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Plasma clearance of cadmium and zinc in non-acclimated and metal-acclimated trout

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Abstract

Adult rainbow trout were pre-exposed to a sublethal concentration of waterborne cadmium (Cd, 26.7 nmol/l) or waterborne zinc (Zn, 2294 nmol/l) for 30 days to induce acclimation. A single dose of radiolabeled Cd (64.4 nmol/kg) or Zn (183.8 nmol/kg) was injected into the vascular system of non-acclimated and Cd- or Zn-acclimated trout through indwelling arterial catheters. Subsequently, repetitive blood samples over 10 h and terminal tissue samples (liver, heart, bile, stomach, intestine, kidney, gills, muscle, and spleen) were taken to characterize the effect of metal acclimation on clearance kinetics in vivo. Plasma clearance of Cd in Cd-acclimated fish (0.726 ± 0.015 and 0.477 ± 0.012 ml/min per kg for total and newly accumulated Cd, respectively), was faster than that in non-acclimated trout (0.493 ± 0.013 and 0.394 ± 0.009 ml/min per kg). Unlike plasma Cd, the levels of Cd in red blood cells (RBCs) were 1.2–2.2 times higher in Cd-acclimated fish than in non-acclimated fish. At 10 h post-injection, the liver accumulated the highest proportion ($\sim$ 22%) of the injected Cd dose in both non-acclimated and Cd-acclimated fish but did not account for the difference in plasma levels of Cd between two groups. Plasma clearance of Zn (~ 0.23 ml/min per kg for new Zn) was substantially lower than Cd clearance. Pre-acclimation to waterborne Zn reduced the new Zn levels in RBCs, but did not affect the clearance of Zn from blood plasma or tissue burdens of Zn in fish. Bile concentrations of both Cd and Zn were elevated in acclimated fish, but total bile burden accounted for $< 1\%$ of the injected metal dose. The results suggest that the detoxification process of injected plasma Cd is stimulated by pre-acclimation to waterborne Cd, and that Zn levels are homeostatically controlled in both non-acclimated and acclimated trout.

Keywords: Waterborne; Homeostasis; Chronic; Kinetics; Red blood cells

1. Introduction

Cadmium (Cd) and zinc (Zn) are trace elements that are common constituents of industrial effluents. Cd is a non-nutrient metal and toxic to fish even at low concentrations, while Zn is a micronutrient with an active role in many enzyme systems, yet toxic to aquatic organisms at high

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concentrations (Sorensen, 1991; Vallee and Falchuk, 1993; Wood, 2001). The essential need for Zn has led to an ability of fish to regulate Zn levels in the body, although the actual mechanisms for Zn homeostasis are not well understood (see Hogstrand and Wood, 1996 for review). Cd accumulates in sensitive organs like gills, liver, and kidney of fish in an unregulated manner (Brown et al., 1986; Wicklund Glynn, 1990; McGeer et al., 2000b). Zn is homeostatically regulated in the liver and kidney, and found to accumulate mostly in the skin, muscle and bone of fish (Wicklund Glynn, 1991).

Upon uptake, the delivery of metals to internal organs occurs as a result of metal transport via the blood. The blood plasma contains diverse proteins (Scott and Bradwell, 1984; Golaz et al., 1993) which participate in metal binding and subsequent transport to internal organs for utilization, storage, and excretion (see Roesijadi and Robinson, 1994 for review). Given that the dynamics of internalized metals are a part of metal regulation and detoxification, it seems likely that they may be influenced by physiological responses of animals for acclimation during chronic exposure to both nutrient and non-nutrient metals. As postulated by McDonald and Wood (1993), the general responses for acclimation result in an increased tolerance of fish to metals through a "damage-repair mechanism." For example, there is a disruption of ion regulation immediately after exposure to Cd, Cu, or Zn, followed by a restoration during prolonged exposure (McGeer et al., 2000a). The primary site for immediate damage is thought to be the gill, while the internal mobilization of metal binding proteins such as metallothioneins (MT) may be an important factor in the restoration phase through detoxification and storage of metal in tissues (Hogstrand and Huax, 1991). Indeed in mammals, the Cd–MT complex is released from the liver and redistributed to the kidney through the blood circulatory system for long-term storage (Nordberg and Nordberg, 1987). Similarly, Cu-containing ceruloplasmin synthesized in the mammalian liver is released to the blood to provide Cu to extrahepatic tissues (Linder and Hazegh-Azam, 1996).

To understand the mechanisms of metal homeostasis and toxicity in fish, the effect of acclimation to waterborne Cd, Zn, and Cu on gill binding, ion regulation, uptake and endocrine function of these metals has been studied recently (Ricard et al., 1998; Alsop et al., 1999; Hollis et al., 1999, 2000; McGeer et al., 2000a,b; Taylor et al., 2000). In general, a significant effect of acclimation on physiological status of fish and tolerance to toxic metals has been observed. For example, a recent study with rainbow trout revealed that plasma Cu clearance and biliary Cu excretion are stimulated in Cu-acclimated fish compared with non-acclimated fish, probably as a strategy for Cu regulation by fish (Grosell et al., 2001).

To date, no studies have been conducted to investigate the effect of Cd or Zn acclimation on the plasma clearance of the metals in fish. Clearance may be interpreted as a volume of reference fluid (e.g. plasma) cleared of the chemical per unit time; the general process of modeling of the pharmacokinetics of chemicals in aquatic animals is reviewed by Barron et al. (1990). In mammals, clearance kinetics of Cd and Zn following intravascular injection have been described by Klaassen and Kotsonis (1977) and Frazier (1980), and in fish by Schultz et al. (1996) and Barron et al. (2000).

In the present study, we characterized the plasma clearance of Cd and Zn in rainbow trout fitted with dorsal aortic catheters for repetitive blood sampling. The fish were either chronically pre-exposed or not-pre-exposed to sublethal water levels of the metal of interest in order to reveal the effect of metal acclimation on clearance kinetics. Cd and Zn were selected to compare acclimation effects between a non-nutrient and a nutrient metal. Metals were injected as radiolabeled cationic salts into the vascular system through the indwelling aortic catheter. Before injection, fish were exposed to waterborne non-radioactive metal for 30 days at a level (3 μg Cd/l or 150 μg Zn/l) used before in our laboratory to induce acclimation (Hogstrand et al., 1995; Galvez et al., 1998; Hollis et al., 1999; McGeer et al., 2000a,b). The metal concentration–time profiles of plasma and red blood cells (RBCs) were analyzed with an exponential decline function and a two-compart-

ment model as appropriate. Tissue samples were taken to follow the disposition of metal cleared from plasma.

2. Materials and methods

2.1. Experimental fish

Adult rainbow trout *Oncorhynchus mykiss* weighing between 275 and 455 g (370 ± 50 g; $n = 70$) were used in all experiments. They were obtained from Humber Springs Trout Hatchery (Mono Mills, Ont., Canada) and acclimated to laboratory conditions for at least 2 weeks before experimental use. Fish were held in 500-l tanks, each supplied with a minimum of 2.5 l/min flow-through of moderately hard dechlorinated Hamilton City tapwater (0.3 nM or 0.033 μg Cd; 47 nM or 2.98 μg Cu; 270 nM or 17.65 μg Zn; 1 mM Ca; 0.6 mM Na; 0.7 mM Cl; hardness: 120–140 mg/l as $CaCO_3$). The water temperature and pH were kept at 12 ± 1 °C and 8.0 ± 0.2, respectively, throughout acclimation and subsequent procedures. The fish were fed dry, floating trout pellets (5 point, Martin's Feed Mills, Ont., Canada) at a ration of 1.5% of their body weight three times per week. The fish were not fed for 48 h prior to surgery.

