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Acid-base, plasma ion and blood gas changes in rainbow trout during short term toxic zinc exposure

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Summary. 1. Rainbow trout exposed to waterborne zinc at an acutely lethal level (1.5 mg/l) or at a lower concentration close to the 4 d LC_{50} (0.8 mg/l) exhibited contrasting physiological responses in artificial soft water (ASW).

2. The changes in acid-base status and other blood parameters during the acute zinc exposure (1.5 mg/l) in ASW resulted from a rapid cascade of events in which hypoxemia, probably due to gill damage, resulted in tissue hypoxia and a mixed acidosis which were rapidly fatal. Changes in Hct, MCHC, Cl^- and lactate reflected the acidosis. Hypoxia rather than acidosis was the primary lethal mechanism.

3. Lower level zinc exposure (0.8 mg/l) over a 3 day period resulted in a slight alkalosis, despite a rise in Pa_{CO_2}. No changes were observed in plasma concentrations of Na^+, Cl^- or K^+, and Pa_{O_2} remained high. Possible causes for the alkalosis are discussed. Some mortality occurred, suggesting that toxic mechanisms other than hypoxemia may have operated.

4. Zinc accumulated in whole blood to a greater extent during the 3 day low level exposure than the short term, high level exposure, suggesting that toxicity in the latter reflected an external effect of zinc. Zinc accumulated only in plasma with no penetration of the RBC's.

Introduction

The toxic potential of zinc to fish is well known. This potential varies with changes in water quality, in particular increasing with decreasing hardness and alkalinity although the underlying reasons are unclear (see reviews by Skidmore 1964; European Inland Fisheries Advisory Commission 1973; Spear 1981; and Spry et al. 1981). The lethal mechanism of acute zinc toxicity to fish is probably one of hypoxia as indicated by decreases in arterial oxygen tension (Pa_{O_2}) and oxygen utilization by rainbow trout (*Salmo gairdneri*) exposed to 40 mg/l Zn (Skidmore 1970). Brafield and Matthiessen (1976) found that sticklebacks (*Gasterosteus aculeatus*) exposed to 1 mg/l Zn in calcium-free water had highly erratic oxygen uptake which declined shortly before death. Gill damage consisted of massive delamination of the respiratory epithelium and eventual collapse of branchial perfusion (Skidmore and Tovell 1972). Fusion of secondary lamellae, generalized cellular disintegration and a doubling of the water to blood diffusion distance were also reported (Matthiessen and Brafield 1973). The impairment of oxygen transfer associated with this damage resulted in tissue hypoxia, as evidenced by increases of lactate concentration in liver and skeletal muscle in terminally sampled rainbow trout (Burton et al. 1972; Hodson 1976). The effects of acute exposures on ion regulation are equivocal, as blood osmotic concentration either remained within the normal range in trout exposed to 40 mg/l Zn (Skidmore 1970) or was greatly reduced in channel catfish (*Ictalurus punctatus*) at 12–30 mg/l Zn (Lewis and Lewis 1971).

The physiological effects of lower levels of zinc which are not acutely lethal have been little studied. A sublethal exposure of rainbow trout to zinc at around the 48 h LC_{50} (1.4 mg/l) revealed a decrease in Pa_{O_2} and arterial pH (pHa) over 24 h (Sellers et al. 1975); blood lactate was not measured. Exposure to 0.3–2.0 mg/l Zn had minimal effect on serum osmolality and electrolytes in the same species, although various gill ATPases were stimulated (Watson and Beamish 1980).

Abbreviations: *SITS* 4-acetamido-4′-iso-thiocyanatostilbene-2,2′disulphonic acid; *Hct* hematocrit; *MCHC* mean cell hemoglobin concentration; *ASW* artificial soft water; C_{P_r} plasma total protein; *Hb* hemoglobin

The aim of the present study was to characterize fully the blood acid-base, ion, and oxygen delivery disturbances caused by zinc using cannulated rainbow trout and clinical methods which have been successfully applied in other studies (Cameron and Randall 1972; Eddy 1976; Wood et al. 1977; McDonald et al. 1980). Two concentrations of zinc were chosen, an acutely lethal one (less than 24 h survival), and a lower one near the 96 h LC_{50}. In view of evidence that zinc toxicity increases with decreasing hardness and alkalinity (cf. Spear 1981; Spry et al. 1981), the tests were conducted in artificial soft water (ASW) similar in composition to that of Ontario's Precambrian shield lakes. The acidification of this area by acid precipitation enriched zinc and other heavy metals in these waters, causing a concern over their possible effect (Spry et al. 1981).

Materials and methods

Fish and experimental water. Rainbow trout (*Salmo gairdneri*) from Spring Valley Trout Farm, Petersburg Ontario, were held in carbon-dechlorinated, aerated, flowing Hamilton city tapwater (hardness ~ 140 mg/l as $CaCO_3$). Trout were fed a commercial pelleted diet three times per week but were fasted during acclimation and testing. Fingerlings (2–4 g) were used in toxicity bioassays, while yearlings (150–400 g) were used in the physiological studies.

