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

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Abstract

The physiological responses of adult rainbow trout to a high level (30000 pg l⁻¹) of waterborne silver complexed by thiosulfate [Ag(S₂O₃)₂⁻], as occurs in photoprocessing effluent, were compared with the responses to a low level of ionic Ag⁺ (10 µg l⁻¹ as AgNO₃). Ag(S₂O₃)₂⁻ was synthesized by combining one mole part AgCl with four mole parts of Na₂S₂O₃; responses to an equivalent level of Na₂S₂O₃ alone (1.11 mM) were examined as a control. Under flow-through conditions in moderately hard freshwater, 97% occurred as Ag(S₂O₃)₂⁻, 3% as AgS₂O₃, and a negligible fraction as ionic Ag⁺ (<0.003 µg l⁻¹). Whereas 10 pg l⁻¹ Ag (as AgNO₃) caused a variety of internal disturbances related to losses of plasma Na⁺ and Cl⁻, 3000-fold greater Ag(S₂O₃)₂⁻ 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 NaNO₃ controls. Plasma Na⁺, Cl⁻, Ca²⁺, K⁺, lactate, arterial blood Po₂, and Pco₂, and hematology were essentially unaffected. Nevertheless, by Day 6, total gill Ag was 3-fold greater than during AgNO₃ 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 AgNO₃ 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.

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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 AgNO₃) 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, hemococoncentration, 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(S₂O₃)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(S₂O₃)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 (Onchorhyncus mykiss; Hogstrand et al., 1995) have indicated that Ag(S₂O₃)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(S₂O₃)ₙ⁻ (30 000 µg l⁻¹ as Ag) on adult rainbow trout, parallel to our previous study with a much lower level of AgNO₃ (10 µg l⁻¹ as Ag; Wood et al., 1996). Ag(S₂O₃)ₙ⁻ is not available commercially; in order to simulate photoprocessing effluent, Ag(S₂O₃)ₙ⁻ (highly soluble) was generated by mixing 1 mol part AgCl (highly insoluble) to 4 mol parts Na₂S₂O₃ (highly soluble). To check for possible toxic effects of thiosulfate alone, this same high concentration of Na₂S₂O₃ was run
as a control. A second objective was to determine whether exposure to such a high level of Ag(S₂O₃)₂⁻ 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 AgNO₃ 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 (Onchorhynchus mykiss; 250–450 g) were obtained, fed, held, acclimated to identical temperature (15 ± 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 [Na₂S₂O₃] and experimental [Ag(S₂O₃)₂⁻] series were run simultaneously and replicated three times, yielding total n of 17 for the Na₂S₂O₃ and 18 for the Ag(S₂O₃)₂⁻ 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(S₂O₃)₂⁻ for 6 days and then sampled. The Na₂S₂O₃ stock was 1.850 M Na₂S₂O₃·5H₂O (BDH) whereas the Ag(S₂O₃)₂⁻ stock was 0.4625 M AgCl (Johnson Mathey Ltd.) plus 1.850 M Na₂S₂O₃·5H₂O (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(S₂O₃)₂⁻ (as Ag, i.e. 0.278 mM) and 1.11 mM Na₂S₂O₃.

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(S₂O₃)₂⁻ 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 AgNO₃ 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(S₂O₇)₃ exposures was 30310 ± 690 µg l⁻¹ (n = 48), very close to the nominal value of 30,000 µg l⁻¹. MINEQL⁺ speciation yielded 97.1% Ag(S₂O₇)₃⁻ (29,431 µg l⁻¹), 2.9% AgS₂O₅⁻ (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 Na₂S₂O₇ exposures. The addition of Na₂S₂O₇·5H₂O 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(S₂O₇)₃ exposures, and to 2.60 mM ± 0.05 mM (n = 45) in the Na₂S₂O₇ exposures.

