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Glutathione and methylation of inorganic arsenic in hamsters

Miyuki Hirata,* Akira Hisanaga, Akiyo Tanaka and Noburu Ishinishi
Department of Hygiene, Faculty of Medicine, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812, Japan

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The effect of glutathione (GSH) concentrations in livers and kidneys of hamsters on the toxicity and methylation of arsenite in these animals was studied. No significant changes in hepatic and renal GSH concentrations were observed after a single arsenite administration (5 mg As kg^{-1}, p.o.). When buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, was given (4 mmol kg^{-1}, i.p.) two hours before administration of arsenite, hepatic and renal GSH concentrations were more severely and persistently depressed than in the case of BSO administration not followed by arsenite. Hamsters treated with BSO plus arsenite suffered from severe nephrotoxicity (acute renal failure) characterized by increases in plasma creatinine and urea nitrogen and by proximal tubular necrosis. Concurrently, transient hepatotoxicity was observed in the BSO plus arsenite group. Neither arsenite alone nor BSO alone produced liver or kidney injury. The BSO plus arsenite-treated animals excreted in the urine only 3.5% of the arsenic dose during the 72 h period after administration of arsenite, probably because of a decrease in urine volume caused by kidney injury, whereas the arsenite-only group excreted 27%. In addition, BSO pretreatment influenced the relative proportion of arsenic metabolites excreted in the urine during the first 24 h after administration. Urinary metabolites in the BSO plus arsenite group were predominantly inorganic arsenic. These results suggest that GSH provides protection against arsenic toxicity.

Keywords: Arsenite, glutathione depletion, acute poisoning, nephrotoxicity, hepatotoxicity, methylation

* Author to whom correspondence should be addressed

INTRODUCTION

Inorganic arsenic (arsenite and arsenate) is readily methylated in mammals to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). The methylation can be considered to be a detoxication, because methylated arsenic compounds have a lower toxicity and a lower affinity for tissue constituents than inorganic arsenic.[1–4] Although the complete mechanism of the methylation of inorganic arsenic has not been elucidated, this methylation may involve initially a reduction of arsenate to arsenite when arsenate is administered.[5–8] The methylation may take place in the liver by transfer of methyl groups from *S*-adenosylmethionine to arsenic through the mediation of methyltransferase.[8–10]

The sources of the electrons needed for the reduction of pentavalent to trivalent arsenic compounds have received little attention. Glutathione (GSH: viz. reduced glutathione) is the most abundant (~ 10 μmol g^{-1})[11] naturally occuring non-protein thiol in the body, and its various forms [GSH, oxidized GSH (GSSG) and mixed disulfides such as protein-SSG] are involved in numerous biochemical reactions within cells. GSH has important physiological roles such as conjugation of electrophilic compounds, donation of a γ-glutamyl group in amino acid transport in the γ-glutamyl cycle, and service as a reservoir of cystein.[12–14] The fundamental role of GSH may be the protection of thiol groups present in tissue from oxidative stress by xenobiotics.

Arsenite has a high affinity for thiol groups, especially dithiol groups of proteins,[15–19] a factor probably related to the toxic effects of arsenic (Fig. 1). Though arsenite is considered to compete with GSH for protein thiols, the relationship between GSH status and arsenic metabolism has not been defined. The present study was undertaken to determine whether the

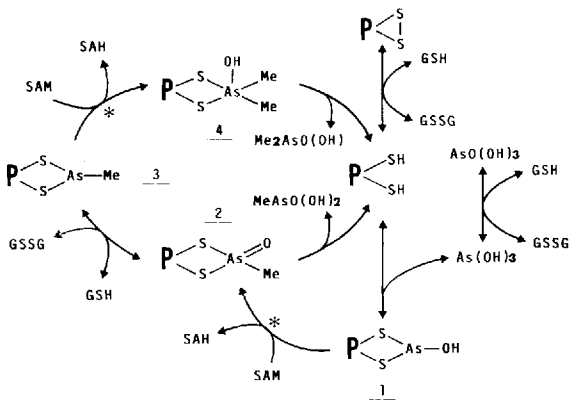

Figure 1 Schematic illustration of possible mechanisms of GSH mediation during arsenic methylation. *P*-protein; *methyltransferase; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine.