2.2. Acclimation to cadmium and zinc

For acclimation, fish were exposed to Cd and Zn separately for 1 month. Fish from the main holding tanks were divided into four groups, each placed in a separate 300-l tank ($n = 25$). Water flow to each tank was maintained at approximately 1.8 l/min throughout the acclimation period. Two tanks were designated for Cd (one control tank and one "Cd-acclimation" tank) and the other two for Zn (one control tank and one "Zn-acclimation" tank). The Cd-acclimation and Zn-acclimation tanks received water from a head tank via one of two mixing chambers. The stock solutions of either $Cd(NO_3)_2 \cdot 4H_2O$ (48.3 mg/l; Fisher Scientific, Ont., Canada) or $ZnSO_4 \cdot 7H_2O$ (1.4 g/l; Anachemia, Montreal, Quebec, Canada) were acidified with HNO_3 (0.1%; trace metal analysis grade; BDH Chemicals, Toronto, Ont., Canada) and delivered to the mixing chambers by a separate Mariotte bottle so as to achieve a nominal Cd concentration of 26.7 nmol/l (3 μg/l) in the Cd-acclimation tank and a nominal Zn concentration of 2294 nmol/l (150 μg/l) in the Zn-acclimation tank. Water flow and dosing rates were monitored daily and adjusted if necessary. Water samples were taken every second day, acidified with HNO_3 (1%), and analyzed for Cd and Zn levels in water against certified standards (Fisher Scientific) by graphite or flame atomic absorption spectroscopy (Varian, SpectrAA-220, Mississauga, Ont., Canada). Actual measured concentrations were close to nominal in the experimental tanks (Cd: 30.7 ± 5.3 nmol/l, $n = 20$); Zn: 2403 ± 410 nmol/l, $n = 15$). Background concentrations in the control tanks remained low (Cd: 1.3 ± 0.3 nmol/l, $n = 10$; Zn: 288 ± 35 nmol/l, $n = 10$).

2.3. Cannulation

After being exposed for 1 month, 5–8 fish from each group were cannulated via the dorsal aorta for intravascular injection of Cd or Zn and for blood sampling. For cannulation, fish were anaesthetized with neutralized MS-222 (0.1 g/l) and placed on a surgery table, where the gills were continuously irrigated with water. A catheter of PE-50 polyethylene tubing (Intramedic, Becton Dickinson, Sparks, MD, USA) was implanted into the dorsal aorta as described by Soivio et al. (1972) and filled with heparinized (50 USP units/ml, Na-heparin, Sigma, St. Louis, MO, USA) Cortland saline (Wolf, 1963). After cannulation, each fish was placed in an aerated experimental-chamber (the "fish box"; 2.5 l) supplied with flowing water (250 ml/min) and allowed to recover from surgery for at least 36 h. For Cd- and Zn-acclimated fish, the same metal concentrations as used for acclimation were still maintained in the flowing water to ensure constant exposure to Cd or Zn even during surgery and the recovery period.

2.4. Experimental procedure

After recovery from surgery, an initial control blood sample (200 μl) was obtained via the dorsal aorta catheter and replaced with Cortland saline (Wolf, 1963). The waterborne Cd or Zn exposure of the metal acclimated groups was then terminated to ensure that the only difference between non-acclimated and metal-acclimated fish after this point of time was the metal acclimation. The fish box was closed while aeration was maintained, and the volume was set to a known value (2.5 l) with control water. Fish were immediately injected intravascularly with radioactive Cd ($^{109}CdCl_2$ in 0.5M HCl; specific activity: 116.6 kBq/μg; NEN Life Science Products, Boston, MA, USA) or Zn ($^{65}ZnCl_2$ in 0.5M HCl; specific activity: 77.4 kBq/μg; NEN Life Science Products). The ^{109}Cd and ^{65}Zn solutions were diluted in 140 mM NaCl solution and adjusted to a pH of 7.8 by gradual addition of NaOH to obtain a final concentration of 1850 kBq/ml (145.6 nmol total Cd/ml; 367.7 nmol total Zn/ml). The fish were injected with 0.5 ml/kg of the radiolabeled NaCl solution using a Hamilton syringe (0.5 ml), resulting in doses of 64.4 nmol/kg Cd and 183.8 nmol/kg Zn.

Blood samples were drawn according to the method described by Grosell et al. (2001) to determine the plasma clearance of Cd and Zn. Approximately 300 μl of blood was removed repeatedly via the catheter at 10, 20, 40, 60, 90, 120, 180, 300, 420, and 600 min after injection. To flush the catheter, a 200-μl blood sample was drawn before sampling and reinjected immediately after sampling. Afterwards, 300 μl of Cortland saline (Wolf, 1963) was injected into the blood stream via the same catheter to replace the volume of sampled blood. The catheter was finally refilled with heparinized Cortland saline (100 μl) to prevent clotting. All blood samples were centrifuged (3 min at $14\,000 \times g$) to separate plasma from blood cells, and analyzed for radioactivity and total metal concentration in both compartments of the blood. To determine the radioactivity of ^{109}Cd and ^{65}Zn that was excreted by the fish, water samples (10 ml) were collected every hour from the experimental chamber and counted for γ-radioactivity.

After terminal blood sampling at 600 min, the fish were killed immediately by an overdose of MS-222 (0.6 g/l) and dissected to obtain tissue samples. The liver, heart, gill basket, stomach, intestine including pyloric caeca, gall bladder including bile content, kidney, and spleen were obtained as whole organs. For muscle, the skin was removed from one side of the fish and a sample of white muscle (8–13 g) was collected. A sample of fat (1–10 g) was collected from the deposit as available along the alimentary tract. For determining the background concentration of Cd and Zn in the tissues before injection, six non-acclimated and six metal-acclimated fish were sampled directly from the holding tanks, and tissue samples were obtained as above. Radioactive tissue samples were counted for γ-radioactivity and all tissue samples (radioactive and non-radioactive) were digested for the determination of total metal concentrations.

2.5. Measurements and calculations

All plasma, tissue and water samples were counted in a Minaxi-γ Auto-gamma 5530 counter (Canberra Packard, Mississauga, Ont., Canada) to determine the radioactivity of ^{109}Cd or ^{65}Zn. After counting, tissue samples were digested in approximately five volumes of 1 N HNO_3 (trace metal grade, Fisher Scientific) at 60 °C for 48 h. The total metal content of plasma and tissue digests was measured by atomic absorption spectrophotometer (SpectrAA-220, Varian) with graphite furnace atomization for Cd and with flame atomization for Zn. Absorbance of the appropriately diluted unknown samples (duplicate or triplicate) was related to the absorbance of a series of known standard samples made from certified stock solutions of Cd and Zn (Fisher Scientific) to obtain total metal concentrations expressed on the basis of per-gram wet tissue or per-milliliter plasma.

Concentrations of radioactive metals in tissues or plasma were converted to absolute values (newly accumulated Cd or Zn) following the "previous compartment specific-activity approach" to account for the post-exposure changes of specific activity (radioactivity per mole of Cd or Zn) in the compartment from which the tissue

accumulates the metals. This method has been used previously for different metals (e.g. Cu, Zn, and Cd) of which the background concentrations in plasma and tissues are high and variable because they are essential for animals and/or because of prior acclimation of animals to the metals (Grosell et al., 1997; Alsop et al., 1999; Szebedinszky et al., 2001). In other words, the specific activity of the injected isotope is diluted by endogenous metal already present in the plasma or tissue, and this is taken into account in the calculation. The approach assumes equilibrium between the Cd or Zn (both radioactive and non-radioactive) in the tissue from the bolus injection, and stable Cd or Zn present in the fish before the injection.

The newly accumulated Cd or Zn (nmol/g) was calculated from the following equation:

$$\text{New Cd or Zn} = a(bc^{-1})^{-1} \quad (1)$$

where a is the ^{109}Cd or ^{65}Zn radioactivity in the compartment of interest, b is the ^{109}Cd or ^{65}Zn radioactivity in the previous compartment, c is the total Cd or Zn concentration in the previous compartment, all expressed on the same per-unit weight or volume basis. The injection stock was considered to be the previous compartment for the plasma and RBCs, while the plasma was considered to be the previous compartment for the ambient water and other tissue samples except hepatic bile for which the liver was assumed to be the previous compartment.

