Development of Acclimation-Induced Tolerance and Resistance To Acute Cadmium Toxicity in *Catostomus commersoni*

by

David Alexander Duncan

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements for

the Degree of Master of Science

Department of Zoology

University of Manitoba

Sept., 1982

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ABSTRACT

Tolerance and resistance to acute Cd toxicity in (Catostomus commersoni) increased as a consequence of previous metal exposure. The order of metal toxicity to white suckers was Hg>Cd>Zn>>Se. The 96-h LC50 values for control suckers were 0.69 mg Hg/L, 1.1 mg Cd/L, 2.2 mg Zn/L and 31.4 mg Se/L. Toxicity curves clearly showed that white suckers exposed to sufficiently elevated Cd, Hg, or Zn levels subsequently survived longer than control suckers in Cd toxicity tests. The 96-h Cd LC50 increased by 61% or 127% following 1 wk exposure to 0.40 or 0.72, respectively, of the 96-h Cd LC50 for control suckers. The 96-h Cd LC50 also increased by 260% following a 1 wk exposure to 0.22 mg Hg/L. The 12- and 24-h Cd LC50 values were increased, but the 48-, 72- and 96-h values were not, following a 1 wk exposure to 0.20 or 0.89 mg Zn/L. Exposure to 0.22 mg Cd/L, 0.001 mg Hg/L, 0.10 mg Se/L, or 1.9 mg Se/L prior to toxicity testing had little or no effect on acute Cd toxicity. Possible consequences of increased tolerance to metals were discussed including compensatory adjustments which result in poor recruitment into the population, accumulation of potentially toxic metals, and increased survival in metal-impacted areas. Results were discussed in relation to mechanisms, which might be responsible for the decreased Cd toxicity, including decreased uptake, increased excretion, redistribution of metals to less sensitive target sites, and/or induced synthesis of metallothionein (MTN).

Analyses of white suckers' liver and gill cytosol by a Cd-displacement and a metal-summation technique revealed alteration of *in vitro* Cd-binding capacity and substantial redistribution of metals which resulted from a 1 wk sublethal exposure to Cd (0.66 mg/L), Hg (0.19 mg/L), or Zn (0.89 mg/L). The *in vitro* Cd-binding capacity of the cytosol fraction (Pk II) with molecular weight of 8 750 to 14 500 daltons (which would include MTN) was increased in liver from Cd- or Zn-exposed suckers and also in gill from Hg-exposed suckers. The *in vitro* Cd-binding capacity of the high molecular weight cytosol fraction (Pk I) was decreased in liver from metal-exposed fish and in gill from Cd-exposed fish. The metal-summation technique proved to be more appropriate than the Cd-displacement technique for investigating the role of metal-binding protein, such as MTN, in acclimation to Cd toxicity. Liver and gill cytosol from control suckers contained 31.4 and 3.4 nmol of MTN/g of tissue, respectively; as estimated by the summation of Zn, Cu, Cd, and Hg levels and assuming 8 g-atoms of metals were associated with each mole of MTN. MTN content of liver and gill cytosol was increased in suckers exposed to a sublethal concentration of Zn and Cd, respectively and exposure of suckers to a sublethal Hg concentration increased MTN content in both liver and gill cytosol. The need for specific investigations on MTN polymorphism, metal-MTN binding affinities, and the role of other metal-binding proteins, such as Cu-chelatin, in inhibiting Cd toxicity is discussed.

ACKNOWLEDGMENTS

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CHAPTER I

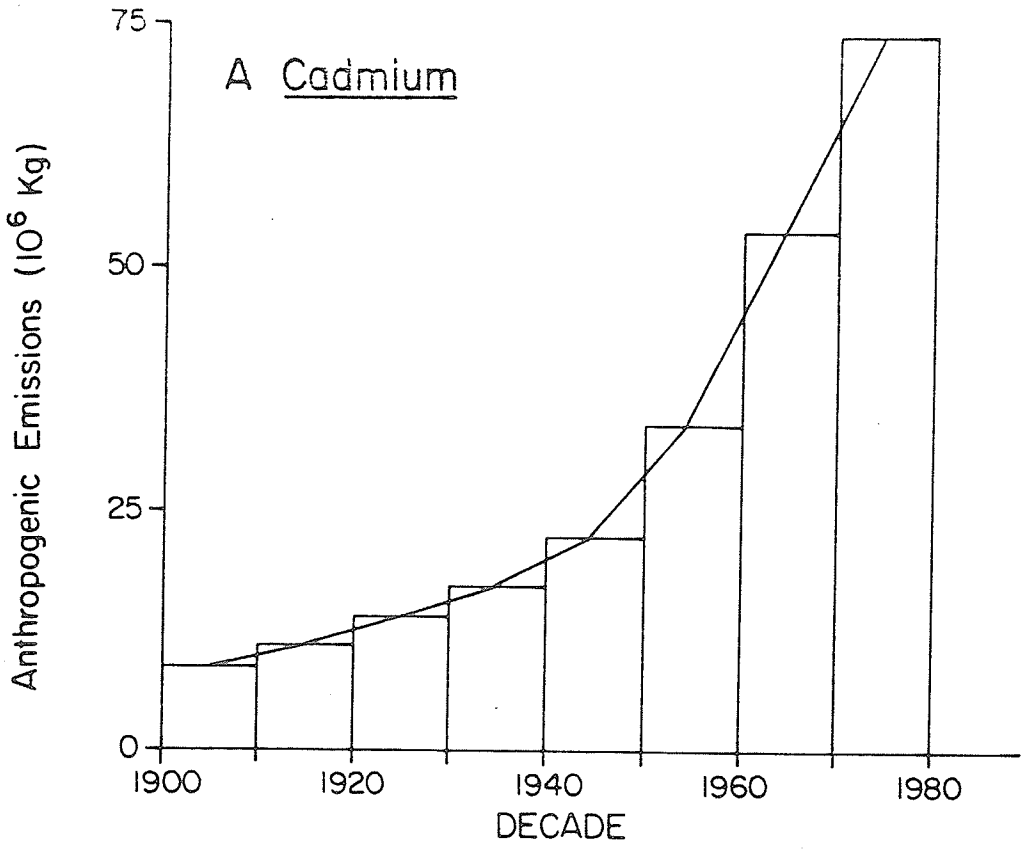
General Introduction

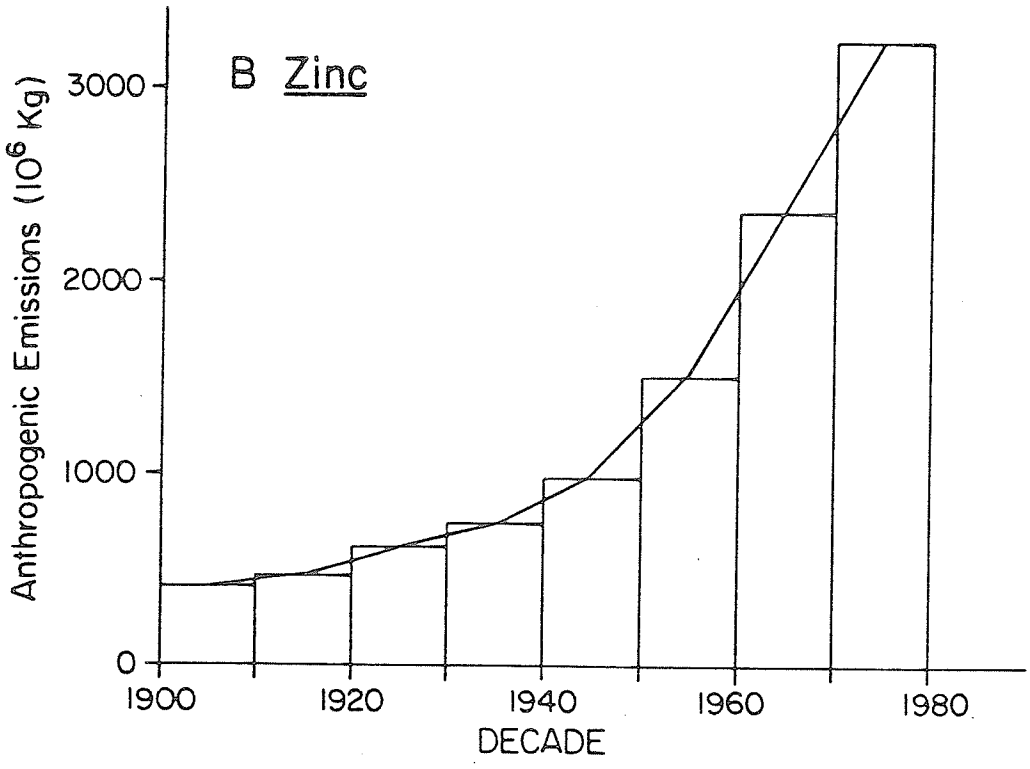
GENERAL INTRODUCTION

Metals enter the environment from both natural and anthropogenic sources. Natural sources include volcanic activity, weathering, forest fires, vegetation, wind-blown dusts and sea-aerosol generation (Friberg et al. 1979; Lantzy and MacKenzie 1979; Nriagu 1979). Anthropogenic sources include fossil fuel combustion, mining and smelting operations, processing and manufacturing operations (e.g. the production of iron, steel or cement), municipal and industrial waste disposal and agricultural practices which increase soil erosion and utilize fertilizers and pesticides (Friberg et al. 1979; Goldberg et al. 1981; Muhlbaier and Tisue 1981; Nriagu 1979; Schroeder 1971).

Human activities clearly effect the release and distribution of heavy metals in the environment (Brown 1976; Friberg et al. 1979; Harris and Hohenemser 1978; Hueck 1975; NAS 1981; NRCC 1981). Cadmium (Cd) and zinc (Zn) global emissions from anthropogenic sources exceed those from natural sources by approximately 900% and 700% respectively (Nriagu 1979; Taylor and DeMayo 1980). Recent rapid increases in anthropogenic emissions of Cd and Zn (Fig. 1A and B) emphasize the potential impact of human activities on the global concentrations of these elements (Nriagu 1979). Recent increases in metal input from the atmosphere via long-range transport and deposition processes are evident in glacial snows and lake sediments distant from any point-source of metals (Dickson 1980; Jeffries and Snyder 1981; Weiss et al. 1975). Total atmospheric emissions of selenium (Se) from anthropogenic sources are 34 times emissions from natural sources (Lantzy and MacKenzie 1979). Anthropogenic

Figure 1. The exponential increase in global anthropogenic emissions of Cd (A) and Zn (B) since the begining of this century. Adapted from Nriagu (1979).


A Cadmium
Anthropogenic Emissions (10^6 Kg)
75
50
25
0
1900
1920
1940
1960
1980
DECADE


B Zinc
Anthropogenic Emissions (10^6 Kg)
3000
2000
1000
0
1900
1920
1940
1960
1980
DECADE

emissions of Hg range from 275 times (Lantzy and MacKenzie 1979) to 20% of (Reeder et al. 1979) the natural Hg emissions. Reeder et al. (1979) proposed that only in localized situations do anthropogenic sources contribute more than natural sources to Hg levels in the environment.

There have been numerous reports of localized metal pollution caused by human activities. The most familiar cases occurred in Japan with diseases such as Minamata and itai-itai which resulted from exposure to elevated Hg and Cd levels respectively (Harada 1978; Kobayashi 1978). Elevated metal levels have been recorded in the area of base-metal smelters (Jeffries and Snyder 1981; McFarlane and Franzin 1978; Van Loon and Beamish 1977). Hueck (1975) reported that 94% of Hg and Zn concentrations and 98% of Cd concentrations in the Rhine River had anthropogenic sources. Human activities often result in increased heavy metal concentrations, especially in aquatic ecosystems (Dickson 1980; Forstner and Wittmann 1979; Lantzy and MacKenzie 1979).

Considering these increases and their causes it is appropriate that the acute toxicity of elements such as Cd, Zn, Hg and Se, has received considerable attention and has been documented for numerous fish species (Table 1). Salmonids appear to be the fish species most sensitive to Cd and Zn toxicity. Spear (1981) reported the degree of tolerance to Zn toxicity in three taxonomic orders of fish as Perciformes> Cypriniformes>Clupeiformes. In many instances increased water hardness and/or decreased temperature will decrease metal toxicity (Brown 1968; Sprague 1970). These investigations showed substantial variation in the acute toxicity of Cd, Zn, Hg or Se among species.

Production of a low molecular weight (6 000-7 000 daltons)

Table 1. Relative Toxicities of Cd, Zn, Hg, and Se

Toxicity of Cd, Zn, Hg and Se to various freshwater species of fish. Toxicity is expressed as the 96-h LC50 value in mg metal/L.

Species	Toxicity				Reference
	Cd	Zn	Hg	Se	
Ictalurus punctatus	3.5	-	-	19	Cardwell et al. 1976; Rausina et al. 1975
Lepomis cyanellus	2.8	-	-	-	Pickering and Henderson 1966
Carrasius auratus	2.1-2.8	-	-	37	Cardwell et al. 1976; McCarty et al. 1978; Pickering and Henderson 1966
Jordanella floridae	2.5	1.5	-	-	Spehar 1976
Lepomis macrochirus	1.9-2.5	5.4	-	-	Pickering and Henderson 1966; Rausina et al. 1975
Lepomis gibbosus	1.5	20	.30	-	Rehwoldt et al. 1972
Lebistes reticulatus	1.3	-	-	-	Pickering and Henderson 1966
Roccus saxatilis	1.1	6.8	.09	-	Rehwoldt et al. 1972
Pimephales promelas	.63-1.0	-	-	1.0-7.3	Cardwell et al. 1976; Halter et al. 1980; Pickering and Henderson 1966
Anguilla rostrata	.82	14	.14	-	Rehwoldt et al. 1972
Cyprinus carpio	.24	7.8	.18	35	Rehwoldt et al. 1972; Sato et al. 1980
Fundulus diaphanus	.11	19	.11	-	Rehwoldt et al. 1972
Catostomus commersoni	-	-	-	34	Klaverkamp et al. 1982a
Perca flavescens	-	-	-	12	Klaverkamp et al. 1982a
Salmo gairdneri	.001-.12	.14-.18	.21-.28	-	Chapman 1978a; Chapman and Stevens 1978; Lloyd 1960; Macleod and Pessah 1973; Matida et al. 1971; Rausina et al. 1975; Sinley et al. 1974
Oncorhynchus tshawytscha	.004	.46	-	-	Chapman 1978a

cytoplasmic protein termed metallothionein (MTN) has been implicated as a potential biochemical strategy utilized by some organisms to moderate the toxic effects of such heavy metals as Cd, Hg, Zn and Cu (Cherian and Goyer 1978; Kojima and Kagi 1978). MTN was first isolated from equine kidney (Margoshes and Vallee 1957). Since then it has been identified in a wide range of organisms and several tissues (Kagi and Nordberg 1979). MTN has been characterized as a sulfur-rich protein with a high affinity for metals (6-9 g-atoms of metal/mole of MTN). The amino acid composition of MTN from mammalian systems is 30% cysteine with no aromatic amino acids (Kagi et al. 1974; Kojima et al. 1976). The biological function of MTN is not yet clear, though suggestions include detoxication and storage of metals, metabolism of essential metals and transport of both metals and amino acids (Cherian 1974; Evans 1979; Kagi et al. 1974; Kagi and Vallee 1960; Richards and Cousins 1977; Webb 1979). MTN content can be greatly increased in tissues by sublethal exposure to metals such as Cd, Zn, and Hg (Brown et al. 1977; Olafson and Thompson 1974; Vallee 1979) which indirectly supports the hypothesis that MTN functions in metal detoxication.

Catostomus commersoni was an ideal test organism because large numbers of a suitable size were readily available and they were easily maintained under laboratory conditions. Despite the abundance and wide-spread distribution of C. commersoni in North America and considerable knowledge of its biology (Scott and Crossman 1973), toxicity data for this species are lacking. Chapters II and III of this thesis are potential manuscripts for publication. Chapter II provides information on the acute toxicity of Cd, Zn, Hg and Se to white suckers and the

effects of sublethal metal exposure on white suckers' resistance and tolerance to Cd toxicity are examined. The nature of metal binding in liver and gill cytosol from white suckers in the absence and presence of elevated metal concentrations is investigated in Chapter III. The object of this thesis is to provide useful information to researchers interested in biochemical or physiological mechanisms of metal toxicity and detoxication in fish, the development of acclimation-induced tolerance and resistance to metals and in the establishment of water quality criteria. It is hoped this thesis will stimulate further research, both in the laboratory and the field, on the occurrence of, the mechanism(s) involved in and the consequences of acclimation-induced alterations of aquatic organisms' responses to metals.

CHAPTER II

Tolerance and Resistance to Acute Cadmium Toxicity in *Catostomus commersoni*

IIA: INTRODUCTION

Cadmium (Cd) and mercury (Hg), elements with no known biological function, are of major concern due to their potential toxicity and their exponential increase in aquatic environments (Babich and Stotzky 1978; Dickson 1980; Harriss and Hohenemser 1978; Nriagu 1980a; Muhlbaier and Tisue 1981). Selenium (Se) and zinc (Zn), elements essential for biological function, become toxic at high concentrations. Increases in release of these elements to the environment from anthropogenic activities threaten the aquatic biota (Copeland 1970; Norton et al. 1978; Hilton et al. 1980; Nriagu 1980b). The sources, quantities, effects and water quality objectives of these four elements have recently been reviewed (Demayo et al. 1979; Reeder et al. 1979a, b; Taylor and Demayo 1980).

