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Mobilization of exogenous and endogenous selenium to bile after the intravenous administration of environmentally relevant doses of arsenite to rabbits

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Extending our studies of the effect of arsenite on the metabolism of inorganic selenium (selenite and selenate) to lower doses, we intravenously injected New Zealand white rabbits with aqueous solutions of arsenite, selenite, arsenite + selenite, selenate and selenate + arsenite at 50 μg and 5 μg metalloid per kilogram body weight. Bile samples were collected for 25 min, acid-digested and analyzed for total arsenic and selenium by double focusing magnetic sector field inductively coupled plasma mass spectrometry. At both dose levels, and in accord with previous observations, an increased mutual biliary excretion of arsenic and selenium was observed regardless of whether selenium was coadministered with arsenite in the form of selenite or selenate. Based on our previous investigations into the *in vivo* interaction between arsenite and selenite (or selenate), these findings can be rationalized in terms of the biliary excretion of the seleno-bis(*S*-glutathionyl) arsinium ion, $[(GS)_2AsSe]^-$. In addition, the treatment of rabbits with 50 μg arsenic per kilogram body weight in form of arsenite alone also resulted in a significantly increased bile selenium concentration compared with bile from untreated animals ($p < 0.05$), which implies a mobilization of endogenous selenium to bile. Combined, these results establish a causal relationship between the exposure of mammals to arsenite and selenium deficiency. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: arsenic; selenium; metabolism; $[(GS)_2AsSe]^-$; toxicity; bile

INTRODUCTION

The formation of toxicologically important compounds in blood between essential elements (or their metabolites) and simultaneously ingested toxic metals or metalloid compounds has recently been identified as a novel biomolecular mechanism by which environmentally abundant inorganic pollutants can dramatically affect mammalian health.[1] In spite of the large number of possible essential element–toxic element combinations, 'interactions' between essential elements that have to be ingested only in microgram quantities per day for optimum health and environmentally abundant toxic metals and/or metalloid compounds will result in the most dramatic overall health effect.[1–5] Since the dietary requirement of selenium in humans is currently estimated at 50–200 μg day^{-1},[6] and since selenium deficiency increases the susceptibility of humans for cancer,[7] *in vivo* 'interactions' between dietary selenium compounds (or their metabolites) and toxic metals/metalloid compounds are particularly important from a toxicological point of view.[1]

The striking antagonistic 'interaction' between the individually highly toxic metalloid compounds arsenite and selenite was discovered in feeding studies with rats more than 60 years ago.[2,8] Studies aimed at a better understanding

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of this mineral antagonism in rats and rabbits revealed that intravenously administered arsenite dramatically increased the biliary excretion of selenium regardless whether this latter metalloid was administered as selenite[9–11] or selenate.[9,12] Although arsenate also stimulated the biliary excretion of selenium when the molar ratio of arsenate : selenite exceeded 1.5,[12,13] arsenate did not affect the biliary excretion of selenium within 25 min when selenium was intravenously injected in the form of selenate at an equimolar dose.[12]

The combined application of X-ray absorption spectroscopy (XAS) and size-exclusion chromatography (SEC) with simultaneous multi-element-specific detection by inductively coupled argon plasma atomic emission spectroscopy (ICP-AES) eventually revealed that a previously unknown metabolite, the seleno-bis(*S*-glutathionyl) arsinium ion, $[(GS)_2AsSe]^-$, is excreted from the liver to the bile after the intravenous injection of New Zealand white rabbits with selenite followed by arsenite.[11,14] Subsequent studies revealed that $[(GS)_2AsSe]^-$ is also excreted from the liver to bile after the intravenous injection of rabbits with selenate followed by arsenite.[12] The *in vivo* formation of $[(GS)_2AsSe]^-$ thus links the mammalian metabolism of toxic arsenite with that of selenite and selenate. Since $[(GS)_2AsSe]^-$ will have different toxicological properties (on mammalian cells) than selenite, selenate or arsenite, and since the biliary excretion of this compound could lead to selenium deficiency, $[(GS)_2AsSe]^-$ is of fundamental toxicological importance.[11] Our animal studies conducted so far, however, have involved the administration of arsenite, selenite and selenate in the low milligram per kilogram body weight range because the physico-chemical techniques that were employed to identify the metalloid species in bile (namely XAS and SEC–ICP-AES) required these metalloid dose levels.

