volume of 3 ml required by the automated analyzer. All analyses were performed by Belvue Medical Laboratories, Long Beach, using standard clinical chemistry methods for human plasma. The concentrations of ionic constituents (sodium, potassium, chloride, total and ionic calcium, phosphorus), osmolality, nitrogeneous waste (blood urea nitrogen, creatinine, uric acid), protein content (albumin, globulin), and other parameters (glucose, cholesterol, bilirubin) in each plasma sample were determined.

Energy Reserves

The energy reserves of scorpionfish were estimated by measuring the concentrations of two major tissue energy sources (glycogen and total lipid) in liver and muscle. Glycogen was analyzed colorimetrically in samples which had been digested in potassium hydroxide and reacted with phenol and sulfuric acid (Brown and McLeay 1975). Total tissue lipids were determined gravimetrically following chloroform extraction (Bligh and Dyer 1959).

Data Analysis

The significance of effects due to cadmium exposure, exposure time, and fish sex on the blood chemistry and energy reserve data were tested using a three-way analysis of variance (ANOVA). Analyses were performed using the SAS package of computer programs (SAS Institute, Inc., 1982). Some of these parameters had unequal variances between

groups; these inequalities were reduced prior to analysis by logarithmic transformation. Two-way ANOVAs were used to test the significance of changes in enzyme activity that were related to exposure concentration or time.

ANOVAs which had significant effects for exposure level or time were further examined using the Ryan-Einot-Gabriel-Welsch multiple F test to determine which treatment groups were significantly different (SAS Institute, Inc., 1982). The relationship of changes in enzyme activity with variations in ENZ pool metals was examined by calculating correlation coefficients between the enzyme and metals data.

RESULTS

Enzyme Activity

The results of the catalase, Se-GSH-Px, and ALP measurements on the cadmium-exposed scorpionfish are shown in Figure 1. Analyses of liver, kidney, intestine, and gill tissue are shown for fish from the 4- and 8-week exposures only.

Statistical evaluation of the enzyme data using two-way ANOVAs indicated that cadmium exposure resulted in a significant decrease in kidney ALP (Figure 1). A significant interaction with time was also found for this effect, indicating that the effect of cadmium exposure on ALP activity was different at each sampling time. ALP activity was reduced by exposure to 1.0 mg Cd/L after 4 weeks, but not after 8 weeks. ALP activity was also significantly affected by cadmium exposure in the intestine. In this tissue, ALP activity was increased by exposure to 1.0 mg Cd/L. This increase was observed at both sampling times, but was much greater at 4 weeks (Figure 1).

Changes in Se-GSH-Px activity of gill tissue due to cadmium exposure were nearly significant ($p = 0.065$), with the 0.1-mg Cd/L group having the greatest activity. No cadmium-related effects on catalase activity were observed.

Differences in enzyme activity between the two exposure times were frequently found. Significant effects due to exposure time were always found for Se-GSH-Px and were occasionally observed for ALP and catalase. Both increases and decreases in enzyme activity were found with increasing exposure time.

The correlation of enzyme activity with ENZ pool metal levels was calculated in order to determine if enzyme activities in individual fish were related to changes in ENZ-Cd, ENZ-Cu, or ENZ-Zn concentration.