Gradual increases of heavy metals in freshwater ecosystems allows some organisms to acclimatize or adapt to these higher metal concentrations (Beamish 1976; Luoma 1977). The phenomenon of increased tolerance and resistance resulting from exposure to elevated metal concentrations was documented 45 years ago (King 1937). Since that time there have been several reports of aquatic organisms from sites contaminated with heavy metals which are more tolerant to metals than organisms from non-contaminated sources (Paul 1952; Schofield 1965; Stokes et al. 1973; Bryan 1974; Weis et al. 1981). Fish inhabiting environments contaminated with Zn and/or Copper (CU) might adapt to the high concentrations and this may account for apparent overestimations of Zn and Cu toxicity (EIFAC 1977). Resident fish populations were more tolerant of the Zn contamination in some Flin Flon area lakes than expected on the basis of

Zn toxicity tests done in the laboratory (Van Loon and Beamish 1977).

It is perhaps appropriate at this point to define some terminology used in this paper. Acclimation refers to compensatory responses in organisms (e.g. white sucker) resulting from experimental manipulation of a single factor (e.g. metal concentration)(Barrington 1968; Fry 1971). Responses monitored in this study were changes in tolerance and in resistance to acute Cd toxicity resulting from exposure to an elevated metal concentration prior to Cd toxicity testing. Tolerance refers to the ability of an organism to survive indefinitely under a given set of environmental conditions. Resistance refers to the ability of an organism to survive for a limited period in an environment that will eventually exert a lethal effect (Fry 1947; Shepard 1955). Changes in tolerance and resistance to acute Cd toxicity are indicated by alteration of 96-h Cd LC50 values and alteration of survival times in lethal Cd concentrations, respectively.

Questions about the effects of sublethal metal exposure on resistance to and tolerance of acute Cd toxicity in white suckers were addressed in the laboratory to minimize confounding factors present in field situations. The object of this study was to answer the following questions. Is the acute toxicity of Cd to white suckers reduced (as measured by increased survival) if the suckers have previously experienced elevated Cd concentrations? Would such a protective effect be enhanced by increasing the duration of exposure to elevated Cd concentrations prior to toxicity testing? Is Cd toxicity to white suckers reduced if the suckers have previously experienced elevated Hg, Zn or Se concentrations? The answers should help explain the phenomenon of

aquatic organisms inhabiting environments contaminated with metals.

IIB: MATERIALS AND METHODS

White suckers were obtained from lakes 278, 317 and 384 of the experimental lakes area (ELA) in northwestern Ontario (Johnson and Vallentyne 1971) during the spring and summer of 1979. Lakes in this region typically contain only trace amounts of heavy metals (Beamish et al. 1976). Mean fork length and weight (± 1 SD) of suckers were 121±10 mm and 17.7±4.7 g respectively (n=1 981). All fish were held for at least 10 d prior to metal exposure and were fed Silver Cup Trout Chow #3, at 1% of estimated body weight, every second day. Feeding was halted 24 h prior to beginning the acclimation period. Acclimation period refers to the interval immediately prior to Cd toxicity testing during which suckers were exposed to diluent water alone or diluent water with an elevated metal level. Water temperature and photoperiod were maintained at 12.1±0.2°C and 12 h, respectively, throughout the acclimation periods and toxicity tests.

Diluent water was taken from the hypolimnion of lake 239 (Rawson Lake) in ELA. Chemical characteristics (Table 2) were determined monthly according to Stainton et al. (1974). Water samples (250 mL) for metal analyses were collected and immediately acidified with approximately 1 mL of concentrated nitric acid. Cd and Zn concentrations were determined on a Varian AA-5 atomic absorption spectrophotometer (equipped with a BC-6 background corrector) by flameless atomic absorption (carbon rod atomization) or by using an air-acetylene flame, depending on concentration. Hg and Se concentrations were determined as outlined in Klaverkamp et al. (1982a).

Table 2. L239 Water Chemistry

Chemical characteristics and heavy metal content of lake water (L239) used as diluent water and control medium. All units are mg/L except for Cd, Hg, Se, Zn (which are µg/L) and pH.

Parameter	Mean	Standard deviation	Number of Observations
Alkalinity (as $CaCO_3$)	6.35	0.21	7
Cadmium	0.25	0.09	9
Calcium	2.74	0.13	7
Magnesium	0.82	0.02	7
Mercury	0.007	0.002	14
Oxygen	7.90	1.64	7
pH	6.37	0.13	7
Potassium	0.72	0.03	7
Selenium	<0.5	-	5
Sodium	1.36	0.13	7
Total Hardness (as $CaCO_3$)	18.0	0.77	7
Zinc	6.08	2.40	6

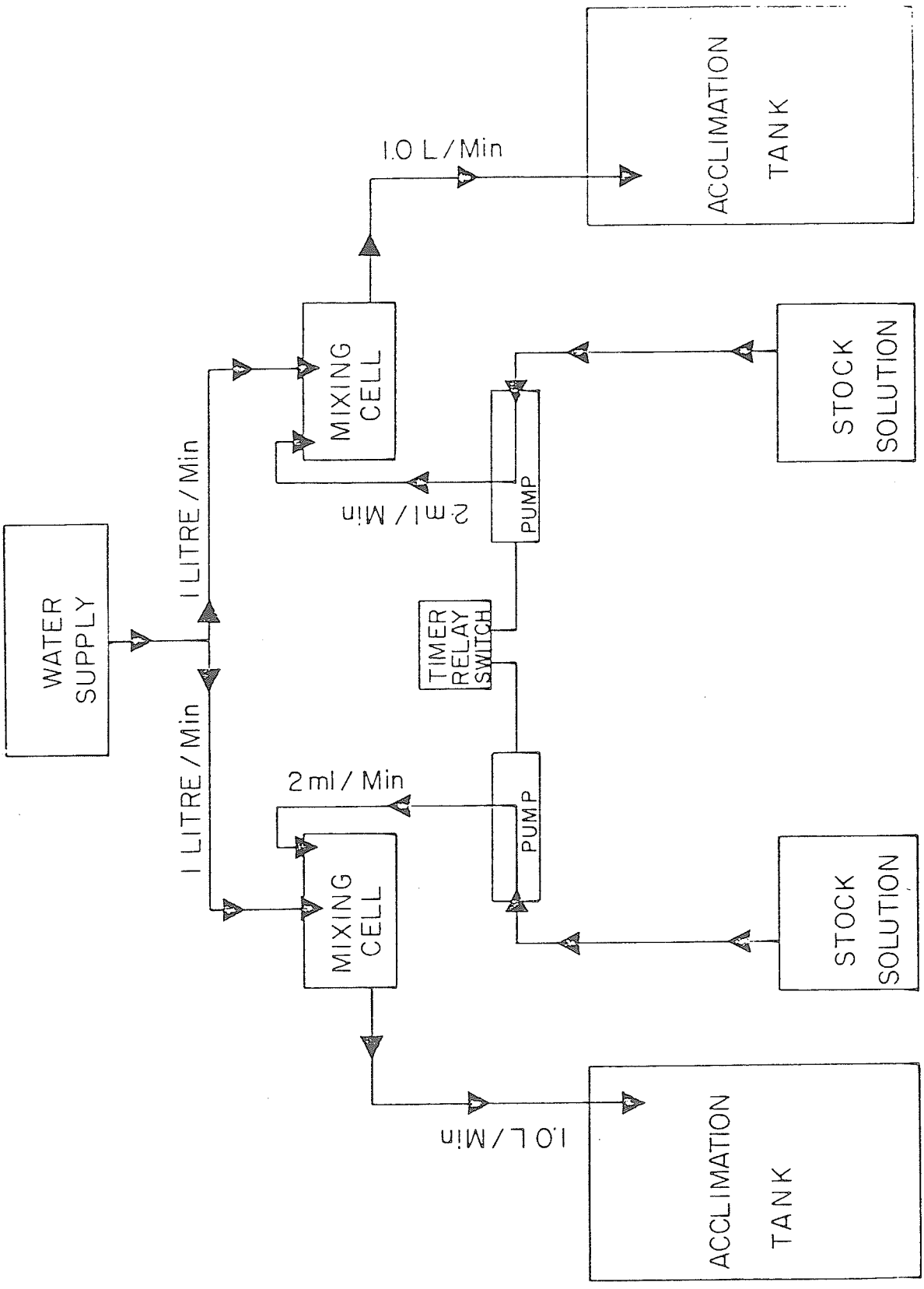
IIB 1): Acclimation Periods

Equal numbers of suckers were randomly transferred to each of four, 150-L fiberglass acclimation tanks for a 1 or a 2 wk period prior to toxicity testing. Loading density was approximately 12 g fish/L and the flow rate (1.0 L/min) provided a 95% replacement time of 7.5 h (Sprague 1973). Two tanks received diluent water alone (suckers from these tanks are referred to as controls) while the other two received diluent water with an elevated concentration of Cd, Zn, Hg or Se. Elevated metal concentrations were maintained by media dispensing pumps (connected to a 30-s interval timer and relay switch) which delivered appropriate volumes of a concentrated stock solution (Fig. 2). Chloride salts of Cd, Zn, and Hg (Fisher Scientific Ltd) and sodium selenite (BDH Chemicals Ltd) were used to prepare stock solutions. Reagent grade chemicals were used throughout the experiment. Water samples for metal analyses were collected at 48 h intervals during the acclimation periods. Average measured metal concentrations during acclimation periods were: 215, 410 or 730 µg Cd/L; 195 or 890 µg Zn/L; 1.03 or 220 µg Hg/L; 100 or 1900 µg Se/L. The coefficients of variation ranged from 1.2 to 23%.

IIB 2): Toxicity Tests

The effects of these elevated metal levels on the tolerance and resistance of C. commersoni to acute Cd toxicity were examined by conducting 100-h Cd toxicity tests. Toxicity tests of 100 h duration were also conducted with Zn, Hg or Se as the toxicant (rather than Cd) and with control suckers as test organisms. The test vessels and toxicant delivery system used for toxicity testing were similar to those

Figure 2. Schematic representation of exposure system used for exposing suckers to sublethal metal concentrations. The loading density in the 150-L acclimation tanks was approximately 12 g fish/L. Appropriate volumes of a concentrated stock solution were mixed with diluent water and a flow rate of 1.0 L/min into the acclimation tanks provided a 95% replacement time of 7.5 h.


WATER SUPPLY
1 LITRE / Min
1 LITRE / Min
MIXING CELL
MIXING CELL
1.0 L/Min
2 ml / Min
2 ml / Min
PUMP
PUMP
TIMER RELAY SWITCH
STOCK SOLUTION
STOCK SOLUTION
ACCLIMATION TANK
ACCLIMATION TANK
1.0 L / Min

described by Harrison et al. (1975). Test vessels had flow rates (100 mL/min) which provided a 95% replacement time of 10 h (Sprague 1973). The toxicant delivery system was a 75% proportional diluter modified to produce a range of concentrations without replication plus 1 control. After a 1 or 2 wk acclimation period, 10 suckers were randomly transferred to each of the appropriate test vessels in two testing systems. Loading density was approximately 8 g fish/L. Toxicant was added to test vessels within 30 min to establish nominal concentrations which were maintained throughout the test by the toxicant delivery system. Allocation of suckers into these two systems allowed simultaneous testing of fish from all 4 acclimation tanks.

Toxicity tests were performed over a range of toxicant concentrations. Water from test vessels was sampled for analyses at approximately 3, 48 and 96 h during each toxicity test. Average measured test concentrations ranged from .60 to 9.4 mg/L for Cd, 0.99 to 12 mg/L for Zn, 0.36 to 4.8 mg/L for Hg, and 3.4 to 44 mg/L for Se. The coefficients of variation ranged from 5.9 to 22%.

Survival time (the interval from addition of toxicant until cessation of all visible movement and lack of response to gentle prodding) was determined by inspection of fish at successive time increments of 0.04 log units, with the exception of the first 3 h during which inspections occurred every 15 min. Dead fish were removed and survival time, fork length and weight recorded. The time at which 50% of test organisms remained alive, i.e. the median survival time (MST), was determined by graphical interpolation (Litchfield 1949). The number of fish responding (i.e. dying) and a slope factor(s) were used to

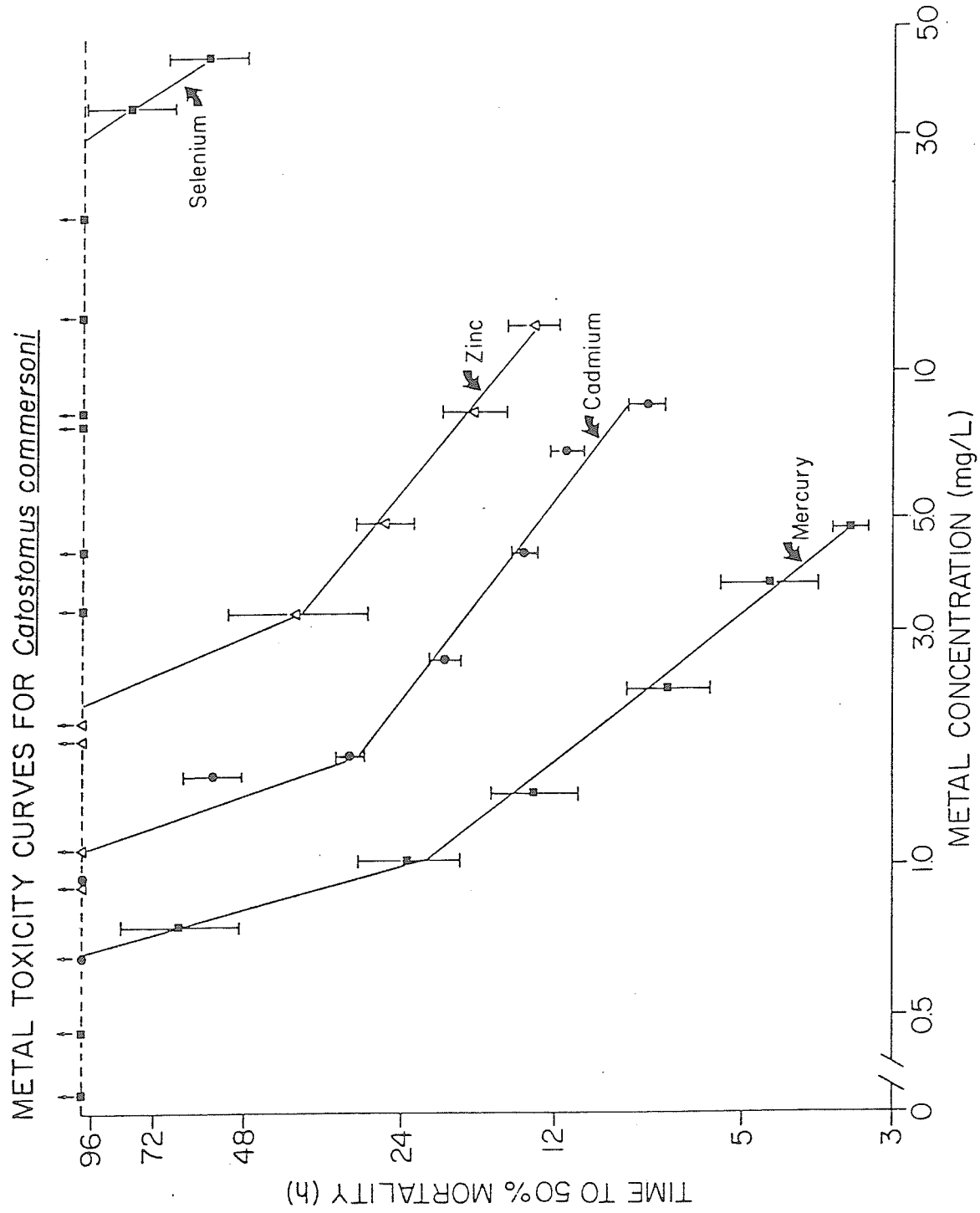
obtain a 95% confidence interval (CI) for the MST (Litchfield, 1949).The graph and slope factors used in determining MST values and their 95% CI for control suckers in Cd toxicity tests appear in Appendix A. Standard toxicity curves were drawn by plotting, on a log scale, MST against metal concentration.

The concentration lethal to 50% of test organisms (LC50) was derived from standard toxicity curves by determining the lethal concentration after specific periods of exposure (12, 24, 48, 72 and 96 h). In the case of Se the 12-, 24- and 48-hr LC50 values were derived by extrapolation of the Se toxicity curve. The accuracy of this extrapolation is confirmed by the similarity to corresponding values reported by Klaverkamp et al. (1982a). Cd LC50 values (and their 95% CI) for control suckers were also calculated from raw data according to the method described by Litchfield and Wilcoxon (1949). A comparison of the two methods used to determine Cd LC50 values for control fish indicated that the values derived from the toxicity curve were within the appropriate 95% CI of the calculated values and differences between corresponding values were less than 10%.