In order to produce an artificial soft water (ASW) similar to the dilute waters of northern Ontario's Precambrian shield lakes, dechlorinated tapwater was diluted ten fold with distilled water or water from a reverse osmosis unit (Culligan Corp) to give a final water hardness of ~ 14 mg/l as $CaCO_3$ (Table 1). Fish were acclimated to ASW (15° C, pH 6.9) for a minimum of one week prior to use (McDonald et al. 1980).

Bioassay. The 96 h LC_{50} for zinc in ASW was determined in static tests using reagent grade $ZnSO_4 \cdot 7H_2O$ at two water pH's, 7.5 and 6. Zinc concentrations ranged from 0.05 to 10 mg/l in equal log intervals. White polypropylene tubs containing 50 l of toxicant were randomized for Zn concentration and ten trout fingerlings were added to each tub using a stratified procedure. The protocol followed that of Sprague (1973) except that solutions were gently aerated, and the minimum water to fish ratio was 0.5 l/g of fish·d (Craig and Beggs 1979). Water samples were taken daily, acidified and total Zn measured on a Jarrel-Ash 800 atomic absorption spectrophotometer. The results of the bioassays were used to select two zinc concentrations for the physiological studies, the first being rapidly lethal over 24 h and the second having little or no acute lethality.

Table 1. Some water quality parameters under control conditions from the experimental system with fish in place for 24 h, means ± SE(n)

Cl^- (mequiv/l)	0.125 ± 0.014 (28)
Na^+ (mequiv/l)	0.111 ± 0.007 (54)
K^+ (mequiv/l)	0.009 ± 0.001 (54)
Ca^{2+} (mequiv/l)	0.22 ± 0.08 (49)
Zn^{2+} (mequiv/l)	None detected (< 50 µg/l)
Temperature (°C)	15.6 ± 0.19 (40)
pH[a]	6.88 ± 0.05 (24)
alkalinity (µequiv/l)[b]	208

[a] Statistics performed on $[H^+]$ and converted to pH
[b] Single measurement

Physiological experiments. In order to follow changes in acid-base and ion status during zinc stress, a cannula was implanted in the dorsal aorta (Smith and Bell 1964) under MS 222 anaesthesia. Fish were allowed to recover for 36–48 h in individual compartments (36 × 6 × 8 cm) of a compound box which held eight fish. Each compartment was supplied with water from a common head tank at a rate of 300 ± 30 ml/min·fish. Effluent water was collected in a sump tank, aerated and returned through a cooling coil to the head tank. Total volume of the system was 115 l, and contained no metal parts. Two systems were arranged in parallel so that water or toxicant could be circulated to the fish with minimum disturbance. Solutions were changed daily. Cannulae were flushed regularly with Cortland saline (Wolf 1963) heparinized with 100 IU/ml of ammonium heparin (Sigma). A control blood sample was taken at time zero, followed by samples at 3 h intervals in acutely lethal zinc solution or at 24 h intervals at the lower zinc level. Consecutive control experiments with the same sampling regime were then done to note the effect of the experimental protocol.

Analytical methods. Arterial blood samples (0.8 ml) were drawn anaerobically into gastight Hamilton syringes. A 100 µl sample was immediately deproteinated in 8% $HClO_3$ and refrigerated for later lactate analysis. The remainder of the sample was used for the measurement of pHa, total CO_2 (Ca_{CO_2}) in both plasma and whole blood, Pa_{O_2}, hematocrit (Hct), hemoglobin (Hb) and plasma ions. For the latter, a subsample was centrifuged at 10,000 *g* for 5 min, and the plasma removed for Na^+, Cl^-, K^+, Ca^{2+}, and protein (C_{Pr}) determination. Finally, an equal volume of heparinized saline was infused to maintain blood volume. Only data from fish whose cannulae functioned throughout the experiment and whose Hct remained above 5% were used. This Hct was used as a lower limit, since anemia itself provokes acidosis of complex origin in trout (Wood et al. 1982).

Ca_{CO_2} (Cameron 1971), Pa_{O_2} and pHa were measured on a Radiometer PHM 27 acid-base analyzer fitted with a gas module. The pH microelectrode was thermostatically maintained at the experimental temperature and was frequently calibrated with Radiometer precision buffers. The P_{O_2} electrode, maintained at the experimental temperature, was calibrated with water-saturated nitrogen and air. Hemoglobin was determined as cyanmethemoglobin using Hycel or Sigma reagents. L-(+)-lactic acid concentration was assayed enzymatically (LDH/NADH; Sigma). Plasma total protein was measured on a Goldberg refractometer (American Optical). Plasma Cl^- levels were measured with a Radiometer CMT-10 titrator, and other ions with EEL (Na^+, K^+) or Coleman (Ca^{2+}) flame photometers. The K^+ standards were swamped with Na^+ to compensate for the high sample Na^+ level.