No differential mortality could be attributed to the Ag(S₂O₇)₃⁻ exposure. Four of 18 trout exposed to Ag(S₂O₇)₃ died (between 48 and 144 h), whereas 3 of 17 trout exposed to Na₂S₂O₇ alone died over the same time course. Inspection of data from

![Fig. 1](image-url)  
**Fig. 1.** The influence of 6 days exposure to 30,000 µg l⁻¹ Ag(S₂O₇)₃⁻ (n = 14–18) or the same total Na⁺ and S₂O₇⁻ concentrations alone (control S₂O₇⁻; 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 AgNO₃, 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 AgNO3, trout exposed to Ag(S2O3)− or to Na2S2O3 alone for 6 days exhibited small but significant decreases in both treatments (Fig. 1). Neither Ag(S2O3)− nor Na2S2O3 alone had any effect on the partial pressures of O2 (Fig. 2A) or CO2 (Fig. 2B) in arterial blood in contrast to the progressive increase in $P_{\text{a}O_2}$ and decrease in $P_{\text{a}CO_2}$ caused by AgNO3. $P_{\text{a}CO_2}$ 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 AgNO3. Thus none of the treatments caused respiratory suffocation.

Arterial blood pH exhibited small increases (ca. 0.05 units; Fig. 3A), in concert with significant elevations of plasma HCO3− (ca. 1.5 mmol l−1; Fig. 3B), at 4, 24 and 48 h of exposure to Ag(S2O3)−. Na2S2O3 had no significant effects at these times. The increases in HCO3− at Days 1 and 2 in Ag(S2O3)−-exposed trout were
Fig. 3. The influence of 6 days exposure to 30 000 μg l⁻¹ Ag(S₂O₅)²⁻ (n = 14-18) or the same total Na⁺ and S₂O₅²⁻ concentrations alone (control S₂O₅²⁻; n = 14-17) on (A) arterial blood pH (pHₐ), (B) plasma HCO₃⁻, and (C) plasma metabolic acid load (ΔH⁺ₚₚ) 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 AgNO₃, from Wood et al. (1996).

Also significant with respect to the Na₂S₂O₃ controls, but the increases in pHₐ were not. At Days 4 and 6, pHₐ and plasma HCO₃⁻ returned to pre-exposure values in the Ag(S₂O₅)²⁻ treatment, whereas pHₐ fell very slightly at Day 4 in the Na₂S₂O₃ treatment. The overall acid–base picture was a moderate metabolic alkalosis (negative plasma ΔH⁺ₚₚ load of about 2 mmol l⁻¹) during the first 48 h of exposure to Ag(S₂O₅)²⁻, corrected by Days 4 and 6 (Fig. 3C). This contrasts with the progressive metabolic acidosis (decreases in pHₐ, HCO₃⁻, and increases in ΔH⁺ₚₚ) seen during exposure to AgNO₃.

Neither Ag(S₂O₅)²⁻ nor Na₂S₂O₃ had any effect on plasma Ca²⁺ (Fig. 4A) or K⁺ (Fig. 4B), similar to the lack of action of AgNO₃ on these parameters. However, unlike AgNO₃, 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(S₂O₅)²⁻-exposed fish (Fig. 5A), increases which were significant with respect to the pre-exposure values, but not with respect to the simultaneous Na₂S₂O₃ control values. Plasma Na⁺ did not change in the fish exposed to Na₂S₂O₃ alone.
Fig. 4. The influence of 6 days exposure to 30,000 μg l\(^{-1}\) Ag(S\(_2\)O\(_3\))\(^{-}\) (n=14–18) or the same total Na\(^+\) and S\(_2\)O\(_3\)\(^{-}\) concentrations alone (control S\(_2\)O\(_3\); 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\(_2\)O\(_3\))\(^{-}\) and Na\(_2\)S\(_2\)O\(_3\) 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\(_2\)O\(_3\))\(^{-}\)-exposed fish, but only at Days 1 and 2 in Na\(_2\)S\(_2\)O\(_3\)-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\(_2\)O\(_3\))\(^{-}\)-exposed fish but not in the Na\(_2\)S\(_2\)O\(_3\)-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).
3.3. Internal silver and metallothionein levels

Exposure to this high level of Ag(S₂O₃)₃⁻ (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; Fig. 7B). This plasma accumulation was 3- to 4-fold greater than that seen during exposure to 10 μg l⁻¹ AgNO₃ (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 Na₂S₂O₃ alone (Fig. 7B). Gill total Ag was significantly elevated after 6 days of Ag(S₂O₃)₃⁻ treatment (Table 2), to about 3-fold the level in the AgNO₃ 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 AgNO₃-exposed trout.