In reality, however, the general population is simultaneously exposed to background concentrations in the microgram per day range of all four common oxy-anions of arsenic and selenium through the ingestion of food and drinking water.[15,16] Geogenic arsenic containing rocks and deposits (which contain arsenic predominantly in the form of sulfidic minerals), for instance, release quantities of inorganic arsenic to groundwater that in some regions of the world dramatically affect human health.[17,18] In fact, an estimated 36 million people in the Bengal Delta alone (India and Bangladesh) currently ingest inorganic arsenic-contaminated drinking water[19] (between 50 and 90% of which is present as arsenite[20,21]) and will eventually suffer from the adverse health effects that are associated with the chronic exposure to these metalloid compounds such a various internal cancers.[22–24] Despite extensive research on the metabolism of inorganic arsenic in mammals, however, the molecular mechanism(s) underlying the inorganic arsenic-induced carcinogenesis in humans is (are) not completely understood.[25]

On the other hand, selenate, organoselenium compounds and selenite have been identified in environmental waste waters.[16] Selenite has been detected in serum following the oral administration of rats with selenite, selenate or selenomethionine[26] and has also recently been identified in serum of adults and children.[27]

Consequently, mammals are inevitably and simultaneously exposed to arsenite and selenite, and the antagonistic 'interaction' that was phenomenologically described between these metalloid compounds (in mammals) more than six decades ago[2,8] could be of fundamental toxicological importance. In the present study, we investigate whether the arsenite-induced biliary excretion of selenium (given as selenite or selenate) can still be observed in New Zealand white rabbits when 10- to 100-fold lower doses of both metalloid compounds are intravenously injected. After treatment with arsenite, selenite, arsenite + selenite, selenate or arsenite + selenate at 50 and 5 μg arsenic and/or selenium per kilogram body weight, bile was collected for 25 min and subsequently analyzed for total arsenic and selenium by double focusing magnetic sector field ICP mass spectrometry (SF-ICP-MS) after digestion with nitric acid.

EXPERIMENTAL

Chemicals

$Na_2SeO_3 \cdot 5H_2O$ (>99%) and hydrochloric acid (Suprapur, 30%) were purchased from Merck (Darmstadt, Germany), $NaAsO_2$ (>99%) was obtained from GFS Chemicals (Columbus, OH, USA) and Na_2SeO_4 was from Sigma (St Louis, MO, USA). Phosphate-buffered saline (pH 7.4) was prepared from PBS tablets (Sigma) and triply distilled water. Suprapure HCl for the adjustment of the pH of the metalloid solutions in PBS to pH 7.4 was purchased from Fischer Chemicals (Pittsburgh, PA, USA). HNO_3 for the digestion procedure was obtained by subboiling distillation.

Animal experiments

The animal experiments were conducted between April and December 2002 at the GSF National Research Center for Environment and Health (animal protocol # 209.1/211-2531-89/01). Male New Zealand white rabbits (1.70–3.40 kg body weight) were purchased from River Charles GmbH (97 633 Sulzfeld, Germany) and maintained on an Altromin rabbit diet (32 770 Lage, Germany) for at least 3 days before the animal experiment. The animals were prepared for the experiment as reported previously,[11] except that anesthesia was achieved by the intramuscular injection of a 2 : 5 (v/v) mixture of xylazin (2%) and ketamin (10%) (0.7 ml of the mixture per kilogram body weight). After the cannulation of the common bile duct with polyethylene tubing and after a constant bile flow had been established, control bile was collected from each animal for approximately 10 min. Immediately thereafter the corresponding aqueous metalloid solution (in PBS) was injected intravenously through the marginal ear vein (in contrast to our previous studies, both metalloids were injected in one solution) and bile was collected for 25 min into ice-cold polypropylene tubes. Only bile samples that were visually free of even