Terminal samples for total blood zinc were taken by caudal puncture at the end of the experiment, or when cannulae became inoperative. Blood zinc levels from acutely poisoned fish were determined by exposing uncannulated yearlings to 1.7 mg/l Zn and sampling by caudal puncture after the fish lost equilibrium, (~ 14 h). Samples were dried, ashed, dissolved in 20% HNO_3, and analyzed by atomic absorption spectrophotometry. Blanks, standards (recovery = 92.3 ± 0.7 (7)%) and spiked samples (recovery = 98.5 ± 3.3 (7)%) were also carried through the

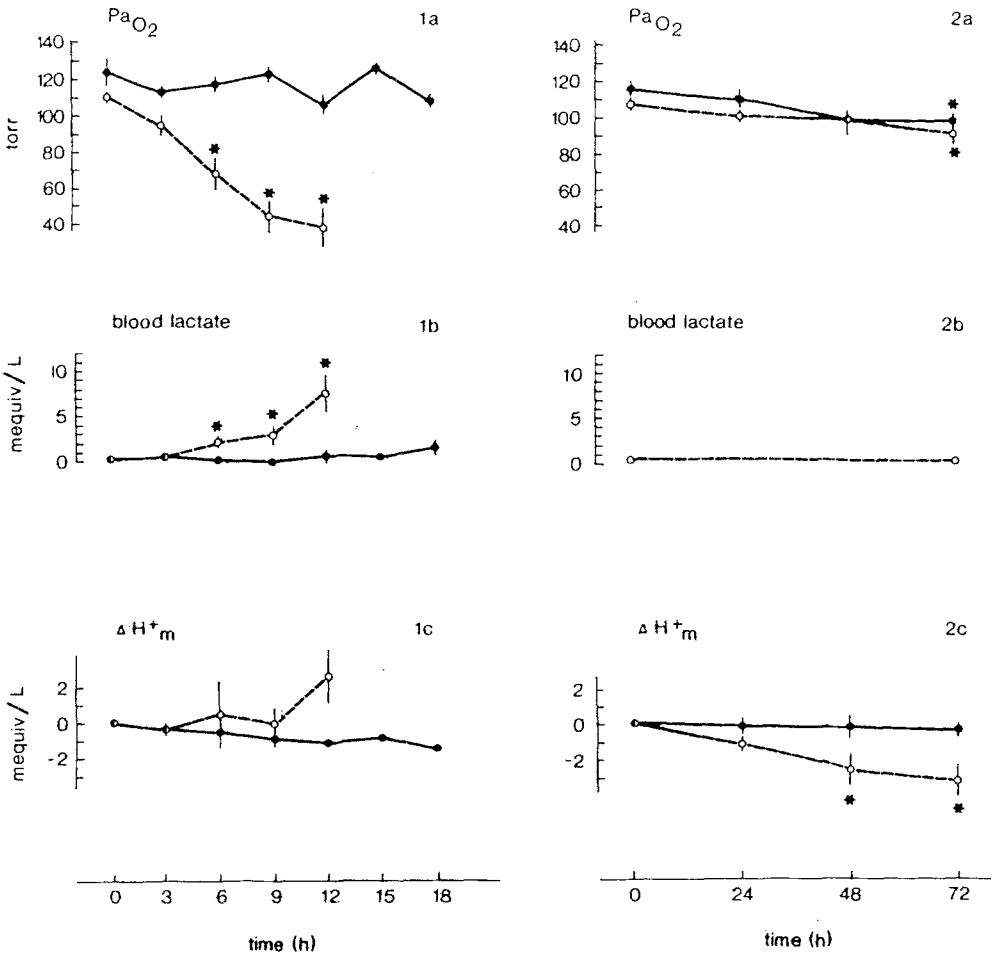

Fig. 1. **a** Arterial oxygen tension, **b** whole blood lactate and **c** metabolic acid load of control trout (-●-) and trout exposed to 1.5 mg/l Zn (--o--) for up to 12 h (mean ± SE). For controls $n=4$, for experimentals n declined with time being 10, 6, 9, 7 and 5 respectively. *Statistics in all figures:* Means marked with an *asterisk* are significantly different from their respective time zero mean ($P<0.05$). The ▲ indicates that time zero means for treatment and control groups are significantly different ($P<0.05$)

Fig. 2. **a** Arterial oxygen tension, **b** whole blood lactate and **c** metabolic acid load of control trout (-●-) and trout exposed to 0.8 mg/l Zn (--o--) for up to 72 h (mean ± SE). For controls $n=9$ for experimentals $n=11$. Statistics as in Fig. 1

procedure. A second blood sample was centrifuged at 10,000 *g* for 5 min and plasma for zinc determination was withdrawn and stored frozen at −20° C. Subsamples were thawed, diluted eight fold and aspirated directly into the atomic absorption spectrophotometer.

Calculations. During the bioassays the cumulative percent mortality was plotted as probits against log time for each zinc concentration. Times to 50% mortality were interpolated and plotted together with 95% confidence limits (Litchfield 1949) against measured Zn concentration on a log/log plot to give a toxicity curve for each water pH. The 96 h LC_{50}'s with 95% fiducial limits were calculated after Litchfield and Wilcoxon (1949).

