The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*) 2. The effects of silver thiosulfate

Chris M. Wood*, Christer Hogstrand[1], Fernando Galvez, R.S. Munger

Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ont. L8S 4K1, Canada

Abstract

The physiological responses of adult rainbow trout to a high level (30 000 μg l^{-1}) of waterborne silver complexed by thiosulfate [Ag(S$_2$O$_3$)$_n^-$], as occurs in photoprocessing effluent, were compared with the responses to a low level of ionic Ag$^+$ (10 μg l^{-1} as AgNO$_3$). Ag(S$_2$O$_3$)$_n^-$ was synthesized by combining one mole part AgCl with four mole parts of Na$_2$S$_2$O$_3$; responses to an equivalent level of Na$_2$S$_2$O$_3$ alone (1.11 mM) were examined as a control. Under flow-through conditions in moderately hard freshwater, 97% occurred as Ag(S$_2$O$_3$)$_2^{3-}$, 3% as AgS$_2$O$_3^-$, and a negligible fraction as ionic Ag$^+$ (< 0.003 μg l^{-1}). Whereas 10 μg l^{-1} Ag (as AgNO$_3$) caused a variety of internal disturbances related to losses of plasma Na$^+$ and Cl$^-$, 3000-fold greater Ag(S$_2$O$_3$)$_n^-$ had very minor effects – a moderate, transient metabolic alkalosis and an apparent expansion of plasma volume. Plasma glucose declined slightly, but this also occurred in Na$_2$S$_2$O$_3$ controls. Plasma Na$^+$, Cl$^-$, Ca^{2+}, K$^+$, lactate, arterial blood P_{O_2} and P_{CO_2}, and hematology were essentially unaffected. Nevertheless, by Day 6, total gill Ag was 3-fold greater than during AgNO$_3$ exposure, while plasma Ag was 3–4 times greater (stabilized by Day 2). There was also two-fold greater Ag accumulation in the liver than during AgNO$_3$ exposure, as well as accumulation in kidney, and an induction of metallothionein in liver, gills, and kidney. We conclude that acute toxic effects of waterborne silver are caused by ionic Ag$^+$ interacting with key functions at the gill surface and not by internal Ag accumulation. Even very high levels of waterborne silver are relatively benign when complexed by thiosulfate.

*Corresponding author. Tel. (+1-905) 525 9140, fax. (+1-905) 522 6066.

[1]*Present address:* T.H. Morgan School of Biological Sciences, 101 Morgan Building, University of Kentucky, Lexington, KY 40506-0225, USA

1. Introduction

The preceding study (Wood et al., 1996) has demonstrated that the extreme toxicity of free ionic Ag^+ (10 µg l^{-1} total Ag tested as $AgNO_3$) to freshwater fish is associated with a disruption of net Na^+ and Cl^- uptake at the gills, resulting in internal ionic and fluid volume disturbances, hemoconcentration, and probable circulatory failure. These effects were attributed to ionic Ag^+ binding to the gill surface, although Ag also entered the fish, as shown by a moderate accumulation in blood plasma and a larger accumulation in the liver. In natural waters, even heavily polluted sites, the levels of free Ag^+ are very low (< 0.2 µg l^{-1}; Chudd, 1983; Lytle, 1984). The great majority of total Ag exists as complexes and particulates (Cooper and Jolly, 1970; Cooley et al., 1988) because of the great avidity of many anionic ligands for Ag^+ and the low solubility of many of the products (Morel and Hering, 1993). In first world countries, the majority of industrial silver discharge originates from photoprocessing operations (Taylor et al., 1980; Lytle, 1984). Silver thiosulfate complexes $[Ag(S_2O_3)_n^-]$ predominate in the effluent due to the use of sodium thiosulfate as an Ag^+ scavenging agent during film development (Bard et al., 1976; Cooley et al., 1988). Thiosulfate is an unusual ligand inasmuch as it binds Ag^+ with great avidity (log K values of 8.8, 13.7 and 14.2 for the mono-, di-, and tri-thiosulfate complexes, respectively), yet the products are extremely soluble, unlike chloride, sulfide, and iodide complexes (Morel and Hering, 1993).

The importance of speciation in metal toxicity is now well recognized (e.g. Meador, 1991; Florence et al., 1992). For most metals, free cationic forms are most toxic, apparently due to interactions at anionic sites at the gill surface, but other forms may also cause damage (Pagenkopf, 1983; McDonald et al., 1989; Wood, 1989; McDonald and Wood, 1993). This raises the question whether dissolved $Ag(S_2O_3)_n^-$ complexes, especially in high concentration, will be at all toxic to fish, and whether they will exert similar physiological effects to free Ag^+. On the one hand, LC50 tests with fathead minnows (*Pimephales promelas*; Terhaar et al., 1972; Leblanc et al., 1984) and rainbow trout (*Oncorhynchus mykiss*; Hogstrand et al., 1995) have indicated that $Ag(S_2O_3)_n^-$ complexes have very low toxicity. On the other, thiosulfate does not entirely prevent Ag accumulation on the gills (Janes and Playle, 1995) or internal accumulation in the fish (Terhaar et al., 1977; Hogstrand et al., 1995).