toxicity and methylation of arsenite in hamsters is influenced by the GSH concentration that was lowered by administration of DL-buthionine-(*S,R*)-sulfoximine (BSO). BSO is one of the inhibitors of γ-glutamylcysteine synthetase and depletes tissue GSH.[20–21]

EXPERIMENTAL

Animals and treatment

Male Syrian Golden hamsters (Kyudo Animal Laboratory, Kumamoto, Japan), 7–9 weeks old, had free access to food (Oriental NMF, Oriental Co., Japan) and water *ad libitum*. After an overnight fast, the animals were divided into three groups. The first group received sodium arsenite (99% pure, Merck; dissolved in distilled water) orally by stomach intubation (5 mg As kg^{-1} body wt.), the second group BSO [DL-buthionine-(*S,R*)-sulfoximine from Sigma, USA], dissolved in distilled water, intraperitoneally (4 mmol kg^{-1}), and the third group BSO (4 mmol kg^{-1}) followed by arsenite (5 mg As kg^{-1}) 2 h later. Four hamsters in each group were sacrificed by cardiac puncture at times ranging from 0 to 74 h after administration.

Determination of tissue GSH concentrations

Hepatic and renal GSH concentrations were estimated by determining non-protein thiol according to Ellman's method[22] as modified by Kawata and Suzuki.[23]

Assessment of liver and kidney injury

Plasma activity of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT), and blood urea nitrogen (BUN) and creatinine in plasma were determined using a Hitachi 736 auto-analyzer. For histopathologic evaluations, pieces of the livers and kidneys from each of the 72 h groups were fixed in 10% formalin, embedded in paraffin, and then stained with hematoxylin and eosin.

Determination of arsenic metabolites in urine

Hamsters (five animals for the arsenite group and seven animals for the BSO plus arsenite group) were kept in individual metabolic cages. The urine was collected every 24 h. After addition of 10 mol dm^{-3} NaOH to a final concentration of 3 mol dm^{-3} NaOH, the basic urine samples were heated in polymethylpentene test tubes at 85°C for 3 h. Inorganic arsenic, monomethyl- (MMA), dimethy- (DMA) and trimethylarsenic compounds (TMA) were reduced to the arsines with $NaBH_4$, and the arsines separated and detected by atomic absorption spectrophotometry according to a modification of the method described by Braman *et al.*[24] The amounts of arsenic compounds excreted in the urine of untreated hamsters were subtracted from the respective values of arsenic metabolites.

Statistics

Student's *t*-test was used for statistical comparisons between the arsenite- and the BSO plus arsenite-treated groups.

RESULTS AND DISCUSSION

GSH concentrations in BSO-, arsenite-, and BSO plus arsenite-treated hamsters

The GSH concentrations in hamsters fasted for 12 h were 9.1 ± 0.4 μmol g^{-1} in the liver and 3.0 ± 0.1 μmol g^{-1} in the kidney (mean ± SE). These values, considered to be the control GSH concentrations, were plotted as zero-hour concentrations in Fig. 2. After a single administration of arsenite (as Na_2HAsO_3) (5 mg As kg^{-1}) the hepatic GSH concentration decreased from 9.1 to 7.1 μmol g^{-1} during the first 3 h, reached 9.8 μmol g^{-1} after 24 h, and