Pa_{CO_2} was calculated from measured plasma Ca_{CO_2} and pHa using the Henderson-Hasselbalch relationship:

$$Pa_{CO_2}=\frac{Ca_{CO_2}}{\alpha_{CO_2}(1+\text{antilog}(\text{pHa}-\text{pK}'_1))} \quad (1)$$

where pK'_1 is the apparent first dissociation constant of carbonic acid, and α_{CO_2} the solubility of CO_2 in plasma at the appropriate temperature from Severinghaus (1965). The HCO_3^- concentration for either plasma or whole blood was determined from:

$$[HCO_3^-]=Ca_{CO_2}-\alpha_{CO_2}\cdot P_{CO_2} \quad (2)$$

(Severinghaus 1965) by substitution of the appropriate Ca_{CO_2}. The metabolic acid load added to or removed from the blood (ΔH^+_m) over any time period was determined after McDonald et al. (1980):

$$\Delta H^+_m=[HCO_3^-]_i-[HCO_3^-]_f-\beta(pH_i-pH_f) \quad (3)$$

where the subscripts i and f refer to initial and final values. The HCO_3^- concentrations are whole blood values. The slope of the non-bicarbonate buffer line (β) was estimated from the Hb concentration using the relationship of Wood et al. (1982):

$$\beta=-1.073[Hb]-2.48. \quad (4)$$

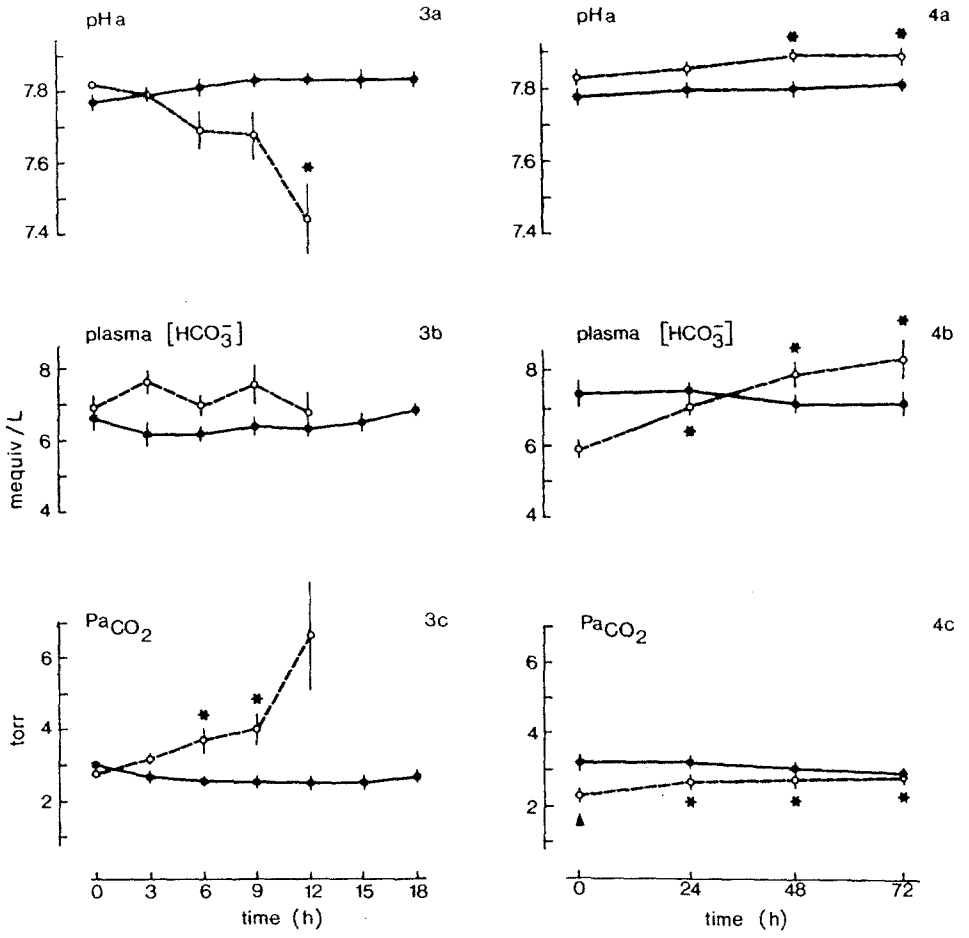

Fig. 3. Acid-base parameters: **a** arterial blood pH, **b** plasma bicarbonate concentration and **c** arterial carbon dioxide tension of control trout (-●-), and trout exposed to 1.5 mg/l Zn (--o--) for up to 12 h (mean ± SE). For controls $n = 4$, for experimentals n decreased from 10 to 6, 9, 7 and 5 respectively. Statistics as in Fig. 1

Fig. 4. Acid-base parameters: **a** arterial blood pH, **b** plasma bicarbonate concentration and **c** arterial carbon dioxide tension of control trout (-●-), and trout exposed to 0.8 mg/l Zn (--o--) for up to 72 h (mean ± SE). For controls $n = 9$, for experimentals $n = 11$. Statistics as in Fig. 1

The total blood metabolic acid load at any time was calculated by summing the ΔH_m^+'s, signs considered, for each period from the time zero sample onward.

Statistics. Values are reported as mean ± one standard error (n). To assess significant differences, treatment means were compared to the time zero mean (i.e. prior to zinc exposure) by two-tailed Student's paired t-test at the 0.05 level, while unpaired t-tests were used to compare treatment means to control means at any given time. Means for blood zinc data were compared using Duncan's new multiple range test (Steel and Torrie 1960).

Results

The major water parameters in the recirculating system after 24 h with fish in place (Table 1) were similar to those given by Beamish and Van Loon (1977) for soft water Ontario shield lakes.