To calculate the relative distribution of the radiolabeled Cd or Zn injected, the total burdens (nmol) of injected Cd or Zn in the blood plasma, RBCs, and sampled tissues were determined using the specific activity of the injection solutions instead of applying the "previous compartment specific-activity approach". Therefore, there was no influence of endogenous Cd or Zn on the calculation. Since there was no significant excretion of ^{109}Cd and ^{65}Zn from fish during the observation time of 10 h ($<1.5\%$ of the injected dose was found in water), the total burdens were converted into relative values as the percentage of injected dose. To calculate the total burden in blood compartments, the relative mass of blood in rainbow trout was assumed to be 6.0% (Bushnell et al., 1998), and the RBCs were assumed to represent 30% of the mass of whole blood (Gallaugher and Farrell, 1998). The rest of the blood mass was assumed to be plasma (70%). The total muscle burden was calculated assuming 60% of body mass of trout to be muscle (Giblin and Massaro, 1973).

2.6. Pharmacokinetic analysis

The data for Cd and Zn clearance from plasma over the experimental period of 10 h were analyzed by a mono-exponential decline function with three parameters of the form:

$$C_p = Ae^{-\alpha t} + B \quad (2)$$

where C_p is the concentration of total or new Cd (or Zn) in the plasma (nmol/ml) at time t, A and B are zero-time intercepts which together $(A+B)$ represent the plasma concentration of the metal at $t=0$, and α is the slope that can be interpreted as the fractional rate of removal of the metal from plasma per unit time (per min). Using a biexponential function did not result in significant parameter estimates. To estimate transfer rate constants between plasma and peripheral tissues, plasma concentration data (total or new Cd, new Zn) were also analyzed by a closed, two-compartment model (Fig. 1, Frazier, 1980; Barron et al., 1990), assuming that Cd or Zn is intra-arterially (*ia*) injected into a central compartment (**I**) from which the metal is exchanged with the peripheral compartment (**II**). The coefficients, k_{12} and k_{21} represent the transfer rate constants between two

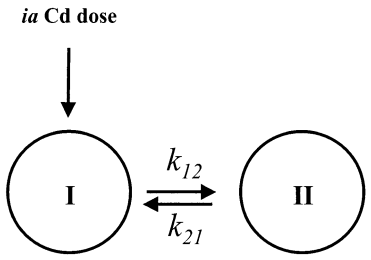

Fig. 1. Schematic of the two-compartment, closed model for analyzing plasma concentration—time profile of Cd and Zn after *ia* injection, assuming that Cd or Zn is introduced into a central compartment (I) and from this compartment the metal is exchanged with the peripheral compartment (II). The transfer rate constants are shown as k_{12} and k_{21}.

compartments. The closed model was chosen since no significant amount of excreted Cd or Zn was found in water. Thus, the experiments primarily investigated the distributive phase of the metal clearance from blood plasma (Frazier, 1980).

A software program for kinetic processes (SAAM II, Washington, USA) was used to formulate the model, and to fit it to the experimental data. Assuming that the compartments are well mixed and kinetically homogeneous, the transfer of the solute (Cd or Zn) between the compartments is assumed to follow first-order kinetics resulting in the following relationships:

$$F_{12} = k_{12} \cdot Q_1 \quad (3)$$

$$F_{21} = k_{21} \cdot Q_2 \quad (4)$$

where F_{12} and F_{21} are mass fluxes (nmol/min) of total or new Cd and Zn from central to peripheral compartment and vice versa, and Q_1 and Q_2 are the amount of the metal (nmol) in the central and peripheral compartments at time t, respectively. Thus the differential equations are

$$dQ_1/dt = [k_{21} \cdot Q_2] - [k_{12} \cdot Q_1] + M_{iv} \quad (5)$$

$$dQ_2/dt = [k_{12} \cdot Q_1] - [k_{21} \cdot Q_2] \quad (6)$$

where M_{iv} is the mass of the metals injected (nmol). While the mass of the metals in the compartments was expressed as the total amount, the model fitted the plasma concentration data (C_p in nmol/ml) using the following equation:

$$C_p = Q_1/V_p \quad (7)$$

where V_p is the apparent volume of the central compartment (ml), which refers to the distribution volume of metals in extracellular fluid in fish and was estimated simultaneously with rate constants as unknown parameters during model fitting. Apparent volume of distribution of the peripheral compartment (V_t), which refers to the distribution volume of the metal in tissue (ml), was calculated from (Barron et al., 1990):

$$V_t = V_p(k_{12}/k_{21}) \quad (8)$$

Finally, Cd or Zn clearance (CL_p: ml/min per kg), interpreted as the volume of plasma cleared of the metal per unit time per unit body weight, was calculated using the equation:

$$CL_p = V_p k_{12} \quad (9)$$

2.7. Statistical analysis

To assess differences between the clearance kinetics of Cd and Zn in plasma and blood cells in nonacclimated and metal-acclimated groups, a two-factor ANOVA, with acclimation and time as the main factors, was used. Student's t-test or Newman–Keuls test was applied to compare terminal tissue levels of total and newly accumulated Cd or Zn between acclimated and nonacclimated fish or to compare them with their controls. All statistical tests and nonlinear regressions were performed using the computer software STATISTICA (StatSoft, Tulsa, OK, USA) or INSTAT (GraphPad, San Diego, CA, USA).

3. Results

3.1. Cd clearance kinetics

Exposure of trout to 30 nmol/l of waterborne Cd resulted in 10% mortality that occurred only in the first week of the 30-day exposure for acclimation. Before *ia* injection, total Cd concentration in plasma was 0.06 ± 0.01 nmol/ml in Cd-acclimated fish and was not detectable in non-acclimated fish. The injection of 64.41 nmol Cd/kg resulted in an approximately 35-fold increase of newly accumulated and total Cd in plasma (levels were essentially identical) in both non-acclimated and Cd-acclimated trout (Fig. 2A and B). Following *ia* injection, more than 70% of the injected Cd was removed from plasma within 2 h. Plasma concentrations of total and new Cd in Cd-acclimated fish were smaller than those in non-acclimated fish, and ANOVA indicated that the difference was statistically significant overall ($P < 0.05$).

No radioactive ^{109}Cd was detected in water over 10 h after injection, meaning that less than 0.5% of the injected dose was excreted from the fish during this period. The concentration of total and newly accumulated Cd in plasma was best described ($r^2 > 0.9$, $P < 0.001$) by the mono-exponential

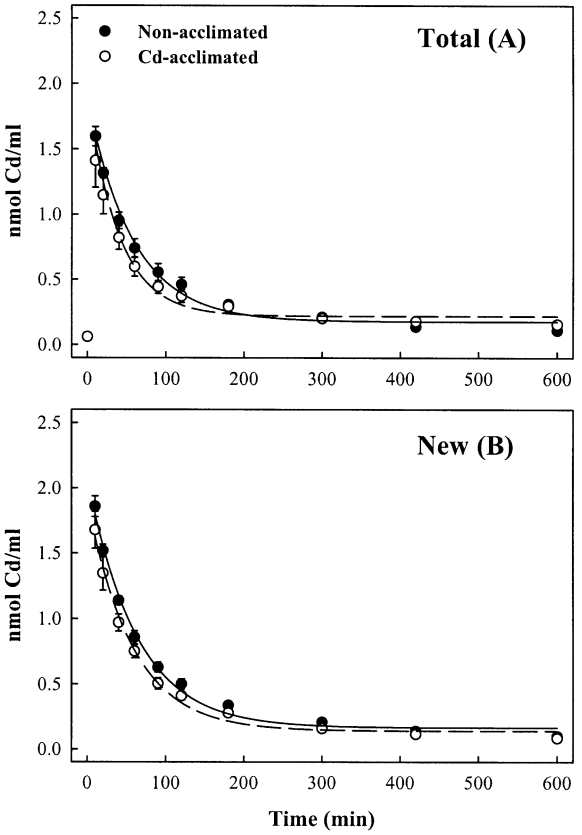

Fig. 2. Plasma concentration—time profile of total Cd (A) and newly accumulated Cd (B) in non-acclimated and Cd-acclimated rainbow trout after an injection of 64.4 nmol radiolabeled Cd/kg into blood. The acclimated fish were pre-exposed to waterborne stable Cd (26.7 nmol/l) for 30 days. Symbols represent experimental values (mean ± S.E., $n = 5–7$). ANOVA revealed significant differences ($P < 0.05$) between non-acclimated and Cd-acclimated fish both for total Cd and newly accumulated Cd. The solid lines and broken lines are the fit of a mono-exponential decline function (Eq. (2), $r^2 > 0.9$; $P < 0.001$) to the data values for non-acclimated and Cd-acclimated fish, respectively.

