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Determination of Silver in Blood, Urine, and Tissues of Volunteers and Burn Patients

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Silver sulfadiazine cream (SSD) has been used successfully in the management of burn wound sepsis. Silver deposition has been found in the skin, gingiva, cornea, liver, and kidney of patients treated with this cream, causing argyria, ocular injury, leukopenia, and toxicity in kidney, liver, and neurologic tissues. Monitoring concentrations of silver in blood and urine of patients receiving this treatment has become necessary, but sensitive and suitable methods adaptable to a clinical laboratory are still needed. We have developed a flameless thermal atomic absorption spectrophotometric method to measure silver concentrations in blood, urine, and other tissues. The detection limit is 0.4 μg/L; the within-run precisions (CV) are 5.16%, 3.83%, and 2.79% for concentrations of 5, 13.5, and 42 μg/L, respectively; and the between-run precisions are 4.3% and 3.2% for concentrations of 13.5 and 42 μg/L. The concentrations of silver in blood, urine, liver, and kidney of subjects without industrial or medicinal exposure are <2.3 μg/L, 2 μg/day, 0.05 μg/g wet tissue, and 0.05 μg/g wet tissue, respectively. In SSD cream-treated burn patients, plasma concentrations may be as great as 50 μg/L within 6 h of treatment and can reach a maximum of 310 μg/L. Silver in urine is detectable after one day of treatment and may reach a maximum of 400 μg/day. After absorption, silver was found to be deposited in various tissues. Tissue silver concentrations in one burn patient who died of renal failure after eight days of treatment were 970, 14, and 0.2 μg/g wet tissue in cornea, liver, and kidney, respectively.

Additional Keyphrases: *trauma · toxicology · atomic absorption spectrophotometry · silver sulfadiazine*

The development of sepsis after thermal injury is the major cause of high mortality in burn patients. Various treatments have been used to prevent and control sepsis (*1, 2*), but none is more successful than the topical use of silver sulfadiazine (SSD) cream (*3–5*), although this treatment is not without adverse effects. Argyria in gingiva and cheek tissues (*6*), nephrotic syndrome (*7*), and leukopenia (*8*) have all been reported in severely burned patients being treated with SSD cream. Silver toxicity is well known among workers in factories manufacturing silver nitrate and silver oxide (*9*), in the photochemical industry (*10*), and in people taking anti-smoking chewing gum containing silver acetate (*11, 12*) and was reported in a patient receiving arthroplasty cement containing silver (*13*). In vitro studies have also shown that silver causes cellular (*14, 15*) and subcellular (*16*) toxicity. The possible absorption of silver in burn patients treated with SSD cream and its consequent toxic effect are thus a cause of concern.

Published data on absorption of silver through topical use are scarce and controversial. Fox et al. (*15*) reported that no significant absorption was detected. Boosalis et al. (*17*) found only moderate increases in the concentration of silver in plasma of burn patients. Given the potential for toxicity from silver, we consider it important to monitor routinely the concentrations of silver in plasma and urine in patients undergoing treatment with SSD cream. We have developed an easy method based on electrothermal atomic absorption spectrometry but without use of the Zeeman background correction and platform recommended elsewhere (*18, 19*). To alleviate the serum matrix effect, we used NH_4NO_3 as the matrix modifier and assayed standards supplemented with bovine serum albumin (BSA), Na^+, K^+, Cl^-, and bicarbonate. We also investigated the effects of matrix modifiers such as nickel and palladium salts.

Materials and Methods

Apparatus: A Model 3030 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT), equipped with deuterium lamp background correction and an attached Model HGA-400 graphite furnace unit, was used in this study. The silver hollow-cathode lamp (Photron Pty., Ltd., Dandenong, Victoria, Australia) with a wavelength of 328.1 nm and a high-density graphite carbon tube (Perkin-Elmer) without platform were used in the assay.