IIC: RESULTS

There is a linear relationship between the MST value and the higher test concentrations when plotted on a logarithmic scale (Fig. 3). The Cd toxicity curve for control suckers is based on pooled data from 7 Cd toxicity tests. There were no detectable differences between individual tests. For Cd, Zn, and Hg toxicity curves a sharp break in the linear relationship is evident at the lower metal concentrations resulting in longer MST values. Figure 3 illustrates that white suckers

Figure 3. Median survivan times (MST) of control suckers during 100-h Hg, Cd, Zn or Se toxicity tests. Vertical bars represent 95% confidence intervals of the MST values.


METAL TOXICITY CURVES FOR Catostomus commersoni
TIME TO 50% MORTALITY (h)
96
72
48
24
12
5
3
0
0.5
1.0
3.0
5.0
10
30
50
METAL CONCENTRATION (mg/L)
Selenium
Zinc
Cadmium
Mercury

exposed to 5 mg of Hg, Cd, Zn or Se/L would have a MST of 2.97, 12.6, 26.4 or >100 h respectively. LC50 values derived from the Cd, Zn, Hg and Se toxicity curves (Table 3) show the order of metal toxicity as Hg>Cd>Zn>>Se.

Toxicity curves (Fig. 4A and B) demonstrate the effects of duration and concentration of sublethal Cd exposure on subsequent acute Cd toxicity. Increasing the acclimation period from 1 to 2 wk had very little effect on MST values. With the exception of one test concentration (1.55 mg Cd/L), MST values of control suckers from the 1 wk acclimation period were within the 95% CI for corresponding values from the 2 wk acclimation period. MST values for suckers exposed to 410 µg Cd/L for 1 or 2 wk (Fig. 4A and B) and 730 µg Cd/L for 1 wk (Fig. 4A), prior to toxicity testing, were consistently greater than corresponding control values. MST values for suckers pre-exposed to 215 µg Cd/L were greater than control values only at test concentrations above 1.55 and 2.45 mg Cd/L for 1 and 2 wk acclimation periods respectively.

Cd LC50 values depend on the sublethal Cd level during the acclimation period (Fig. 5). Exposure of suckers to 410 or 730 µg Cd/L during a 1 wk acclimation period increased both resistance and tolerance to Cd. The 12-, 24- and 96-h Cd LC50 values for suckers pre-exposed to 410 µg Cd/L were 137, 173 and 175%, respectively, of control values. The 12-, 24- and 96-h Cd LC50 values for suckers pre-exposed to 730 µg Cd/L were 352, 287 and 245%, respectively, of control values. Pre-exposure of suckers to 215 µg Cd/L increased Cd resistance but not Cd tolerance as the 12-, 24-, and 96-h LC50 values were 130, 126 and 94%, respectively, of control values.

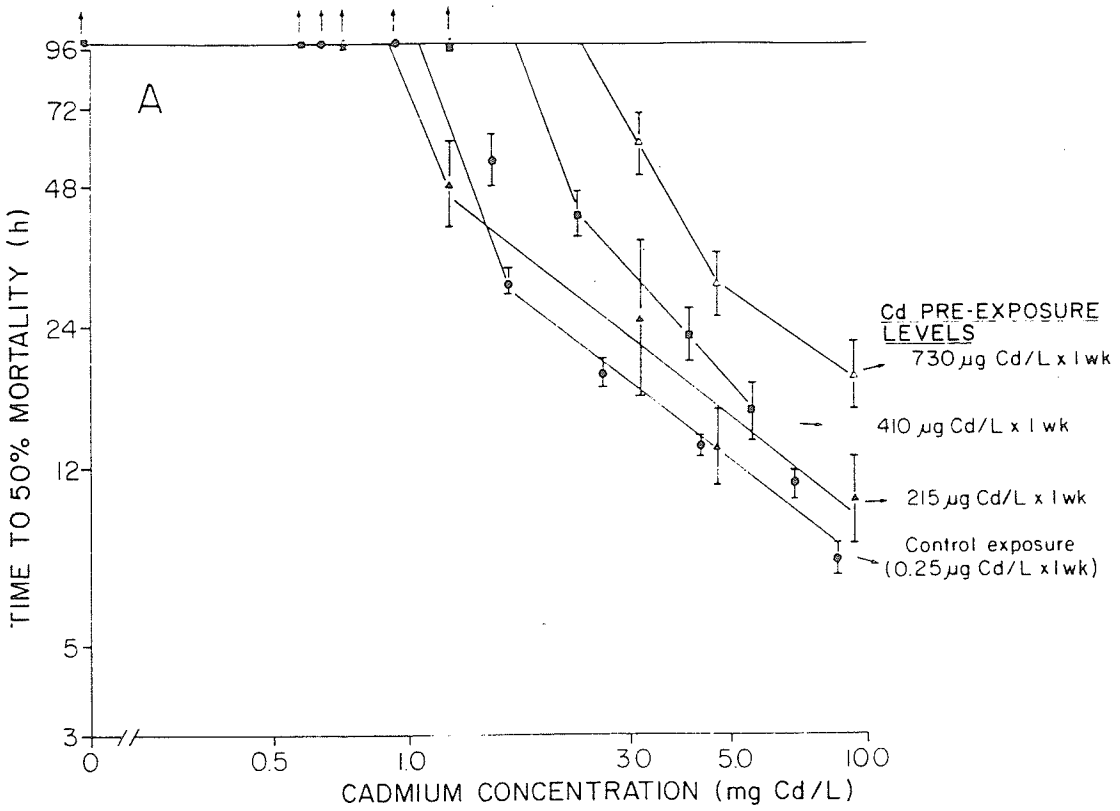
Table 3. Cd, Zn, Hg, and Se LC50 values for C. commersoni.

Metal concentration lethal to 50% of test organisms (LC50). Test organisms were white suckers exposed to diluent water alone for 1 wk prior to toxicity testing (i.e. control suckers).

Time (h)	LC50							
	Cadmium		Zinc		Mercury		Selenium	
	mg/L	µmol/L	mg/L	µmol/L	mg/L	µmol/L	mg/L	µmol/L
12	5.35	48	13.3	200	1.60	8.0	117	1,500
24	2.12	19	5.58	85	1.00	5.0	75.4	950
48	1.41	12	2.96	45	0.830	4.1	48.6	620
72	1.27	11	2.48	38	0.740	3.7	37.6	480
96	1.11	9.9	2.20	34	0.687	3.4	31.4	400

Figure 4. Median survival times (MST) of suckers during 100-h Cd toxicity tests. Suckers were pre-exposed to the sublethal Cd concentrations for either 1 (A), or 2 (B) wk. Vertical bars represent 95% confidence intervals of the MST values.

CADMIUM TOXICITY CURVES FOR SUCKERS PRE-EXPOSED TO CADMIUM



CADMIUM TOXICITY CURVES FOR SUCKERS PRE-EXPOSED TO CADMIUM

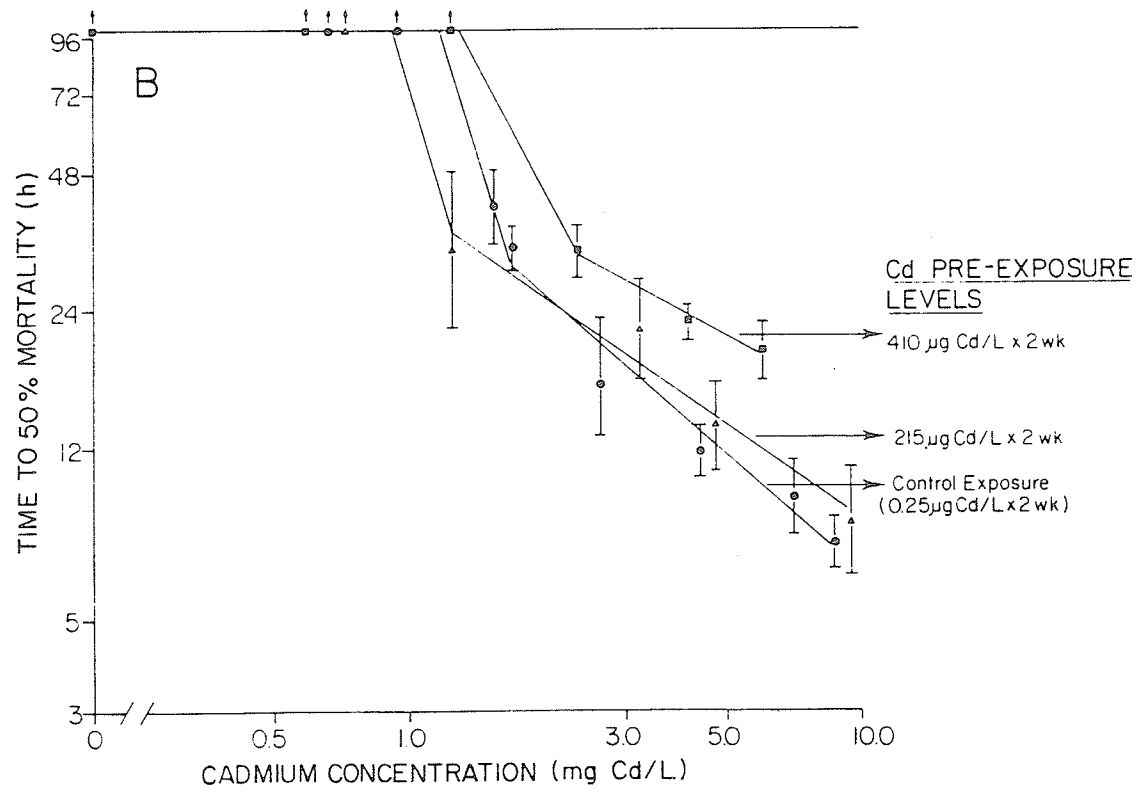
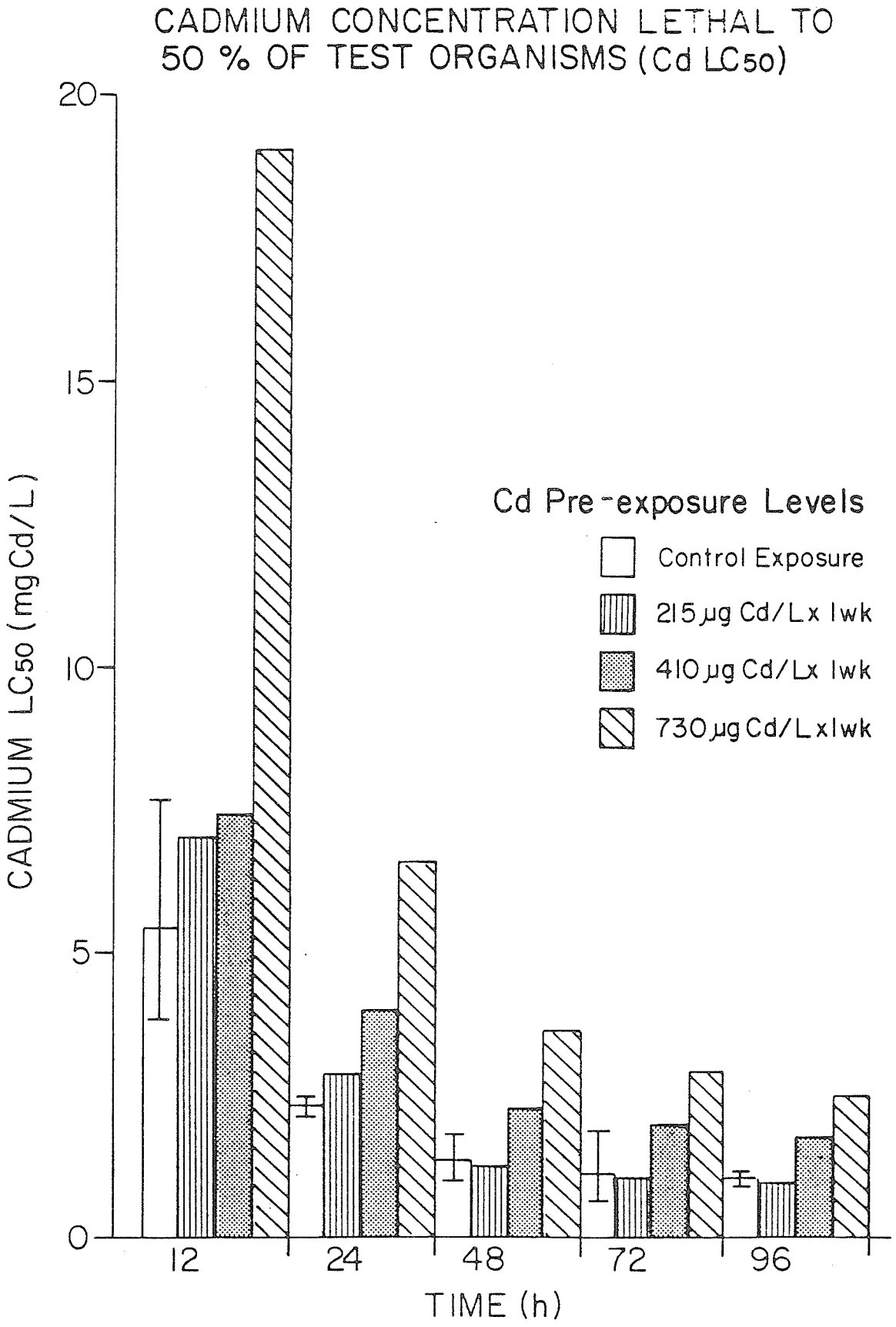


Figure 5. Cadmium LC50 values, at five specific times, for suckers exposed to sublethal Cd concentrations during a 1 wk acclimation period. Vertical bars represent 95% confidence intervals of the LC50 value.


CADMIUM CONCENTRATION LETHAL TO
50 % OF TEST ORGANISMS (Cd LC50)
CADMIUM LC50 (mg Cd/L)
20
15
10
5
0
12
24
48
72
96
TIME (h)
Cd Pre-exposure Levels
Control Exposure
215 µg Cd/L x 1wk
410 µg Cd/L x 1wk
730 µg Cd/L x 1wk

Toxicity curves (Fig. 6A, B and C) demonstrate the effects of sub-lethal Hg, Zn or Se exposure on subsequent acute Cd toxicity. Exposure to 220 µg Hg/L prior to toxicity testing increased both resistance and tolerance to Cd as evidenced by the toxicity curve being shifted up and to the right of the curve for control suckers. MST values for suckers pre-exposed to 195 or 890 µg Zn/L were greater than control values only at test concentrations above 1.55 mg Cd/L. Exposure of suckers to 1.03 µg Hg/L, 100 or 1900 µg Se/L did not produce compensatory responses to acute Cd toxicity.

Suckers pre-exposed to 220 µg Hg/L had 12- and 24-h Cd LC50 values approximately one order of magnitude greater than control values, and a 96-h Cd LC50 value nearly 4 times the control value (Fig. 7A). Cd resistance increased in suckers pre-exposed to 195 or 890 µg Zn/L but Cd tolerance did not (Fig. 7B). The 12- and 24-h Cd LC50 values for suckers pre-exposed to 195 or 890 µg Zn/L were greater than the upper limit of the 95% CI for corresponding control values. The 96-h Cd LC50 values for suckers exposed to 195 or 890 µg Zn/L during the acclimation period were not different from the control value, indicating that these Zn concentrations had no effect on Cd tolerance.

MST values obtained from appropriate Cd toxicity curves at specific concentrations (3.0 and 5.0 mg Cd/L) provided comparisons of changes in Cd resistance (Table 4). Exposure of suckers to elevated Cd or Hg concentrations prior to lethal Cd challenge resulted in up to 3- or 5-fold increases, respectively, in MST values relative to values for control suckers.

Figure 6. Median survival times (MST) of suckers during 100-h Cd toxicity tests. Suckers were pre-exposed to the sublethal Hg (A), Zn (B) or Se (C) concentrations for 1 wk. Vertical bars represent 95% confidence intervals of the MST values.