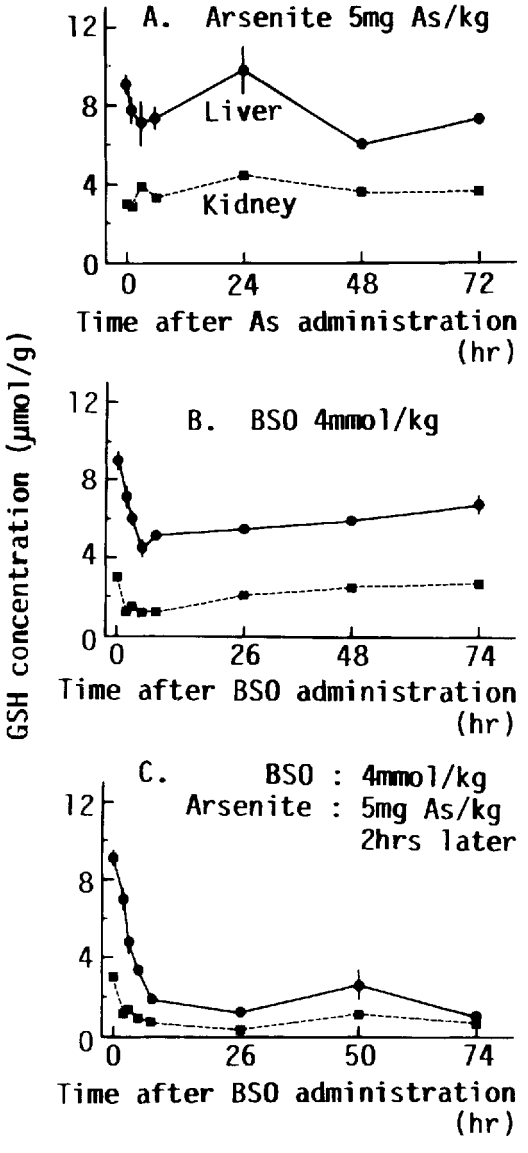

Figure 2 GSH levels in the liver and kidney after arsenite, BSO, and BSO plus arsenite administration (mean ± SE, for four animals).

then decreased again to settle between 6 and 7 μmol g^{-1} during the 48–72 h period (Fig. 2A). The renal GSH concentration remained almost constant (Fig. 2A). After a single dose of BSO (4 mmol kg^{-1}), hepatic and renal GSH decreased during the first 5 h to approximately half of the concentrations found in untreated hamsters and then increased slowly during the next 69 h without recovering completely (Fig. 2B). Similar trends in GSH concentrations were observed in mice after a single BSO administration.[25,26] When arsenite (5 mg As kg^{-1}) was given 2 h after BSO administration (4 mmol kg^{-1}), GSH disappeared almost completely from the liver and the kidney within several hours and remained at low levels until termination of the experiment at 74 h (Fig. 2C).

Nephrotoxicity

Whereas arsenite treatment reduced the 24 h urine volume, BSO plus arsenite administration caused oliguria or anuria (Fig. 3). No urine was present in the bladders of animals given BSO plus arsenite. Nephrotoxicity was quantified by determination of creatinine and urea nitrogen (BUN) in the plasma. Administration of BSO plus arsenite led to acute renal failure characterized by increases in plasma creatinine and BUN (Fig. 4). Neither arsenite nor BSO, when given alone, affected BUN and creatinine levels. Histopathological studies of the kidney from animals exposed to BSO plus arsenite by light microscopy revealed that the epithelial lining cells in the proximal tubules had become necrotic (Fig. 6) and the glomeruli moderately changed. Kidneys from hamsters treated with either arsenite or BSO alone were histologically normal.

Hepatotoxicity

A single-treatment of hamsters with arsenite or BSO had little effect on the plasma GPT and GOT (Fig. 5). However, BSO plus arsenite treatment cause the activities of these enzymes to increase during the first 6 h, to peak within 24 h, and to return to normal 48 h after arsenite administration (Fig. 5). Histopathological

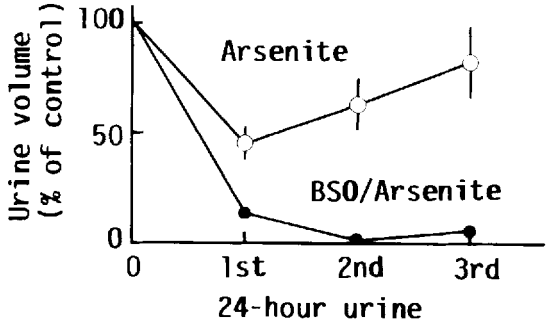

Figure 3 24-h urine volumes from hamsters treated with arsenite (five animals) or BSO plus arsenite (seven animals) (mean ± SE).