Reagents: Water purified in a high-grade Milli-Ro De-ionizer system (Millipore, Melbourne, Australia) with resistance >14 MΩ was used to prepare all reagents. A 0.5 mL/L aqueous solution of Triton X-100 (iso-octylphenoxypolyethoxy ethanol), containing 40 g of NH_4NO_3 per liter as the matrix modifier, was used for the final dilution of all samples, including standards, controls, and patients' specimens. Aqueous solutions containing 2 g of nickel per liter, prepared from $Ni(NO_3)_2 \cdot 6H_2O$, and 4 g of palladium per liter, prepared from $Pd(NO_3)_2 \cdot 2H_2O$, were also tried as alternative matrix modifiers. All chemicals were of analytical grade (BDH Chemicals, Sydney, Australia). High-purity nitrogen was used as purging gas. A solution containing 60 g of BSA (Sigma Chemical Co., St. Louis, MO), 140 mmol of NaCl, and 5 mmol of $KHCO_3$ per liter was used to prepare all working standards.

Standards: For the silver standard, we used the standard solution of silver for atomic absorption spectroscopy (BDH Chemicals), which contained $AgNO_3$

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equivalent to 1 mg (9.270 μmol) of silver per milliliter. The stock standard, 1000 μg/L, was prepared by dilution with 0.1 mol/L HNO_3. Working standards of 2, 5, 10, 20, and 50 μg/L were prepared by diluting the stock standard with the BSA matrix solution.

Control specimens: Two pools of sera containing ~12 and ~40 μg of silver per liter were used as controls. They were prepared by diluting serum of high silver content with normal human serum to the desired ranges. The pooled sera were mixed, filtered, aliquotted (0.5 mL) into capped small plastic sample cups, and stored at −70 °C. They were used at the beginning of each run to standardize the instrument and also during the run to assess possible drift. These control specimens were stable for at least 12 months under these conditions.

Specimen preparation: The blank (BSA matrix solution), standards, control, and patients' plasma and urine specimens were all diluted fivefold with the Triton X-100 solution before assay. Urine samples were well mixed (without centrifugation) before sampling. Tissue specimens were first rinsed with 8.5 g/L NaCl solution to remove possible contamination of blood and were then blot-dried with filter paper. The wet tissue (0.5 g) was roughly diced and then dissolved in 2 mL of 10 mol/L HNO_3 in a 15-mL glass tube by heating the tube at 120 °C for 15 min. After cooling to room temperature, the contents were diluted to 10 mL with water. These tissue preparations were also diluted fivefold with the Triton X-100 solution before assay. Factors for dilutions were included in the final calculation.

Analytical procedure: For this assay, we used the alternative slit (0.7 μm) on the instrument and a 20-μL sample volume. Samples with silver concentrations >50 μg/L were diluted to below that concentration before assay. Automatically integrated peak areas were used for calculation of results. To optimize the procedure, we evaluated ashing temperatures between 300 and 1200 °C, and atomization temperatures between 1100 and 2500 °C, using pooled human serum with a silver content of 13.6 μg/L.

Results

The effect of ashing temperature, with NH_4NO_3 as modifier, on the silver yield is shown in Figure 1. The ashing was incomplete at temperatures <600 °C, and the effect of the matrix background was noticeable. Ashing appeared complete when the ashing temperature was between 650 and 750 °C; when >800 °C, silver loss was noticeable. We chose an ashing temperature of 700 °C for this study. When nickel was included as matrix modifier, a higher temperature of 900 °C for ashing could be achieved. However, this yielded no greater sensitivity and gave no improvement in the recovery, but led to formation of precipitates during the preparation of plasma specimens, so we did not use this approach. An attempt at using palladium nitrate as a modifier was abandoned due to its high silver content.

The survey on the effect of atomization temperature on silver recovery showed that little atomization occurred at temperatures <1100 °C (Figure 2). As the temperature increased to >1100 °C, the silver yield increased, reaching the maximum at 2000 °C. For maximum atomization, we chose 2100 °C for this study. A summary of the heating sequence program of the furnace is shown in Table 1.