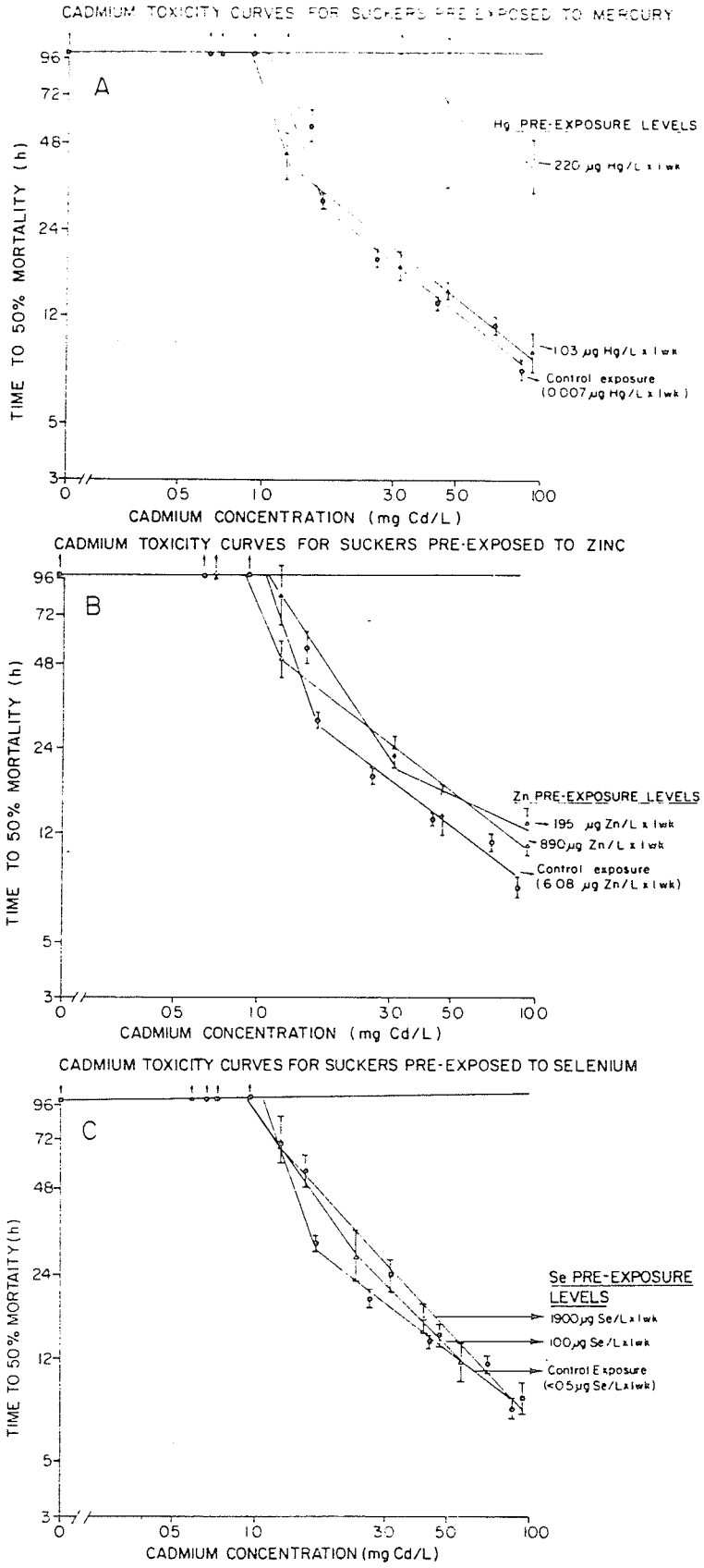

CADMIUM TOXICITY CURVES FOR SUCKERS PRE-EXPOSED TO MERCURY
A
Hg PRE-EXPOSURE LEVELS
220 µg Hg/L x 1 wk
103 µg Hg/L x 1 wk
Control exposure
(0.007 µg Hg/L x 1 wk)
TIME TO 50% MORTALITY (h)
96
72
46
24
12
5
3
0
05
10
30
50
100
CADMIUM CONCENTRATION (mg Cd/L)
CADMIUM TOXICITY CURVES FOR SUCKERS PRE-EXPOSED TO ZINC
B
Zn PRE-EXPOSURE LEVELS
195 µg Zn/L x 1 wk
890 µg Zn/L x 1 wk
Control exposure
(6.08 µg Zn/L x 1 wk)
TIME TO 50% MORTALITY (h)
96
72
48
24
12
5
3
0
05
10
30
50
100
CADMIUM CONCENTRATION (mg Cd/L)
CADMIUM TOXICITY CURVES FOR SUCKERS PRE-EXPOSED TO SELENIUM
C
Se PRE-EXPOSURE LEVELS
1900 µg Se/L x 1 wk
100 µg Se/L x 1 wk
Control Exposure
(<0.5 µg Se/L x 1 wk)
TIME TO 50% MORTALITY (h)
96
72
48
24
12
5
3
0
05
10
30
50
100
CADMIUM CONCENTRATION (mg Cd/L)

Figure 7. Cadmium LC50 values, at five specific times, for suckers exposed to sublethal Hg (A) or Zn (B) levels during a 1 wk acclimation period. Vertical bars represent 95% confidence intervals of the LC50 values.

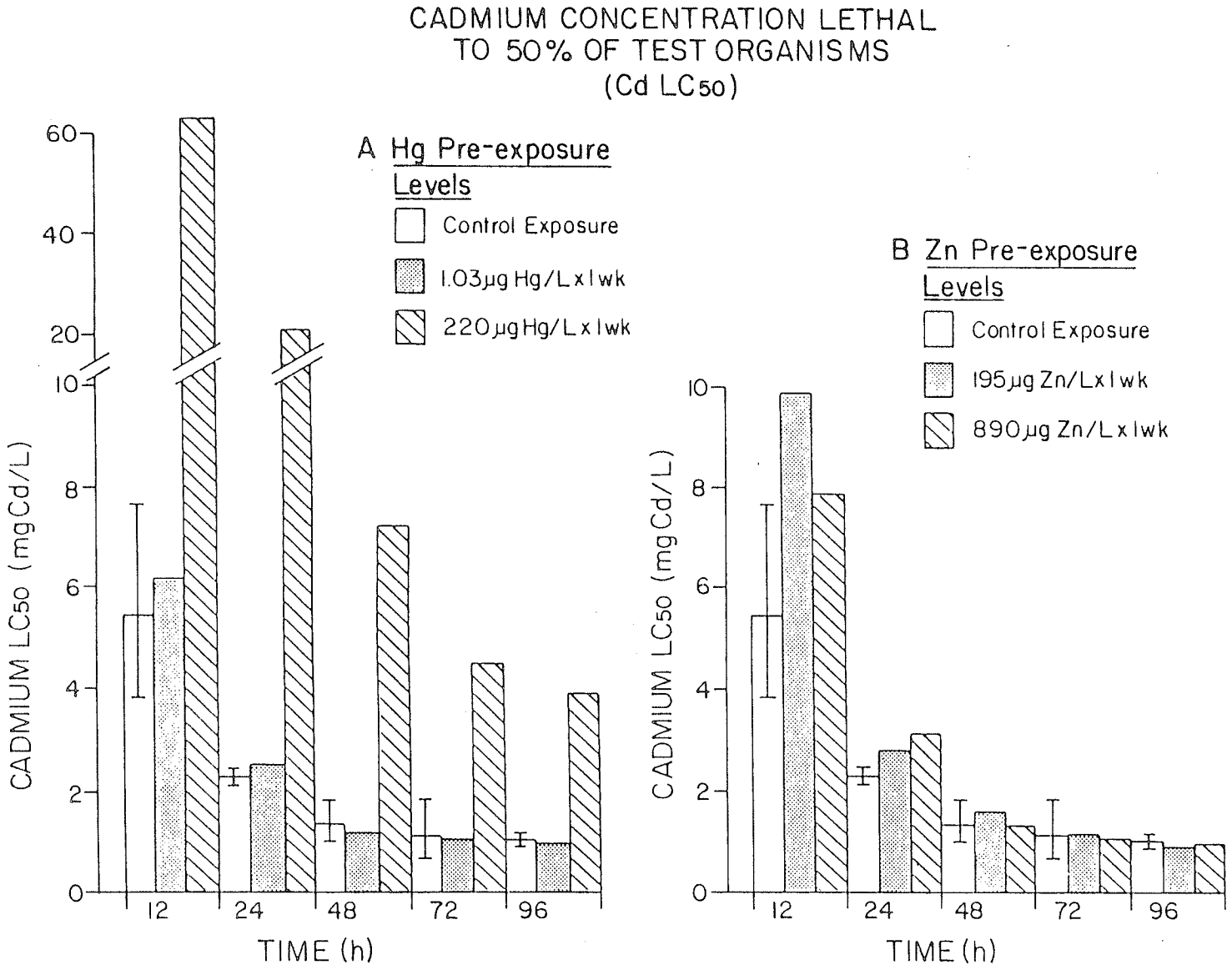

CADMIUM CONCENTRATION LETHAL
TO 50% OF TEST ORGANISMS
(Cd LC50)
A Hg Pre-exposure
Levels
Control Exposure
1.03µg Hg/L x 1wk
220µg Hg/L x 1wk
B Zn Pre-exposure
Levels
Control Exposure
195µg Zn/L x 1wk
890µg Zn/L x 1wk
CADMIUM LC50 (mg Cd/L)
TIME (h)
12
24
48
72
96
0
2
4
6
8
10
20
40
60

Table 4. MST of C. commersoni in Lethal Cadmium Concentrations

Median survival times of white suckers in 5.0 and 3.0 mg Cd/L. Metal concentrations in controls were 0.25 µg Cd/L, 6.08 µg Zn/l, 0.007 µg Hg/L and <0.5 µg Se/L. One toxic unit is approximated by the 96-h Cd LC50 for control suckers. Concentration units are micrograms per litre.

Acclimation Exposure				Median Survival Time (h)			
Metal	Concentration	Time (d)	Toxic Units	5.0 mg Cd/L	% Increase	3.0 mg Cd/L	% Increase
Cd	Control	7	2.45×10^{-4}	12.6	---	18.5	---
"	215	7	0.21	15.8	25	23.8	29
"	410	7	0.40	18.8	48	32.9	78
"	730	7	0.72	33.9	169	57.4	210
Cd	Control	14	2.45×10^{-4}	11.5	---	17.8	---
"	215	14	0.21	14.0	22	22.0	24
"	410	14	0.40	20.7	79	27.8	57
Zn	Control	7	3.0×10^{-3}	12.6	---	18.5	---
"	195	7	0.09	18.1	43	29.8	61
"	890	7	0.40	17.0	35	25.0	36
Hg	Control	7	1.46×10^{-5}	12.6	---	18.5	---
"	1.03	7	1.0×10^{-3}	14.3	14	21.0	13
"	220	7	0.32	65.6	419	99.7	438
Se	Control	7	7.97×10^{-6}	12.6	---	18.5	---
"	100	7	3.0×10^{-3}	12.8	1	23.2	25
"	1900	7	0.06	15.0	19	25.8	39

IID: DISCUSSION

Despite the abundance and widespread distribution of C. commersoni in North America and considerable knowledge of their biology (Scott and Crossman 1973), there is a lack of toxicity data for this species. Relative to other freshwater fish species white suckers are moderately sensitive to the four toxicants ($CdCl_2$, $ZnCl_2$, $HgCl_2$ and Na_2SeO_3) used (Table 1) (Akiyama 1970; Cardwell et al. 1976; Chapman 1978a; Chapman and Stevens 1978; Matida et al. 1971; Pickering and Henderson 1966; Rausina et al. 1975; Rehwoldt et al. 1972; Sato et al, 1980). The Zn 96-h LC50 value (2.20 mg Zn/L) obtained in this study is within 2% of the value calculated by using the equation developed by Spear (1981) based on 22 Zn toxicity tests in waters of varying hardness using "minnow-like fish" as test organisms. Any composite ranking of species' sensitivity to Cd, Zn, Hg or Se, from the scientific literature, would be questionable at best due to variation between test conditions, water characteristics, exposure history of test organisms and chemical form of the toxicant.

Increased tolerance and resistance to acute Cd toxicity resulting from previous exposure to Cd (presented above) has also been demonstrated in other vertebrates. Leber and Miya (1976) demonstrated a concentration dependent increase of Cd tolerance in mice pretreated with sublethal Cd concentrations.Yoshikawa (1970) observed a four-fold increase in survival of mice when challenged with Cd, if they had received a sublethal dose of Cd previously. Terhaar et al. (1965) report on the protective effects of low doses of $CdCl_2$ against subsequent high oral doses of $CdCl_2$ in rats. Pascoe and Beattie (1979) exposed rainbow trout

(Salmo gairdneri) alevins to 0.01 mg Cd/L for 1 wk and observed more than a 10-fold increase in the resultant 48-h Cd LC50 (1.5 mg Cd/L) over the corresponding control value (<0.1 mg Cd/L). Flagfish (Jordanella floridae) and rainbow trout were protected against Cd toxicity by pretreatment with sublethal Cd concentrations during embryonic development (Spehar 1976; Beattie and Pascoe 1978).

A similar protection phenomenon has been reported by researchers working with the essential elements Zn and Cu. In 10 mg Zn/L the MST of rainbow trout pre-exposed for 2 wk to 3.5 or 2.5 mg Zn/L was 500 or 400 min respectively, compared with 290 min for control trout (Lloyd 1960). Rainbow trout held at 0.5 of their Zn incipient lethal level (ILL) for 60 d exhibited a 40% increase in the 48-h Zn LC50 relative to controls (Edwards and Brown 1967). Chapman (1978b) clearly demonstrated that acclimation of juvenile sockeye salmon (Oncorhynchus nerka) to 1/3 the 96-h Zn LC50 for non-acclimated fish resulted in more than a 2-fold increase in the 96-h Zn LC50. Similar to studies using Cd, pre-exposure of embryos to Zn protected against Zn toxicity upon hatching, as demonstrated by Pickering and Vigor (1965), Sinley et al. (1974) and Spehar (1976) using fathead minnows (Pimephales promelas), rainbow trout and flagfish respectively. Saliba and Ahsanullah (1973) and Saliba and Krzyz (1976) found that Artemia pre-exposed to sublethal Cu concentrations exhibited increases in Cu resistance and tolerance only at test concentrations less than 7.5 mg Cu/L. Cu ILL values for rainbow trout parr maintained for 3 wk in 30, 40 or 60% of the Cu ILL for control fish (330 µg Cu/L) were 160, 205 and 190% respectively, of the control value (Dixon and Sprague 1981).

Dixon and Sprague (1981) reported that although most of the protection from Cu toxicity was achieved within the first 7 d of sublethal exposure the protection continued to increase throughout the 21 d exposure period. The results with Cd and white suckers indicate that a 2 wk exposure to sublethal Cd levels does not increase the protection from Cd toxicity over the result from a 1 wk sublethal exposure. This difference between studies might be explained by chemical differences between metals, species differences between organisms, and/or nutritional differences between the two species. In this study the fish were not fed during the pre-exposure period whereas in Dixon and Sprague (1981) they were. Stress resulting from 2 wk without food could be responsible for the decrease in the ability of suckers to withstand Cd challenge thereby masking any further protection that might have been provided by the longer pre-exposure period.

There are few reports of aquatic organisms exhibiting increased tolerance and/or resistance to one metal as a result of previous exposure to a different metal, although there are numerous reports of this phenomenon occurring in mammals. Dixon and Sprague (1981) found that rainbow trout acclimated to Cu exhibited increased resistance to but decreased tolerance of Zn toxicity relative to non-acclimated trout. Pretreatment of rats (Webb 1972a) or mice (Leber and Miya 1976) with Zn resulted in reduced Cd toxicity. In this study, exposure to Zn resulted in increased resistance to Cd but tolerance of Cd was unaffected. Selenium dioxide was found to be an effective antagonist to the toxic effects of Hg_2Cl_2 in the northern creek chub (Semotilus atromaculatus) and the goldfish (Carassius auratus) (Kim et al. 1977;

Heisinger et al. 1979). Parizek (1978) reports selenite as being highly effective in decreasing mortality caused by Cd in rats and mice. In this study selenite does not appear to be very effective in decreasing Cd toxicity, possibly due to the relatively low Se concentration (0.3 and 6% of 96-h Se LC50) during the acclimation period. Yoshikawa (1970), in a thorough paper on the preventive effect of pretreatment with metals on acute metal toxicity in mice, recorded a host of metal interactions which included a Hg pretreatment that resulted in decreased Cd-related mortality. Although no such occurrence has been reported previously for aquatic organisms, in this study nearly a 4-fold increase in Cd tolerance resulted from a 1 wk exposure of suckers to 220 µg Hg/L (32% of 96-h Hg LC50) prior to Cd toxicity testing.

IID 1): Potential Protective Mechanisms

Changes in morphology of structures involved with uptake and/or excretion could affect these functions. In mammals, for example, smooth membranes replaced brush borders in kidney samples from organisms treated with tubulotoxic doses of $HgCl_2$ (Price and Kempson 1975) thereby decreasing both surface area and tubular reabsorption (Magos and Webb 1978). Exposure of fish to sublethal metal levels changed gill structure and altered the uptake and excretion of metals by increasing the number and activity of chloride cells and by increasing mucous production (Matthiessen and Brafield 1973; Reichert et al. 1979; Pierson 1981). Decreased uptake and/or increased excretion of Cd during toxicity testing could cause the increased survival observed in this study.

Another possible cause for this increased survival could be an

ability of pre-exposed suckers to store and withstand higher metal body burdens than control suckers (Stokes et al. 1973; Bryan 1974; Kim et al. 1977; Heisinger et al. 1979). Exposure of organisms to sublethal metal levels significantly alters the tissue distribution of metals (Parizek et al. 1971; Stonnard and Webb 1976; Kim et al. 1977; Bremner and Campbell 1978; Nordberg 1978). Redistribution might divert Cd to less sensitive biochemical storage sites, resulting in increased tolerance and/or resistance.