Our primary objective was to examine the physiological effects of a very high level of $Ag(S_2O_3)_n^-$ (30 000 µg l^{-1} as Ag) on adult rainbow trout, parallel to our previous study with a much lower level of $AgNO_3$ (10 µg l^{-1} as Ag; Wood et al., 1996). $Ag(S_2O_3)_n^-$ is not available commercially; in order to simulate photoprocessing effluent, $Ag(S_2O_3)_n^-$ (highly soluble) was generated by mixing 1 mol part AgCl (highly insoluble) to 4 mol parts $Na_2S_2O_3$ (highly soluble). To check for possible toxic effects of thiosulfate alone, this same high concentration of $Na_2S_2O_3$ was run

as a control. A second objective was to determine whether exposure to such a high level of $Ag(S_2O_3)_n^-$ would result in significant entry of Ag and/or induction of metallothionein (MT) inside the organism. These low molecular weight cysteine-rich proteins play fundamental roles in internal metal regulation and detoxification (Kagi and Schaffer, 1988; Roesijadi, 1992). Very recently, it has been reported that MT may be induced by Ag exposure in mammalian cells (Palmiter, 1994) and intact fish (Hogstrand et al., 1995). However, the entry of Ag into $AgNO_3$ exposed trout in the previous study did not result in MT induction (Wood et al., 1996).

2. Materials and methods

2.1. Experimental animals

Adult rainbow trout (*Oncorhynchus mykiss*; 250–450 g) were obtained, fed, held, acclimated to identical temperature ($15 \pm 1°C$) and water quality, and implanted with dorsal aortic catheters, as outlined by Wood et al. (1996). Experimental chambers were operated exactly as described — volume = 8 l, 50% replacement time = 15 min, with open-circuit flow to waste.

2.2. Experimental protocol

The exposure system, the blood and water sampling regime over the 6 day exposure, and the terminal tissue sampling methods have been described (Wood et al., 1996). Control $[Na_2S_2O_3]$ and experimental $[Ag(S_2O_3)_n^-]$ series were run simultaneously and replicated three times, yielding total n of 17 for the $Na_2S_2O_3$ and 18 for the $Ag(S_2O_3)_n^-$ treatments (for fish with working catheters). Unfortunately, many of the tissue samples were lost because of a freezer failure, so an additional 7 trout (non-cannulated) were exposed to $Ag(S_2O_3)_n^-$ for 6 days and then sampled. The $Na_2S_2O_3$ stock was 1.850 M $Na_2S_2O_3 \cdot 5H_2O$ (BDH) whereas the $Ag(S_2O_3)_n^-$ stock was 0.4625 M AgCl (Johnson Mathey Ltd.) plus 1.850 M $Na_2S_2O_3 \cdot 5H_2O$ (BDH); the stocks were stored in light-shielded bottles and renewed daily. Filtration through 0.45 μm filters indicated that dissolution of the AgCl was 100%. The peristaltic pumps were set to produce nominal concentrations of 30 000 μg l^{-1} $Ag(S_2O_3)_n^-$ (as Ag, i.e. 0.278 mM) and 1.11 mM $Na_2S_2O_3$.

2.3. Analytical methods and calculations

Analytical methods, calculations, and statistical procedures were identical to those of Wood et al. (1996) and followed protocols detailed in earlier publications (McDonald et al., 1980; Milligan and Wood, 1982; Wood et al., 1988a,b; Playle et al., 1989). The one exception was total Ag concentration in the water samples from the $Ag(S_2O_3)_n^-$ exposure which was assayed by flame atomic absorption spectrophotometry (Varian 1275) rather than graphite furnace, in view of the very high levels of Ag present. The speciation of Ag in the exposure water was determined

using measured water chemistry and the aquatic equilibrium program MINEQL$^+$ (Schecher and McAvoy, 1992). In the figures, changes significant within a group relative to the pre-exposure value are marked with an asterisk (*), and differences significant between groups at the same time are noted in the figure legends. For comparison, the mean responses of the fish exposed to 10 μg l^{-1} AgNO$_3$ in the preceding study are indicated with a dotted line.

3. Results

3.1. Water chemistry and survival

The mean measured total Ag concentration during the Ag(S$_2$O$_3$)$_n^-$ exposures was 30 310 ± 690 μg l^{-1} ($n = 48$), very close to the nominal value of 30,000 μg l^{-1}. MINEQL$^+$ speciation yielded 97.1% Ag(S$_2$O$_3$)$_2^{3-}$ (29,431 μg l^{-1}), 2.9% AgS$_2$O$_3^-$ (879 μg l^{-1}), and a negligible amount of free ionic Ag$^+$ (< 0.003 μg l^{-1}). Ag was consistently below the detection limit of 0.5 μg l^{-1} in both the pre-exposure water and the Na$_2$S$_2$O$_3$ exposures. The addition of Na$_2$S$_2$O$_3$·5H$_2$O raised the water total Na$^+$ concentration from the pre-exposure level of 0.53 ± 0.01 mM ($n = 13$) to 2.49 ± 0.04 mM ($n = 51$) in the Ag(S$_2$O$_3$)$_n^-$ exposures, and to 2.60 mM ± 0.05 mM ($n = 45$) in the Na$_2$S$_2$O$_3$ exposures.