function (Eq. (2)). Introducing another exponential term (bi-exponential function) did not fit the data with significant parameter estimates (results not shown). The half-life of the distributive phase of total plasma Cd ($\ln 2/\alpha$) in Cd-acclimated fish was somewhat shorter than that in non-acclimated fish, but the difference was not statistically significant. The distribution half-lives for newly accumulated Cd were more or less similar in both groups (Table 1). The clearance volume (Cl_p) of total and newly accumulated Cd was greater in Cd-acclimated fish, indicating that plasma clearance of Cd in Cd-acclimated fish was significantly faster than in the non-acclimated fish (Table 1). In Cd-acclimated fish, the apparent distribution volume of the central compartment (V_p) was larger in comparison to non-acclimated fish for both total and new Cd. Unlike the central compartment, a larger ($\sim$5-fold) distribution volume of the peripheral compartment (V_t) was only observed for total Cd in Cd-acclimated fish.

3.2. Cd kinetics in red blood cells

With the *ia* injection, the total Cd concentration (mean ± S.E.) in RBCs increased from 0.05 ± 0.01 to 0.49 ± 0.04 nmol/g in non-acclimated fish and from 0.11 ± 0.01 to 0.58 ± 0.07 nmol/g in Cd-acclimated fish (Fig. 3A). This increase ($\sim$5–9-fold) was not, however, as much as the increase in the total plasma Cd levels ($\sim$35-fold). Due to low background Cd, the corresponding increase of newly accumulated Cd in RBCs was similar to that of the total Cd (Fig. 3B). Observed concentrations of both total and new Cd in Cd-acclimated fish were 1.2–2.2 times greater than the levels in non-acclimated fish. As for plasma, the total and new Cd in RBCs declined exponentially and reached close to background levels in non-acclimated and Cd-acclimated fish in 10 h (0.12 ± 0.02 and 0.19 ± 0.03 nmol/g, respectively). The exponential decline model used to describe the plasma concentration of Cd was also adequate to describe the blood cell concentration–time profile of Cd (Fig. 3). Unlike plasma, the distribution rate constant (α) appeared to be smaller (i.e. longer half-life of Cd in blood cells) in Cd-acclimated fish than in non-acclimated fish. However, none of the parameters for RBCs showed statistically different values for Cd-acclimated fish (Table 1).

3.3. Tissue concentrations of Cd

Tissue concentrations of total and newly accumulated Cd in non-acclimated and Cd-acclimated fish obtained 10 h after injection are presented in Fig. 4. In non-acclimated fish, the injected Cd resulted in a significant increase of total Cd in

Table 1
Parameter estimates (±S.E.) for clearance kinetics of total and newly accumulated Cd in the plasma and RBCs of non-acclimated and Cd-acclimated trout

Parameters[a]	Total Cd		New Cd	
	Non-acclimated	Cd-acclimated	Non-acclimated	Cd-acclimated
Plasma				
A (nmol/ml)	1.70±0.06	1.70±0.13	1.92±0.05[c]	1.77±0.09
α (per min)	0.017±0.002	0.025±0.004	0.016±0.001	0.017±0.003
$t_{1/2}$ (min)	40.8±4.8	27.7±4.4	43.3±2.7	40.8±7.2
B (nmol/ml)	0.175±0.029	0.217±0.045	0.164±0.025	0.139±0.038
V_p (ml/kg)	38.07±0.60	42.59±1.30[b]	35.10±0.81[c]	41.77±1.67[b]
V_t (ml/kg)	664.3±43.9	3340.0±95.7[b]	565.0±24.9	626.1±32.4[c]
Cl_p (ml/min per kg)	0.493±0.013	0.726±0.015[b]	0.394±0.009[c]	0.477±0.012[b,c]
Red blood cells				
A (nmol/g)	0.393±0.042	0.419±0.061	0.410±0.068	0.630±0.103
α (per min)	0.019±0.005	0.013±0.005	0.032±0.011	0.023±0.008
$t_{1/2}$ (min)	36.5±9.6	53.3±20.5	21.7±7.4	30.1±10.5
B (nmol/g)	0.130±0.020	0.202±0.038	0.142±0.017	0.187±0.038

[a] Parameters for plasma were estimated by fitting the exponential function (Eq. (2), $r^2 > 0.9$; $P < 0.001$) and the compartment model (Fig. 1) to data presented in Fig. 2. Parameters for blood cells were estimated by fitting the exponential function (Eq. (2), $r^2 > 0.8$; $P < 0.05$) to data presented in Fig. 3. A, B: intercepts of the Eq. (2); α: slope of the Eq. (2); $t_{1/2}$(distribution halftime) = ln 2/α; V_p: apparent volume of the central compartment, V_t: apparent volume of the peripheral compartment; Cl_p: Cd clearance rate from plasma.

[b] Significant difference between non-acclimated and Cd-acclimated trout for total or new Cd ($P < 0.05$; non-acclimated trout: $n = 7$; Cd-acclimated trout: $n = 5$).

[c] Significant difference between total and new Cd in the same acclimation group ($P < 0.05$; non-acclimated trout: $n = 7$; Cd-acclimated trout: $n = 5$).

most of the observed tissues, with the exception of heart, bile, and muscle. In Cd-acclimated fish, tissue total Cd levels were higher in every tissue except muscle, with the greatest increases being seen in kidney and gills > liver > intestine. In the acclimated fish, the injected Cd resulted in no detectable change in tissue Cd burdens, with the exception of the gills, in which total Cd was actually reduced over the period of 10 h after injection, presumably because the waterborne exposure was suspended during this period (Fig. 4A). The highest concentrations (nmol/g) of total Cd was found in the kidney (10.7±1.4), gills (9.0±0.8) and liver (4.9±0.6) of Cd-acclimated fish, with the lowest concentration in the muscle (0.01±0.002) of non-acclimated fish.

The pattern of new Cd accumulation was different from that of total Cd. The highest concentrations of newly accumulated Cd were observed in the liver and kidney of both non-acclimated and Cd-acclimated fish, which contained ~20 and ~5% of the injected Cd dose, respectively (Fig. 4B, Fig. 5). Unlike total Cd, a lower concentration of new Cd was found in the kidney than in the liver. Second to the liver, muscle accumulated almost 12% of the injected dose of Cd, however, in terms of concentration it was the lowest for new Cd due to largest body mass.