The bioassay 96 h LC_{50}'s with their 95% fiducial limits were 0.56 mg/l (0.34–0.93) for pH 7.5 (range, 7.46–7.52) and 0.67 mg/l (0.35–1.29) for pH 6.0 (range, 5.90–6.23). There is essentially no pH effect as shown by the extensive overlap of fiducial limits. Based on these bioassays, the two zinc concentrations chosen for physiological studies were nominally 0.8 and 1.5 mg/l; a 'low' level near the 96 h LC_{50} and an acutely toxic 'high' level.

'High level' exposure

Although there was no mortality in the controls, exposure to 1.5 mg/l Zn was rapidly lethal to all fish. No fish died before 6 h exposure but the number of dead trout rose rapidly after 9 h with

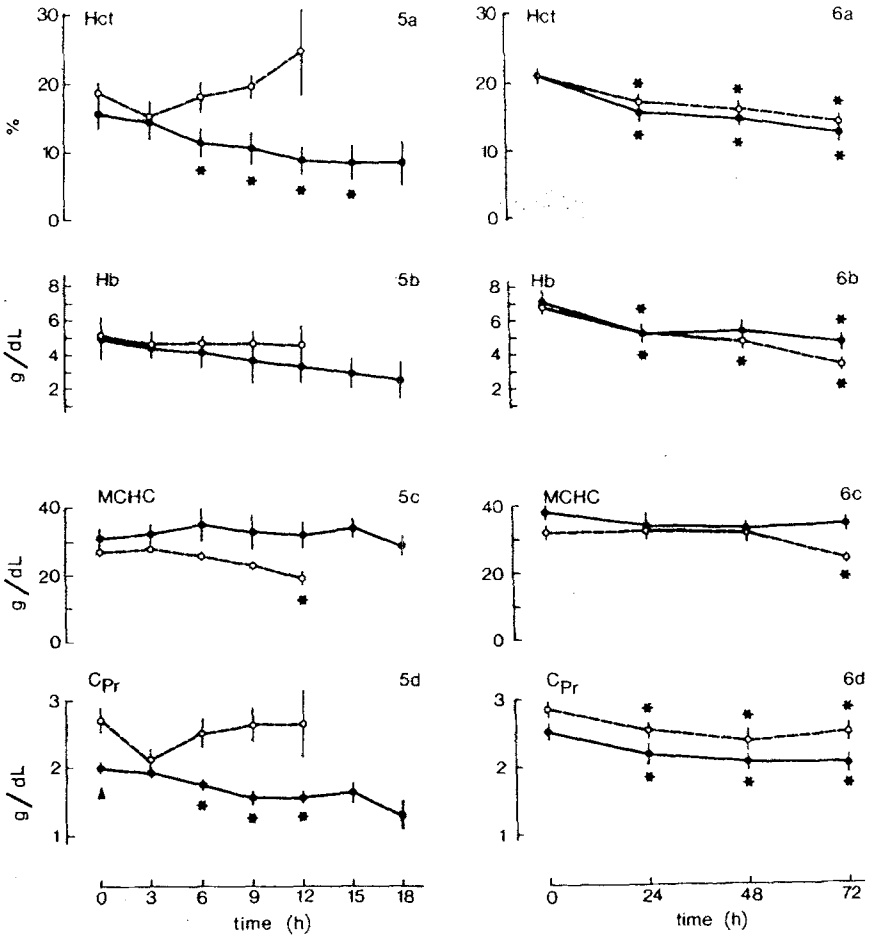

Fig. 5. **a** Hematocrit, **b** hemoglobin, **c** mean cell hemoglobin concentration, **d** plasma total protein of control trout (•–), and trout exposed to 1.5 mg/l Zn (--o--) for up to 12 h (mean ± SE). For controls $n=4$, for experimentals n decreased from 10 to 6, 9, 7 and 5 respectively. Statistics as in Fig. 1

Fig. 6. **a** Hematocrit, **b** hemoglobin, **c** mean cell hemoglobin concentration, **d** plasma total protein of control trout (•–) and trout exposed to 0.8 mg/l Zn (--o--) for up to 72 h (mean ± SE). For controls $n=9$, for experiments $n=11$. Statistics as in Fig. 1

complete mortality by 15 h. The fact that fish deteriorated quickly but at different times is reflected by the larger standard errors and declining N number near the end of the experiments. The Pa_{O_2} was drastically reduced (Fig. 1a). This coincided with a rise in blood lactate (Fig. 1b). Metabolic acid load (ΔH_m^+) was slightly negative in controls but appeared to rise at 12 h of zinc exposure (Fig. 1c). The three parameters which fix the acid-base status of the blood, pHa, Pa_{CO_2}, and plasma HCO_3^- are shown in Fig. 3. Acutely lethal exposure to zinc had pronounced effects, causing a rapid drop in the pHa and rise in Pa_{CO_2} (Figs. 3a, c). Plasma HCO_3^- (Fig. 3b) fluctuated, but showed no significant trend. The fish were clearly acidotic with low arterial oxygen tensions approaching venous levels (~30 Torr) (Holeton and Randall 1967; Wood et al. 1979). Analysis of the pH disturbance on pH/bicarbonate diagrams (Davenport 1974) revealed that the acidosis was of mixed metabolic and respiratory origin with considerable variability in the relative importance of the two components. The respiratory component predominated by 12 h in all fish however. Unlike the control group, Hct did not fall during acutely lethal zinc exposure (Fig. 5a) suggesting a swelling of RBC's accompanying the acidosis. This is supported by a decrease in MCHC ([Hb]/Hct × 100) at 12 h (Fig. 5c). C_{Pr} (Fig. 5d) did not decrease as it did in control fish, suggesting some loss of water from the plasma compartment. The significantly higher control time zero value does not alter this interpretation.

