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The metabolism of methylarsine oxide and sulfide

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Methylarsine oxide and sulfide are more toxic to *Candida humicola* than arsenite; the sulfide is rapidly metabolized to trimethylarsine (Me_3As) and methylarsine ($MeAsH_2$) and the oxide to dimethylarsinic acid [$Me_2AsO(OH)$]. Cell-free extracts of *C. humicola* also convert the oxide to $Me_2AsO(OH)$. The glutathione (RSH) derivative Me_2AsSR is metabolized by *C. humicola* to Me_3As and Me_2AsH, but some other Me_2AsSR' compounds are unaffected. Studies involving the interaction of the arsenic(III) compounds with natural ecosystems and other micro-organisms such as *Scopulariopsis brevicaulis, Straptococcus sanguis, Escherichia coli,* and *Veillonella alcalescens* are described.

Keywords: Arsenic, metabolism, *Candida humicola,* methylarsines, methylarsine oxide, methylarsine sulfide, micro-organisms

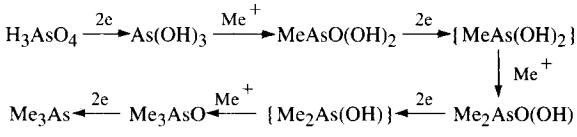
INTRODUCTION

The pathway proposed by Challenger[1,2] for the biomethylation of arsenicals to trimethylarsine (Scheme 1) is made up from two basic steps: reduction of the arsenic(V) species to arsenic(III) species, possibly oxides; and subsequent oxidative methylation to methylarsenic(V) moieties. The mechanism of this two-step cycle remains unclear; however, it is likely that *S*-adenosylmethionine is the source of the methyl group[3,4] and that reduction is facilitated by the ubiquitous sulfhydryl function either in solution on small molecules, or at protein sites.[5,6]

Thus far, in studies connected with Scheme 1, the inorganic species arsenate, arsenite, and the organoarsenic(V) species methylarsonate and dimethylarsinate, have received most attention, and have been shown to react as indicated in the scheme. These arsenicals are known to be present in the environment and the biosphere,[7,8] although there are no reports of the identification of involatile methylated arsenic(III) species. However, as most analytical techniques employed to detect methylated arsenicals rely on reduction of the arsenicals to the volatile methylarsines, $MeAsH_2$ or Me_2AsH, it is not possible to say whether the $MeAsH_2$, for example, is derived from $MeAs^{V}O_3H_2$ or $(MeAs^{III}O)_x$.[7,9] The species $\{MeAs(OH)_2\}$ and $\{Me_2As(OH)\}$ in Scheme 1 probably do not exist as such; these arsenic(III) compounds are better represented as $(MeAsO)_x$ and $[Me_2As]_2O$, and in an anoxic environment the compounds may be $(MeAsS)_x$, $MeAs(SR)_2$, Me_2AsSR, etc.[10]

Our interest in $(MeAsO)_x$, $(MeAsS)_x$, and a range of dimethylthioarsinites arises from their implication as intermediates in Scheme 1. Prior to the present investigation it had not been established whether methylarsenic(III) compounds could act as substrates for biological methylation processes, although the reductive processes occurring between thiols and methylarsenic(V) oxo species which produce $MeAs(SR)_2$ and Me_2AsSR derivatives indicate that these may be biologically available.[5,6]

Methylarsine sulfide [$(MeAsS)_x$] is a very potent fungicide (Rhizoctol).[11–17] For example, mercury-resistant *Pyrenophora arenae,* responsible for leaf-spot and seedling blight in seed oats, is best controlled by methylarsine sulfide.[12] Although practically insoluble in water, it disappears within six months of application as a fungicide; that is, in some way it is mobile in the environment.



Scheme 1

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EXPERIMENTAL

The dimethylthioarsinites of cysteine, glutathione, mercaptoethanol, and thioglycollic acid were prepared as previously reported.[6]

Synthesis of methyl[^{74}As] arsine oxide and methyl[^{74}As] arsine sulfide

[^{74}As] Arsenate, of initial specific activity 1.0 mCi nmol^{-1}, was obtained from Amersham Corporation. Arsenate (200 μCi) was reduced to arsenite by reaction for 40 min with freshly prepared reducing solution (1.0 cm^3) made up as follows: 0.28 g sodium metabisulphite, 0.2 cm^3 10% aqueous sodium thiosulfate, 0.25 cm^3 7.5 mol dm^{-3} sulfuric acid, 1.5 cm^3 water.[18] This radioactive arsenite solution was diluted with [^{75}As]arsenite (1 g As_2O_3 in 3 cm^3 10 mol dm^{-3} sodium hydroxide) and the total arsenite was methylated by refluxing the solution with 6.25 cm^3 methyl iodide for 24 h. Addition of ethanol (15–20 cm^3) precipitated disodium methylarsonate from the reaction mixture. This solid was collected by filtration and divided into two parts: one part to be reduced to methylarsine oxide, and the other to be converted into the sulfide.