A biochemical storage mechanism receiving considerable attention in research to-date is the induced synthesis of a small molecular weight protein termed metallothionein (MTN). This protein is believed to function in the detoxication and storage of metals such as Cd, Zn, Hg and Cu (Kagi and Nordberg 1979). Several studies have shown that Cd, Hg and Zn induce the biosynthesis of MTN in fish tissues (Bouquegneau et al. 1975; Marafante 1976; Noel-Lambot et al. 1978). Current investigations in our laboratory include the occurrence and nature of metal binding proteins in fish exposed to sublethal metal concentrations in the laboratory and from metal impacted areas. Production of MTN may be induced (an important trait of any detoxication system) by the presence of heavy metals as indicated by aquatic organisms from environments contaminated with heavy metals possessing increased MTN levels, relative to organisms from non-contaminated sites (Brown et al. 1977). Comprehensive biochemical, physiological and behavioral studies are required to establish the fundamental mechanism(s) involved in, and the "biological costs" for, the development of compensatory responses resulting from metal exposure.

IID 2): Significance of Acclimation

The ability of an organism to increase its tolerance and/or resistance to metals, as a consequence of previous exposure to metals, is significant not only to aquatic organisms and their communities but also to consumers and managers of aquatic resources. A population of white suckers inhabiting a lake impacted with high Cd, Zn and Cu levels from a near-by smelter exhibited increased egg resorption, decreased egg size, reduced spawning success (McFarlane and Franzin 1978) and increased resistance to acute Cd toxicity (Klaverkamp et al. 1982b) relative to a population from a lake with much lower metal levels. These compensatory adjustments in adults, however, were offset by poorer recruitment of young fish into the population. Communities in which organisms are forced to adapt to a toxicant, and in which tolerance develops,typically contain only a few species (Ferguson 1970; Luoma 1977). Tolerant populations are able to survive in habitats lethal to non-tolerant populations; thus gene flow into the former may be prevented or, at least, severely limited. This environmentally induced isolation, enforced by a toxicant barrier, may in time lead to the creation of a new species. Metal accumulation in tolerant or resistant organisms from heavily contaminated sites may represent a potential danger to consumers in higher trophic levels, including man.

Adaptation and/or acclimatization by aquatic organisms to metals is a major hindrance to the accurate application of laboratory data to environmental situations. A complete understanding of an organism's capacity to withstand metal exposure necessitates descriptions of both resistance and tolerance. High metal concentrations resulting from

accidental leaks from tailings ponds, toxic effluents encountered while migrating to spawning grounds, or contaminated melt-water in spring require relatively short term survival capabilities (i.e. increased resistance). High metal concentrations resulting from atmospheric deposition (from smelting operations or fossil fuel burning) or continual contamination from mining or industrial wastes require the organism to withstand increased metal concentrations indefinitely (i.e. increased tolerance) if it is to survive in the contaminated habitat. The "biological costs" of fish developing compensatory responses to metals and of implementing the mechanism(s) necessary for this development, should be evaluated thoroughly. Aquatic resource managers involved with setting water quality criteria must consider many factors which influence an organism's responses to a toxicant, including previous exposure to that and other toxicants.

CHAPTER III

Metal Distribution In Liver and Gill Cytosol from *Catostomus commersoni*

IIIA: INTRODUCTION

The tolerance of and resistance to cadmium (Cd) by white suckers increased after acclimation to elevated metal concentrations (Duncan and Klaverkamp 1982). Tolerance, as indicated by the maximum Cd concentration in which fish survive in for longer than 100 h, was increased approximately 2.3- or 3.6-fold in fish exposed to sublethal Cd or Hg concentrations respectively. Resistance, expressed as survival times in lethal Cd concentrations, also increased approximately 3.1-, 5.4- or 1.6-fold in fish exposed to sublethal concentrations of Cd, Hg or Zn respectively.

Klaverkamp et al. (1982b) recently reviewed studies on acclimation to heavy metal toxicity by fish and on the occurrence and nature of the protein metallothionein (MTN). MTN is located in the cytosolic fraction (cytosol) of tissues contains a high concentration of cysteine, and has been implicated as a biochemical chelator of metals such as Cd, Hg, Zn and Cu so that the toxicity of these metals is reduced (Cherian and Goyer 1978; Kojima and Kagi, 1978). The occurrence of, and the similarities in, MTN from Neurospora to man are indicative of its involvement in essential biological processes within the cell. One such process might be regulating metabolism of essential (Cu and Zn) and non-essential (Cd and Hg) metals (Evans 1979; Vallee 1979; Webb 1972b). Exposure to sublethal concentrations of these metals induces increased synthesis of MTN which may not be involved only in acclimation to metal toxicity, but also in producing adverse effects through alterations in the distribution of metals in cytosolic fractions (Coombs 1975).

The object of this study was to provide information on the nature

of metal binding in liver and gill cytosol from C. commersoni both in the absence and in the presence of elevated Cd, Zn or Hg concentrations. Specifically, the study was designed to answer the following three questions. Is the methodology for MTN analysis, developed for use with mammalian systems, suitable for detecting MTN in liver and gill cytosol from suckers which have not been exposed to elevated metal concentrations? Is the partitioning of Zn, Cu, Cd and Hg in the cytosol of these organs affected by exposures to sublethal concentrations of Cd, Hg or Zn? Do these exposures increase MTN concentrations in liver and gill cytosol? In answering these questions this study serves as a preliminary experiment on methodology for the analysis of MTN and on the role of this protein in acclimation by fish to heavy metal toxicity.

IIIB: MATERIALS AND METHODS

White suckers were collected from minnow traps in lake 815 of the experimental lakes area (E.L.A.) in northwestern Ontario (Johnson and Vallentyne 1971) and held in lake 239 hypolimnion water at 12.1±0.2 °C and a 12 h light/12 h dark photoperiod for at least 10 d prior to use. Mean fork length and weight (± 1 SD) were 142±9 mm and 28.7±5.6 g, respectively (n=40). Suckers were transferred to a flow-thru (1.0 L/min) exposure system and exposed to lake 239 water, alone (controls), or with an elevated metal concentration for seven days. A detailed description of dilution water characteristics and exposure system was given above (Table 2 and Fig. 2).

Cd, Zn and Hg concentrations (± 1 SD) of lake 239 water were 0.25±.09, 6.1±2.4 and <0.01 µg metal/L, respectively. Elevated Cd, Zn or Hg concentrations (± 1 SD) of 660±50, 890±15, or 190±15 µg metal/L,

respectively (n=4) were used because similar concentrations produced the greatest Cd-acclimation response in a previous study (Duncan and Klaverkamp 1982) At the end of each exposure gill filaments from 7 suckers and livers were removed from 10 suckers (i.e. 3 additional fish were required to provide enough liver tissue) were removed and pooled for analyses.

IIIB 1): Cytosol Preparation

Pooled tissue samples were prepared for MTN analyses by rinsing in ice-cold ammonium formate buffer (AFB) (0.01 M NH_4COOH, 0.02% NaN_3, pH 8.0) and stored at -20°C until needed. The tissue sample was homogenized in 4 volumes of AFB using a glass Potter-Elvehjem homogenizer equipped with a motorized teflon pestle. The homogenate was centrifuged at 10 000 RCF for 10 min at 2°C (on a Sorvall Superspeed RC2-B) and the resultant supernatant (SN) further centrifuged at 100 000 RCF for 60 min (on a Sorvall Ultracentrifuge OTD-2) at 2°C, and the pellet discarded to obtain the cytosol.

IIIB 2): Gel Fractionation and Metal Analyses

Gel filtration was used to fractionate tissue cytosol. The fractions were analyzed according to a Cd-displacement technique using ^{109}Cd (Chen and Ganther 1975; Probst et al. 1977a) and also according to a metal-summation technique (Brown et al. 1977; Noel-Lambot et al. 1978). To prepare a "Cd-saturated" cytosol for use in the Cd-displacement technique the cytosol from 1/2 of the pooled tissue sample was added with vigorous vortex mixing to an equal volume of $^{109}CdCl_2$ solution, consisting of 1 µmol Cd, and incubated on ice for 45 min. Carrier-free $^{109}CdCl_2$ (New England Nuclear Ltd.) was mixed with carrier

to produce a solution with a specific activity of 0.25 μCi/μmol Cd. To prepare a "Cd-unsaturated" cytosol for use in the metal-summation technique the remaining half of the pooled tissue sample was subjected to standard cytosol preparation procedures (described above).

Four mL of either "Cd-saturated" or "Cd-unsaturated" cytosol were applied to a 1.6 cm Sephadex G-75 column (calibrated using a low molecular weight gel filtration calibration kit from Pharmacia Fine Chemicals) and eluted with AFB at a flow rate of 25.5 ml/h. Details of column calibration are given in Appendix B. The column's void volume (Vo) equalled 72.0 mL and the total bed volume (Vt) equalled 183 mL. Thirty fractions of 7.5 mL each were collected for protein analysis according to the method of Lowry et al. (1951) and for determination of metal concentrations.

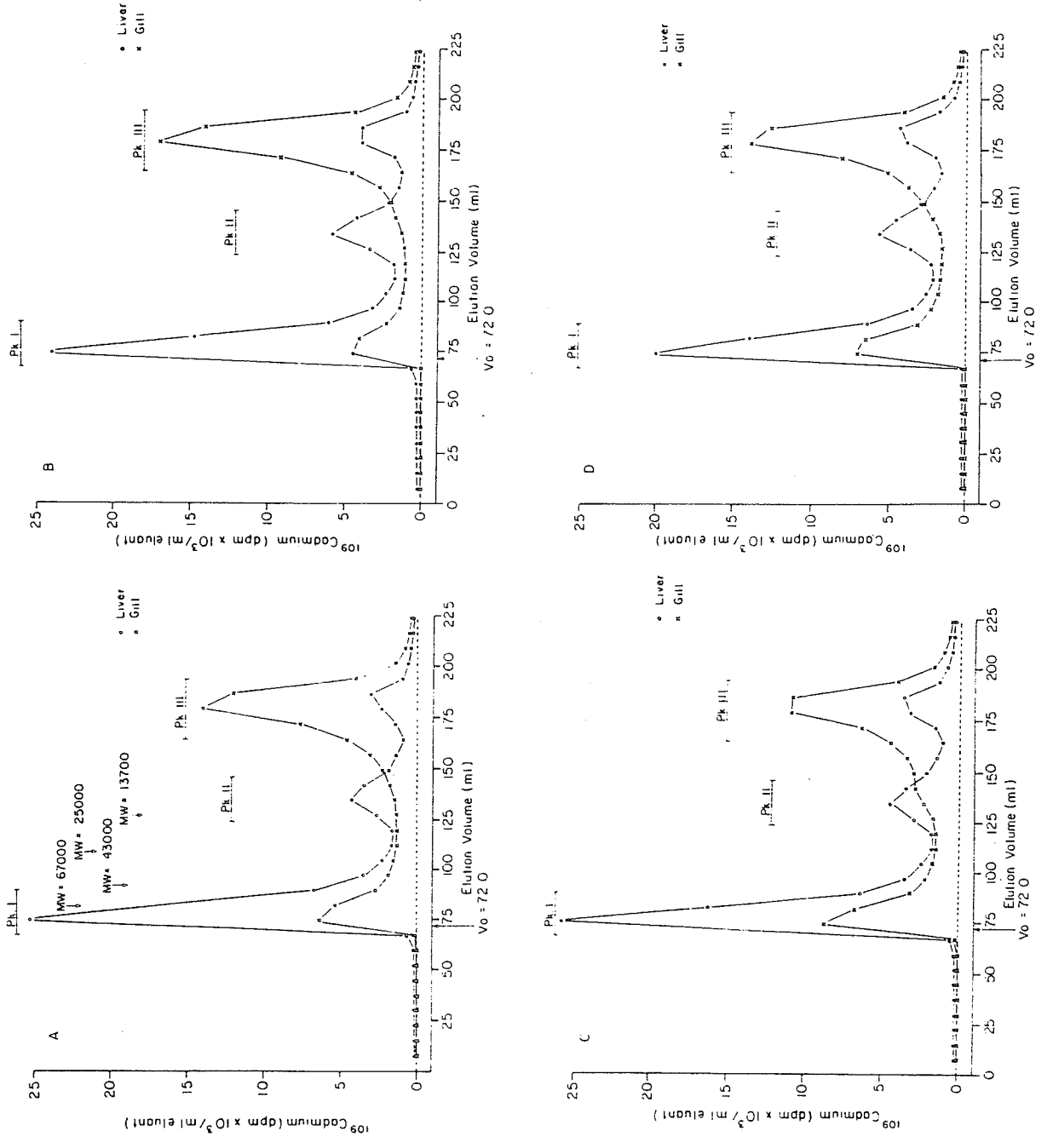
All fractions from the "Cd-saturated" cytosols were analyzed for ^{109}Cd activity using a Beckman LS7500 liquid scintillation counter. Cd concentrations in these fractions were calculated from the specific activity of the $^{109}CdCl_2$ solution. Only fractions 10-12, 17-19 and 23-26 (described as Peaks I, II, and III respectively in the Results section) from the "Cd-unsaturated" cytosol were analyzed for protein and metal concentrations. Cd and Zn concentrations in these fractions were measured using a Varian AA-5 atomic absorption spectrophotometer. Hg concentrations were measured using a Perkin Elmer 403 atomic absorption spectrophotometer. Cu concentrations were measured using a SMI III DC argon-plasma-emission spectrophotometer.

IIIC: RESULTS

IIIC 1): "Cd-saturated" Cytosol (Cd-displacement technique)

Elution profiles of "Cd-saturated" cytosol (Fig. 8A, B, C and D)

Figure 8. Elution profiles on Sephadex G-75 column of "Cd-saturated" liver (∘____∘) and gill (x____x) cytosol from control suckers (A), and suckers exposed to a sublethal concentration of Cd (B), Hg (C) or Zn (D). Tissue homogenates were incubated for 45 min in an equal volume of a 1 μmol $^{109}CdCl_2$ solution with a specific activity of 0.25 μCi/μmol Cd.

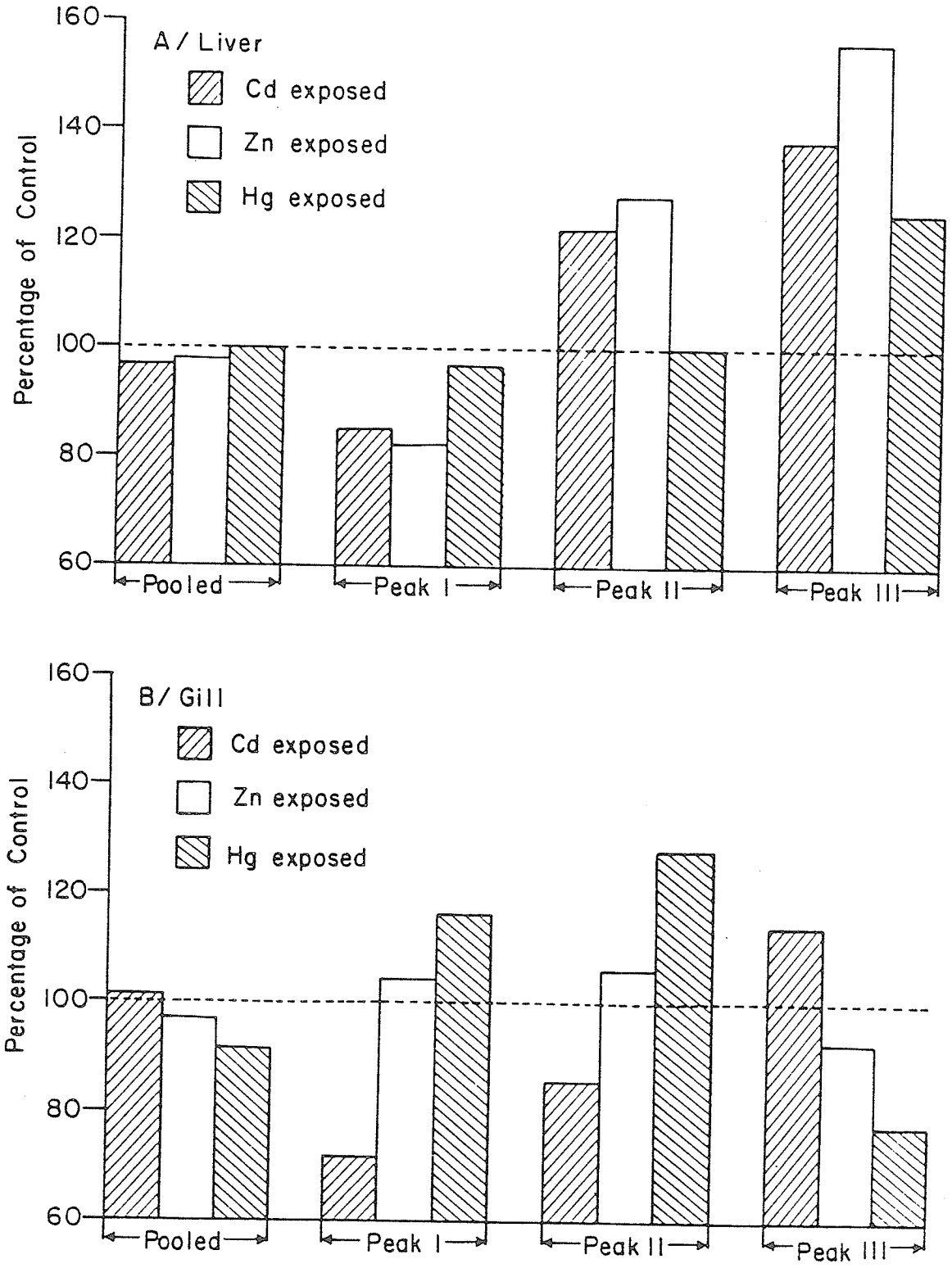

A
B
C
D
Liver
Gill
MW = 67000
MW = 25000
MW = 43000
MW = 13700
Pk I
Pk II
Pk III
Vo = 72.0
Elution Volume (ml)
109Cadmium (dpm x 10³/ml eluant)

indicate three major Cd binding fractions present in liver, and possibly gill, cytosol of C. commersoni. Peak I represents high molecular weight proteins including cellular metallo-enzymes. Peak II corresponds to metallothionein-like heavy-metal binding proteins which would include MTN. This peak exhibited substantial in vitro Cd binding in liver cytosol but was detectable only in gill cytosol from fish exposed to Hg. Peak III (the "salt fraction")represents low molecular weight cytosolic material (Brown et al. 1977) and excess metal ions. The maximum amount of ^{109}Cd in Peaks I, II, and III is eluted with substances having a molecular weight of >80 000, 10 300, and <3 000 respectively.