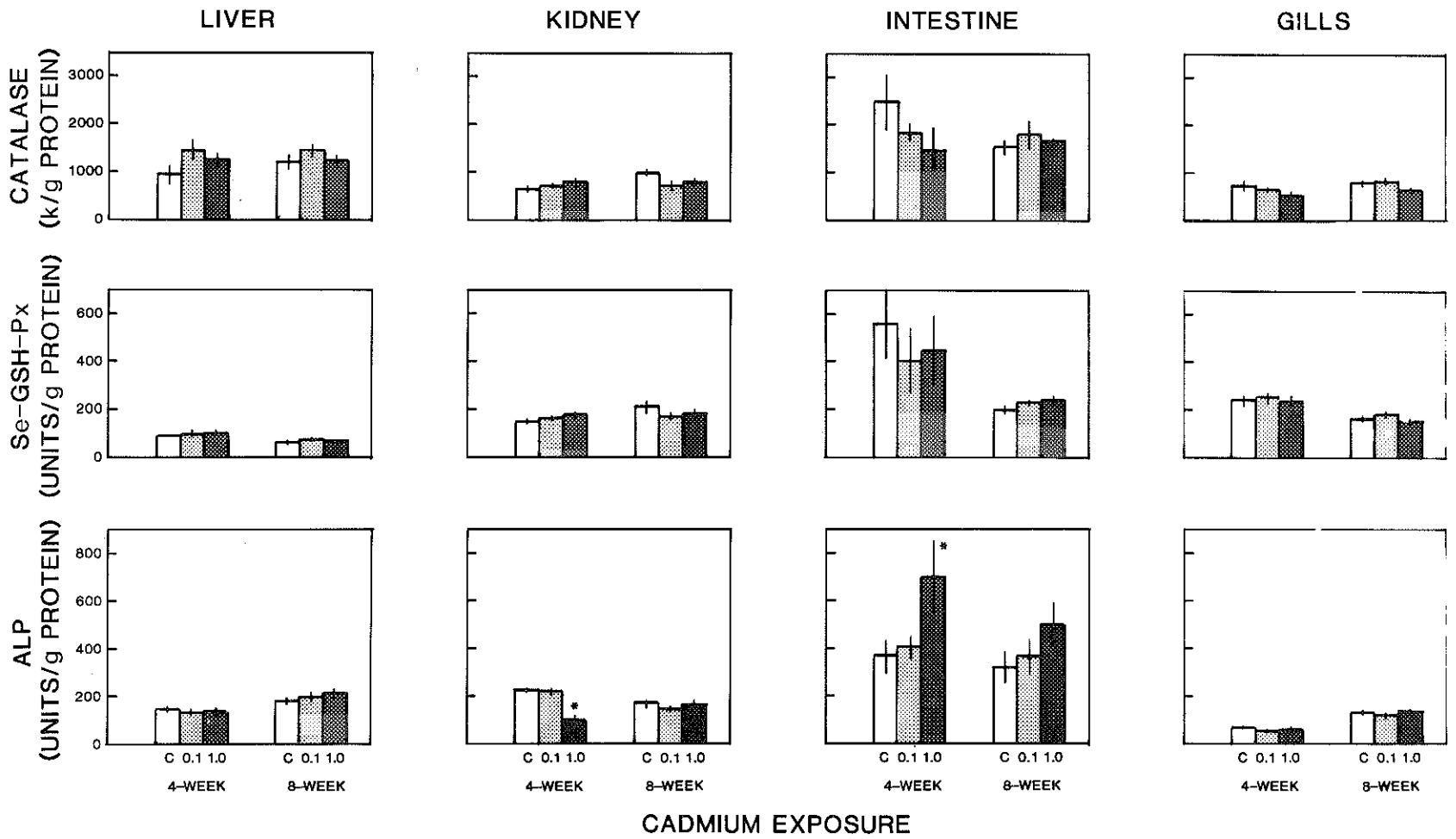

Figure 1. Changes in the activities of the enzymes catalase, selenium-dependent glutathione peroxidase (Se-GSH-Px), and alkaline phosphatase (ALP) in scorpionfish tissues following exposure to 0 (control), 0.1, or 1.0 mg Cd/L. Values are mean ± standard error (n = 7). Cadmium exposure resulted in a change in the activity of ALP only (indicated by asterisks).

The correlation analyses indicated that only changes in ALP activity were related to changes in ENZ-Cd concentration. A negative correlation was present for the 4-week kidney data, indicating that ALP activity was lowest in fish with elevated ENZ-Cd concentrations. Positive correlations were found for liver ALP at both times. No correlations were evident between ALP and ENZ-Cd for the intestine or gills. Though correlations with ENZ-Cd were absent, the activities of SE-GSH-Px and catalase were often positively correlated with copper and zinc concentrations in the ENZ pool. Increased Se-GSH-Px activity in scorpionfish was always related to increases in ENZ-Cu and/or ENZ-Zn concentration.

The presence of blood in the tissue cytosols was noticeable for the liver, kidney, and gills. The relative influence of blood enzymes on the enzyme measurements was estimated by measuring enzyme activity in three samples of whole blood from the 16-week exposure. The catalase and Se-GSH-Px activities present in these samples (Table 1) were similar to or greater than those measured in the other tissues (Figure 1). The relative activity of ALP in blood has not yet been determined.