No differential mortality could be attributed to the Ag(S$_2$O$_3$)$_n^-$ exposure. Four of 18 trout exposed to Ag(S$_2$O$_3$)$_n^-$ died (between 48 and 144 h), whereas 3 of 17 trout exposed to Na$_2$S$_2$O$_3$ alone died over the same time course. Inspection of data from
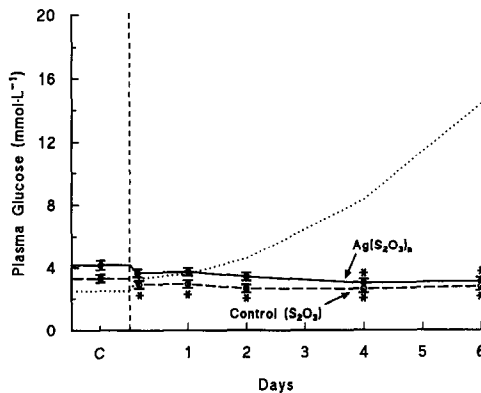

Fig. 1. The influence of 6 days exposure to 30 000 μg l^{-1} Ag(S$_2$O$_3$)$_n^-$ ($n = 14$–18) or the same total Na$^+$ and S$_2$O$_3^{2-}$ concentrations alone (control S$_2$O$_3^{2-}$; $n = 14$–17) on plasma glucose levels in rainbow trout. Means ± 1 S.E.M. Asterisks indicate means significantly different ($p < 0.05$) from the pre-exposure value ('C') in that treatment. There were no significant differences at any time between the two treatments. The dotted line indicates the mean response to 10 μg l^{-1} AgNO$_3$, from Wood et al. (1996).
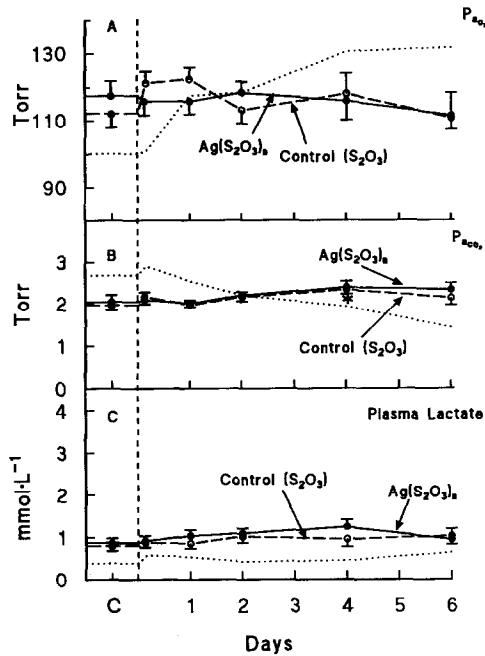
Fig. 2. The influence of 6 days exposure to 30 000 $\mu g \cdot l^{-1}$ Ag(S$_2$O$_3$)$_n^-$ ($n = 14$–18) or the same total Na$^+$ and S$_2$O$_3^{2-}$ concentrations alone (control S$_2$O$_3^{2-}$; $n = 14$–17) on (A) arterial blood O$_2$ tension (P_{aO_2}), (B) CO$_2$ tension (P_{aCO_2}), and (C) plasma lactate levels in rainbow trout. Means ± 1 S.E.M. Asterisks indicate means significantly different ($p < 0.05$) from the pre-exposure value ('C') in that treatment. There were no significant differences at any time between the two treatments. The dotted line indicates the mean response to 10 μg l^{-1} AgNO$_3$, from Wood et al. (1996).

those fish which died indicated no differences from those which survived, so their values were included in all means up to the time of death.

3.2. Physiological responses

There was no evidence of a stress response. In marked contrast to the 5-fold rise in plasma glucose seen earlier with AgNO$_3$, trout exposed to Ag(S$_2$O$_3$)$_n^-$ or to Na$_2$S$_2$O$_3$ alone for 6 days exhibited small but significant decreases in both treatments (Fig. 1). Neither Ag(S$_2$O$_3$)$_n^-$ nor Na$_2$S$_2$O$_3$ alone had any effect on the partial pressures of O$_2$ (Fig. 2A) or CO$_2$ (Fig. 2B) in arterial blood in contrast to the progressive increase in P_{aO_2} and decrease in P_{aCO_2} caused by AgNO$_3$. P_{IO_2} remained uniformly high at 145–155 Torr (not shown). There was also no change in plasma lactate (Fig. 2C); in this respect the results were similar to AgNO$_3$. Thus none of the treatments caused respiratory suffocation.

Arterial blood pH$_a$ exhibited small increases (ca. 0.05 units; Fig. 3A), in concert with significant elevations of plasma HCO$_3^-$ (ca. 1.5 mmol l^{-1}; Fig. 3B), at 4, 24 and 48 h of exposure to Ag(S$_2$O$_3$)$_n^-$. Na$_2$S$_2$O$_3$ had no significant effects at these times. The increases in HCO$_3^-$ at Days 1 and 2 in Ag(S$_2$O$_3$)$_n^-$-exposed trout were