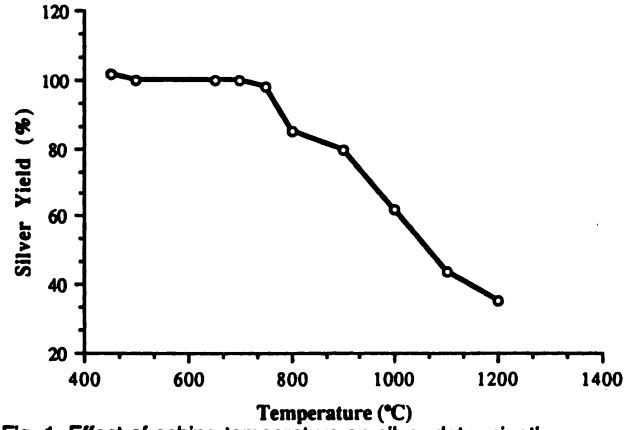

Fig. 1. Effect of ashing temperature on silver determination
Low percentage yield beyond 700 °C indicates loss of silver during the ashing stage

Standard Curve and Detection Limit

A representative standard curve is shown in Figure 3. The absorbance was linearly related to concentration up to at least 75 μg/L (the actual concentration of silver is 15 μg/L in the assay sample after fivefold dilution with Triton X-100 solution). The detection limit of silver measurement was determined by repeated analyses of a specimen of low silver content that yielded a ratio of mean/SD ≤2. So defined, the detection limit was 0.4 μg/L [mean = 0.39, SD = 0.161 μg/L (n = 22); ratio = 2.42; range = 0.12–0.68 μg/L]. The lowest standard of 2.0 μg/L is thus considerably above the detection limit.

Assay Precision Assessment

The within-run and between-run precision studies were performed with pooled plasma specimens containing silver at selected concentrations. The within-run precision was determined at three concentrations: 5, 13.5, and 42 μg/L; between-run precision was determined at two concentrations: 13.5 and 42 μg/L. Results

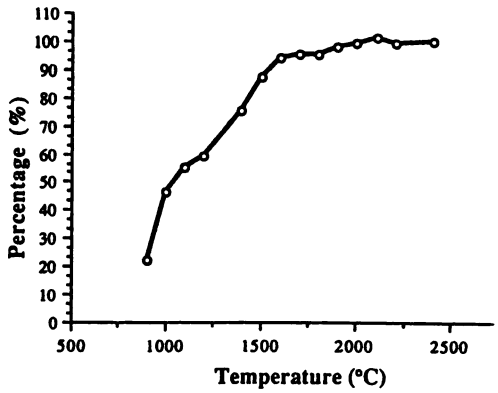

Fig. 2. Effect of atomization temperature on silver determination
Low percentage yield at <2000 °C indicates incomplete atomization

Table 1. Heating Program on the HGA-400

Step	Temp, °C	Ramp time, s	Hold time, s	Gas flow, mL/min	Read
1	100	20	20	100	—
2	140	10	20	100	—
3	700	10	30	100	—
4	2100	0	3	No	Yes
5	2650	1	3	100	—

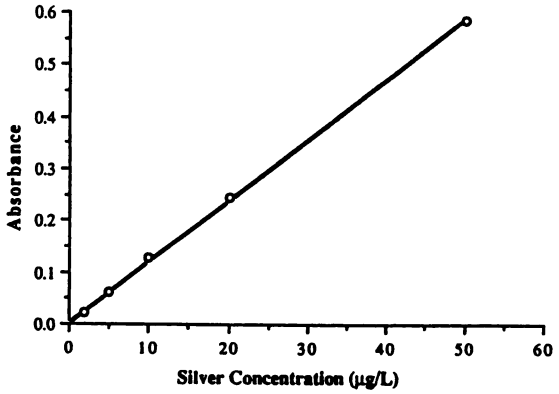

Fig. 3. A typical standard curve for the silver assay
Silver concentration represents concentration before fivefold dilution with the 0.5 mL/L Triton X-100 solution before analysis

are shown in Table 2. The between-run imprecisions were slightly greater than the within-run variations; nevertheless, all were within our acceptable value for a CV of 5%.

Accuracy and Analytical Recovery Studies

Assay accuracy was determined by comparing a serially diluted Spectrometric Solution for Silver (NIST Standard Reference Material; National Institute for Standards and Technology, Gaithersburg, MD) with our standard solution. The values expected and those measured were very close, within the variation of assay precision (Table 3).

Recovery studies were done by assaying a patient's serum with known amounts of silver added. The increase in values was compared with the quantities added. Recoveries were satisfactory and ranged between 94% and 99%, with a mean of 97.5% (Table 4).