Blood Chemistry

Fish plasma samples from each of the exposure groups at 0, 4, 8, and 16 weeks were analysed for chemical composition (Table 2). Some of the plasma constituents, such as sodium and potassium, had a narrow range of variation, while others (glucose, cholesterol) were much less consistent. Three-way ANOVAs on each of the plasma constituent concentrations showed that some components were significantly affected by cadmium exposure, exposure time, or fish sex (Table 3). Cadmium concentration was found to affect the blood urea nitrogen (BUN), creatinine, and calcium (total and ionic) content of plasma. Examination of the overall trend of these effects with multiple F tests indicated that BUN in fish exposed to 1.0 mg Cd/L was significantly lower than the control or 0.1-mg Cd/L groups (Table 3). For creatinine, total calcium, and ionic calcium, concentrations in the 0.1-mg Cd/L fish were greater than those for the 1.0-mg Cd/L fish. Significant differences between the 0.1-mg Cd/L and control groups were also found for the calcium concentrations. One-way ANOVAs on these four parameters at each sampling time showed that none of the cadmium effects were significant at 4 weeks. At 8 weeks, changes in BUN and ionic calcium were significant, while BUN, ionic calcium, and total calcium were all significantly affected at 16 weeks. The overall change in creatinine with cadmium exposure was not strong enough to be significant at an isolated sampling time.

Independent of the cadmium effects, the concentrations of nine plasma constituents (glucose, creatinine, sodium, chloride, cholesterol, albumin, globulin, osmolality, ionic calcium) varied significantly between

Table 1. Selenium-dependent glutathione peroxidase (Se-GSH-Px) and catalase activities in scorpionfish blood after 16 weeks' exposure to cadmium (n = 1 for each exposure level). These enzyme activities were greater than those for other scorpionfish tissues in most cases.

Exposure	Se-GSH-Px (u/g protein)	Catalase (k/g protein)
Control	584	2096
0.1 mg Cd/L	504	1121
1.0 mg Cd/L	490	1700

exposure times (Table 3). Variable patterns of change were observed for each parameter. With regard to sex, the female fish had significantly higher values for total calcium, total protein, albumin, globulin, and ionic calcium than the males.

Energy Reserves

Analyses of the lipid and glycogen concentrations of liver and muscle have been completed for the 0-, 4-, and 8-week samples. Considerable variability in the glycogen concentrations of both muscle and liver was observed (Figure 2). A significant increase in glycogen was found for fish exposed to 0.1 mg Cd/L for 4 weeks. Liver lipid concentrations were relatively high (30%) and were unaffected by cadmium (Figure 2). Male fish, however, had significantly higher liver lipid concentrations (34%) than females (24%). A trend (not statistically significant) towards increased muscle lipid concentration with cadmium exposure was also apparent (Figure 2).

DISCUSSION

Enzyme Activity

Of the three enzymes examined in this study, only alkaline phosphatase showed changes which were related to cadmium exposure. Reductions in kidney ALP activity at 4 weeks were correlated with increases in ENZ-Cd, suggesting a temporary effect at the molecular level due to cadmium accumulation. These data are consistent with evidence that the vertebrate kidney is very sensitive to chronic cadmium exposure (Singhal and Merali 1979) and that cadmium-induced kidney damage is accompanied by reduced ALP activity (Piscator and Axelsson 1970). It is not clear, however, if this ALP reduction represented damage to

Table 2. Constituent concentrations in plasma of cadmium-exposed scorpionfish.