Concentrations of major plasma ions showed no major changes due to zinc exposure when compared with control fish (Fig. 7). Plasma chloride

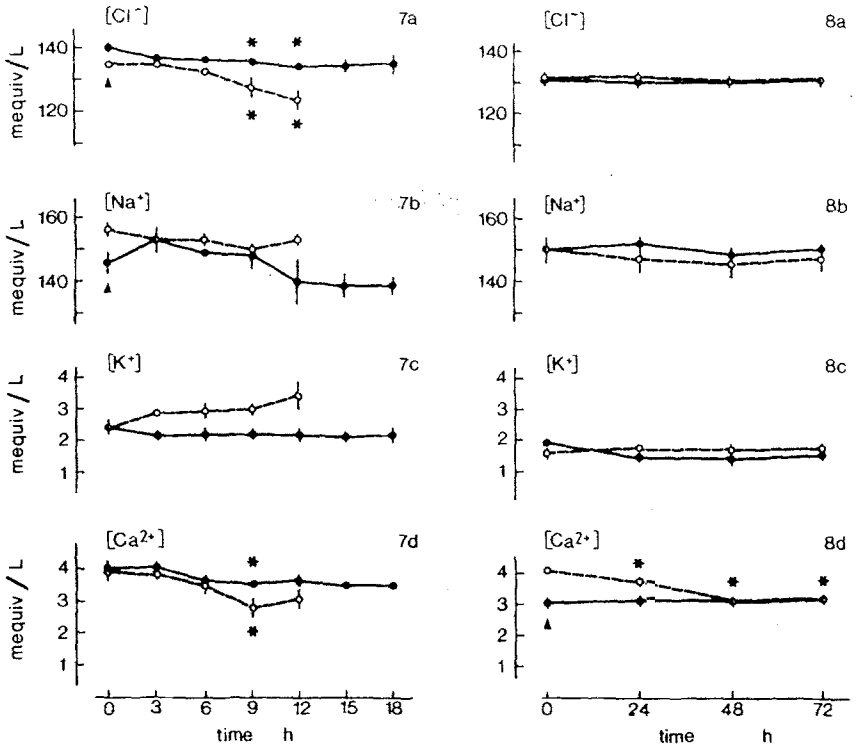

Fig. 7. Major plasma ions: **a** chloride, **b** sodium, **c** potassium, **d** calcium in control trout (-●-) and trout exposed to 1.5 mg/l Zn (--o--) for up to 12 h (mean ± SE). For controls $n=4$, for experimentals n decreased from 10 to 6, 9, 7 and 5 respectively. Statistics as in Fig. 1

Fig. 8. Major plasma ions: **a** chloride, **b** sodium, **c** potassium, **d** calcium in control trout (-●-) and trout exposed to 0.8 mg/l Zn (--o--) for up to 72 h (mean ± SE). For controls $n=9$, for experimentals $n=11$. Statistics as in Fig. 1

in control fish was significantly depressed at 9 and 12 h of exposure but subsequently recovered while during zinc exposure it fell to a greater extent and did not recover (Fig. 7a). There were unexplained significant differences between time zero plasma sodium for both treated and control trout (Fig. 7b). Despite this, there was no effect of zinc exposure. Plasma potassium (Fig. 7c) was similarly unaffected. Although plasma calcium was significantly depressed at 9 h of zinc exposure (Fig. 7d) the response of the controls was similar suggesting a sampling rather than a treatment effect.

'Low level' exposure

While some deaths occurred in control fish during these experiments, they were totally due to low Hct (<3%) or mishap. Apart from a small drop in Pa_{O_2} (Fig. 2a), sampling effects in the control group were restricted to the expected declines in Hct, Hb, and C_{Pr} (Fig. 6a, b, d). However, in zinc-exposed fish, only about one fifth of the total mortality of 39% was due to low Hct or mishap, with the remainder apparently due to zinc.

Exposure to the 'low level' of zinc, 0.8 mg/l, for a longer period of time (3 d) gave very different results than did acute exposure to 1.5 mg/l Zn. Alkalosis was the dominant physiological response. The metabolic acid load (Fig. 2c) showed a clear decline from control values reflecting the accumulation of HCO_3^- in the plasma. Blood lactate levels (Fig. 2b) were unchanged. Pa_{O_2} (Fig. 2a) fell slightly and uniformly in both groups but remained greater than 90 Torr, a partial pressure which should still saturate the hemoglobin (Cameron 1971). The arterial pH rose significantly (Fig. 4a) due to the accumulation of HCO_3^- (Fig. 4b) but the alkalosis was reduced by half by the simultaneous rise in Pa_{CO_2} (Fig. 4c). Reasons for the differing initial values of HCO_3^- and Pa_{CO_2} between treated and controls (Fig. 4b, c) are unknown and although this obviates comparisons of control with treated groups in this case, the response due to zinc is a clear accumulation of total CO_2 in the plasma and a concomitant rise in pHa. The Hct and C_{Pr} both fell due to saline replacement (Fig. 6a, b). Plasma chloride, sodium and potassium were unaffected by zinc exposure (Fig. 8a, b,