Liver cytosol from control suckers incorporated 1.12, 0.24 and 0.17 µmol Cd/g liver in Peaks I, II, and III, respectively, as a result of in vitro incubation with $^{109}CdCl_2$. Corresponding values for gill cytosol were 0.37, 0.12 and 0.95 µmol Cd/g gill. Cd levels incorporated by liver and gill cytosol, from metal-exposed suckers, resulting from in vitro incubation with $^{109}CdCl_2$, are presented in Appendix C.

Figure 9A illustrates the alteration of Cd concentration in liver cytosol from metal exposed suckers as compared with the Cd concentration in liver cytosol from control suckers. A 22 or 28% increase of in vitro Cd-binding was evident in Peak II from liver cytosol of suckers previously exposed to a sublethal concentration of Cd or Zn respectively. There was a concommitant 15 or 18% decrease of in vitro Cd-binding in Peak I from the liver cytosol of these suckers. Exposure of suckers to a sublethal Hg concentration had little or no effect on in vitro Cd-binding in either Peak I or II. There was a 38, 56, or 25%

Figure 9. Comparison of Cd levels in "Cd-saturated" liver (A) and gill (B) cytosol from metal-exposed suckers to levels from control suckers. Cd levels in liver cytosol from control suckers were 1.12, 0.24 and 0.17 μmol Cd/g liver liver in Peaks I, II and III, respectively. Corresponding levels in gill cytosol from control suckers were 0.37, 0.12, and 0.95 μmol Cd/g gill.


A / Liver
Cd exposed
Zn exposed
Hg exposed
Percentage of Control
160
140
120
100
80
60
Pooled
Peak I
Peak II
Peak III
B / Gill
Cd exposed
Zn exposed
Hg exposed
Percentage of Control
160
140
120
100
80
60
Pooled
Peak I
Peak II
Peak III

increase of in vitro Cd-binding in Pk III of liver cytosol from suckers exposed to Cd, Zn or Hg respectively.

Figure 9B illustrates the alteration of Cd concentration in gill cytosol from metal exposed fish as compared with the Cd concentration in gill cytosol from control suckers. Exposure of suckers to a sublethal Cd concentration resulted in a 28 and 16% decrease of in vitro Cd-binding in Peaks I and II, respectively and a corresponding increase of 14% in Pk III. Exposure of suckers to Hg resulted in a 16 and 28% increase of in vitro Cd-binding in Peaks I and II, respectively and a corresponding decrease of 22% in Pk III. There was less than a 10% alteration of in vitro Cd-binding in Peaks I, II and III of gill cytosol from suckers exposed to Zn.

IIIC 2): "Cd-unsaturated" Cytosol (metal summation technique)

Zn and Cu are the major metal constituents of liver and gill cytosol from control suckers (Table 5). Liver cytosol contains much higher metal levels than gill cytosol, consistant with the known function of liver in metabolic processes requiring metallo-enzymes. The metal content in Pk II of liver cytosol is 9.4 times the corresponding metal content of gill cytosol. Zn is the primary metal in Pk I of both liver and gill cytosol. Zn is also the predominant metal in Pk II of gill cytosol, but Cu is the primary metal in Pk II of liver cytosol. Zn and Cu content are about equal in Pk III of liver and gill cytosol. Cd and Hg are relatively minor constituents of cytosol from these organs. Zn, Cu, Cd and Hg levels in liver and gill cytosol from metal-exposed fish are presented in Appendix D.

Sublethal exposure of suckers to Cd altered metal levels in the

Table 5. Metal Levels in Liver and Gill Cytosol from Control Suckers.

Metal	Concentration (nmol/g tissue)							
	Liver				Gill			
	PkI	PkII	PkIII	Pooled	PkI	PkII	PkIII	Pooled
Zn	258	32.1	16.6	307	163	18.5	10.5	192
Cu	62.7	213	21.0	297	32.6	7.2	10.2	50.0
Cd	1.7	5.3	1.3	8.3	0.7	0.7	1.9	3.3
Hg	0.3	0.3	0.4	1.0	0.3	0.3	0.4	1.0
Sum	323	251	39.3	613	197	26.7	23.0	246

three major peaks from liver and gill cytosol (Fig. 10A and B). Increases of Cd levels which ranged from 3 times, to over an order of magnitude greater than, control levels were observed in Peaks I and II, from both liver and gill cytosol. Decreases of 74 and 49% in Zn levels were evident in Peak II from liver and gill cytosol, respectively. With the exception of PK II from liver cytosol Cu levels were increased up to a maximum, observed in Pk II of gill cytosol, of 8.4 times.

Sublethal exposure of suckers to Hg altered metal levels in the 3 major peaks from liver and gill cytosol (Fig. 11A and B). Increases of Hg levels ranging from 17.4 times to more than 2 orders of magnitude greater than control levels occurred in Peaks I and II, from both liver and gill cytosol. Concommitant with increases of Hg levels in Pk I from liver and gill cytosol were 1.7- and 3.3-fold increases, respectively, of Cd levels. A 55% decrease of Cd levels was evident in Peak II of liver cytosol. Similar to the Cd exposure, Hg exposure caused an increase (4.6-fold) of Cu and a decrease (55%) of Zn in Peak II from gill cytosol. Contrary to the Cd exposure, Hg exposure caused nearly a 3-fold increase of Zn levels in Peak II from liver cytosol.

The effects on metal distribution in liver and gill cytosol from suckers exposed to a sublethal Zn concentration are illustrated in Figure 12 (A and B). The exposure to Zn increased Zn levels in Peaks I, II and III from liver cytosol by 36, 125 and 82% respectivly. Peak II from gill cytosol exhibited a 28% reduction in Zn level but there was little change of Zn levels in Peaks I or III when compared with control levels. Similar to the Hg exposure, Zn exposure caused a 59% decrease of Cd levels in Peak II from liver cytosol but nearly a 3-fold increase

Figure 10. Comparison of metal levels in liver (A) and gill (B) cytosol from Cd-exposed (0.66 mg Cd/L) suckers with levels from control suckers. Metal levels from control suckers are given in Table 5.

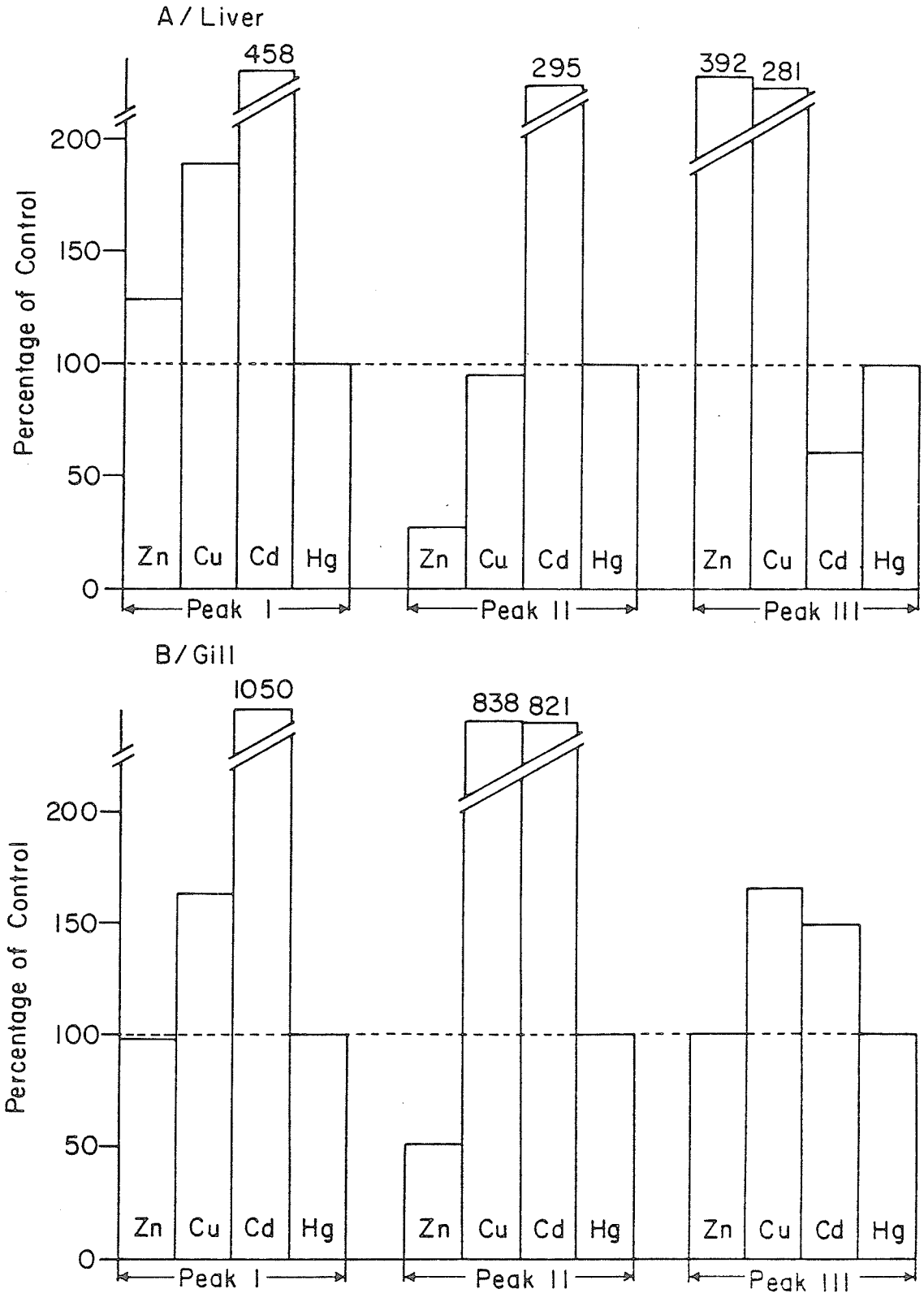

A / Liver
458
295
392
281
200
150
100
50
0
Percentage of Control
Zn
Cu
Cd
Hg
Peak I
Peak II
Peak III
B / Gill
1050
838
821
200
150
100
50
0
Percentage of Control
Zn
Cu
Cd
Hg
Peak I
Peak II
Peak III

Figure 11. Comparison of metal levels in liver (A) and gill (B) cytosol from Hg-exposed (0.19 mg Hg/L) suckers with levels from control suckers. Metal levels from control suckers are given in Table 5.

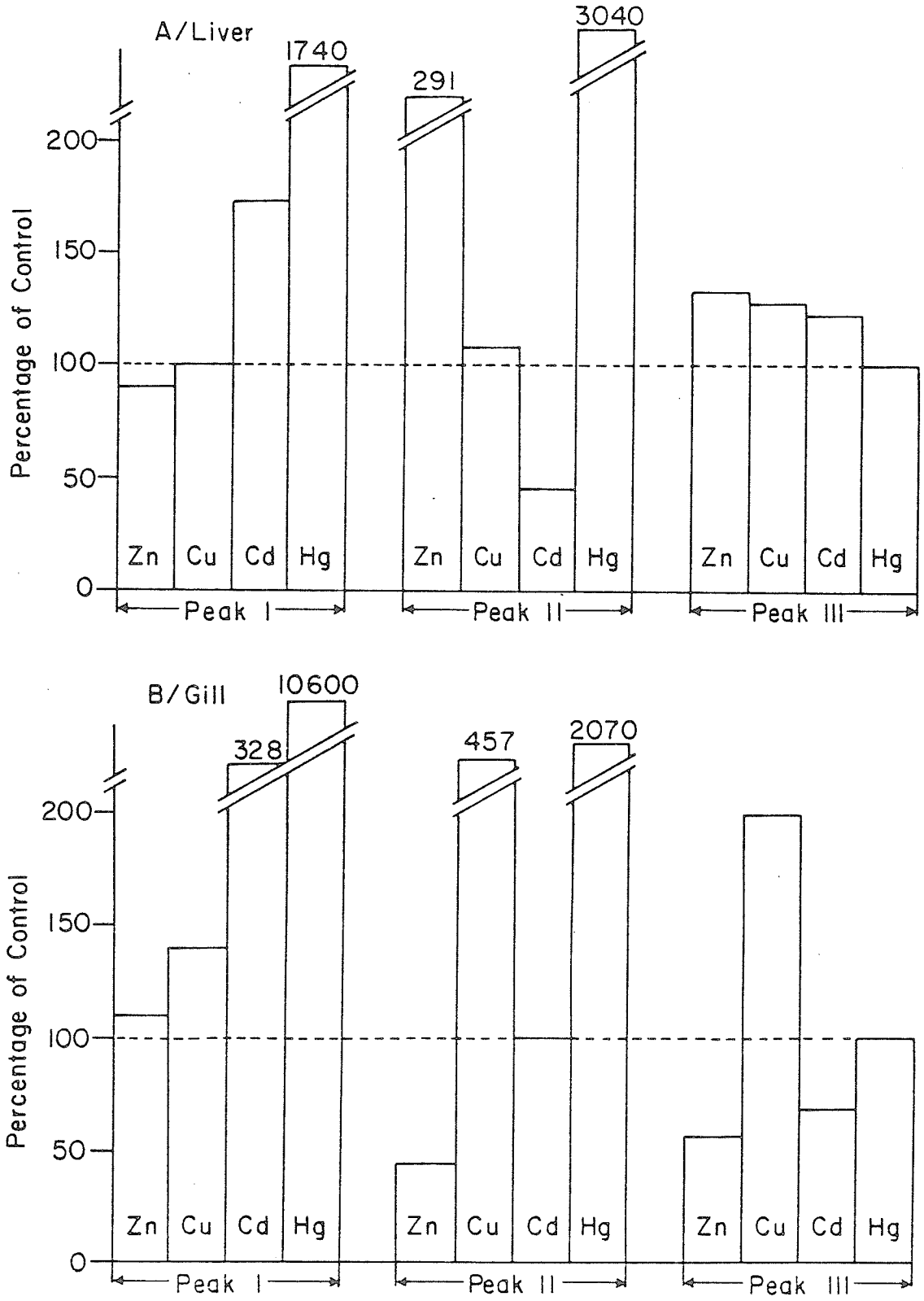
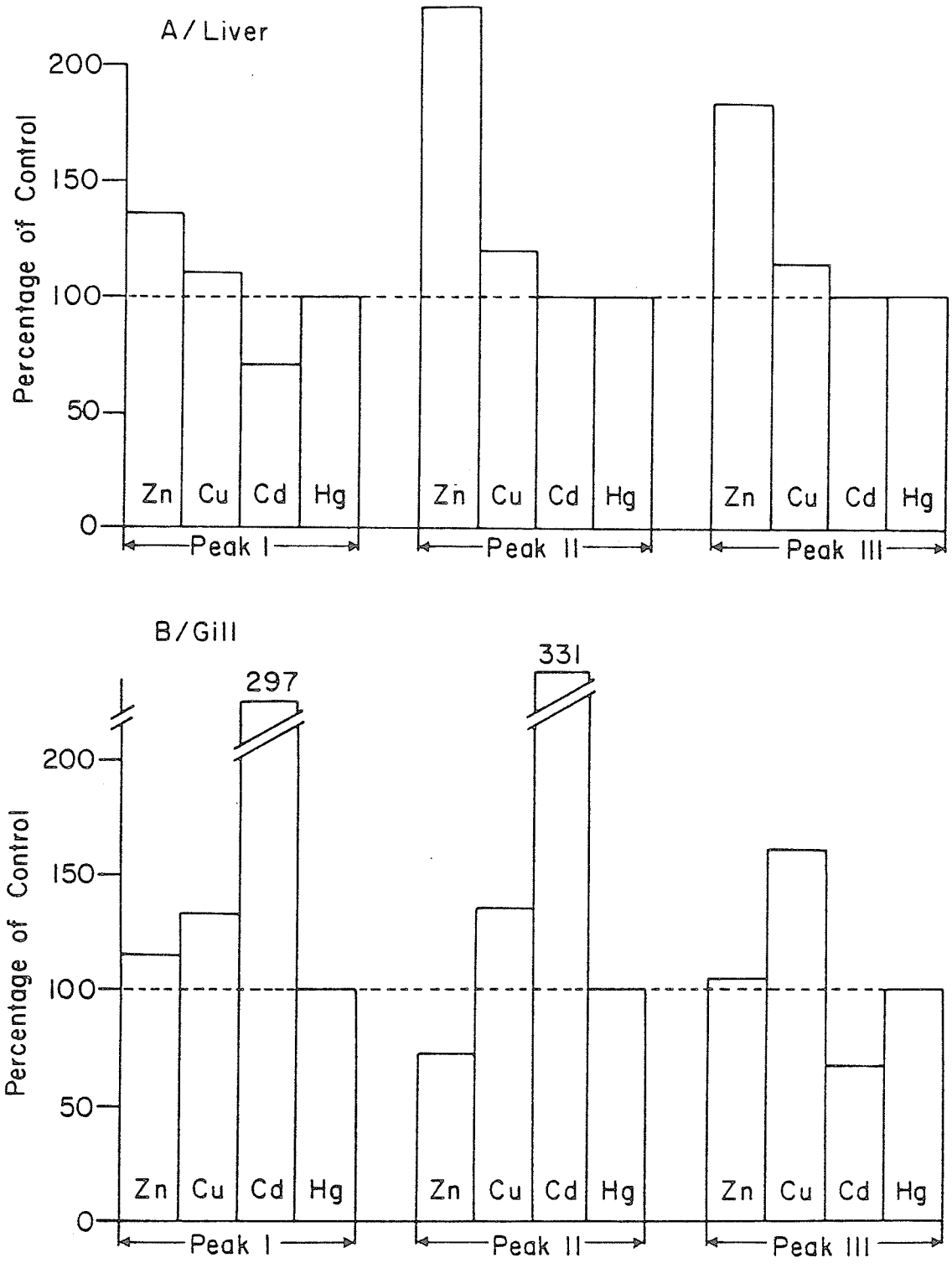