Parameter		Preexposure	4 Weeks			8 Weeks			16 Weeks		
			Control	0.1 mg/L	1.0 mg/L	Control	0.1 mg/L	1.0 mg/L	Control	0.1 mg/L	1.0 mg/L
Sodium	(meq/L)	182 ± 4	174 ± 5	177 ± 6	176 ± 3	176 ± 4	176 ± 7	177 ± 4	182 ± 4	183 ± 7	181 ± 8
Postassium	(meq/L)	3.8 ± 0.5	3.2 ± 0.9	3.9 ± 0.6	4.2 ± 0.6	4.2 ± 0.6	4.1 ± 0.5	4.0 ± 0.5	4.2 ± 0.5	3.5 ± 0.9	3.4 ± 1.2
Chloride	(meq/L)	182 ± 18	146 ± 6	146 ± 6	145 ± 4	145 ± 4	141 ± 8	144 ± 7	151 ± 4	151 ± 7	147 ± 9
Phosphorus	(mg/dl)	9.6 ± 1.3	9.0 ± 1.2	10.1 ± 2.0	9.2 ± 2.1	7.9 ± 1.0	8.8 ± 1.3	8.8 ± 0.9	8.7 ± 1.5	9.1 ± 1.0	10.4 ± 2.0
Calcium	(mg/dl)	11.8 ± 3.4	10.4 ± 2.2	11.2 ± 2.3	11.3 ± 3.7	9.8 ± 1.4	13.3 ± 4.1	10.4 ± 2.1	10.4 ± 0.9	13.1 ± 4.1	9.4 ± 0.9
Ionic calcium	(mg/dl)	6.5 ± 1.5	6.2 ± 0.7	6.6 ± 1.3	6.0 ± 1.7	7.1 ± 0.8	9.1 ± 2.4	7.2 ± 1.3	7.4 ± 0.7	8.8 ± 2.1	6.4 ± 0.5
Osmolality	(mos/L)	343 ± 9	328 ± 14	331 ± 10	330 ± 4	329 ± 8	329 ± 14	331 ± 7	341 ± 9	343 ± 12	339 ± 17
BUN	(mg/dl)	0.6 ± 1.0	7.7 ± 17.8	0.3 ± 0.8	2.0 ± 2.4	1.4 ± 1.0	2.0 ± 1.2	0.3 ± 0.8	2.3 ± 1.3	2.2 ± 0.4	0.7 ± 1.0
Creatinine	(mg/dl)	0.3 ± 0.1	0.4 ± 0.2	0.6 ± 0.3	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.4 ± 0.3
Uric acid	(mg/dl)	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.4	1.0 ± 0.4	1.0 ± 0.4	0.6 ± 0.4	0.6 ± 0.1	0.8 ± 0.2	0.8 ± 0.3	1.3 ± 0.2
Glucose	(mg/dl)	70 ± 55	14 ± 16	26 ± 14	24 ± 25	16 ± 7	29 ± 29	21 ± 7	38 ± 27	24 ± 12	43 ± 26
Cholesterol	(mg/dl)	160 ± 28	166 ± 67	209 ± 27	185 ± 24	197 ± 94	221 ± 54	172 ± 44	230 ± 65	249 ± 92	228 ± 92
Albumin	(g/dl)	1.4 ± 0.4	1.8 ± 0.5	2.0 ± 0.4	1.8 ± 0.4	1.5 ± 0.5	1.8 ± 0.5	1.7 ± 0.3	1.7 ± 0.2	1.9 ± 0.4	1.8 ± 0.2
Globulin	(g/dl)	3.2 ± 0.6	2.8 ± 1.0	3.1 ± 0.5	3.2 ± 0.3	2.9 ± 1.1	3.1 ± 0.6	3.1 ± 0.6	3.5 ± 1.3	3.8 ± 0.5	3.6 ± 0.7

Table 3. Results of multiple comparison tests on scorpionfish blood plasma constituents. Test results are only given for those constituents that were shown by three-way ANOVA to be significantly affected by cadmium exposure, time, or sex. Mean values are listed below the exposure level, sex, or time group designations. Groups which are underscored by the same line are not significantly different from each other ($p<0.05$).

	Cd Exposure Level (mg/L)			Sex		Time (Weeks)			
Parameter	0.1	0	1.0	F	M				
Glucose[a]						0	16	8	4
						47.3	29.9	18.3	14.7
BUN[a]	1.96[b]	1.56	0.30						
Creatinine[a]	0.41[c]	0.35	0.27			16	4	0	8
						0.47	0.44	0.32	0.18
Sodium[a]						0	16	8	4
						183.1	181.8	176.4	175.7
Chloride[a]						0	16	4	8
						181.3	149.6	145.6	143.1
Total calcium[a]	12.2[d]	10.4	10.2	13.3	9.68				
Ionic calcium[a]	7.96[b]	6.73	6.51	8.18	6.44	8	16	0	4
						7.68	7.35	6.40	6.17
Cholesterol						16	8	4	0
						234.8	196.6	185.9	160.0
Total protein				5.59	4.56				
Albumin				2.04	1.55	4	16	8	0
						1.87	1.77	1.64	1.41
Globulin				3.55	3.02	16	0	8	4
						3.61	3.21	3.04	3.00
Osmolal calcium[a]						0	16	8	4
						342.8	340.4	329.7	329.4

[a] Approximate means calculated from the antilog of the logarithmically transformed data.
[b] Significant difference at 8 and 16 weeks only.
[c] Significant difference overall, but not at individual exposure times.
[d] Significant difference only at 16 weeks.