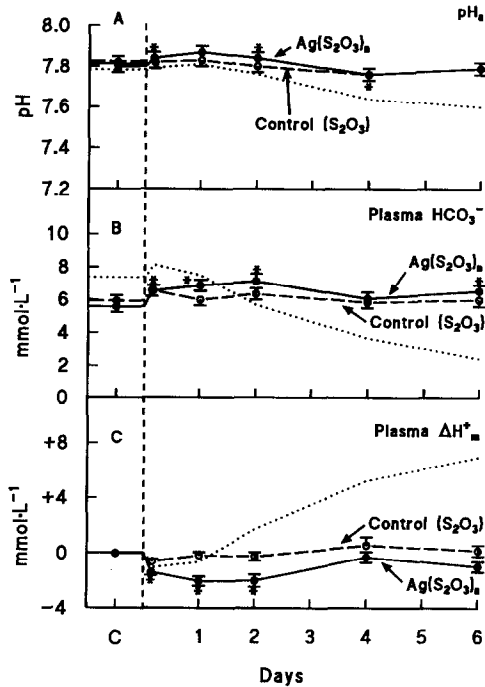Fig. 3. The influence of 6 days exposure to 30 000 μg l^{-1} $Ag(S_2O_3)_n^-$ ($n = 14$-18) or the same total Na^+ and $S_2O_3^{2-}$ concentrations alone (control $S_2O_3^{2-}$; $n = 14$-17) on (A) arterial blood pH (pH_a), (B) plasma HCO_3^-, and (C) plasma metabolic acid load (ΔH_m^+) in rainbow trout. Means ± 1 S.E.M. Asterisks indicate means significantly different ($p < 0.05$) from the pre-exposure value ('C') in that treatment. There were no significant differences at simultaneous times between the two treatments, except at Days 1 and 2 in (B) and (C). At Days 4 and 6 in (A), the means for the two treatments were identical. The dotted line indicates the mean response to 10 μg l^{-1} $AgNO_3$, from Wood et al. (1996).

also significant with respect to the $Na_2S_2O_3$ controls, but the increases in pH_a were not. At Days 4 and 6, pH_a and plasma HCO_3^- returned to pre-exposure values in the $Ag(S_2O_3)_n^-$ treatment, whereas pH_a fell very slightly at Day 4 in the $Na_2S_2O_3$ treatment. The overall acid–base picture was a moderate metabolic alkalosis (negative plasma ΔH_m^+ load of about 2 mmol l^{-1}) during the first 48 h of exposure to $Ag(S_2O_3)_n^-$, corrected by Days 4 and 6 (Fig. 3C). This contrasts with the progressive metabolic acidosis (decreases in pH_a, HCO_3^-, and increases in ΔH_m^+) seen during exposure to $AgNO_3$.

Neither $Ag(S_2O_3)_n^-$ nor $Na_2S_2O_3$ had any effect on plasma Ca^{2+} (Fig. 4A) or K^+ (Fig. 4B), similar to the lack of action of $AgNO_3$ on these parameters. However, unlike $AgNO_3$, these treatments did not lower plasma Na^+ (Fig. 5A) and Cl^- levels (Fig. 5B). Plasma Na^+ actually rose by about 4 mmol l^{-1} over the first two days in the $Ag(S_2O_3)_n^-$-exposed fish (Fig. 5A), increases which were significant with respect to the pre-exposure values, but not with respect to the simultaneous $Na_2S_2O_3$ control values. Plasma Na^+ did not change in the fish exposed to $Na_2S_2O_3$ alone.
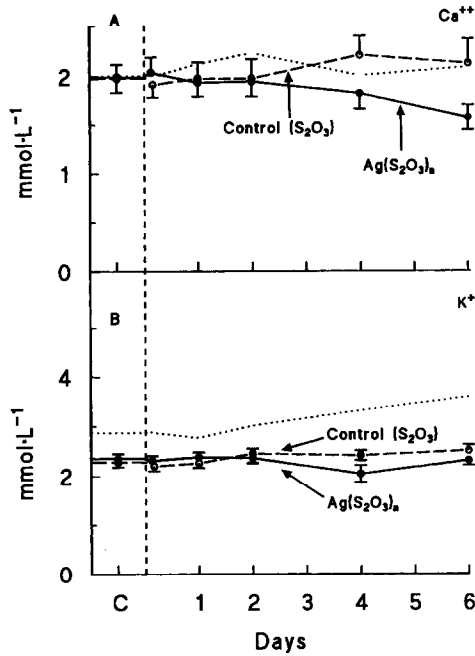
Fig. 4. The influence of 6 days exposure to 30 000 μg l^{-1} $Ag(S_2O_3)_n^-$ ($n = 14$–18) or the same total Na^+ and $S_2O_3^{2-}$ concentrations alone (control $S_2O_3^{2-}$; $n = 14$–17) on (A) plasma Ca^{2+} and (B) plasma K^+ levels in rainbow trout. Means ± 1 S.E.M. Asterisks indicate means significantly different ($p < 0.05$) from the pre-exposure value ('C') in that treatment. There were no significant differences at any time between the two treatments. The dotted line indicates the mean response to 10 μg l^{-1} $AgNO_3$, from Wood et al. (1996).

The hematological picture reflected the relative constancy of the major plasma osmolytes Na^+ and Cl^-. Thus hematocrit (Fig. 6A) and hemoglobin (Fig. 6B) declined with repeated blood sampling in the expected fashion in both $Ag(S_2O_3)_n^-$ and $Na_2S_2O_3$ treatments. This contrasts with the *lack* of change in these parameters during $AgNO_3$ exposure, attributed to fluid loss from the plasma. In similar contrast, plasma protein concentration decreased rather than increased. Protein was significantly depressed with respect to pre-exposure values at all sample times from Day 1 onwards in $Ag(S_2O_3)_n^-$-exposed fish, but only at Days 1 and 2 in $Na_2S_2O_3$-exposed trout. This difference between treatments was significant at Days 4 and 6.