traces of blood were used for the determination of total arsenic and selenium by SF-ICP-MS (in order to avoid the contamination of bile with blood-derived metalloids). The treatment groups were as follows (all amounts are per kilogram body weight): 50 μg As^{III} (four animals), 50 μg Se^{IV} (three animals), 50 μg $As^{III} + Se^{IV}$ (three animals), 50 μg Se^{VI} (two animals) and 50 μg $As^{III} + Se^{VI}$ (three animals); 5 μg As^{III} (four animals), 5 μg Se^{IV} (three animals), 5 μg $As^{III} + Se^{IV}$ (three animals), 5 μg Se^{VI} (two animals) and 5 μg $As^{III} + Se^{VI}$ (three animals). All experiments were carried out between 9 : 00 and 11 : 30 a.m. to exclude the effects of diurnal variations of the hepatic glutathione levels and all animals were alive and well at the end of the experiment. All bile samples were immediately frozen and stored at −20 °C until they were digested and analyzed by SF-ICP-MS. Differences in the total bile selenium concentration between untreated and treated animals were evaluated using a two-sided Student *t*-test; $p < 0.05$ is statistically significant.

Analysis of total arsenic and selenium by SF-ICP-MS

Determinations of total arsenic and selenium were performed using double focusing magnetic SF-ICP-MS, using the ELEMENT1 (Finnigan MAT, Germany). Pneumatic nebulization (Meinhard), a water-cooled spray chamber (Scott type), a peristaltic pump (0.9 ml min^{-1}) and an ASX-400 sample changer (Cetac, USA) were used for sample introduction. Commercially available nickel cones were used in the interface. Aliquots of the completely thawed and mixed bile samples (100 μl from the 50 μg metalloid/kilogram body weight dose level and 200 μl from the 5 μg metalloid/kilogram body weight dose level) were digested by pressure ashing. The device used was a SEIF-Apparatus (Seif Aufschlußtechnik, Germany) with quartz sample ampoules to avoid sample contamination. 1.0 ml HNO_3 was added to either 100 or 200 μl of bile; the ampoules were then sealed and treated for 10 h in an oven at 180 °C. Afterwards, the clear digest was diluted with H_2O to 10.0 ml with Ultrapure (MilliQ) H_2O (Millipore, Germany). After the addition of an internal standard (50 μl 100 ppb rhodium per 5 ml) the samples were analyzed by SF-ICP-MS using a calibration curve. The highest resolution that can be achieved with this instrument was necessary for the determination of the elements of interest and was approximately 11.000. Blank values (HNO_3) were run beside the samples and a certified reference material (CRM 278), certified at 5.9 ± 0.2 mg arsenic per kilogram and 1.66 ± 0.04 mg selenium per kilogram, was used to check the accuracy of the method. The mean values of seven determinations were 6.2 ± 0.3 mg arsenic per kilogram and 1.7 ± 0.2 mg selenium per kilogram.

RESULTS

SF-ICP-MS was used to quantify arsenic and selenium in the collected bile samples. The mean arsenic and selenium concentrations in bile before any metalloid was injected (also referred to as the bile background arsenic concentration and the bile background selenium concentration) were 1.1 ± 0.8 μg l^{-1} and 7.4 ± 3.7 μg l^{-1} ($n = 37$) respectively. The arsenic and selenium concentrations in bile after various treatments of the animals (As^{III}, Se^{IV}, $As^{III} + Se^{IV}$, Se^{VI} and $Se^{VI} + As^{III}$ at 50 μg or 5 μg metalloid per kilogram body weight) are summarized in Table 1. Table 2 depicts the total arsenic and selenium excreted in bile and as percentage of the injected metalloid dose.