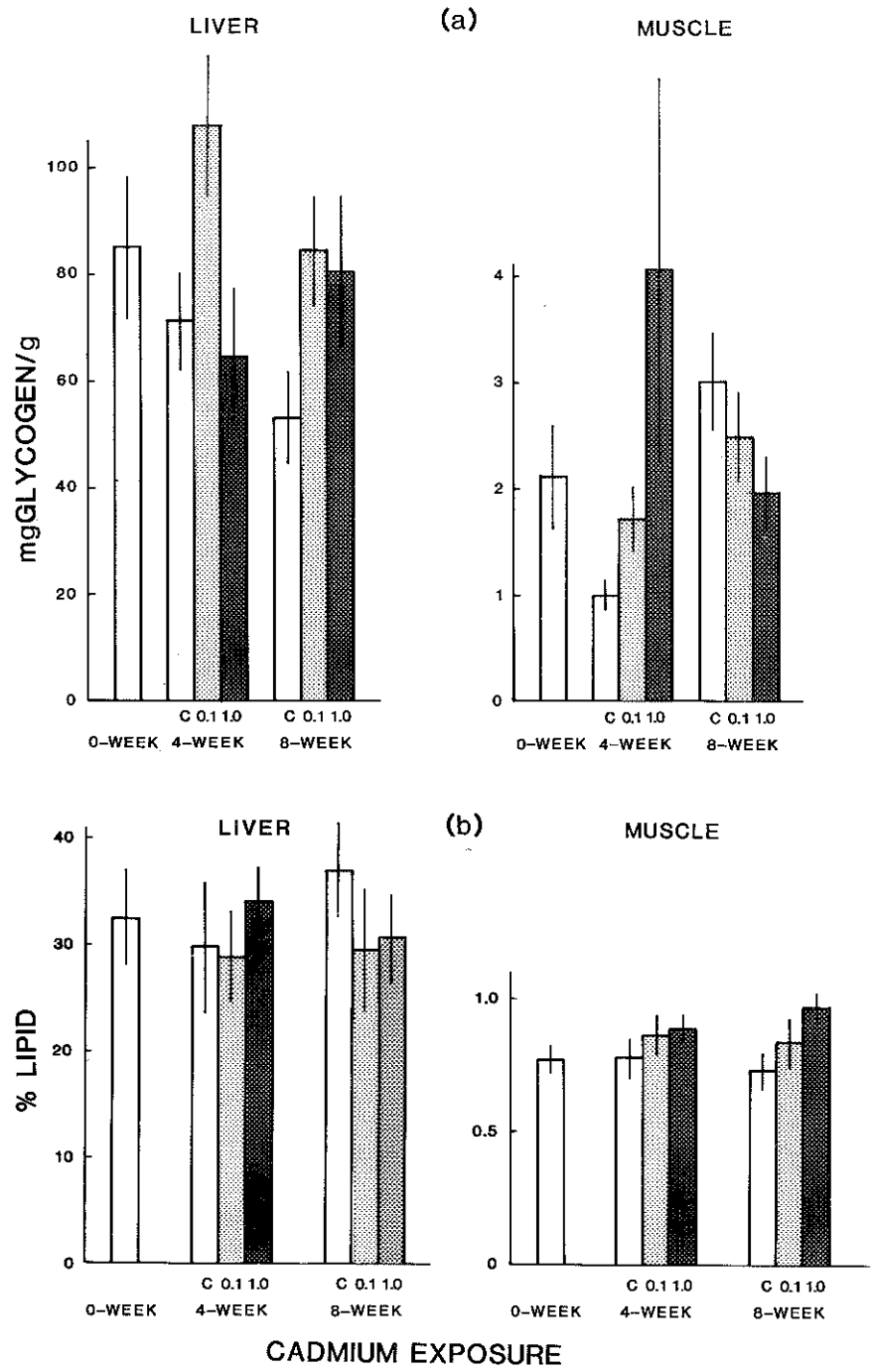

Figure 2. Changes in scorpionfish energy reserve data, during exposure to cadmium (mean ± standard error; n = 7). a) Of liver and muscle glycogen, only 4-week liver glycogen was significantly affected by cadmium exposure. b) Neither liver nor muscle lipid was significantly affected by cadmium exposure.

scorpionfish kidneys, since histological analysis of these samples did not indicate cadmium-related pathology (Perkins and Rosenthal, this volume) and the blood chemistry results do not suggest impaired kidney function at the 1.0-mg Cd/L exposure (discussed subsequently). The exact mechanism of the reduction in kidney ALP activity is not known, but may involve an antagonistic effect of Cd on the normal cellular functions of zinc. ALP is a zinc metalloenzyme, and metal substitution studies have shown that replacement of ALP zinc with cadmium abolishes enzyme activity (Chlebowski and Coleman 1976).