Table 2. Mean zinc levels (±SE(n)) in whole blood (μequiv/g) and plasma (μequiv/ml) of rainbow trout. Means which are underlined are *not* significantly different ($P<0.05$)

Water Zn (mg/l)	Control	0.8	1.7[a]
Exposure time (h)	120	120	14
Whole blood Zn	0.17 ±0.03 (4)	0.57 ±0.02 (9)	0.44[b] ±0.04 (5)
Plasma Zn	0.11 ±0.05 (3)	0.52 ±0.04 (11)	0.77 ±0.07 (5)
RBC Zn	0.67 ±0.26 (2)	0.72 ±0.49 (9)	0.12 ±0.09 (4)
RBC Zn / plasma Zn	4.28 ±2.09 (2)	1.11 ±0.07 (9)	0.18 ±0.09 (4)

[a] Acutely lethal exposure; sampled by caudal puncture at overturn

[b] Significantly different from the corresponding plasma [Zn] ($P<0.01$)

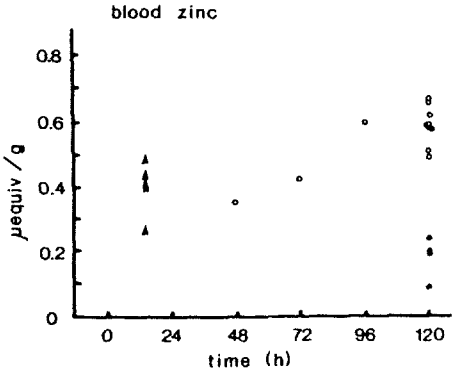

Fig. 9. Scatter diagram of whole blood zinc concentration from control (●) rainbow trout and those exposed to 0.8 mg/l zinc (○) or 1.7 mg/l zinc (▲)

c) while calcium decreased in zinc-exposed fish but remained unchanged in controls (Fig. 8d). The significant difference between control and treatment time zero values complicates interpretation however.

Blood zinc

Under control conditions, whole blood zinc levels were less than 0.2 μequiv/g with a much greater concentration of zinc in the red cells than in the plasma, presumably reflecting the presence of zinc metalloenzyme carbonic anhydrase within the erythrocytes (Table 2). During 'low level' exposure to 0.8 mg/l Zn, whole blood zinc levels progressively increased 3–4 fold in surviving fish (Fig. 9), reaching a level by 3 days significantly higher even than that in fish dying of 'high level' exposure to 1.7 mg/l Zn after ~14 h (Fig. 9, Table 2). However in contrast, plasma levels were higher in the short term 'high level' exposure than in the long term 'low level' exposure (Table 2). There was little if any penetration of zinc into the red cell as shown by the RBC zinc concentration which showed no significant difference across treatments.

Discussion

Exposure of yearling rainbow trout to two different zinc concentrations in ASW, the first near the 96 h LC_{50} and the second approximately 2 times greater, elicited two very different responses. The high level exposure to 1.5 mg/l Zn provoked a well-defined series of events which undoubtedly constitute the mechanism of toxic action in all acute exposures to zinc at concentrations greater than the LC_{50}. Arterial oxygen tension decreased precipitously, as reported by Skidmore (1970) at very high waterborne Zn (40 mg/l), and by Sellers et al. (1975) at their 48 h LC_{50} value of 1.43 mg/l. At the observed Pa_{O_2} of 40 Torr, oxygen loading at the gill would be minimal, especially in view of the simultaneous acidosis. Root and Bohr effects (Riggs 1970) would also impede oxygen uptake and the possibility of direct effects of zinc on the oxygen carrying characteristics of hemoglobin cannot be discounted. The ensuing hypoxemia necessitated a dependance upon glycolysis as demonstrated by the rise in blood lactate concentration. Increased blood lactate is a common consequence of environmental hypoxia (Holeton and Randall 1967; Burggren and Cameron 1980) while lethal zinc exposures are known to increase lactate in other tissues, notably muscle and liver (Burton et al. 1972; Hodson 1976). These observations are consistent with the mechanism proposed by Skidmore and Tovell (1972) in which lamellar damage caused increased water to blood diffusion distance ending in collapse of branchial perfusion and death from hypoxia. This results directly from an external effect of zinc on the gills rather than an effect mediated through zinc accumulation in the blood. Indeed, blood zinc levels from dying fish in the high level exposure were lower than those from surviving fish in the low level exposure (Fig. 9), and the latter showed no evidence of hypoxia.