MCHC was not significantly different between treatments at Day 6 (Table 1) or over the time course of the experiment (not shown), indicating constancy of individual red cell volume. There was no evidence of splenic discharge; relative spleen weight and hemoglobin content were similar between treatments. However relative plasma volume increased significantly (by 23%) over the 6 day experiment in the $Ag(S_2O_3)_n^-$-exposed fish but not in the $Na_2S_2O_3$-exposed fish. These responses contrast with the marked splenic discharge and 40% fall in plasma volume seen during $AgNO_3$ treatment (cf. Table 1 of Wood et al., 1996).
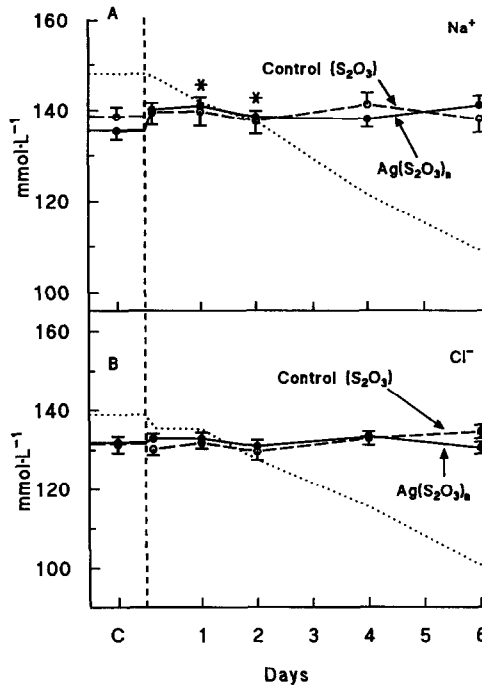
Fig. 5. The influence of 6 days exposure to 30 000 μg l^{-1} Ag(S$_2$O$_3$)$_n^-$ ($n = 14$–18) or the same total Na$^+$ and S$_2$O$_3^{2-}$ concentrations alone (control S$_2$O$_3^{2-}$; $n = 14$–17) on (A) plasma Na$^+$ and (B) plasma Cl$^-$ levels in rainbow trout. Means ± 1 S.E.M. Asterisks indicate means significantly different ($p < 0.05$) from the pre-exposure value ('C') in that treatment. There were no significant differences at any time between the two treatments. The dotted line indicates the mean response to 10 μg l^{-1} AgNO$_3$, from Wood et al. (1996).

3.3. Internal silver and metallothionein levels

Exposure to this high level of Ag(S$_2$O$_3$)$_n^-$ (30 310 μg l^{-1} ≅ 280 μmol l^{-1}) caused an increase in plasma total Ag from about 1 μmol l^{-1} (ca. 110 μg l^{-1}) to about 8 μmol l^{-1} (ca. 900 μg l^{-1}; Fig. 7B). This plasma accumulation was 3- to 4-fold greater than that seen during exposure to 10 μg l^{-1} AgNO$_3$ (ca. 0.1 μmol l^{-1}; Fig. 7A). In both cases, the response reached a plateau by Day 2 onwards. There was no build-up of plasma Ag during exposure to Na$_2$S$_2$O$_3$ alone (Fig. 7B). Gill total Ag was significantly elevated after 6 days of Ag(S$_2$O$_3$)$_n^-$ treatment (Table 2), to about 3-fold the level in the AgNO$_3$ treatment (cf. Table 2 of Wood et al., 1996). In accord with the greater elevation of plasma and gill levels, kidney and especially liver elevations were much greater than seen in AgNO$_3$-exposed trout.

Whereas metallothionein (MT) induction did not occur in response to AgNO$_3$, MT was clearly induced by Ag(S$_2$O$_3$)$_n^-$ in liver, kidney, and gills. Gills exhibited the lowest induction while the highest MT levels occurred in liver, the tissue which accumulated the greatest concentration of Ag (Table 2).
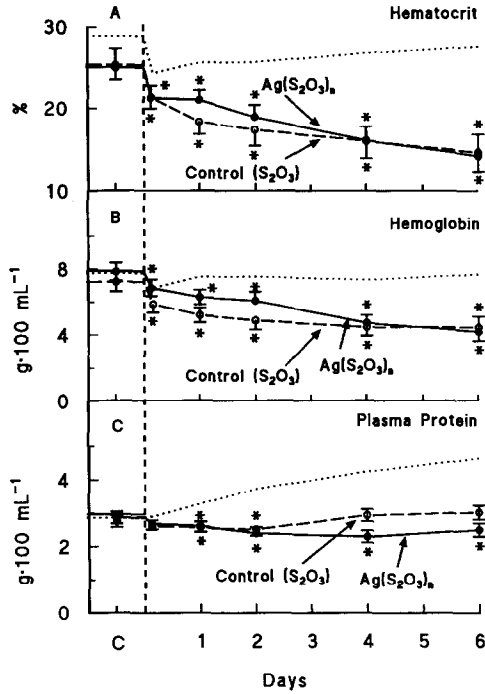
Fig. 6. The influence of 6 days exposure to 30 000 $\mu g \ l^{-1}$ $Ag(S_2O_3)_n^-$ ($n = 14$–18) or the same total Na^+ and $S_2O_3^{2-}$ concentrations alone (control $S_2O_3^{2-}$; $n = 14$–17) on (A) blood hematocrit, (B) blood hemoglobin concentration, and (C) plasma protein concentration in rainbow trout. Means ± 1 S.E.M. Asterisks indicate means significantly different ($p < 0.05$) from the pre-exposure value ('C') in that treatment. There were no significant differences at simultaneous times between the two treatments, except at Days 4 and 6 in (C). The dotted line indicates the mean response to 10 $\mu g \ l^{-1}$ $AgNO_3$, from Wood et al. (1996).