Molecular effects resulting from the 1.0-mg Cd/L exposure were also observed in the intestine, but as elevated ALP activities. Although these changes were observed in the tissue with the highest ENZ-Cd concentrations, ENZ-Cd and ALP activities were not correlated; this finding suggests that the effects were not caused by a direct interaction of ENZ-Cd with this enzyme, but may instead have reflected toxic effects at other cellular sites in the intestine. This increase in ALP activity may have been the result of a greater rate of enzyme synthesis in response to an increased need for phosphorous uptake by intestinal cells (Rueter 1983).

The lack of significant changes in the catalase activity of scorpionfish tissues was unexpected, since other studies have shown this enzyme to be inhibited in cadmium-exposed fish (Pruell and Englehardt 1980; Jackim et al. 1970). Pruell and Englehardt measured liver catalase and the concentration of cadmium in the ENZ pool. These authors found a 30% catalase inhibition in fish with a liver ENZ-Cd concentration of about 8 mg/kg wet weight. This value is much higher than the highest ENZ-Cd levels (0.5 mg/kg) found in scorpionfish exposed to cadmium in the present study. The lack of catalase inhibition in scorpionfish suggests that the increases in ENZ-Cd were not high enough to inhibit the enzyme.

Measurements of catalase and Se-GSH-Px activity in gill and kidney cytosol may not accurately reflect the status of these enzymes in the tissues. Samples of blood cytosol showed relatively high catalase and Se-GSH-Px activities. If gill and kidney cytosols are assumed to be about 50% contaminated by blood, then the enzyme activities contributed by the blood would account for most of the activity found in these tissues.

Blood Chemistry

The clinical chemistry measurements made on fish plasma provided valuable information about the effects of cadmium exposure and the general status of the fish. While all of the 16 parameters for which we obtained useful data are important measures of human health, some of these plasma components may not be relevant for fish or may respond in

an unexplainable fashion. A general interpretation of the significance of changes in these parameters to fish health is presented in Table 4.

Four blood plasma constituents were found to be affected by cadmium exposure, especially after 8 and 16 weeks. BUN, a byproduct of protein metabolism, was the only plasma component significantly affected in fish at 1.0 mg Cd/L, where its concentration was reduced. BUN is formed primarily in the liver and is excreted mainly through the gills of fish (Wood 1958; Forster and Goldstein 1969). An alteration in either of these processes or a reduced protein absorption rate may have been responsible for the reduction in BUN. The other blood chemistry, enzyme, and histological results do not provide any further explanation for the change in BUN.

The other three plasma constituents affected by cadmium exposure (creatinine, total calcium, ionic calcium) all showed a similar pattern of higher concentrations in the 0.1-mg Cd/L group. Fish exposed to 1.0 mg Cd/L were not significantly different from the controls. Creatinine, a metabolic byproduct released from skeletal muscle, is probably excreted solely by the kidney (Hickman and Trump 1969). Increased plasma creatinine is generally interpreted in humans as indicative of reduced kidney filtration rate. However, impairment of scorpionfish kidney function due to cadmium accumulation was probably not the cause of the elevated creatinine levels for the following reasons: the lowest creatinine levels were found at the highest cadmium exposure, other blood chemistry changes indicating kidney dysfunction were not found, and histological examination of the kidneys of these fish did not indicate effects related to cadmium exposure (Perkins and Rosenthal, this volume).

Increased plasma calcium concentrations were consistently observed in fish exposed to 0.1 mg Cd/L. The calcium status of fish can be affected by changes in intestinal absorption, altered excretion (fecal and urinary), and bone reabsorption (Holmes and Donaldson 1969). Calcium content of the blood is a reflection of these processes and is under precise hormonal control in humans and probably also in fish. Hormonal control of scorpionfish plasma calcium is suggested by the elevated concentrations observed in female fish. The changes in plasma calcium and creatinine showed the same pattern (increase at low toxicant exposure levels) as has been reported for other physiological parameters, such as growth (Stebbing 1981). This phenomenon, known as hormesis, is attributed to an overcompensation by physiological control mechanisms to inhibition. Overcompensation may be brief, with physiological rates rapidly returning to normal, or it may persist for many days. This latter situation seems to have occurred in the present experiment.