Whereas metallothionein (MT) induction did not occur in response to AgNO₃, MT was clearly induced by Ag(S₂O₃)₃⁻ 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 μg l⁻¹ Ag(S₂O₃)₆⁻ (n = 14–18) or the same total Na⁺ and S₂O₃²⁻ concentrations alone (control S₂O₃²⁻; 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 μg l⁻¹ AgNO₃, from Wood et al. (1996).

4. Discussion

Ag(S₂O₃)₆⁻ was synthesized from Na₂S₂O₃·5H₂O plus AgCl, rather than

Table 1
Parameters influencing hematological status in rainbow trout exposed to 30 000 μg l⁻¹ Ag(S₂O₃)₆⁻ or the same total Na⁺ and S₂O₃²⁻ concentrations alone (control S₂O₃²⁻) for 6 days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control S₂O₃²⁻</th>
<th>Ag(S₂O₃)₆⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCHC (g Hb ml RBC⁻¹)</td>
<td>0.300 ± 0.016</td>
<td>0.294 ± 0.009</td>
</tr>
<tr>
<td>Relative plasma volume (%)</td>
<td>96.0 ± 4.7%</td>
<td>123.2 ± 10.9%b</td>
</tr>
<tr>
<td>[Spleen weight/body weight] × 100</td>
<td>0.28 ± 0.01</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Spleen hemoglobin (g spleen⁻¹)</td>
<td>0.162 ± 0.025</td>
<td>0.171 ± 0.028</td>
</tr>
</tbody>
</table>

Means ± 1 S.E.M (n = 9–13).

MCHC=mean cell hemoglobin concentration.

b p < 0.05
Fig. 7. The influence of 6 days exposure to (A) 10 μg l⁻¹ Ag (0.1 μM) as AgNO₃ (n=8-12) or (B) 30000 μg l⁻¹ Ag (278 μM) as Ag(S₂O₃)²⁻ (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 S₂O₃²⁻ concentrations alone (control S₂O₃²⁻; 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).

AgNO₃, 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 NO₃⁻ which otherwise
Table 2
Total Ag and metallothionein levels in tissues of rainbow trout exposed to 30000 µg l⁻¹ Ag(S₂O₅)²⁻ or the same total Na⁺ and S₂O₅²⁻ concentrations alone (control S₂O₅²⁻) for 6 days

<table>
<thead>
<tr>
<th>Total Ag (µg kg wet tissue⁻¹)</th>
<th>Control S₂O₅²⁻</th>
<th>Ag(S₂O₅)²⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>99 ± 9 (14)</td>
<td>880 ± 90* (14)</td>
</tr>
<tr>
<td>Liver</td>
<td>9440 ± 3020 (4)</td>
<td>73150 ± 10500* (8)</td>
</tr>
<tr>
<td>Gills</td>
<td>70 ± 29 (5)</td>
<td>3180 ± 430* (9)</td>
</tr>
<tr>
<td>Kidney</td>
<td>2210 ± 370 (4)</td>
<td>7340 ± 860* (7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metallothionein (mg kg wet tissue⁻¹)</th>
<th>Liver</th>
<th>Gills</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90.81 ± 21.41 (5)</td>
<td>13.71 ± 2.51 (5)</td>
<td>7.64 ± 0.45 (5)</td>
</tr>
<tr>
<td></td>
<td>151.08 ± 12.81* (9)</td>
<td>27.68 ± 3.64* (9)</td>
<td>52.22 ± 13.44* (9)</td>
</tr>
</tbody>
</table>