4. Discussion

$Ag(S_2O_3)_n^-$ was synthesized from $Na_2S_2O_3 \cdot 5H_2O$ plus AgCl, rather than

Table 1
Parameters influencing hematological status in rainbow trout exposed to 30 000 $\mu g \ l^{-1}$ $Ag(S_2O_3)_n^-$ or the same total Na^+ and $S_2O_3^{2-}$ concentrations alone (control $S_2O_3^{2-}$) for 6 days

	Control $S_2O_3^{2-}$	$Ag(S_2O_3)_n^-$
MCHC[a] (g Hb ml RBC^{-1})	0.300 ± 0.016	0.294 ± 0.009
Relative plasma volume (%)	96.0 ± 4.7%	123.2 ± 10.9%[b]
[Spleen weight/body weight] × 100	0.28 ± 0.01	0.31 ± 0.03
Spleen hemoglobin (g $spleen^{-1}$)	0.162 ± 0.025	0.171 ± 0.028

Means ± 1 S.E.M ($n = 9$–13).
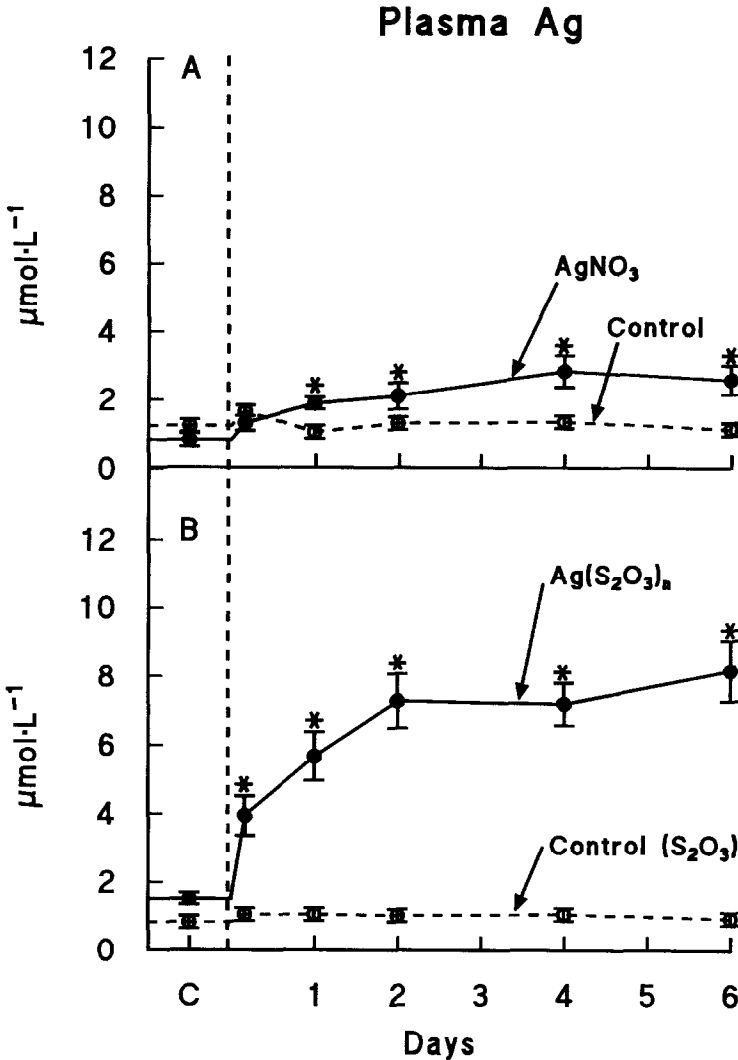[a] MCHC=mean cell hemoglobin concentration.
[b] $p < 0.05$

Plasma Ag

Fig. 7. The influence of 6 days exposure to (A) 10 µg l^{-1} Ag (0.1 µM) as AgNO$_3$ (n=8–12) or (B) 30 000 µg l^{-1} Ag (278 µM) as Ag(S$_2$O$_3$)$_n^-$ (n=13–18) on plasma total Ag concentrations in rainbow trout. Means ±1 S.E.M. Control treatments were either (A) continued exposure to pre-exposure water (n = 4–8) or (B) exposure to the same total Na$^+$ and S$_2$O$_3^-$ concentrations alone (control S$_2$O$_3^{2-}$; n = 10–16). Means ±1 S.E.M. Asterisks indicate means significantly different ($p < 0.05$) from the pre-exposure value ('C') in that treatment. Means in the Ag exposures were also significantly different from the respective simultaneous control means at these times, except at Day 2 in (A). Data in panel (A) are from the study of Wood et al. (1996).