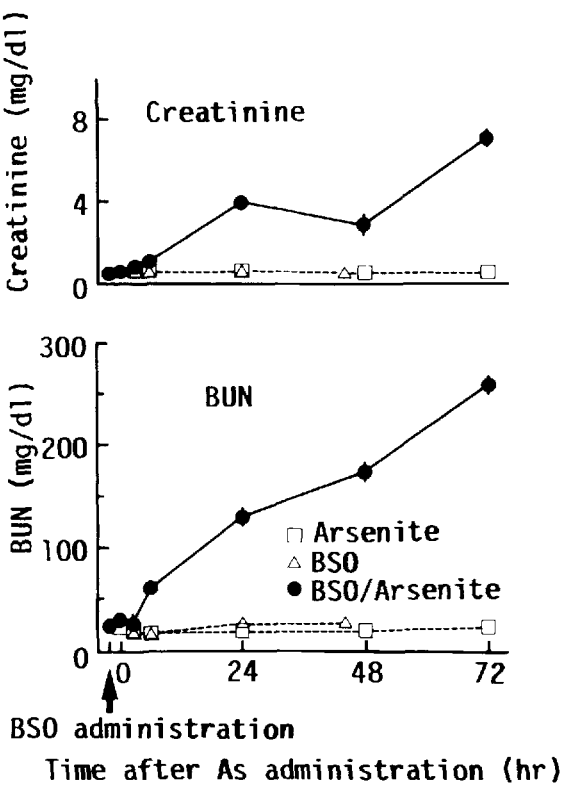

Figure 4 Blood urea nitrogen (BUN) and creatinine levels in plasma of hamsters after administration of arsenite, BSO, and BSO plus arsenite (mean ± SE, four animals).

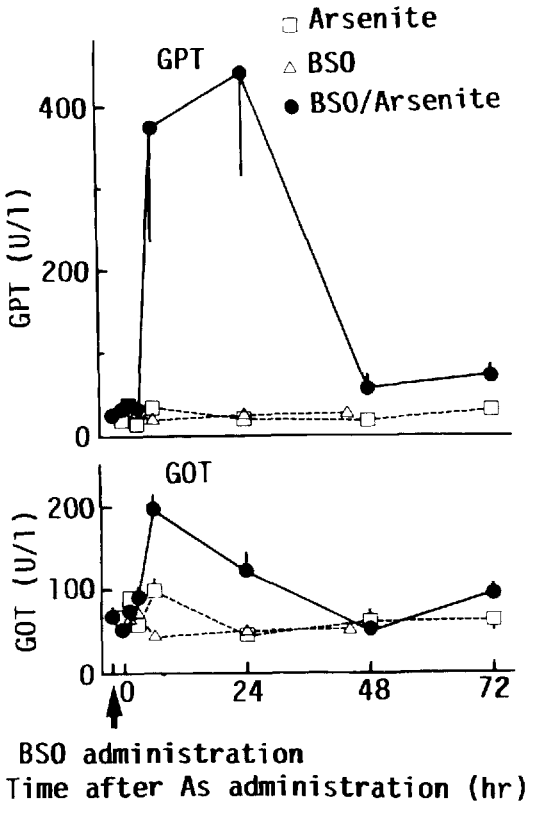

Figure 5 GPT and GOT activity in plasma of hamsters after administration of arsenite, BSO and BSO plus arsenite (mean ± SE, four animals).

examinations of liver sections by light microscopy revealed no significant differences among the livers of the arsenite, BSO, and BSO plus arsenite-treated animals.

Arsenic excretion in the urine

The BSO plus arsenite-treated hamsters excreted during 72 h only 3.5% of the administered arsenic, whereas the arsenite-treated hamsters excreted 27% (Table 1). Inorganic arsenic, monomethylated arsenic, and dimethylated arsenic were found in the urine of arsenite- and the BSO plus arsenite- treated groups. Trimethylated arsenic was detected in the urine of both groups, but the yield in the BSO plus arsenite group was regarded as 0%. The yields of these arsenic species are also expressed as a percentage of the total arsenic in each 24-h urine (Fig. 7). In the BSO plus arsenite group, three of seven animals excreted urine every day, so that data from these three animals was used for statistical analysis. Inorganic arsenic accounted for almost all of the arsenic in the first 24-h urine of the BSO plus arsenite group (Fig. 7). Although inorganic arsenic was also predominant in the first 24-h urine of the arsenite group, considerable amounts of methylated arsenic species were also found. The urine of the BSO plus arsenite group contained only traces of methylated arsenic compounds. Inorganic arsenic concentrations in the urine decreased and dimethylated arsenic concentrations increased with time in both groups. Dimethylated arsenic is the most common form of arsenic in the second and third 24-h urines. The ratios of the concentrations of each of the arsenic species in both the second and third 24-h urines for the arsenite group are not statisically different from the ratios for the BSO plus arsenite group.