A/Liver
1740
291
3040
200
150
100
50
0
Percentage of Control
Zn
Cu
Cd
Hg
Peak I
Peak II
Peak III
B/Gill
10600
328
457
2070
200
150
100
50
0
Percentage of Control
Zn
Cu
Cd
Hg
Peak I
Peak II
Peak III

Figure 12. Comparison of metal levels in liver (A) and gill (B) cytosol from Zn-exposed (0.89 mg Zn/L) suckers with levels from control suckers. Metal levels from control suckers are given in Table 5.


A / Liver
Percentage of Control
200
150
100
50
0
Zn
Cu
Cd
Hg
Peak I
Peak II
Peak III
B/Gill
297
331
Percentage of Control
200
150
100
50
0
Zn
Cu
Cd
Hg
Peak I
Peak II
Peak III

of Cd levels in Peak I from gill cytosol. A 3.3-fold increase of Cd level was evident in Peak II of gill cytosol from suckers exposed to the sublethal Zn concentration.

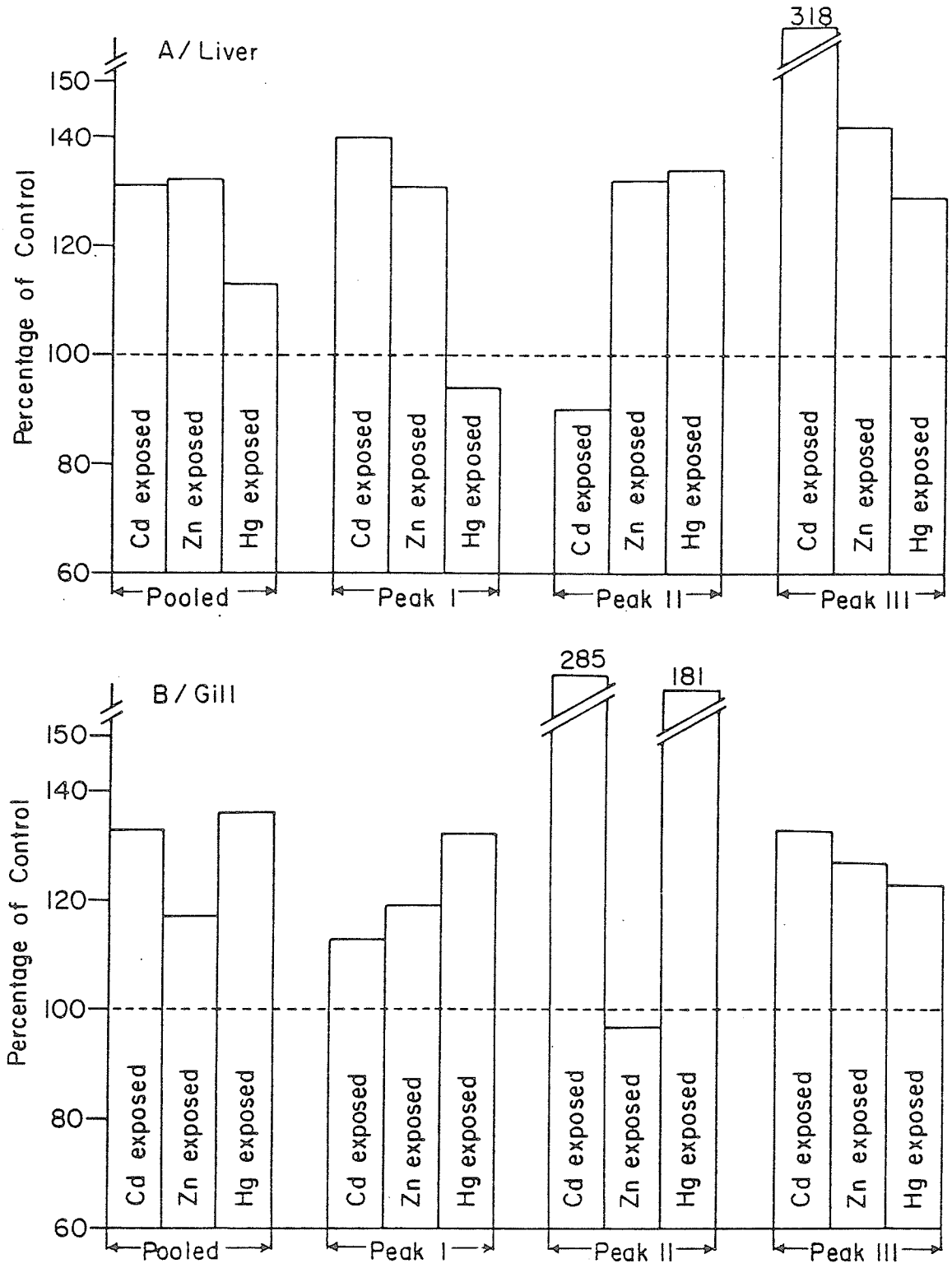
Exposure of suckers to Cd, Hg or Zn generally increased "total" metal concentrations in liver and gill cytosol (Fig. 13A and B). Largest increases of "total" metal concentrations were observed in Pk III from liver cytosol of Cd-exposed suckers and in Pk II from gill cytosol of Cd- and Hg-exposed suckers.

IIID: DISCUSSION

This investigation addressed three questions which arose from experiments which demonstrated increased resistance and/or tolerance to acute Cd toxicity in white suckers previously exposed to Cd, Hg or Zn. Emphasis was placed on detecting and quantifying MTN, an inducible protein capable of inhibiting Cd toxicity (Cherian and Goyer 1978; Kojima and Kagi 1978), with methods commonly employed in other biological systems.

The first question evaluated the applicability of Cd-displacement and metal-summation techniques in identifying and quantifying MTN in cytosol from control suckers. In liver, the Cd-displacement technique demonstrated 3 distinct ^{109}Cd-binding peaks commonly observed in mammals (Chen and Ganther 1975; Probst et al. 1977b) and other fish species (Klaverkamp et al. 1982b). The intermediate Cd-binding fraction (Pk II) had a molecular weight ranging from 8 750 to 14 500 daltons with maximum Cd-binding occurring at 10 300 daltons. This value is in close agreement with molecular weight determinations of MTN by gel filtration in other investigations (Probst et al. 1977b, Yamamoto et al. 1978;

Figure 13. Comparison of "total" metal levels (i.e. the sum of Zn, Cu, Cd and Hg levels) in liver (A) and gill (B) cytosol from metal-exposed fish with levels from control suckers. The sum of Zn, Cu, Cd and Hg levels from control fish are also presented in Table 5.


A / Liver
318
150
140
120
100
80
60
Percentage of Control
Cd exposed
Zn exposed
Hg exposed
Pooled
Peak I
Peak II
Peak III
B / Gill
285
181
150
140
120
100
80
60
Percentage of Control
Cd exposed
Zn exposed
Hg exposed
Pooled
Peak I
Peak II
Peak III

Reichert et al. 1979; Roesijadi 1980; Weidow et al. 1982). The actual molecular weight of MTN is thought to be considerably lower than this estimate due to the non-globular shape of the protein (Kaji and Nordberg 1979).

Liver cytosol was also analyzed by a metal-summation technique. Metals included for analyses were limited to Zn, Cu, Cd, and Hg, because they are the principal metals bound to MTN (Kaji and Vallee 1960; Pulido et al. 1966; Noel-Lambot et al. 1978). It is also generally accepted that there are approximately 8 g-atoms of metal per mole of MTN (Pulido et al. 1966; Wesser et al. 1973; Noel-Lambot et al. 1978; Kagi and Nordberg 1979; Overnell and Coombs 1979). Using this value, liver cytosol from control suckers contained 31.4 ηmol of MTN/g of liver. This value is within 10% of the MTN concentration reported for liver from eels (Anguilla anguilla) (Noel-Lambot et al. 1978).

The Cd-displacement technique failed to show a Cd-binding peak, corresponding to MTN, in gill filaments from control suckers. Noel-Lambot et al. (1978), using a metal-summation technique, did not observe MTN in gill cytosol from eels. In this study the metal-summation technique indicated 3.4 ηmol of MTN/g of gill in cytosol from control suckers. This value is nearly an order of magnitude less than MTN content in liver cytosol from control suckers.

Although the Cd-displacement technique requires less time to perform, it is based on the assumption that Cd added in vitro will displace all metals bound to MTN (Chen and Ganther 1975; Noel-Lambot et al. 1978). This technique may underestimate MTN content because Cd may not displace MTN-bound Cu. Investigations using other biological systems

(Pulido et al. 1966; Noel-Lambot et al. 1978; Olafson and Sims, 1979; Thompson et al. 1982) have demonstrated that the order of metal binding affinity for MTN is Hg>Cu>Cd>Zn. This order indicates than an isotope of Hg may be more suitable than one of Cd in a metal-displacement method of MTN analysis. It is interesting to note that the order of metal-MTN binding affinity for Hg, Cd and Zn is the same as the effectiveness of acclimation to these metals in reducing acute Cd toxicity.

The metal-summation technique requires more time in analyzing individual metals in each of the gel filtration fractions, but the approach is not based on assumptions of metal affinity and displacement. While the increases in MTN estimates obtained using this technique are generally thought to indicate induced MTN synthesis (Olafson and Thompson 1974; Marafante 1976; Cherian and Goyer 1978; Talbot and Magee 1978), the site of this synthesis has not been determined (Johnson and Foulkes 1980), as it may occur elsewhere in the organism with subsequent MTN transport to a different organ. In general, this technique probably provides a more accurate estimation of MTN concentration and gives considerable insight into metal distributions and interactions. The metal-summation technique should be the method of choice for investigations on the role of metal-binding proteins, such as MTN, in acclimation to Cd toxicity.

The second question considered in this study was on the effects of sublethal metal exposure on the partitioning of metals in gill and liver cytosol from C. commersoni. For obvious reasons, the metal-summation technique provided the information required for investigation of metal interactions, whereas the Cd-displacement technique lacked such

provision. Pk II (MTN) in gill cytosol from fish exposed to sublethal Cd, Zn or Hg concentrations exhibited decreased Zn and increased Cu levels. In liver cytosol, however, the MTN fraction exhibited decreased Zn levels only in Cd-exposed fish and little or no change of Cu levels in any of the sublethal exposures. Cd and Hg levels were elevated in Pk II of both gill and liver cytosol from suckers exposed to these 2 metals. Surprisingly, gill cytosol from Zn-exposed fish had decreasd Zn and increased Cd levels in Pk II, while liver cytosol from these fish exhibited increased Zn and decreased Cd levels in Pk II. It is interesting to note that although Cd generally displaces Zn in the MTN fraction (Pulido et al. 1966; Cherian and Goyer 1978; Olafson and Sim 1979; Thompson et al. 1982) and that Pk II of liver cytosol from Hg-exposed suckers had elevated Zn levels, the Cd-displacement technique did not demonstrate increased Cd-binding in this fraction from Hg-exposed fish.

Differences in metal interactions observed between organs and metals may result from differences in forms of MTN or metal-MTN binding affinities, from metal-binding proteins other than MTN, or from a combination of these factors. Polymorphism of MTN has been reported in humans (Pulido et al. 1966), horses (Kagi et al. 1974), rabbits (Nordberg et al. 1972), rats (Shaikh and Lucis 1971; Kimura et al. 1974; Winge et al. 1975a), fish (Noel-Lambot et al. 1978), crustacea (Olafson et al. 1979; Weidow et al. 1982) and oligochaetes (Thompson et al. 1982). While it is apparent that sub-cellular Cu dynamics were altered by exposure to Cd, Hg, and Zn, the reasons for these changes are unknown. An inducible Cu binding protein having a molecular weight of approximately 8 000 daltons has been described as Cu-chelatin in recent investigations

(Premakumar et al. 1975; Winge et al. 1975b; Brady et al. 1979; Roesijadi 1980). Differences between Cu-chelatin and MTN are evident in amino acid analyses, and ion-exchange chromatography (Winge et al. 1975b; Kagi and Nordberg 1979). In clams, Cu was associated with two low molecular weight proteins, one similar to MTN and the other to Cu-chelatin (Roesijadi 1980).

Table 6 presents a summary of the metal interactions in liver and gill cytosol from suckers exposed to sublethal concentrations of Cd, Zn or Hg. The limits representing an increase or decrease were arbitrarily set at ±25% of control values. It is apparent that redistribution of metals was not limited to Pk II, but occurred in all 3 peaks. The increases of metal levels in Pk I from metal-exposed fish is generally thought to indicate a "spillover" of metals from MTN to larger molecular weight enzymes and the onset of toxicological damage (Brown et al. 1977; Brown and Parsons 1978; Engel and Fowler 1979). Much of the support for the idea of a "spillover" comes from studies in which tissue homogenates were subjected to heat which denatured the high molecular weight proteins in Pk I and reduced metal content of this fraction (Winge et al. 1975b) but did not alter the MTN fraction which is heat-stable. Subjecting tissue homogenates to heat treatment would seem to be inappropriate if metal distribution between the various cytosolic fractions, other than MTN, are to be investigated.

The third question asked whether sublethal Cd, Hg or Zn exposures produced increased MTN concentrations in gill and/or liver cytosol. Using the Cd-displacement technique, exposure of suckers to Cd or Zn produced increased ^{109}Cd-binding in Pk II of liver cytosol (Fig. 9A),

Table 6. Metal Interactions in Liver and Gill Cytosol

The redistribution of metals in liver and gill cytosol from suckers acclimated to a sublethal concentration of Cd, Zn, or Hg for 1 wk. Increases in metal levels of 1.25 to 2.49, 2.50 to 10.0, and >10.0 times metal levels in control suckers are represented by "↑", "↑↑" and "↑↑↑" respectively. Similarly, decreases of 0.75 to 0.50 and 0.49 to 0.25 times control levels are represented by "↓" and "↓↓" respectively. Metal levels in exposed-suckers which ranged from 0.76 to 1.24 times metal levels in control suckers were designated as unchanged and represented by "-".

Acclimation Metal	Fraction	Shifts in Metal Levels									
		Liver					Gill				
		Zn	Cu	Cd	Hg	Sum	Zn	Cu	Cd	Hg	Sum
Cadmium	Pk I	↑	↑	↑↑	-	↑	-	↑	↑↑↑	-	-
	Pk II	↓↓	-	↑↑	-	-	↓	↑↑	↑↑	-	↑↑
	Pk III	↑↑	↑↑	↓	-	↑↑	-	↑	↑	-	↑
	Pooled	↑	↑	↑↑	-	↑	-	↑↑	↑↑	-	↑
Zinc	Pk I	↑	-	↓	-	↑	-	↑	↑↑	-	-
	Pk II	↑	-	↓↓	-	↑	↓	↑	↑↑	-	-
	Pk III	↑	-	-	-	↑	-	↑	↓	-	↑
	Pooled	↑	-	↓	-	↑	-	↑	↑	-	-
Mercury	Pk I	-	-	↑	↑↑↑	-	-	↑	↑↑	↑↑↑	↑
	Pk II	↑↑	-	↓↓	↑↑↑	↑	↓↓	↑↑	-	↑↑↑	↑
	Pk III	↑	↑	-	-	↑	↓	↑	↑	-	-
	Pooled	-	-	-	↑↑↑	-	-	↑	↓	↑↑↑	↑

and Hg exposure produced a corresponding increase in gill cytosol (Fig. 9B). Exposures to Hg and to Zn increased estimates of MTN (calculated from the metal-summation technique) in liver cytosol (Fig. 13A), and Hg and Cd exposures increased these estimates of MTN in gill cytosol (Fig. 13B).