Treatment of rabbits with 50 μg metalloid per kg body weight

The intravenous injection of rabbits with arsenite resulted in bile with an arsenic concentration of 105 μg l^{-1}, which is approximately 100-fold higher than the bile background arsenic concentration. The bile selenium concentration in these samples was 15.5 μg l^{-1}. The standardized difference between this selenium concentration and the selenium concentration of the control group (37 bile samples collected from untreated animals) was 2.21 and corresponds to a *p*-value of 0.0328. Therefore, the bile selenium concentration after arsenite treatment is significantly higher than the background bile selenium concentration. The intravenous injection of rabbits with selenite resulted in bile with a selenium concentration of 255 μg l^{-1} (16-fold higher than the bile background selenium concentration). The intravenous injection of rabbits with a solution containing arsenite and selenite resulted in a bile arsenic concentration of 1550 μg l^{-1} and a bile selenium concentration of 1805 μg l^{-1}. Compared with the concentrations of arsenic and selenium in bile after the individual treatment of rabbits with arsenite or selenite, these metalloid concentrations are

Table 1. Total arsenic and selenium concentrations in bile after treatment of rabbits with 50 or 5 μg metalloid per kilogram body weight in the form of As^{III}, Se^{IV}, $As^{III} + Se^{IV}$, Se^{VI} and $Se^{VI} + As^{III}$. Bile was collected for 25 min and three rabbits were used in each treatment group unless stated otherwise

Injected dose (per kilogram body weight)	Bile [As] (μg l^{-1})	Bile [Se] (μg l^{-1})
50 μg As^{III}[a]	105 ± 42	15.5 ± 6.1
50 μg Se^{IV}	0.8 ± 0.1	255 ± 25
50 μg $As^{III} + Se^{IV}$	1550 ± 575	1805 ± 595
50 μg Se^{VI}[b]	0.8	96
50 μg $Se^{VI} + As^{III}$	450 ± 175	455 ± 140
5 μg As^{III}[a]	5.6 ± 0.9	8.0 ± 1.4
5 μg Se^{IV}	1.1 ± 0.4	41 ± 8
5 μg $As^{III} + Se^{IV}$	60 ± 24	115 ± 88
5 μg Se^{VI}[b]	0.6	13.3
5 μg $Se^{VI} + As^{III}$	35 ± 16	42 ± 18

[a] Four rabbits per group.
[b] Two rabbits per group.

Table 2. Calculated total arsenic and selenium in bile after treatment of rabbits with 50 or 5 μg metalloid per kilogram body weight in the form of As^{III}, Se^{IV}, $As^{III} + Se^{IV}$, Se^{VI} and $Se^{VI} + As^{III}$ using a bile flow of 50 mg kg^{-1} min^{-1},[14] a collection time of 25 min and an average rabbit weight of 2.5 kg

Injected dose	As in bile (μg)	Proportion of i.v. dose (%)	selenium in bile (μg)	Proportion of i.v. dose (%)
125 μg As^{III}	0.3	0.3	<0.1	—
125 μg Se^{IV}	—	—	0.8	0.6
125 μg $As^{III} + Se^{IV}$	4.8	3.9	5.6	4.5
125 μg Se^{VI}	—	—	0.3	0.2
125 μg $Se^{VI} + As^{III}$	1.4	1.1	1.4	1.1
12.5 μg As^{III}	<0.1	0.1	—	—
12.5 μg Se^{IV}	—	—	0.1	0.8
12.5 μg $As^{III} + Se^{IV}$	0.2	1.5	0.4	2.7
12.5 μg Se^{VI}	—	—	<0.1	0.1
12.5 μg $Se^{IV} + As^{III}$	0.1	0.9	0.1	0.9

approximately 15- and 7-fold higher and correspond to a molar As : Se ratio of 0.91. Expressing the total metalloid excreted in bile (within 25 min) as the percentage of the injected dose shows that the simultaneous administration of arsenite and selenite to rabbits increased the biliary excretion of arsenic by 3.6% and that of selenium by 3.9% compared with the corresponding metalloid concentrations in bile after the injection with arsenite or selenite alone (Table 2).