A majority of the blood plasma constituents varied significantly between

Table 4. Possible significance of changes in blood chemistry to organ function or fish health. Most of these parameters can also be affected by seasonal changes. Changes in blood urea nitrogen (BUN), creatinine, and calcium were found following cadmium exposure.

Parameter	Significance of Decreased Value	Significance of Increased Value
Glucose	Nutritional deficiency; liver disease	Chronic or acute stress
BUN	Liver damage; change in protein metabolism	Decreased excretion from gill or kidney
Creatinine	Unclear	Decreased kidney glomerular filtration
Sodium	Excessive loss through kidney	Decreased gill excretion
Chloride	Excessive loss through kidney	Decreased gill excretion; stress
Potassium	Stress; decreased gill intake	Leakage from damaged cells
Calcium	Decreased intestinal absorption; hormone change	Decreased kidney loss; hormone change
Cholesterol	Dietary change; altered lipid metabolism	High fat diet; stress
Protein	Kidney damage; nutritional imbalance	Dietary change
Bilirubin	Normally low	Liver damage
Phosphorus	Unclear	Kidney damage
Uric acid	Liver damage; change in protein metabolism	Decreased kidney glomerular filtration
Osmolality	Excessive loss of electrolytes	Decreased kidney or gill electrolyte excretion

the 0-, 4-, 8-, and 16-week exposure times. Many different patterns of change were present, suggesting that there was not a single or simple explanation for these variations. Alterations in blood plasma

due to the experimental system were indicated by two parameters, glucose and cholesterol. Plasma glucose values were significantly increased in the preexposure (0-week) fish. Increased blood glucose levels often result from hormonal changes in response to stress (Chavin and Young 1970). These data indicate that the 4-week acclimation period allotted before the experiment was begun was not sufficient to allow the scorpionfish to adapt to the laboratory environment.

Plasma cholesterol concentration increased during the experiment in both control and cadmium-exposed fish. This change may represent either a normal seasonal change in scorpionfish metabolism during the 4-month experiment or an artifact caused by stress or dietary imbalances. Increased blood cholesterol in fish has been reported to result from both chronic stress (Larsson and Fange 1977) and high-fat diets (Farrel and Munt 1983).

Energy Reserves

The chronic exposure of scorpionfish to cadmium did not result in reductions in the lipid or glycogen content of liver or muscle tissue. An increase in liver glycogen in fish exposed to 0.1 mg Cd/L for 4 weeks was found and may be related to the increased plasma calcium and creatinine levels also observed at this concentration. These data indicate that the metabolic cost of cadmium detoxification by scorpionfish at these exposure levels had a very small influence on the scorpionfish's overall metabolism.

CONCLUSIONS

The responses to cadmium shown by the scorpionfish in this experiment, together with measurements of the accompanying changes in the cytosolic distribution of cadmium (Bay et al., this volume), have increased our understanding of the relationship between cadmium detoxification and biological effects. Physiological changes (hormesis) occurred at 0.1-mg Cd/L exposure, a level that did not increase cadmium concentrations in the ENZ pools of scorpionfish tissue, except for gills. As these effects were observed in blood plasma, the specific tissues responsible for the changes cannot be determined. These physiological responses may have occurred before changes in the cytosolic distribution of cadmium occurred.

Changes in cytosolic cadmium distribution were related to ALP changes in the kidney (decrease) and intestine (increase) of fish exposed to 1.0 mg Cd. Because these changes in enzyme activity were observed at the highest exposure level, a direct response to cadmium accumulation is indicated and may represent a toxic effect. These results support

our hypothesis that the measurement of contaminants in the ENZ pool is a useful indicator of biological effects. Measurement of cytosolic cadmium distribution also appears to be a sensitive tool, as changes in ENZ-Cd occurred before histological changes were observed (Perkins and Rosenthal, this volume).

That cadmium-related changes were found in only one of the three enzymes examined and varied with tissue type demonstrates the complexity of the relationship between ENZ-Cd concentration and biological effects. Additional information is needed before we can accurately evaluate the relationship between changes in cytosolic cadmium distribution and toxic effects.

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