Although preliminary in nature, this study provides considerable information on metal-binding characteristics of liver and gill cytosol from C. commersoni. Increased MTN production may be involved in acclimation to acute Cd toxicity by suckers, but additional research is required to understand critical organs and fundamental processes of Cd toxicology in fish and to correlate MTN induction to inhibition of adverse effects produced by Cd. The substantial shifts of Cd, Zn, Hg and Cu levels in liver and gill cytosol from metal-exposed fish exemplify the importance of multi-element analyses and of examining various organs. Since Zn and Cu are essential elements for many metallo-enzymes (Friedberg 1974; Vouk 1979), the biochemical consequences of metal interactions warrant further investigation. This study illustrates the need for specific investigations on MTN polymorphism, metal-MTN binding affinities, and the role of other metal-binding proteins, such as Cu-chelatin, in inhibiting Cd toxicity.

CHAPTER IV

General Summary

GENERAL SUMMARY

1. The order of acute metal toxicity to C. commersoni was Hg>Cd>Zn>>Se.

2. Tolerance and resistance to acute Cd toxicity by white suckers was increased by sublethal exposure to sufficiently elevated Cd or Hg concentrations, but only resistance was increased by sublethal Zn exposure. Sublethal exposure of suckers to Se had little or no effect on survival times of these fish during subsequent Cd toxicity tests.

3. Extending the sublethal Cd exposures from 1 to 2 wk did not provide suckers with any further protection from acute toxicity during Cd toxicity tests.

4. A metal-summation technique is more appropriate than a Cd-displacement technique for investigating the role of metal-binding proteins, such as MTN, in acclimation to Cd toxicity.

5. Sublethal exposure of C. commersoni to Cd, Hg, or Zn resulted in substantial redistribution of metals in liver and gill cytosol. With the exceptions of Pk II and Pk I from fish exposed to Cd and Hg, respectively, Zn levels in liver cytosol from metal-exposed fish were increased. With the exception of Pk II from Hg-exposed suckers, Cd levels were increased in gill cytosol from suckers exposed to Cd, Zn or Hg. These sublethal metal exposures also increased Cu levels in peaks I, II and III of gill cytosol.

6. The in vitro Cd-binding capacity of Pk II was increased (relative to control values) in liver cytosol from Cd- or Zn-exposed suckers and also in gill cytosol from Hg-exposed suckers.

7. MTN levels, estimated from the sum of Zn, Cu, Cd and Hg levels

in Pk II, increased (relative to levels from control suckers) in liver cytosol from Zn- or Hg-exposed suckers and also in gill cytosol from fish exposed to a sublethal Cd or Hg concentration.

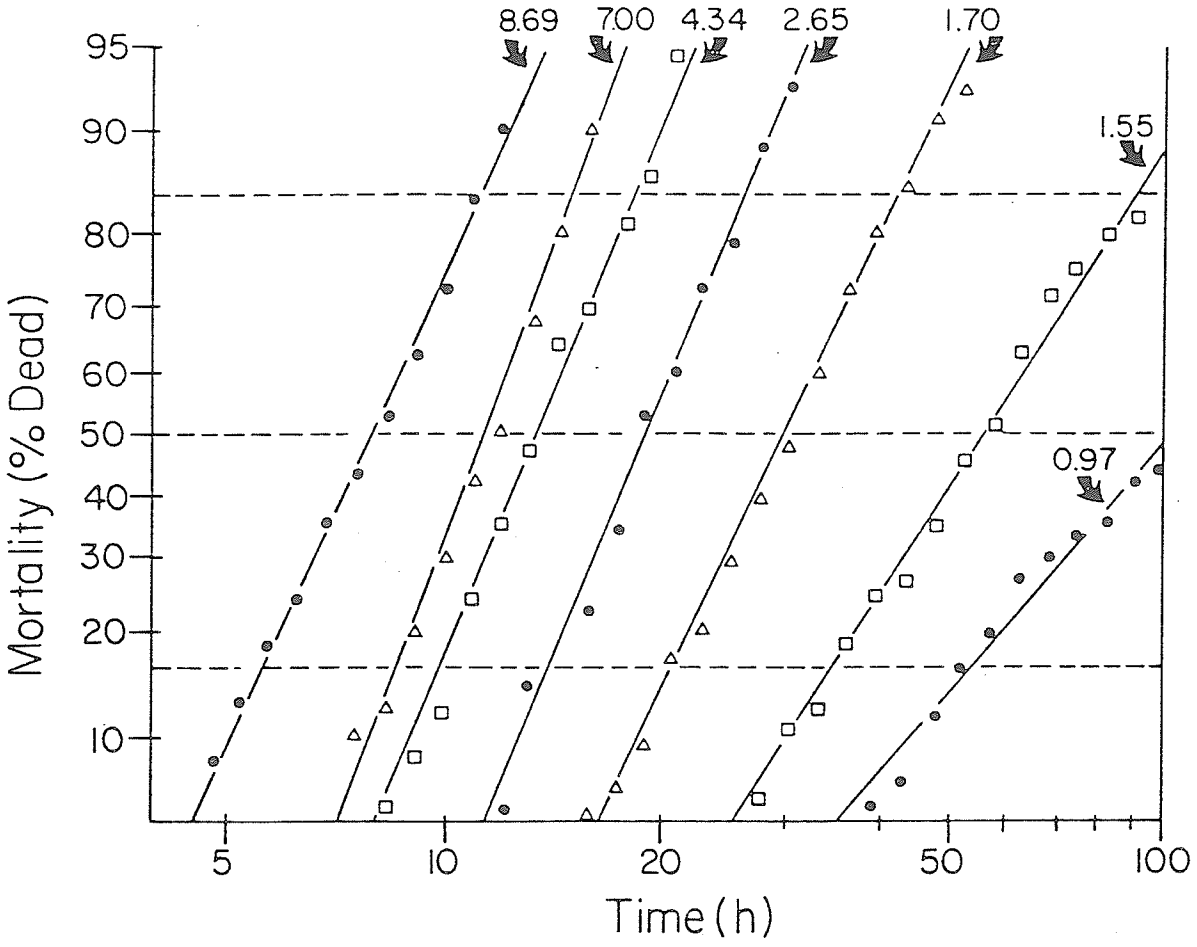
APPENDIX A
Application of Litchfield's (1949) Method to Experimental Data from Control Suckers

The cumulative per cent mortality is plotted against time on logarithmic probability paper for each Cd test concentration and lines are fitted to these data with a straight edge (Fig. 14). MST values equal the time at which the line intercepts 50% mortality. The Slope Function (S) is equivalent to the standard deviation and is calculated according to the formula: $S = [(ET_{84}/MST)+(MST/ET_{16})]:2$, where ET_{84} and ET_{16} signify the time at which the line intercepts 84 and 16% mortality respectively. The upper and lower 95% confidence limits for parameters are derived by multiplying and dividing, respectively, the MST and S values by a factor (f) obtained by means of a nomograph presented in Litchfield (1949). the MST and S values, with their 95% CI, for control suckers in Cd toxicity tests are given in Table 7.

Table 7. Median Survival Time (MST) and Slope Function (S) for Control Suckers in Cd Toxicity Tests

Test Concentration (mg Cd/L)	MST	95% CI	S	95% CI
8.69	7.75	7.16- 8.39	1.41	1.33-1.49
7.00	11.2	10.4 -12.0	1.31	1.24-1.38
4.34	13.5	12.8 -14.2	1.36	1.30-1.41
2.65	19.2	17.9 -20.7	1.36	1.29-1.43
1.70	29.7	27.8 -31.6	1.43	1.36-1.49
1.55	55.4	48.4 -63.5	1.61	1.44-1.80
0.97	>100	- - -	-	- - -
0.67	>100	- - -	-	- - -
Control	>100	- - -	-	- - -

Figure 14. Resistance times of control suckers in various lethal cadmium concentrations. Lethal concentrations, indicated in body of graph, are in mg Cd/L.


8.69
7.00
4.34
2.65
1.70
1.55
0.97
95
90
80
70
60
50
40
30
20
10
Mortality (% Dead)
5
10
20
50
100
Time (h)

APPENDIX B

Calibration of Gel Chromatography Column

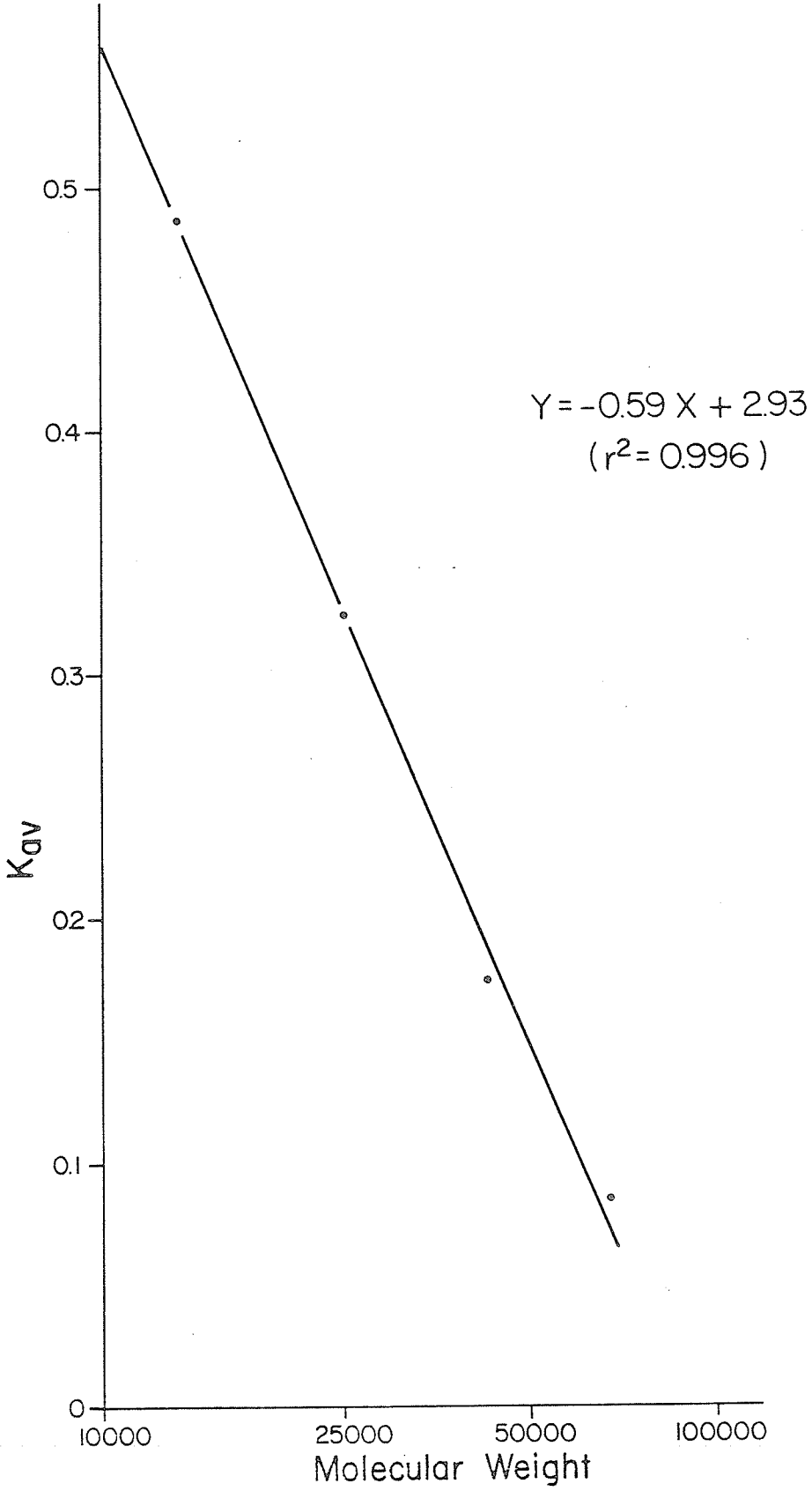
The gel chromatography column used in these experiments was calibrated with 4 low molecular weight (LMW) proteins (supplied by Pharmacia Fine Chemicals). Chromatographic properties of the LMW proteins are presented in Table 8. A calibration curve was prepared by plotting Kav values of the LMW proteins versus the logarithm of their molecular weights (Fig. 15). The regression equation for the calibration curve was $Y = -0.59X + 2.93$, where Y = Kav value and X = logarithm of molecular weight ($r^2 = 0.996$ and $n = 4$). Calculated Kav values of unknown substances are used to determine their molecular weight from the calibration curve.

Table 8. Chromatographic Properties of LMW Proteins

Protein	Molecular Weights	Ve (ml)	Ve/Vo	Kav
Albumin	67 000	81.4	1.13	0.084
Ovalbumin	43 000	91.4	1.27	0.174
Chymotrypsinogen A	25 000	107.8	1.50	0.323
Ribonuclease A	13 700	126.0	1.75	0.487

Where: Ve = elution volume; Kav = (Ve-Vo)/(Vt-Vo); Vo = column's void volume= Ve for Blue Dextran 2000 = 72 mL; Vt = total bed volume of column

Figure 15. Molecular weight calibration curve for gel chromatography column. Standard proteins were Albumin, Ovalbumin, Chymotrypsinogen A and Ribonuclease A. Y=Kav and X=log molecular weight.


0.5
0.4
0.3
0.2
0.1
0
K_{av}
Y = -0.59 X + 2.93
(r^2 = 0.996)
10000
25000
50000
100000
Molecular Weight

APPENDIX C

Cd Levels in "Cd-saturated" Cytosol of Liver and Gill from *C. commersoni*

The amount of Cd incorporated from the *in vitro* $^{109}CdCl_2$ incubation by liver and gill cytosol was dependant upon previous metal exposure (Table 9). Cdconcentrations were calculated using the specific activity of the $^{109}CdCl_2$ solution (0.25 µCi/µmol Cd).

Table 9. Cd Levels in "Cd-saturated" Cytosol

Acclimation Metal	Cd Concentration (µmol/g tissue)							
	Liver				Gill			
	PkI	PkII	PkIII	Pooled	PkI	PkII	PkIII	Pooled
Cd	0.95	0.29	0.24	1.48	0.26	0.10	1.09	1.46
Zn	0.92	0.31	0.26	1.49	0.38	0.13	0.88	1.39
Hg	1.08	0.24	0.21	1.53	0.43	0.15	0.74	1.32
Control	1.12	0.24	0.17	1.53	0.37	0.12	0.95	1.44

APPENDIX D

Metal Levels in "Cd-unsaturated" Cytosol of Liver and Gill from C. commersoni.

Zn, Cu, Cd and Hg concentrations in liver and gill cytosol was dependant uponprevious metal exposure (Table 10). With the exception of Pk II from liver and gill cytosol of Cd- and Zn-exposed suckers, respectively, sublethal metal exposure increased the sum of metal levels.

Table 10. Metal Levels in "Cd-unsaturated" Liver and Gill Cytosol.

Accli- mation metal	Cytosol Fraction	Metal Concentration (nmol/g tissue)									
		Liver					Gill				
		Zn	Cu	Cd	Hg	Sum	Zn	Cu	Cd	Hg	Sum
Cd	PkI	327	117	7.65	0.32	452	160	53.0	7.66	0.32	221
	PkII	8.24	202	15.6	0.32	227	9.46	60.5	5.97	0.33	76.3
	PkIII	65.1	59.1	0.79	0.43	125	10.4	16.9	2.87	0.43	30.7
	Pooled	400	378	24.0	1.08	804	180	130	16.5	1.08	328
Zn	PkI	350	69.2	1.18	0.32	421	187	43.5	2.16	0.32	233
	PkII	72.3	256	2.16	0.32	331	13.3	9.81	2.41	0.32	25.8
	PkIII	30.2	23.8	1.29	0.43	55.7	11.0	16.4	1.30	0.43	29.2
	Pooled	453	349	4.63	1.08	808	211	69.7	5.87	1.08	288
Hg	PkI	232	62.8	2.88	5.63	303	177	45.2	2.39	34.2	259
	PkII	93.4	231	2.40	9.83	337	8.21	33.0	0.72	6.68	48.6
	PkIII	22.0	26.7	1.59	0.43	50.8	6.06	20.5	1.30	0.43	28.3
	Pooled	347	321	6.87	15.9	691	191	98.7	4.42	41.3	335
Control	PkI	258	62.7	1.67	0.32	322	163	32.6	0.73	0.32	196
	PkII	32.1	213	5.28	0.32	251	18.5	7.22	0.73	0.32	26.8
	PkIII	16.6	21.0	1.30	0.43	39.3	10.5	10.2	1.91	0.43	23.0
	Pooled	307	297	8.25	1.08	612	192	50.2	3.33	1.08	246

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