The analysis of bile collected from rabbits that had been injected with selenate revealed an unchanged background arsenic concentration and a selenium concentration of 96 μg l^{-1}. The simultaneous injection of rabbits with arsenite and selenate and the subsequent analysis of the collected bile samples resulted in a bile arsenic concentration of 450 μg l^{-1} and a selenium concentration of 455 μg l^{-1}. This corresponds to a four fold increased arsenic and a five fold increased selenium concentration compared with the bile metalloid concentrations after the individual treatment of rabbits with arsenite or selenate. Moreover, the molar As : Se ratio in these bile samples was 1.03. Expressing the total metalloid excreted in bile (within 25 min) as the percentage of the injected dose shows that the simultaneous administration of arsenite and selenate increased the biliary excretion of arsenic and that of selenium by 0.9% (compared with the biliary excretion following the injection of rabbits with arsenite or selenate).

Treatment of rabbits with 5 μg metalloid per kg body weight

Rabbits injected with arsenite excreted bile with an arsenic concentration of 5.6 μg l^{-1} and a selenium concentration of 8.0 μg l^{-1}. Even though this bile arsenic concentration is significantly higher than the bile background arsenic concentration, the bile selenium concentration remains at the bile background selenium concentration. The injection of rabbits with selenite did not change the bile arsenic concentration from its background concentration, but it did lead to a significantly increased bile selenium concentration of 41 μg l^{-1}. When arsenite and selenite were injected together, the collected bile samples had an arsenic concentration of 60 μg l^{-1} and a selenium concentration of 115 μg l^{-1}. These metalloid concentrations are 13- and 3-fold higher than those that were obtained after the individual treatment of rabbits with arsenite or selenite (corrected with the background metalloid concentrations) and correspond to an As : Se molar ratio of 0.53. Expressing the total amount of metalloid excreted in bile (within 25 min) as a percentage of the injected dose clearly shows that the joint administration of rabbits with arsenite and selenite increased the biliary excretion of arsenic by 1.3% and that of selenium by 1.9% (compared with the biliary excretion following the injection of rabbits with arsenite or selenite).

The injection of rabbits with selenate did not increase the bile arsenic concentration from its background concentration, but resulted in a slightly increased bile selenium concentration of 13.3 μg l^{-1} compared with the bile background selenium concentration (since data from only two animals were collected, no statistical analysis was performed). The co-administration of arsenite and selenate significantly increased the bile arsenic concentration to 35 μg l^{-1} and the selenium concentration to 42 μg l^{-1}. This corresponds to a eight fold increase of the bile arsenic concentration and a seven fold increase of the bile selenium concentration compared with the metalloid concentrations that were obtained after the individual administration of arsenite and selenate (and corrected with the background metalloid concentrations). The As : Se molar ratio in these bile samples was 0.88. When expressed as a percentage of the administered dose, the joint administration to rabbits of arsenite and selenate increased the biliary excretion of both arsenic and selenium by 0.7% (compared with the biliary excretion following the injection of rabbits with arsenite and selenate).

DISCUSSION

Extending the *in vivo* 'interaction' of arsenite and selenite to lower doses

After the intravenous injection of rabbits with 0.63 mg selenium per kilogram body weight (as selenite) followed 3 min later by 0.60 mg arsenic per kilogram body weight (as arsenite), we previously obtained a bile arsenic concentration of 20.9 mg l^{-1}, a bile selenium concentration of 21.6 mg l^{-1} (within 25 min) and a molar As : Se ratio in bile of 0.97 using X-ray fluorescence spectroscopy.[11] These bile metalloid concentrations were several-fold increased compared with those obtained after the intravenous injection of rabbits with the same doses of arsenite or selenite alone.[11]