3.4. Zn clearance kinetics

Exposure of trout to 2403 nmol/l of waterborne Zn during the 30 day-acclimation period produced no mortality. Before the *ia* injection, the endogenous levels (mean±S.E.) of plasma Zn in non-acclimated and Zn-acclimated trout were 78.7±18.2 and 114.4±34.8 nmol/ml, respectively. The injection of radiolabeled Zn (183 nmol/kg) resulted in an initial increase of total plasma Zn in non-acclimated and acclimated fish, but the total Zn levels in both groups were not different and remained more or less constant during 10 h of post-injection observation period (98.3±9.3–133.4±14.4 nmol/ml; Fig. 6A). Following *ia*

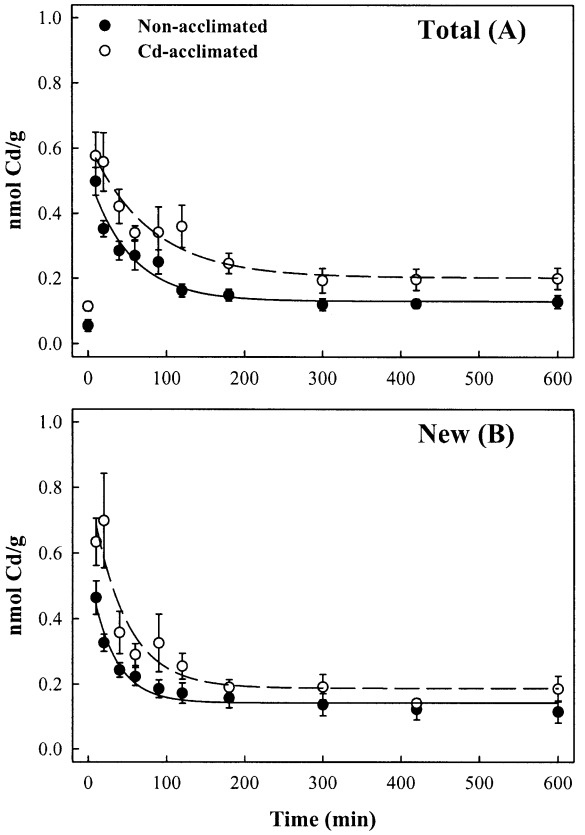

Fig. 3. RBC concentration—time profile of total Cd (A) and newly accumulated Cd (B) in non-acclimated and Cd-acclimated rainbow trout after an injection of 64.4 nmol radiolabeled Cd/kg into blood. The acclimated fish were pre-exposed to waterborne stable Cd (26.7 nmol/l) for 30 days. Symbols represent experimental values (mean ± S.E., $n = 5–7$). ANOVA revealed significant differences ($P < 0.001$) between non-acclimated and Cd-acclimated fish both for total Cd and newly accumulated Cd. The solid lines and broken lines are the fits of a mono-exponential decline function (Eq. (2), $r^2 > 0.8$; $P < 0.05$) to the data values for non-acclimated and Cd-acclimated fish, respectively.

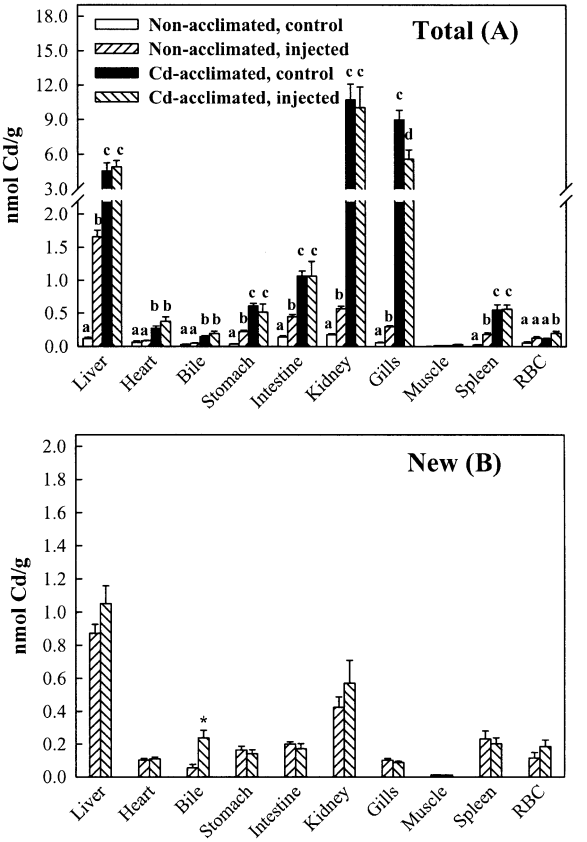

Fig. 4. A: Tissue concentrations of total Cd (nmol/g w wt) in non-injected, non-acclimated control fish, in non-injected, Cd-acclimated control fish, and in both non-acclimated and Cd-acclimated rainbow trout 10 h after an injection of 64.4 nmol radiolabeled Cd/kg into blood. B: Tissue concentrations of newly accumulated Cd (nmol/g w wt) in the same samples of non-acclimated and Cd-acclimated fish. The acclimated fish were pre-exposed to waterborne stable Cd (26.7 nmol/l) for 30 days. Results are presented as mean ± S.E. ($n = 5–7$). For total Cd (A), data points with different letters are significantly different ($P < 0.05$) for the same tissue. *, Significantly different from non-acclimated fish for the same tissue ($P < 0.05$). RBC: red blood cells.

injection, observed plasma concentrations (mean ± S.E.) of newly accumulated Zn declined exponentially from 5.5 ± 0.3 nmol/ml at 10 min to 1.0 ± 0.06 nmol/ml at 10 h in non-acclimated fish and from 5.7 ± 0.4 nmol/ml at 10 min to 0.8 ± 0.03 nmol/ml at 10 h in Zn-acclimated trout (Fig. 6B).

As for ^{109}Cd, only a negligible amount of radioactive ^{65}Zn was found in water over the 10-h observation period (< 1.5% of the injected dose), revealing that ^{65}Zn was not excreted by the fish during this period. Plasma clearance of newly accumulated Zn could be described ($r^2 > 0.9$, $P < 0.001$) by the same functional model (Eq. (2)) and the closed two-compartment model used for Cd (Fig. 1). However, the clearance rate of injected Zn in plasma (Cl_p) was approximately half of the Cd clearance. The half-lives of the distributive phase of new plasma Zn ($\ln 2/\alpha$) in non-acclimated and Zn-acclimated fish were 73

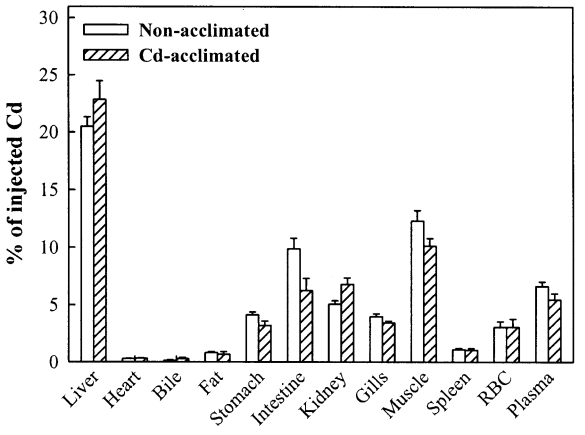

Fig. 5. Relative distribution of Cd as a percentage of the injected dose (64.4 nmol/kg Cd) to sampled tissues in non-acclimated and Cd-acclimated fish. Samples were taken 10 h after injection into blood. The acclimated fish were pre-exposed to waterborne stable Cd (26.7 nmol/l) for 30 days. Results are presented as mean ± S.E. ($n = 5–7$). There were no statistically significant differences between non-acclimated and Cd-acclimated fish. RBC: red blood cells.

and 69 min, respectively, indicating that pre-exposure to Zn had no effect on Zn clearance (Table 2). The apparent distribution volume of the central compartment (V_p) was similar in both Zn-acclimated and non-acclimated fish, but that of the peripheral compartment (V_t) in Zn-acclimated fish was slightly and significantly larger.