Means ± 1 S.E.M.
*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 S₂O₅²⁻ to Ag⁺ would ensure negligible Ag⁺ free in solution, yet avoid excessively high S₂O₅²⁻ levels which might be toxic in themselves. The test concentration of Ag(S₂O₅)²⁻ 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 Na₂S₂O₅·5H₂O (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(S₂O₅)²⁻ (30 000 µg l⁻¹ = 22% of the LC50) was lower than that for AgNO₃ (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 AgNO₃-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(S₂O₅)²⁻, 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(S₂O₅)²⁻.

Nevertheless, approximately 20% of the trout exposed to either Ag(S₂O₅)²⁻ (4 of 18) or Na₂S₂O₅ alone (3 of 17) died in the present tests, so the mortality cannot be attributed to Ag(S₂O₅)²⁻ 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 AgNO₃ (Wood et al., 1996), Ag(S₂O₃)₃⁻ did not cause a rise in plasma glucose (Fig. 1), an increase in PₐO₂ (Fig. 2A), a decrease in PₐCO₂ (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 AgNO₃ (10 µg l⁻¹) were seen at a 3000-fold greater concentration of Ag(S₂O₃)₃⁻ (30000 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(S₂O₃)₂⁻, log K = 13.7; 2.9% = 879 µg l⁻¹ as Ag₂S₂O₇⁻, log K = 8.8) are relatively benign.

Despite this general lack of effect, Ag(S₂O₃)₃⁻ did cause a moderate metabolic alkalosis (rise in plasma pH and HCO₃⁻, negative ∆H⁺ 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(S₂O₃)₃⁻ 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(S₂O₃)₃⁻. 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(S₂O₃)₃⁻ 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(S₂O₃)₃⁻ (Terhaar et al., 1977; Hogstrand et al., 1995).

Ag presumably enters across the gills. One possible mechanism is that the negatively charged Ag(S₂O₃)₃⁻ 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 $\text{AgS}_2\text{O}_3^-$ formation is 8.8 (Morel and Hering, 1993). $\text{AgS}_2\text{O}_3^-$ comprised 2.9% of the total Ag concentration in the present exposure water.

This dissociation of $\text{Ag}^+$ from $\text{AgS}_2\text{O}_3^-$ 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 $\text{Ag(S}_2\text{O}_3)_n^-$ (30 000 $\mu$g l$^{-1}$) were about 3-fold those after 6 days exposure to $\text{AgNO}_3$ (10 $\mu$g l$^{-1}$; Wood et al., 1996). However if the gill Ag burden simply reflects ionic $\text{Ag}^+$ bound to the same gill ligands in both circumstances, we would have anticipated greater toxic symptoms in $\text{Ag(S}_2\text{O}_3)_n^-$-exposed fish. This did not occur. One important difference may be that metallothionein (MT) induction occurred in the gills of $\text{Ag(S}_2\text{O}_3)_n^-$-exposed trout (Table 2), and not in the gills of $\text{AgNO}_3$-exposed trout (Wood et al., 1996). Therefore the location (surface-bound versus intracellular) and chemical form of $\text{Ag}^+$ (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 $\text{Ag(S}_2\text{O}_3)_n^-$-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 $\text{Ag(S}_2\text{O}_3)_n^-$ 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 ($\text{Lepomis macrochirus}$) and largemouth bass ($\text{Micropterus salmoides}$; 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 $\text{Ag(S}_2\text{O}_3)_n^-$ than when dissociated into free ionic $\text{Ag}^+$ from $\text{AgNO}_3$. None of the physiological disturbances seen with ionic $\text{Ag}^+$ occur when $\text{Ag}^+$ 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 $\text{AgNO}_3$ (i.e. ionic $\text{Ag}^+$), 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 $\text{Ag(S}_2\text{O}_3)_n^-$ occurs in the relatively high concentrations present in undiluted photoprocessing effluent, it is unlikely to be a source of acute toxicity to fish.
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