We repeated this previous study, using the same metalloid compounds (but injecting both metalloids in one solution) at doses of 50 and 5 μg arsenic and/or selenium per kilogram body weight, and essentially confirmed the increased mutual biliary excretion of arsenic and selenium at these much lower dose levels (compared with the biliary excretion after the injection of rabbits with arsenite or selenite alone; Tables 1 and 2). A decrease of the administered metalloid dose level from ~600 μg[11] to 50 and 5 μg metalloid/kilogram body weight (this study) decreased the bile As : Se molar ratio from 0.97 to 0.91 and to 0.53. At the high dose level (50 μg metalloid/kilogram body weight) the simultaneous administration of the metalloid compounds increased the biliary excretion of arsenic by 3.6% (of the administered dose) and that of selenium by 3.9% (of the administered dose). At the lower dose level (5 μg metalloid/kilogram body weight) the increase of the biliary excretion of arsenic was 1.4% (of the administered dose) and that of selenium was 1.9% (of the administered dose). Combined, these results are in accord with data reported for rats that were treated in a similar fashion[9] and can be rationalized in terms of the biliary excretion of $[(GS)_2AsSe]^-$.[11] The observed decrease of the bile As : Se molar ratio with a decrease of the administered metalloid dose (see above) could be explained by an increased excretion of a selenium metabolite, such as GS–Se–SG,[28] in addition to $[(GS)_2AsSe]^-$ at the lower dose levels.

Extending the *in vivo* 'interaction' of arsenite and selenate to lower doses

After the intravenous injection of rabbits with 2.52 mg selenium per kilogram body weight (as selenate) followed 3 min later by 2.40 mg arsenic per kilogram body weight (as arsenite), we previously found several-fold increased bile arsenic and selenium concentrations compared with those observed after the treatment of rabbits with the individual metalloid compounds.[12] The analysis of bile (after treatment with selenate followed by arsenite) by XAS revealed a bile As : Se molar ratio of 1.25 (mean from two animals; a small quantity of an arsenic species, e.g. $(GS)_3As$, was also present) and clearly identified $[(GS)_2AsSe]^-$ in bile. Thus, the *in vivo* interaction between arsenite and selenate is biochemically related to that between arsenite and selenite[11,12] and involves the enzymatic reduction of selenate,[29] most likely in hepatocytes.

In the present study we essentially confirmed this increased mutual excretion of arsenic and selenium after the intravenous administration to rabbits with much lower dose levels of arsenite and selenate (50 or 5 μg arsenic and selenium per kilogram body weight) compared with the corresponding metalloid concentrations (and the excreted dose) after the individual administration of the metalloid compounds to rabbits (Tables 1 and 2). With a decrease of the administered dose of selenate and arsenite from ~2450 μg[12] to 50 and 5 μg metalloid/kilogram body weight (this study), the As : Se molar ratio decreased from approximately 1.25 to 1.03 and to 0.88, which is comparable to the trend observed after the injection of rabbits with smaller doses of arsenite and selenite (see above). At the high dose level, the simultaneous administration of the metalloid compounds increased the biliary excretion of arsenic and selenium by approximately 0.9% of the administered dose. The bile As : Se molar ratio in these samples was 1.03. In combination with the reported biliary excretion of $[(GS)_2AsSe]^-$ after the administration of 10-fold higher metalloid doses,[12] these results suggest that $[(GS)_2AsSe]^-$ is also excreted at this dose level. At the lower dose level, the increase of the biliary excretion of arsenic and that of selenium (compared with the corresponding control group) was 0.8% (of the administered dose) and the bile As : Se molar ratio was 0.88. This bile As : Se molar ratio suggests that a selenium metabolite (e.g. GS-Se-SG)[28] is excreted in addition to $[(GS)_2AsSe]^-$ which is in accord with the results that were obtained after the simultaneous administration of arsenite and selenite (see above).