Biochemical aspects

The liver and kidney were selected for study, because the kidney is the organ that eliminates much of the arsenic from the body[27,28] and the liver is also the

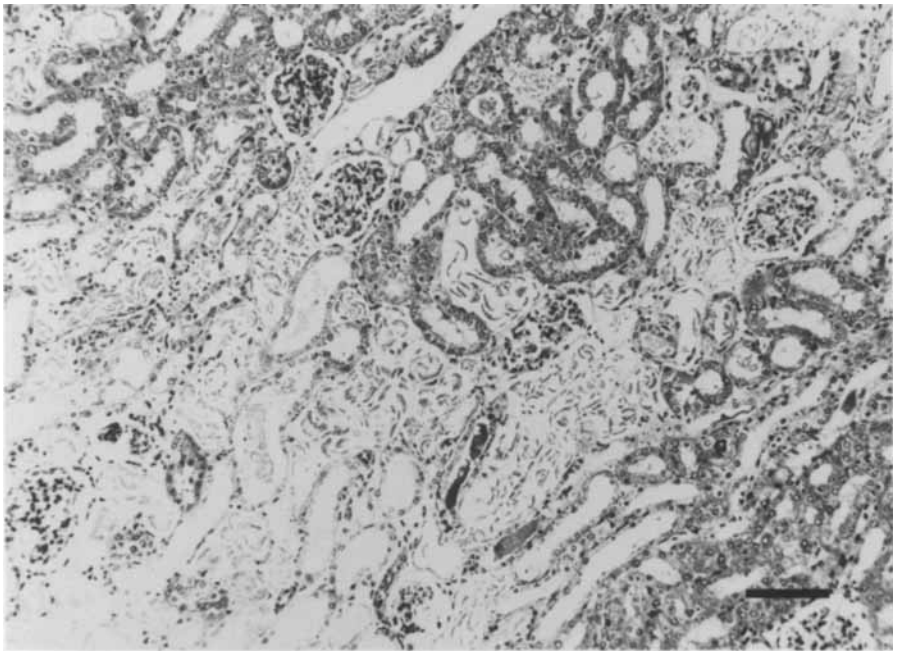

Figure 6 Renal proximal tubule cell (H. E. stain) from a hamster 72 h after BSO plus arsenite administration (scale bar = 100 μm).

organ in which arsenic may be methylated.[7–10] Both organs accumulate arsenic to high levels.[27,28] Inorganic arsenic has been reported to damage the liver and the kidneys in acutely poisoned humans[29–32] and mammals.[33] Whether renal failure is due to toxic effects of arsenic on the kidney, systemic damage, or a combination of both, is unknown.

Sequential administration of BSO and arsenite to hamsters caused transient hepatotoxic and permanent, severe, nephrotoxic effects. The proximal tubular regions of the kidneys are preferentially injured with concomitant reduction of the volume of excreted urine. The GSH concentrations in the livers and kidneys of BSO plus arsenite-treated hamsters decline precipitously. Hamsters treated only with BSO (4 mmol kg^{-1}) or only with a sublethal dose (5 mg As kg^{-1}) of arsenite remained healthy. The GSH levels were not significantly lower in the arsenite-treated than in the untreated hamsters. These results suggest that GSH protects organisms from arsenite-induced injury.

A mechanism of arsenic methylation is illustrated schematically in Fig. 1. GSH converts protein disulfides into thiol groups and reduces arsenate, $AsO(OH)_3$, to arsenite, $As(OH)_3$ (both shown in acidic form). Arsenite reacts with protein thiol groups to form 1,3-dithia-2-arsa-heterocycles (**1**). The heterocyclic arsenic compound is then methylated in a reaction using *S*-adenosylmethionine (SAM) as the methyl donor and a methyltransferase as the catalyst. Most of the monomethylated pentavalent arsenic intermediate (**2**) is reduced by GSH to a monomethylated trivalent arsenic compound (**3**) which is then methylated to a dimethylated pentavalent arsenic derivative (**4**).

Methylarsonic acid [$MeAsO(OH)_2$] and dimethylarsinic acid [$Me_2AsO(OH)$] are generated by hydrolysis of the methylated heterocycles (**2**,**4**). These methylarsenic compounds — much less toxic than arsenite — are then excreted in the urine. The hydrolysis of the heterocycles containing arsenic (**1**,**2**,**4**) liberates the protein thiols that now can bind another molecule of arsenite and make it ready for methylation and detoxification (Fig. 1).