Table 2. Within-Run and Between-Run Precision of the Silver Assay

	Mean, µg/L	SD, µg/L	CV, %
Within run			
1	4.97	0.26	5.16
2	13.61	0.52	3.83
3	42.37	1.18	2.79
Between runs			
1	13.37	0.58	4.30
2	42.28	1.28	3.02

Table 3. Accuracy Survey of Silver Determination

Silver concn, µg/L: Expected	Obtained	Accuracy, %
2.00	2.08	104
5.00	5.04	101
10.00	9.77	98
20.00	20.21	101
50.00	49.78	99

Table 4. Analytical Recovery of Added Silver

Silver concn, µg/L: Expected	Obtained	Recovery, %
2.00	1.89	95
5.00	4.86	96
10.00	9.89	99
20.00	20.23	101
50.00	49.39	99
		Average 98

Plasma and Urine Silver Concentrations in Metropolitan Dwellers

Plasma samples from 26 individuals who lived in the Melbourne metropolitan area (population $>3 \times 10^6$), whose occupations were not related to silver manufacturing and who had no history of silver medication, were analyzed for silver. Silver concentrations were all <1 µg/L. Analysis of 24-h urine collections from the same individuals showed that urinary excretion of silver was <2 µg/day, except for two cases with values of 2.3 and 2.1 µg/day. These findings are lower than those reported by DiVincenzo et al. (*20*), who showed that normal human serum and urine contained silver concentrations <5 µg/L.

Studies of Plasma and Urine Silver Concentrations in Burn Patients

Blood and urine specimens collected on admission to the hospital served to establish baselines for silver content. After the application of SSD cream, blood specimens were collected for silver assay at 6 h, and on days 1, 2, 3, 5, 7, 10, and 14, and then biweekly until the patients were discharged. Urine specimens (24 h) were collected on days 1, 2, 5, and 8 for silver determination.

Silver content in the blood increased quickly after application of SSD cream (Figure 4). After 6 h, all patients who suffered from burns of >5% of total body surface area showed a marked increase in silver concentration, to as much as 69 µg/L. Peak concentrations of silver in plasma, depending on the surface area burned, were reached in 10 ± 3 (mean ± SD) days, ranging from seven to 17 days; the maximum plasma concentration of silver in this series of patients was 310 µg/L. Our findings differ from those of Boosalis et al. (*17*), who reported much greater normal baseline concentrations of plasma silver and only slightly increased silver concentrations in SSD cream-treated patients. This difference probably reflects the type of instrument used:

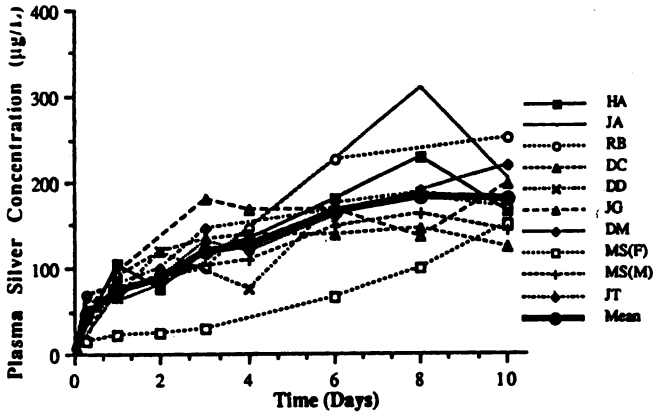

Fig. 4. Silver concentration in plasma of patients in the first 10 days after receiving SSD cream treatment
The *heavy line* indicates the mean

Boosalis et al. (*17*) used flame atomic absorption spectrophotometry, which is less sensitive than the flameless thermal furnace type we used.

Urinary excretion of silver is summarized for nine patients in Figure 5, which shows that absorbed silver is readily excreted in the urine. After only 24 h of treatment with SSD cream, mean urinary silver excretion was considerably increased to 11 (SD 9) µg/day, more than fivefold that of normal subjects. Peak concentrations of silver in urine were reached in 6.7 (SD 2.1) days (range five to 12 days). The highest value found, 558 µg/day in one patient, was less than the 1100 µg/day reported by Boosalis et al. (*17*). Unlike their group of patients, no one we studied had >35% of total body surface burned.