3.5. Zn kinetics in red blood cells

The background concentrations of total Zn in the RBCs of non-acclimated and Zn-acclimated fish were 145 ± 3 and 156 ± 3 nmol/g, respectively, and were not changed by the Zn injection. The concentrations of total Zn were also not affected by Zn acclimation and remained more or less constant during 10-h post-injection observation period (Fig. 7A). The concentrations (mean ± S.E.) of new Zn in RBCs, however, declined exponentially from 1.05 ± 0.19 to 0.25 ± 0.02 nmol/ml in non-acclimated fish and from 0.91 ± 0.13 to 0.21 ± 0.02 nmol/ml in Zn-acclimated fish over 10 h following the *ia* injection (Fig. 7B). RBC levels for new Zn were only 1/3–1/6 of plasma levels in both non-acclimated and acclimated groups (e.g. Fig. 6B). While RBC levels of new Cd in Cd-

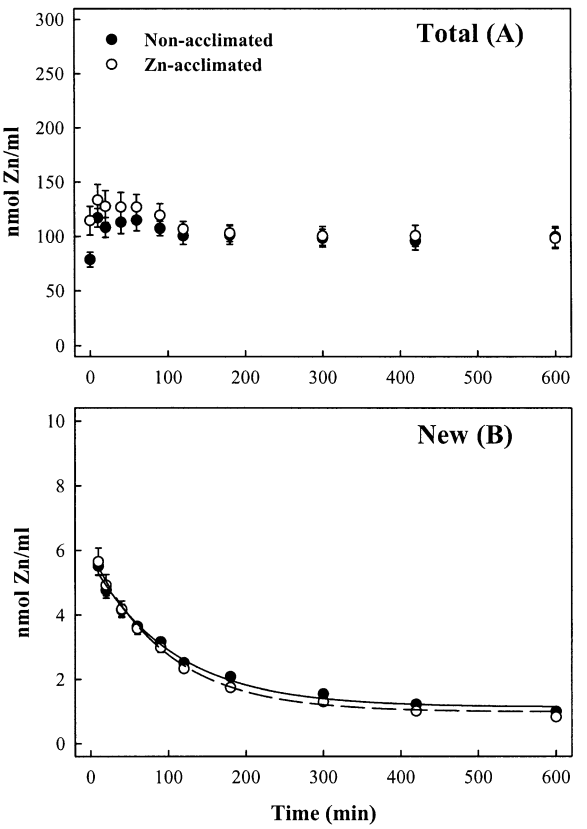

Fig. 6. Plasma concentration—time profile of total Zn (A) and newly accumulated Zn (B) in non-acclimated and Zn-acclimated rainbow trout after an injection of 183.8 nmol radiolabeled Zn/kg into blood. The acclimated fish were pre-exposed to waterborne stable Zn (2294 nmol/l) for 30 days. Symbols represent experimental values (mean ± S.E., $n = 6–8$). There were no statistically significant differences between non-acclimated and Zn-acclimated fish. The solid line and broken line are the fits of a mono-exponential decline function (Eq. (2), $r^2 > 0.9$; $P < 0.001$) to the data values for newly accumulated Zn in non-acclimated and Zn-acclimated fish, respectively.

acclimated fish were greater than those in non-acclimated fish (Fig. 3B), RBC levels of new Zn in Zn-acclimated fish were smaller than those in non-acclimated fish (Fig. 7B), and ANOVA indicated that the overall differences between acclimated and non-acclimated fish were statistically significant ($P < 0.05$).

The blood cell concentration–time profile of new Zn could be described by the exponential decline model (Eq. (2), Fig. 7B). The distribution rate constant (α) was apparently greater (i.e.

Table 2
Parameter estimates (±S.E.) for clearance kinetics of newly accumulated Zn in the plasma and RBCs of non-acclimated and Zn-acclimated trout

Parameters[a]	Non-acclimated	Zn-acclimated
Plasma		
A (nmol/ml)	4.56±0.15	5.00±0.19
α (per min)	0.0095±0.0008	0.010±0.001
$t_{1/2}$ (min)	73.0±6.1	69.3±6.9
B (nmol/ml)	1.13±0.11	0.985±0.132
V_p (ml/kg)	33.88±0.50	34.51±0.78
V_t (ml/kg)	141.4±4.0	186.5±4.3[b]
Cl_p (ml/min per kg)	0.227±0.004	0.234±0.006
Red blood cells		
A (nmol/g)	0.77±0.09	0.80±0.07
α (per min)	0.0096±0.0035	0.014±0.003
$t_{1/2}$ (min)	71.8±26.1	49.6±10.8
B (nmol/g)	0.29±0.07	0.25±0.04

[a] Parameters for plasma were estimated by fitting the exponential function (Eq. (2), $r^2 > 0.9$; $P < 0.001$) and the compartment model (Fig. 1) to data presented in Fig. 6B. Parameters for blood cells were estimated by fitting the exponential function (Eq. (2), $0.49 < r^2 < 0.69$; $P < 0.01$) to data presented in Fig. 7B. A, B: intercepts of the Eq. (2); α: slope of the Eq. (2); $t_{1/2}$ (distribution halftime) = $\ln 2/\alpha$; V_p: apparent volume of the central compartment, V_t: apparent volume of the peripheral compartment; Cl_p: Zn clearance rate from plasma.

[b] Significantly different from non-acclimated trout for plasma Zn ($P < 0.05$; non-acclimated trout: $n = 8$; Zn-acclimated trout: $n = 6$).

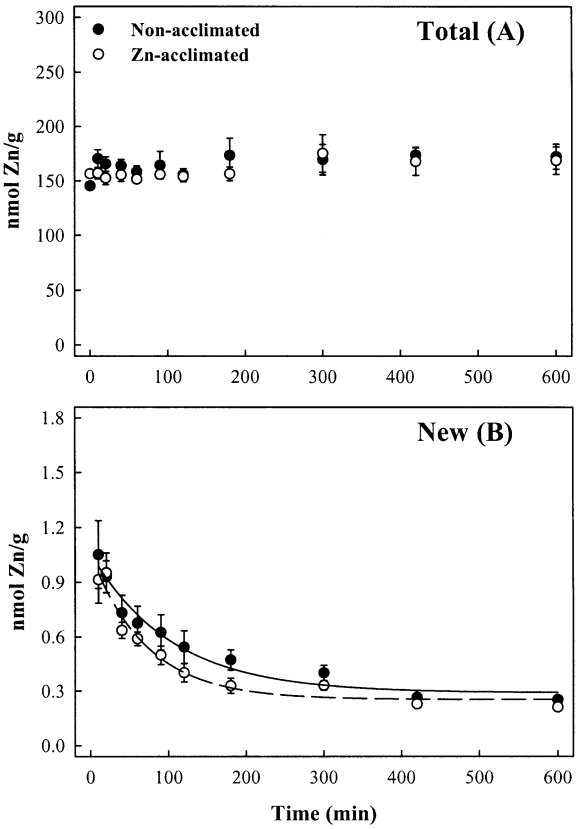

Fig. 7. RBC concentration—time profile of total Zn (A) and newly accumulated Zn (B) in non-acclimated and Zn-acclimated rainbow trout after an injection of 183.8 nmol radiolabeled Zn/kg into blood. The acclimated fish were pre-exposed to waterborne stable Zn (2294 nmol/l) for 30 days. Symbols represent experimental values (mean±S.E., $n = 6$–8). Symbols represent experimental values (mean±S.E., $n = 5$–7). ANOVA revealed significant differences ($P < 0.05$) between non-acclimated and Zn-acclimated fish for newly accumulated Zn. The solid lines and broken lines are the fits of a mono-exponential decline function (Eq. (2), $0.49 < r^2 < 0.69$; $P < 0.01$) to the data values for non-acclimated and Zn-acclimated fish, respectively.

shorter half-life of Zn) in Zn-acclimated fish than in non-acclimated fish. However, none of the parameters for RBCs showed statistically different values for Zn-acclimated fish (Table 2).

3.6. Tissue concentrations of Zn

Tissue concentrations of total and newly accumulated Zn in non-acclimated and Zn-acclimated fish obtained 10 h after Zn injection are presented in Fig. 8. Exposure to 2403 nmol/l of waterborne Zn during the 30-day acclimation did not result in any significant change in total Zn levels in the tissues analyzed. Furthermore, there were no significant increases of total Zn levels of tissues in either non-acclimated fish or in Zn-acclimated fish attributable to Zn injection (Fig. 8A). The highest levels of total Zn, both in the control and injected fish, were found in the intestine (6.9–9.1 μmol/g), which was followed by the stomach (3.4–4.3 μmol/g) and the gills (0.7–1.6 μmol/g) (Fig. 8A). The bile level of total Zn, however, significantly increased from 76 to 204 nmol/g in non-acclimated and from 36 to 267 nmol/g in Zn-acclimated fish after injection, indicating that there was a substantial hepatobiliary excretion of Zn in fish.