Antagonistic 'interactions' between arsenite and selenite/selenate are involved in the toxicity of arsenite

Since the chronic ingestion of approximately 200–250 μg of inorganic arsenic per day will eventually result in cancer in humans,[30] and since the rabbit seems to be the species that is most similar to man with regard to the metabolism of inorganic arsenic,[31] our findings provide direct experimental evidence for an involvement of *in vivo* 'interactions' between arsenite and selenite/selenate in the toxicity of arsenite in mammals. Conversely, these 'interactions' can be practically exploited to alleviate the chronic toxicity of inorganic arsenic in humans by dietary supplementation with sodium selenite or with high-selenium yeast, as has already been successfully demonstrated in rats[32] and humans.[33,34] With millions of people being currently exposed to arsenite-contaminated drinking water, this simple treatment could potentially save many people from the adverse health effects of arsenite by exploiting an evolved mammalian excretory pathway.[35] An analogous treatment has already been successfully exploited to dramatically decrease the tissue concentration of mercury in fish (by 85%) after the addition of selenite to mercury-polluted lakes.[36]

CONCLUSION

After the simultaneous administration of rabbits with 5 μg arsenic per kilogram body weight (given as arsenite) and 5 μg selenium per kilogram body weight (given as selenite or selenate) we observed a significantly increased biliary excretion of total arsenic and selenium compared to the control groups. These findings are in agreement with the previously reported *in vivo* formation and biliary excretion of $[(GS)_2AsSe]^-$ (after the administration to rabbits with approximately 100-fold higher doses of arsenite and selenite)[11,12,37] and suggest that $[(GS)_2AsSe]^-$ is also formed *in vivo* and excreted in bile after the exposure to environmentally relevant doses of arsenite or arsenite and selenate. These results demonstrate that *in vivo* 'interactions' between dietary selenium compounds and simultaneously administered toxic metals are likely to be involved in the chronic toxicity of toxic metals and metalloids in mammals.[1] Thus, the *in vivo* formation of $[(GS)_2AsSe]^-$ could be involved in the carcinogenicity of inorganic arsenic and is of relevance with regard to the observed inhibition of recombinant arsenite-methyltransferase by selenite,[38] the suppression of selenite-induced necrosis in leukemia HL-60 cells by arsenite,[39] the modulation of the anticarcinogenic action of selenite by arsenite,[40] and other recently reported findings.[41,42] $[(GS)_2AsSe]^-$ must therefore be considered as a key intermediate in the carcinogenicity of inorganic arsenic in mammals.