AgNO$_3$, to duplicate the composition of photoprocessing effluent where silver enters from halide salts (Bard et al., 1976; Cooley et al., 1988). The use of AgCl avoided physiological disturbances associated with the ca. 0.3 mM NO$_3^-$ which otherwise

Table 2
Total Ag and metallothionein levels in tissues of rainbow trout exposed to 30 000 μg l^{-1} Ag(S$_2$O$_3$)$_n^-$ or the same total Na$^+$ and S$_2$O$_3^{2-}$ concentrations alone (control S$_2$O$_3^{2-}$) for 6 days

		Control S$_2$O$_3^{2-}$	Ag(S$_2$O$_3$)$_n^-$
Total Ag (μg kg wet tissue^{-1})	Plasma	99 ± 9 (14)	880 ± 90[a] (14)
	Liver	9440 ± 3020 (4)	73 150 ± 10 500[a] (8)
	Gills	70 ± 29 (5)	3180 ± 430[a] (9)
	Kidney	2210 ± 370 (4)	7340 ± 860[a] (7)
Metallothionein (mg kg wet tissue^{-1})	Liver	90.81 ± 21.41 (5)	151.08 ± 12.81[a] (9)
	Gills	13.71 ± 2.51 (5)	27.68 ± 3.64[a] (9)
	Kidney	7.64 ± 0.45 (5)	52.22 ± 13.44[a] (9)

Means ± 1 S.E.M.
[a] $p < 0.05$.

would have been present (Wood and Munger, unpublished results). Cl$^-$ at these concentrations is benign to fish. Speciation calculations demonstrated that a 4:1 ratio of S$_2$O$_3^{2-}$ to Ag$^+$ would ensure negligible Ag$^+$ free in solution, yet avoid excessively high S$_2$O$_3^{2-}$ levels which might be toxic in themselves. The test concentration of Ag(S$_2$O$_3$)$_n^-$ chosen (ca. 30 000 μg l^{-1}) represented 22% of the 7 day LC50 for juvenile rainbow trout in the same water quality (Hogstrand et al., 1995). The concentration of added Na$_2$S$_2$O$_3$·5H$_2$O (1.11 mM) remained far below the highest level (37 mM) tested by Hogstrand et al. (1995) which was non-toxic to juvenile trout.

Relative to the respective LC50's, the test concentration chosen for Ag(S$_2$O$_3$)$_n^-$ (30 000 μg l^{-1} = 22% of the LC50) was lower than that for AgNO$_3$ (10 μg l^{-1} = 110% of the LC50 in Wood et al., 1996). This choice was based on several factors. From preliminary exposures with juvenile trout, we predicted that at this concentration, similar total Ag levels would build up on the gills; higher levels were actually seen (compare gill levels in Table 2 versus values for AgNO$_3$-exposed trout in Table 2 of Wood et al., 1996). Secondly, the level chosen was already far above that ever likely to occur in the environment. For example, undiluted effluent from 48 US photoprocessing plants in the late 1970's averaged only 1100 μg l^{-1} Ag(S$_2$O$_3$)$_n^-$, with a maximum of 3700 μg l^{-1} (Cooley et al., 1988). Effluents today have much lower concentrations because Ag recovery has improved. Finally, the test concentration was dictated by economic reality. AgCl is extremely expensive, and it would have been financially prohibitive to perform flow-through exposures at the LC50 concentration of Ag(S$_2$O$_3$)$_n^-$.

Nevertheless, approximately 20% of the trout exposed to either Ag(S$_2$O$_3$)$_n^-$ (4 of 18) or Na$_2$S$_2$O$_3$ alone (3 of 17) died in the present tests, so the mortality cannot be attributed to Ag(S$_2$O$_3$)$_n^-$ itself. In our experience, cannulated trout occasionally die of unknown causes, but 20% mortality is unexpected. More likely, it reflected toxicity of the thiosulfate anion, although the mechanism is unknown. There

were no differences in physiological parameters in those fish which died. The only physiological effect attributable to the thiosulfate anion was a slight fall in plasma glucose (Fig. 1). This fall suggests either a reduction in stress below pre-exposure levels, and/or a disturbance in the mechanisms of normal blood glucose homeostasis. Fish in these exposures appeared particularly quiet, so perhaps the thiosulfate anion exerts a slight anaesthetic effect.

In contrast to $AgNO_3$ (Wood et al., 1996), $Ag(S_2O_3)_n^-$ did not cause a rise in plasma glucose (Fig. 1), an increase in P_{aO_2} (Fig. 2A), a decrease in P_{aCO_2} (Fig. 2B), or a metabolic acidosis (Fig. 3). Most importantly, it did not cause a fall in plasma Na^+ and Cl^- (Fig. 5), an associated hemoconcentration (Fig. 6), a reduction of the plasma volume (Table 1), or a contraction of the spleen (Table 1). In fact none of the physiological responses caused by $AgNO_3$ (10 µg l^{-1}) were seen at a 3000-fold greater concentration of $Ag(S_2O_3)_n^-$ (30 000 µg l^{-1}), even though much greater amounts of total Ag accumulated in plasma (Fig. 7) and internal organs (Table 2). These finding reinforce the conclusion that free ionic Ag^+ is the highly toxic form of silver and exerts its toxicity at the gill surface rather than internally (Wood et al., 1996). As long as Ag^+ is absent (< 0.003 µg l^{-1} here), very high levels of strongly complexed Ag (97.1% = 29 431 µg l^{-1} as $Ag(S_2O_3)_2^{3-}$, log K = 13.7; 2.9% = 879 µg l^{-1} as $AgS_2O_3^-$, log K = 8.8) are relatively benign.