Silver Deposition in Tissues

One patient, age 81 years, had 25% of the total body surface burned and died of renal failure after eight days of treatment with SSD cream. Postmortem studies of silver content in the liver, kidney, and cornea showed that silver deposition was quite high in these tissues (Table 5). The corneal tissue had the highest concentration, followed by the liver and the kidney, with 970, 14, and 0.2 µg/g wet tissue, respectively. Silver content in the liver and kidney tissues taken from two individuals with no record of treatment with SSD cream was all <0.05 µg/g wet weight. Corneal samples from these two patients were not available for comparison.

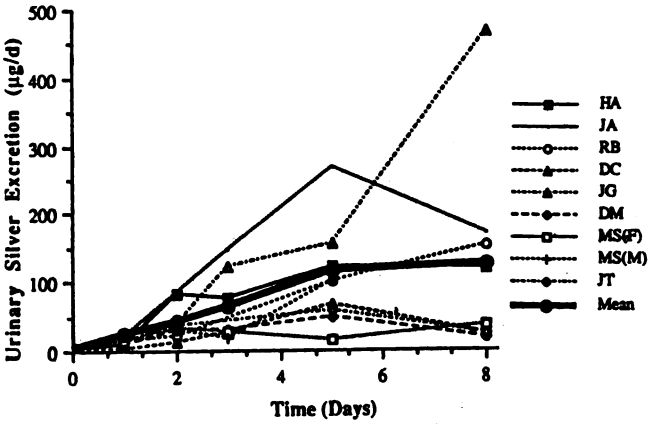

Fig. 5. Silver concentration in the urine of patients in the first eight days after receiving SSD cream treatment
The *heavy line* indicates the mean

Table 5. Silver Content (µg/g wet wt.) in Postmortem Tissues

Patient	Liver	Kidney	Cornea
1[a]	14	0.2	970
2[b]	0.05	0.02	—
3[b]	0.03	0.03	—

[a] Burn patient who received treatment with SSD cream for eight days before dying.
[b] Patients who had no history of receiving medication containing silver.

Discussion

Normal concentrations of silver in human serum, urine, and tissues are very low (*21*). Common laboratory techniques such as spectrophotometry (*22*) and flame atomic absorption spectrometry (*17*) are not suitable for assay of silver in human blood, urine, or tissue because of their low sensitivities. Alvarez-Coque et al. (*22*) used diethyldithiocarbamate in their spectrophotometric determination of silver in aqueous solution; their detection limit was quoted as 250 µg/L, 625-fold the detection limit of 0.4 µg/L we report. Therefore, their method cannot be used as a reference method for silver in tissues and fluids. Boosalis et al. (*17*) used a flame atomic absorption spectrophotometer for their study of serum and urinary silver concentrations in thermally injured patients. They report that the concentration of silver in normal human serum may be as much as 200 µg/L; hence, not many of their patients were shown to have increased silver concentrations—probably because of the relatively insensitive method used in their assay.

The method presented here is sensitive enough to demonstrate that normal human serum contains <2 µg of silver per liter and to determine readily any increase of silver concentration in the blood or urine due to medication. Complex equipment and complicated techniques such as neutron activation (*23*) and atomic absorption spectrometry of atomized plasma (*24*) can be used, but these are usually unavailable in the clinical laboratory. Recently, electrothermal atomic absorption spectrophotometry was used for silver analysis in human blood (*18*). The method involved an instrument equipped with Zeeman background correction and techniques such as standard additions and the use of a platform in the carbon tube. We found that by using a standard electrothermal atomic absorption spectrometer equipped with deuterium background correction, we achieved enough sensitivity and did not require standard additions or a platform for measuring silver in blood, urine, and tissues. The serum matrix effect was overcome by the presence of NH_4NO_3 and was balanced by using standards supplemented with BSA, Na^+, K^+, Cl^-, and bicarbonate. The use of nickel or palladium as matrix modifiers was not necessary, because these gave no improvement in ana-

lytical recovery. In addition, the commercial product of palladium compound was found to contain silver in a high enough concentration to be unsuitable for the assay.

The method presented here is simple and easy to operate, and the sensitivity and precision remain suitable for routine use. Silver is readily absorbed through topical application. As early as 6 h post-application, silver concentrations in plasma reached >50-fold the normal concentration. Plateau values were reached in three to four days. Although silver is cleared through the urine, it is also deposited in liver, kidney, and corneal tissue, as shown in one patient who died eight days after admission. The physiological and pathological effects of silver deposition in a larger group of patients are being documented and will be reported separately.

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