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REFERENCES

1. Gailer J. *Appl. Organometal. Chem.* 2002; **16**: 701.
2. Moxon AL, DuBois KP. *J. Nutr.* 1940; **19**: 477.
3. Parizek J, Ostadalova I. *Experientia* 1967; **23**: 142.
4. Ganther HE, Goudie C, Sunde ML, Kopecky MJ, Wagner P, Oh S-H, Hoekstra WG. *Science* 1972; **175**: 1122.
5. Kar AB, Das RP, Mukerji B. *Proc. Natl. Inst. Sci. India* 1960; **26**: (Suppl. B): 40.
6. Daniels L. *Biol. Trace Elem. Res.* 1996; **54**: 185.
7. Clark LC, Cantor KP, Allaway WH. *Arch. Environ. Health* 1991; **46**: 37.
8. Moxon AL. *Science* 1938; **88**: 81.
9. Levander OA, Baumann CA. *Toxicol. Appl. Pharmacol.* 1966; **9**: 106.
10. Gregus Z, Gyurasics Á, Koszorús L. *Environ. Toxicol. Pharmacol.* 1998; **5**: 89.
11. Gailer J, George GN, Pickering IJ, Prince RC, Ringwald SC, Pemberton JE, Glass RS, Younis HS, DeYoung DW, Aposhian HV. *J. Am. Chem. Soc.* 2000; **122**: 4637.
12. Gailer J, George GN, Pickering IJ, Prince RC, Younis HS, Winzerling JJ. *Chem. Res. Toxicol.* 2002; **15**: 1466.
13. Levander OA, Baumann CA. *Toxicol. Appl. Pharmacol.* 1966; **9**: 98.
14. Gailer J, Madden S, Buttigieg GA, Denton MB, Younis HS. *Appl. Organometal. Chem.* 2002; **16**: 72.
15. Cullen WR, Reimer KJ. *Chem. Rev.* 1989; **89**: 713.
16. Conde JE, Alaejos MS. *Chem. Rev.* 1997; **97**: 1979.
17. Nickson RT, McArthur JM, Ravenscroft P, Burgess WG, Ahmed KM. *Appl. Geochem.* 2000; **15**: 403.
18. Nickson R, McArthur J, Burgess W, Ahmed KM, Ravenscroft P, Rahman M. *Nature* 1998; **395**: 338.
19. Nordstrom DK. *Science* 2002; **296**: 2143.
20. Chatterje A, Das D, Mandal BK, Chowdhury TR, Samanta G, Chakraborti D. *Analyst* 1995; **120**: 643.
21. Harvey CF, Swartz CH, Badruzzaman ABM, Keon-Blute N, Yu W, Ali MA, Jay J, Beckie R, Niedan V, Brabander D, Oates PM, Khandaker NA, Islam S, Hemond HF, Ahmed MF *Science* 2002; **298**: 1602.
22. Chen CJ, Kuo TL, Wu MM. *Lancet* 1988; **1**: 414.
23. Kitchin KY. *Toxicol. Appl. Pharmacol.* 2001; **172**: 249.
24. Smith AH, Lopipero PA, Bates MN, Steinmaus CM. *Science* 2002; **296**: 2145.
25. Goering PL, Aposhian HV, Mass MJ, Cebrian M, Beck BD, Waalkes MP. *Toxicol. Sci.* 1999; **49**: 5.
26. Wang ZJ, Zhou J, Peng A. *Biol. Trace Elem. Res.* 1992; **33**: 135.
27. Michalke B Selenspeziation mit SAX–ICP-MS und RPLC–ICP-MS. In *Ionenanalyse mit modernen Trenntechniken*, Fischer K, Jensen D (eds). Dionex: Idstein, Germany, 2002; 50–60.
28. Gyurasics Á, Perjési P, Gregus Z. *Biochem. Pharmacol.* 1998; **56**: 1381.
29. Schroder I, Rech S, Krafft T, Macy JM. *J. Biol. Chem.* 1997; **272**: 23 765.
30. Marcus WL, Rispin AS. Threshold carcinogenicity using arsenic as an example. In *Advances in Modern Environmental Toxicology*, Cothern CR, Mehlmans MA, Marcus WL (Eds). Princeton Publishing: Princeton, NJ, 1988; 133–158.
31. Vahter M. *Appl. Organometal. Chem.* 1994; **8**: 175.
32. Biswas S, Talukder G, Sharma A. *Mutat. Res.* 1999; **441**: 155.
33. Wang W, Wang L, Hou S, Tan J, Li H. *Curr. Sci.* 2001; **81**: 1215.
34. Yang L, Wang W, Hou S, Peterson PJ, Williams WP. *Environ. Geochem. Health* 2002; **24**: 359.
35. Ishikawa T. *Trends Biochem. Sci.* 1992; **17**: 463.
36. Paulsson K, Lundbergh K. *Sci. Total. Environ.* 1989; **87**: 495.
37. Gailer J, George GN, Pickering IJ, Buttigieg GA, Denton MB, Glass RS. *J. Organometal. Chem.* 2002; **650**: 108.
38. Walton FS, Waters SB, Jolley SL, LeCluyse EL, Thomas DJ, Styblo M. *Chem. Res. Toxicol.* 2003; **6**: 261.
39. Zheng H. *Biol. Trace Elem. Res.* 2001; **83**: 1.
40. Ip C, Ganther H. *Carcinogenesis* 1988; **9**: 1481.
41. Yeh JY, Cheng LC, Liang YC, Ou BR. *Endothelium* 2003; **10**: 127.
42. Csánaky I, Gregus Z. *Toxicology* 2003; **186**: 33.