Despite this general lack of effect, $Ag(S_2O_3)_n^-$ did cause a moderate metabolic alkalosis (rise in plasma pH and HCO_3^-, negative ΔH_m^+ load; Fig. 3) during the first 48 h of exposure. This was likely a 'strong ion' effect (Stewart, 1978; Wood, 1989, 1992) associated with the increase in plasma Na^+ (Fig. 5A). Perhaps $Ag(S_2O_3)_n^-$ stimulates the coupled Na^+ uptake/'acid' excretion transport at the gills (Wood, 1989, 1992). An apparent expansion of plasma volume (Table 1) related to a fall in plasma protein concentration (Fig. 6C) was also attributable to $Ag(S_2O_3)_n^-$. The mechanism could reflect changes in permeability at the gills, kidney, or plasma/interstitial fluid/intracellular fluid interface.

Even though physiological disturbances were minimal, Ag did enter the fish in substantial amounts during this high $Ag(S_2O_3)_n^-$ exposure. Plasma total Ag levels stabilized at about 8 fold control values by Day 2, but were still only about 3% of those in the exposure water (Fig. 7B). Much higher concentrations occurred in liver and kidney (Table 2), suggesting that these organs serve as sinks which may help to keep plasma levels low. Two previous studies also reported that Ag accumulates internally, especially in the liver, when fish are exposed to high levels of $Ag(S_2O_3)_n^-$ (Terhaar et al., 1977; Hogstrand et al., 1995).

Ag presumably enters across the gills. One possible mechanism is that the negatively charged $Ag(S_2O_3)_n^-$ complex itself enters through an anion transport pathway such as the Cl^- uptake mechanism (Wood, 1992). Another is that Ag^+ dissociates from the thiosulfate anion at the gill surface, and then enters by the same mechanism as Ag^+. Theoretically, this could occur if anionic binding sites on the gill surface have a greater avidity for Ag^+ than does the thiosulfate anion. While this might seem unlikely, Janes and Playle (1995) recently concluded that the log K for Ag^+-gill binding is about 10.0 in juvenile rainbow trout, whereas the log K

value for $AgS_2O_3^-$ formation is 8.8 (Morel and Hering, 1993). $AgS_2O_3^-$ comprised 2.9% of the total Ag concentration in the present exposure water.

This dissociation of Ag^+ from $AgS_2O_3^-$ to an Ag-gill ligand could explain why total Ag accumulated on the gills (Table 2; see also Hogstrand et al., 1995 and Janes and Playle, 1995), despite the presence of excess thiosulfate in the water. Gill Ag burdens (Table 2) after 6 days exposure to $Ag(S_2O_3)_n^-$ (30 000 µg l^{-1}) were about 3-fold those after 6 days exposure to $AgNO_3$ (10 µg l^{-1}; Wood et al., 1996). However if the gill Ag burden simply reflects ionic Ag^+ bound to the same gill ligands in both circumstances, we would have anticipated greater toxic symptoms in $Ag(S_2O_3)_n^-$-exposed fish. This did not occur. One important difference may be that metallothionein (MT) induction occurred in the gills of $Ag(S_2O_3)_n^-$-exposed trout (Table 2), and not in the gills of $AgNO_3$-exposed trout (Wood et al., 1996). Therefore the location (surface-bound versus intracellular) and chemical form of Ag^+ (bound to anionic sites or bound in a thiolate cluster on MT) may differ between the two exposures. There is a clear need for detailed work on the mechanism(s) by which Ag binds to and crosses the gill epithelium.

The significant induction of MT in $Ag(S_2O_3)_n^-$-exposed adult trout agrees with findings of Hogstrand et al. (1995) on juvenile trout; both the speed (< 1 week) and extent of induction are remarkable relative to other metals tested in fish. Hogstrand et al. (1995) attributed this to both the high levels of Ag which enter during $Ag(S_2O_3)_n^-$ exposure, and to the potency of Ag in activating MT synthesis, as also seen in mammalian cells (Palmiter, 1994). In addition to its traditional role of internal metal detoxification and immobilization, MT may be involved both the normal and abnormal metabolism of multiple metals (Kagi and Schaffer, 1988; Roesijadi, 1992). Conceivably, Ag loading could alter the metabolism of other metals. In this regard, body Zn levels declined as internal levels of Ag built up during long term exposures in bluegills (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*; Coleman and Cearley, 1974). Loss of Zn from the Zn–MT pool could explain these results because Ag can displace Zn from MT (Kagi and Schaffer, 1988).

To conclude, freshwater rainbow trout tolerate at least a 3000-fold greater concentration of Ag when complexed as $Ag(S_2O_3)_n^-$ than when dissociated into free ionic Ag^+ from $AgNO_3$. None of the physiological disturbances seen with ionic Ag^+ occur when Ag^+ is complexed with thiosulfate, even though much greater amounts of total Ag accumulate internally. Present environmental guidelines and regulations for silver in freshwaters are based on tests with $AgNO_3$ (i.e. ionic Ag^+), yet applied on the basis of total recoverable Ag (e.g. Taylor et al., 1980; EPA, 1980, 1986; Anonymous, 1992). They do not recognize the important influence of complexation in ameliorating toxicity. Even when $Ag(S_2O_3)_n^-$ occurs in the relatively high concentrations present in undiluted photoprocessing effluent, it is unlikely to be a source of acute toxicity to fish.

Acknowledgements

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