The associated acidosis, also reported by Sellers et al. (1975), was found in the present study to be a mixed acidosis, slightly more respiratory than metabolic in nature. The metabolic component (ΔH_m^+) was much smaller than the large rise in blood lactate suggesting that a portion of the H^+ from the lactic acid may have been retained intracellularly, similar to the differential release of lactate and H^+ seen in severely exercised rainbow trout (Turner et al. 1983). The respiratory compo-

nent, a doubling of the Pa_{CO_2}, could reflect increased resistance to diffusion of gases by the damaged branchial epithelium which is probably the major cause of decreased Pa_{O_2}. However, it is unlikely that this is the sole cause of the Pa_{CO_2} rise. Given the very high solubility of CO_2, some 30 fold greater than O_2 (Dejours 1975) and assuming a respiratory exchange ratio near unity, the observed decrease in Pa_{O_2} of 70 Torr should be accompanied by a rise in Pa_{CO_2} of ~2.3 Torr. This value is only 60% of the observed increase suggesting the occurrence of other influences. One factor might be a zinc-induced inhibition of carbonic anhydrase. This enzyme is located in gill tissue and thought to be necessary for CO_2 excretion (Maetz 1971). Although itself a zinc metalloenzyme, carbonic anhydrase is inhibited in vitro by zinc. The concentration causing 50% inhibition of the enzyme was 1.4 m equiv/l (Christensen and Tucker 1976).

The changes in the other blood parameters were generally consistent with acidosis. The maintenance of Hct, despite removal of RBC's, was due to erythyrocyte swelling as shown by decreased MCHC. Maintenance of C_{Pr} suggests loss of water from the plasma volume more likely correlated with hypoxic stress (Swift and Lloyd 1974) than directly with acidosis. Such hemoconcentration would also tend to maintain the Hct. The decrease in plasma Cl^- might be due to a shift of Cl^- into RBC's due to the effect of lowered plasma pH on the Donnan distribution for Cl^- (Funder and Wieth 1966) and/or a penetration of Cl^- into the intracellular compartment to balance the efflux of lactate. Such an exchange is suggested by Turner et al. (1983), who found that the rise in blood lactate concentration coincided with a nearly equivalent fall in plasma Cl^- in severely exercised trout.

The acidosis was for the most part a consequence of the hypoxia, and a contributing rather than a causative factor in acute zinc mortality. Rainbow trout routinely experience pHa depressions below 7.4 immediately following exhaustive exercise (Turner et al. 1983), a condition from which they generally recover (Wood et al. 1983).

In distinct contrast to the changes caused by an acutely lethal zinc exposure, the low level exposure to 0.8 mg/l Zn for 3 days resulted in an opposite change in acid-base state with no detectable change in ionic status. Instead of an acidosis as seen previously, an alkalosis developed. The hypoxemia, which provoked the acidosis, was lacking and the absence of an increase in lactate was proof of adequate oxygen delivery. The mortality in these fish was however higher than that in controls. This suggests that there was some lethal mechanism not involving hypoxia, or that the hypoxia developed so rapidly that it was missed in the sampling protocol. The latter possibility is unlikely, since all the parameters which changed significantly during 'low level' zinc stress did so gradually. In one case, Pa_{O_2} was 108 Torr six hours before death, a value slightly higher than the respective day 0 value.

The observed accumulation of base in the blood might be caused by the endogenous production of ammonia or a perturbation of the normal acid-base regulation in the fish, particularly the electroneutral exchanges of Cl^- for HCO_3^- and Na^+ for H^+ in the gill (Maetz and Garcia-Romeu 1964; Maetz et al. 1976; Cameron 1978). Concentrations of waterborne zinc, which are not sufficiently toxic to cause widespread structural damage to the gill, may upset these exchanges. Alternatively, the effect could be mediated by the observed accumulation of zinc in the blood. A net accumulation of base need not cause detectable changes in plasma Na^+ or Cl^- levels. For example, Perry et al. (1981) used SITS to block anion uptake, which resulted in a slight alkalosis with no change in plasma Cl^-. An additional cause of alkalosis might be increased acid excretion by the kidney (Wood and Caldwell 1978).

The compensatory accumulation of CO_2 diminished the alkalosis by about 60%. Fish cannot easily regulate Pa_{CO_2} for the physical reasons alluded to above (Rahn 1966). Accumulation of CO_2 when oxygen delivery is apparently adequate suggests that limitations of perfusion or diffusion are not responsible. Perhaps inhibition of carbonic anhydrase is again involved, as hypothesized for the "high level" exposure.

Comparative data for zinc in plasma, RBC's, and whole blood of fish are few. Recalculation of Skidmore's (1970) data for trout exposed to 40 mg/l Zn suggests that zinc taken up from the water preferentially accumulated in plasma, as seen in both exposures of the present study (Table 2). The phenomenon may be exaggerated in the high level exposure (1.7 mg/l Zn) by erythrocytic swelling due to acidotic hypoxemia, which would tend to concentrate zinc in the plasma and dilute it in the RBC's. However preferential plasma accumulation might also be expected because of the general cation impermeability of the erythrocytes and the affinity of plasma proteins for divalent cations.

In summary, the acid-base response of rainbow trout to acutely toxic zinc exposure is clear and consistent with death due to hypoxia caused by severe gill damage from an external action of zinc. Perturbations of ionic status occurred only for plasma chloride, were not particularly large and

are seemingly of minor importance. However zinc has other effects upon the physiology of trout which are masked by acutely lethal exposure. This was seen in the low level exposure where zinc accumulated to a greater extent in the blood, yet changes in the acid-base status were opposite to those encountered in the high level exposure. These changes were well within the limits normally tolerated by rainbow trout but nevertheless, some mortality occurred. The toxic mechanism operating under these conditions lies outside gross changes in blood acid-base, gas, or ion status acting instead perhaps at the cellular level.

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