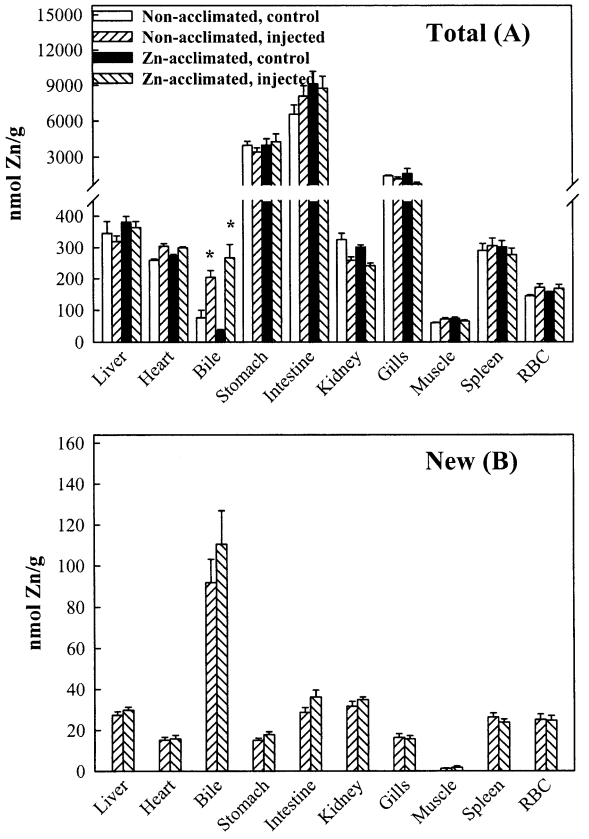

Fig. 8. A: Tissue concentrations of total Zn (nmol/g w wt) in non-injected, non-acclimated control fish, in non-injected, Zn-acclimated control fish, and in both non-acclimated and Zn-acclimated rainbow trout 10 h after an injection of 183.8 nmol radiolabeled Zn/kg into blood. B: Tissue concentrations of newly accumulated Zn (nmol/g w wt) in the same samples of non-acclimated and Zn-acclimated fish. The acclimated fish were pre-exposed to waterborne stable Zn (2294 nmol/l) for 30 days. Results are presented as mean ± S.E. ($n = 6$–8). *, Significant difference from corresponding control values for the same tissue ($P < 0.05$). RBC: red blood cells.

The tissue levels of new Zn did not show the similar trend as found for total Zn, but again they indicated no marked difference between the non-acclimated and acclimated groups (Fig. 8B). The tissue levels were also not as markedly different as found for the tissue levels of new Cd of Fig. 4B. Among the analyzed tissues, the highest concentrations of new Zn (56–171 nmol/g; calculated based on the specific activity of ^{65}Zn in the liver) were found in the bile of non-acclimated and Zn-acclimated fish and the levels were approximately 3–63 times greater than the new Zn levels in the other tissues (Fig. 8B). However, the radiolabeled Zn mass in bile accounted for less than 1% of the injected Zn dose. After 10 h of injection, the highest proportion of the dose was still detected in the plasma (15.8–25.7%), which was followed by the muscle (6.2–13.0%) and intestine (5.4–10.4%) (Fig. 9).

4. Discussion

The results of the present study demonstrate that the acute plasma clearance and tissue specific uptake of Cd versus Zn in trout are different regardless of the Cd or Zn pre-acclimation status. In both non-acclimated and acclimated groups, plasma clearance of Cd ($t_{1/2} = 28$–43 min; $Cl_p =$ 0.39–0.73 ml/min per kg) was almost twice as fast as Zn clearance ($t_{1/2} = 69$–73 min; $Cl_p = 0.23$ ml/min per kg; Tables 1 and 2). This is in agreement with one mammalian study that reported a 5-fold faster clearance of Cd than Zn from rat plasma following intravascular injection of the metals (Frazier, 1980). The slower clearance of Zn may

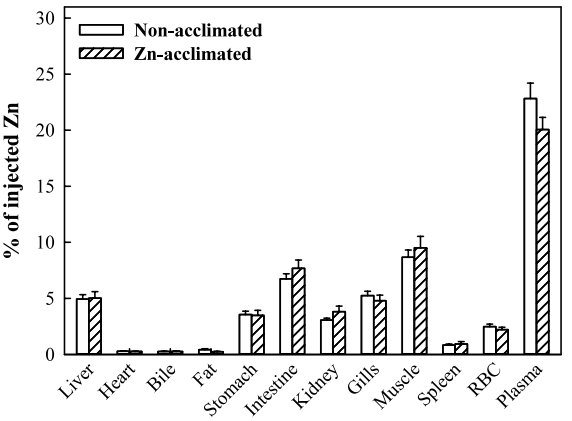

Fig. 9. Relative distribution of Zn as a percentage of injected dose (183.8 nmol/kg Zn) to sampled tissues in non-acclimated and Zn-acclimated fish. Samples were taken 10 h after an injection into blood. The acclimated fish were pre-exposed to waterborne stable Zn (2294 nmol/l) for 30 days. Results are presented as mean ± S.E. ($n = 6$–8). There were no statistically significant differences between non-acclimated and Zn-acclimated fish. RBC: Red blood cells.

be attributed to the much larger endogenous Zn pool in the fish tissue (Fig. 8A) that leads to a longer time for the delivery of radiolabeled Zn in the plasma proteins to metal binding proteins in the tissues. The serum albumin is usually considered to be the primary transport protein of Zn and Cd in vertebrates, although it exhibits only weak, nonspecific binding to these metals (Scott and Bradwell, 1984; Trisak et al., 1990). Histidine-rich glycoprotein (HRG), α_2-macroglobulin, transferrin, IgA, IgG, and prealbumin are other plasma proteins that are reported to have the ability to bind Cd in vertebrates including fish (Guthans and Morgan, 1982; Scott and Bradwell, 1984; Golaz et al., 1993; De Smet et al., 2001). The differences in the specificity and binding affinity of the proteins for Cd and Zn, which are yet to be characterized for rainbow trout, may also be a possible reason for the difference in clearance rate.

Although there is a difference between the clearance of Cd and Zn, both metals were cleared from the plasma rapidly. Almost 72% of the injected Cd and 52% of the injected Zn were delivered from plasma to tissues in 2 h, suggesting an efficient detoxification or homeostatic regulation of these metals in trout as found for Cu (Grosell et al., 2001).

In the present study, the concentration–time profile for both plasma Cd and Zn could be described by an exponential decline function with one exponential term A and one constant term B (Eq. (2)), indicating that most of the plasma Cd ($A = \sim 90\%$; Table 1) and Zn ($A = \sim 80\%$; Table 2) were cleared exponentially from a single plasma pool of the metals to the tissues during the 10-h observation period. This predominant plasma pool may be associated with one or more proteins exhibiting similar kinetics. Our data also revealed another small pool of the metals in the plasma as indicated by the second term B that is probably too tightly bound to proteins to show any significant decline during this short period.

In agreement with our analysis, Frazier (1980) used a similar exponential function to describe the plasma clearance of Cd and Zn in the rat over 15 min. In contrast, a biexponential or multiexponential drop of blood or plasma Cd and Zn has been documented in fish by other authors (Schultz et al., 1996; Barron et al., 2000). These authors, however, studied the kinetics of the metals for much longer periods of time (~ 1 year) and demonstrated three phases of metal pharmacokinetics: (1) a rapid initial distribution of metals outside the vascular system to highly perfused organs like liver and kidney within a few hours, as shown by α (Tables 1 and 2) in the present study; (2) a redistribution phase of metals from tissues to a slowly exchangeable pool, as shown by the constant term B in the present study; and (3) an extremely slow turnover phase that resulted in virtually no excretion of wholebody Cd and Zn in ~ 50 and 2 days, respectively. That no excretion of ^{109}Cd and ^{65}Zn was found in our study not only agrees with other similar studies but also justifies the use of the two-compartment closed model for the analysis of our data (Fig. 1).