Because arsenite administration — expected to decrease GSH concentrations according to the scheme shown in Fig. 1 — did not significantly decrease GSH levels (Fig. 2A), homeostatic mechanisms may exist in the liver and the kidneys that provide sufficient GSH for the generation of protein thiols from the pool of circulatory disulfides.[12–14,34] The GSH-generated protein thiols allow the arsenite to be methylated and excreted as observed with the arsenite-treated hamsters.

Table 1 Excretion of arsenic species in the 72-h urines of arsenite- and BSO plus arsenite-treated hamsters[a] (mean ± SE)

	100[Amount of As (arsenic-species) in urine] / Amount of As (arsenite) administered	
Arsenic species	Arsenite group (5-animal)	BSO plus arsenite Group (7-animal)
Inorganic As	12.7±0.7	2.1±0.5**
MMA	2.8±0.6	0.1±0.1*
DMA	11.1±0.6	1.3±0.6**
TMA	0.6±0.2	0.0*
Total As	27.0±1.3	3.5±1.1**

[a] The background values (inorganic As, 0.19 μg; MMA, 0.05 μg; DMA, 0.69 μg; TMA, 1.14 μg) were subtracted from the amounts of inorganic As, MMA, DMA and TMA excreted in the urine. *$P<0.01$; **$P<0.001$.

BSO has no other apparent effect than inhibition of GSH synthesis.[26] A BSO-treated animal exposed to arsenite does not produce the above-mentioned protein thiols required for the removal of the arsenic compound from its cells. Consequently the arsenite concentration in critical organelles will increase, and arsenite will react with thiol groups in other enzymes and thus damage the cell, as observed in the BSO plus arsenite-treated hamsters.

BSO is not the only compound that reduces GSH concentrations. Administration of heavy metals[23,35–37] or various organic compounds[38–43] is also known to lower GSH levels. Therefore, particular attention should be given to likely potentiating effects of such agents on the toxicity of arsenite. In the BSO plus arsenite group the sustained depletion of intracellular GSH may greatly potentiate cytotoxicity.

CONCLUSIONS

Our data are consistent with the hypothesis that GSH is needed to protect cells from damage by arsenite. Low levels of GSH prevent the efficient methylation of arsenite and cause arsenite to accumulate in the cells. The high arsenite concentrations lead to cell damage particularly in the proximal tubules. Agents that reduce GSH levels may increase the toxic effects of arsenite.

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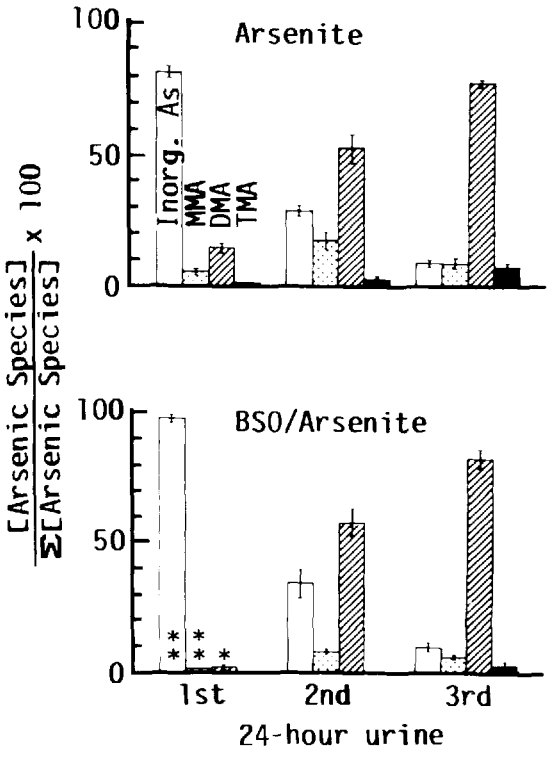

Figure 7 Urinary excretion of arsenic species by arsenite-treated (five animals) and BSO plus arsenite-treated (three animals) hamsters (mean ± SE). Asterisks indicate the levels of significance for the differences between the arsenite- and BSO plus arsenite-treated groups: * $P<0.01$; ** $P<0.001$.

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