There are no previous studies examining the potential effects of acclimation on the plasma clearance of Cd and Zn. The present study demonstrated for the first time an enhanced clearance of Cd in Cd-acclimated fish, indicating that chronic exposure to waterborne Cd stimulated the removal of injected plasma Cd in fish. A recent study in our laboratory with trout that received Cu via an intravascular infusion also demonstrated a stimulated clearance of plasma Cu in Cu-acclimated fish (Grosell et al., 2001). The liver appeared to play the vital role in this elevated clearance as a part of Cu homeostasis, by scavenging plasma Cu and stimulating the excretion of Cu by 2–3-fold in hepatobiliary pathway.

The liver is also thought to be the critical organ for detoxification in response to acute Cd loading, as a whole, and for Cd clearance from the plasma, in particular, in mammals (Frazier, 1980; Sendelbach and Klaassen, 1988) and fish (Wicklund Glynn, 1991; Schultz et al., 1996; Castaño et al., 1998). In the present study, the highest relative accumulation of total injected ^{109}Cd dose was also found in the liver, but the difference between non-acclimated ($20.5 \pm 0.8\%$) and Cd-acclimated fish ($22.8 \pm 1.6\%$) was not significant (Fig. 5). The relative contribution of other tissues sampled 10 h after injection did not show any significant increase of Cd accumulation in Cd-acclimated fish either (Fig. 5). In rainbow trout, urinary

excretion of Cd after 56 days was negligible (Harrison and Klaverkamp, 1989). In the present study, the bile concentration of new Cd in Cd-acclimated fish (0.24 ± 0.8 nmol/ml) was 4-fold higher in comparison to non-acclimated fish, suggesting that the Cd-acclimation increases the hepatobiliary excretion of Cd (Fig. 4B). However, the total amount of radiolabeled Cd accumulated in the gall bladder bile over 10 h was $< 0.5\%$ of the injected Cd dose in both non-acclimated and Cd-acclimated fish (Fig. 5). Thus the bile burdens were not enough to explain the difference (11–31%) in the total amount of radioactive plasma Cd between the two groups at observed time points.

Although the Cd concentrations in plasma were greater in non-acclimated fish than in acclimated fish, the opposite results were observed in blood cells, with approximately 1.2–2.2-fold greater levels of new and total Cd in the RBCs of Cd-acclimated fish (Fig. 3). This may have been responsible, at least partially, for the difference in plasma Cd levels. Cd accumulation in the RBCs of mammals was found to be small immediately after an intravascular injection (within 1–2 h), but later increased significantly reaching a maximum value between 2.5 and 5 days (Nordberg et al., 1971; Garty et al., 1981; Tanaka et al., 1985). A similar type of Cd peak in RBCs was also observed in catfish with a maximum concentration reached at 12 days (Schultz et al., 1996). In these studies, animals were not pre-acclimated to Cd, and the time-dependent elevation was attributed to the distribution of Cd to RBCs following the induction of binding proteins (MT) in immature RBCs (erythroblasts) in response to injected Cd (Tanaka et al., 1985). In the present study, the Cd-acclimated trout were pre-exposed to Cd for 30 days before *ia* dosing. As a result, the MT content in RBCs of Cd-acclimated fish was presumably greater than that (if any) in non-acclimated fish. Thus, pre-exposure of trout to Cd may influence the binding characteristics of Cd in blood compartments and thereby the metal kinetics.

Another factor that may have affected the plasma concentrations of Cd is the distribution volume or size of the central compartment (V_p) which was apparently greater in Cd-acclimated fish than in non-acclimated fish (Table 1). In pharmacokinetic analysis of a chemical, the distribution volume of a compartment depends on the chemical and the species, and on both the anatomical volume of and partitioning/binding to the tissues in the compartment (Gibaldi and Perrier, 1982). The 5-fold larger volume of the peripheral compartment (V_t) for total Cd in Cd-acclimated trout (Table 1) conceivably reflects the extensive binding of Cd in highly perfused organs particularly gills, liver, and kidney during waterborne acclimation (Fig. 4A). The effect of acclimation on the volume of the central compartment may have been a reflection of greater Cd binding in RBCs and/or to increases in binding sites, e.g. MT, in the cells of those highly perfused organs.

During pre-exposure to waterborne Cd, trout accumulated the highest concentration of total Cd in the kidney with the liver ranking third. Ten h after Cd injection, however, the highest burden of new Cd was found in the liver (Fig. 4A and B). This indicates that both the liver and kidney play an important role in Cd clearance, but the kidney is the eventual storage site in a chronic exposure condition. This time-dependent dispositional pattern of Cd is consistent with previous reports in rats (Webb, 1986; Sudo et al., 1994), fish (Schultz et al., 1996; McGeer et al., 2000b), and turtle (Rie et al., 2001). It is suggested that the liver responds to Cd toxicity by synthesizing the binding protein MT, and that the Cd–MT complex is released from the liver and redistributed to the kidney, where long-term storage can occur (Sendelbach and Klaassen, 1988; see Rie et al., 2001).

In contrast to Cd, no acclimation effect on the plasma clearance or tissue-specific accumulation of Zn was observed (except RBCs), but rather a strong homeostatic control of this nutrient element in rainbow trout was demonstrated in the present study. The levels of total Zn in plasma remained more or less constant during 10 h after Zn injection (Fig. 6A), though this can be reasoned as a so-called "masking effect" of the high concentration of endogenous Zn on the kinetics of low-dose Zn administration (Alsop et al., 1999). Pertinent to this argument, the new plasma Zn measured based on radiolabeled ^{65}Zn in plasma showed an exponential decline in both non-acclimated and Zn-acclimated fish, but again there was

no difference between these two groups (Fig. 6B). Pre-exposure to waterborne Zn at 2403 nmol/l for 30 days did not cause any marked difference in the accumulation of Zn in the analyzed tissues in comparison to non-injected fish or control fish (Fig. 8). Only in the case of RBCs were the new Zn levels significantly smaller in Zn-acclimated fish than in non-acclimated fish (Fig. 7B), but the difference was not as much as to affect plasma new Zn levels (Fig. 6B). Moreover, the smaller Zn levels in Zn-acclimated fish may be attributed to Zn regulation in RBCs by fish because Zn is an important component of the metalloenzyme carbonic anhydrase in blood cells (Sorensen, 1991). Consistent with our results, effective Zn regulation with negligible internal accumulation in rainbow trout chronically exposed to waterborne Zn has been shown previously (Spry et al., 1988; McGeer et al., 2000b).

In the injected fish, the concentrations of Zn in the bile increased in both non-acclimated and acclimated fish in comparison to their respective control fish (Fig. 8). This might be caused by stimulated biliary excretion of Zn, triggered as a part of Zn regulation by fish after Zn injection.

5. Conclusions

The present study demonstrates that the plasma clearance and tissue specific uptake of *ia* injected Cd or Zn in fish are different, whether or not the fish is previously acclimated to waterborne Cd or Zn. Plasma clearance of Cd is almost twice as fast as Zn clearance and is enhanced in Cd-acclimated fish, indicating that chronic exposure to waterborne Cd stimulates the detoxification process. On the contrary, no acclimation effect on the plasma clearance or tissue-specific accumulation of Zn is evident, but this nutrient element is already under strong homeostatic control. Since plasma proteins play the major role for metal binding and delivery to tissues, thorough understanding of their composition and binding characteristics are needed in order to understand the difference in the internal metal handling by fish. While albumin-like proteins with a molecular mass of $\sim$66 kDa are considered major binding proteins of Cd and Zn in salmonids, no or little albumin was found in carp (De Smet et al., 1998), thus species specific difference in transport proteins and clearance kinetics of essential and non-essential metals requires further attention. Vertebrate blood cells do not appear to be involved in metal transport because metals in these cells are not readily exchangeable with receptors at sites of utilization (Roesijadi and Robinson, 1994). However, the present study suggests that pre-exposure of fish to Cd increases the Cd burden in RBCs and thereby influences the metal kinetics in plasma. The importance of RBC Cd burden as a chronic biomarker for Cd exposure, and the possible role of the induced protein metallothionein for enhanced Cd burden in RBCs warrant future investigation.

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