For methylarsine oxide, ^{74,75}As-labeled methylarsonate was dissolved in a minimum amount of warm water, then sulfur dioxide was bubbled through the solution until saturation occurred. The solution was boiled for 2 min, quickly cooled to 4°C, and neutralized with sodium carbonate. It was evaporated to dryness and the methylarsine oxide was extracted with benzene. Removal of the benzene left a white, foul-smelling solid. This was redissolved in water and eluted from a Bio-Gel P-2 column (height, 0.6 m; i.d., 0.015 m). The purity was assessed by ^{1}H NMR spectroscopy, mass spectrometry (which showed the solid to exist as a tetramer), and electrophoresis–autoradiography (see below). The yield of $(MeAsO)_x$ was 53%.

The second portion of methylarsonate was dissolved in 10 cm^{-3} of 0.5 mol dm^{-3} sulfuric acid containing 0.3 g potassium iodide. Sulfur dioxide was bubbled through the mixture for 20 min. On cooling, yellow needles of $MeAsI_2$ formed, which were isolated by filtration. The solid was redissolved in 10 cm^3 of warm water, and the solution was treated with hydrogen sulfide for 15 min. The resulting white precipitate of $(MeAsS)_x$ was filtered, washed with cold water and recrystallized from ethanol (30% yield).

Analysis: Calc. for $(MeAsS)_x$: C, 9.84; H, 2.48; S, 26.28%. Found: C, 10.00; H, 2.33; S, 25.95%.

Mass spectrometry indicated a trimeric structure in the solid state. Electrophoresis and autoradiography confirmed the only arsenic-74 species present as [^{74}As] MeAsS.

Detection of arsenic-containing species

(a) Volatile

Two different techniques were used to detect the presence of volatile arsenic-containing products. A Bendix Gas Chromatograph Model 2500 equipped with a flame ionization detector was used if sufficient quantities of arsine were present. Methylarsine, dimethylarsine, and trimethylarsine could all be detected, and were distinguished from each other by using a 2 m × 0.25 mm i.d. column packed with 10% OV-101 on Chromosorb W/AW, a carrier flow rate of 50 cm^3 min^{-1}, and an oven temperature of 50°C. The retention times for methyl-, dimethyl-, and trimethyl-arsines were 47 s, 69 s, and 85 s, respectively. The lowest detectable concentration of trimethylarsine by this procedure was 24 ng cm^{-3}.

Lower arsine levels were detected by using ^{74}As-labeled substrates. Volatile [^{74}As]arsines were found to be effectively trapped (chemofocused) by glass microfiber paper soaked in 5% mercuric chloride and suspended in the head-space of cultures metabolizing arsenicals.[19] Total volatile arsine was measured by counting the mercuric chloride trap after suspension in Bray's scintillation fluid. Arsine concentrations of as low as 1 ng cm^{-3} can be detected, although the arsines cannot be distinguished from each other unless sufficient material is collected to allow identification by other techniques. Thus, if sufficient arsine is produced, small crystals of the arsine–mercuric chloride adduct are formed on the microfiber paper. When the crystals are heated to 100°C the adduct decomposes into volatile arsines and mercuric chloride, which can be analyzed by GC. Alternatively, the crystals can be analyzed directly by mass spectrometry.[19]

(b) Non-volatile

A variety of chromatographic procedures were employed to identify non-volatile arsenicals. After

incubation of a biological system with methyl[^{74}As]arsine oxide or sulfide, centrifugation removed cells and debris from the supernatant. If the latter contained the majority of arsenic-74 label, it was concentrated by evaporation of water. If the pellet had a significant arsenic-74 content, it was treated with hot 90% ethanol to extract the arsenic compounds. The ethanolic solution was dried, and the residue redissolved in water. Either or both of the concentrated supernatant or extract was applied to a Bio Gel P2 column (0.6 m height × 0.015 m i.d.) and eluted with distilled water. Those fractions (1.5 cm^3) containing arsenic-74 were identified by counting aliquots of each fraction. The void volume of the column was 47 cm^3 and the elution volumes of standard arsenate, arsenite, methylarsine oxide, and dimethylarsenic acid were 65, 105, 95, and 80 cm^3, respectively. The elution volume from the P2 column alone was not sufficient proof of the identity of the arsenicals. After separation by the P2 gel, the fractions were subjected to thin-layer electrophoresis and/or thin-layer chromatography. Details of electrophoresis are given elsewhere.[20] If arsenate is assigned an R_f value of 1, the corresponding values for arsenite, methylarsine oxide, methylarsine sulfide, dimethylarsinic acid, and methylarsonic acid are −0.16, −0.25, 0, 0.33, and 0.60, respectively.

Thin-layer chromatography was carried out on cellulose sheets with ethyl acetate, acetic acid, and water (3:2:1) as liquid phase. After air-drying, the chromatogram was developed by exposure to X-ray film for 1−2 weeks. The R_f values obtained were: arsenate, 0.43; arsenite, 0.33; methylarsine oxide, 0.73; methylarsonic acid, 0.73; and dimethylarsinic acid, 0.83.

Organisms

Scopulariopis brevicaulis and *Candida humicola* were grown aerobically at 25°C on Cox and Alexander's minimal salts−glucose media.[21,22] *Lactobacillus brevis, Streptococcus sanguis, Pseudomonas aeruginosa, Escherichia coli,* and *Bacillus subtilis* were all grown aerobically at 25°C on Trypticase Soy Broth (Difco). *Veillonella alcalescens* was grown anaerobically at 25°C on media consisting of Trypticase (5 g), yeast extract (3 g), 70% sodium lactate (25 cm^3) per litre of water (pH 7.0). *Fusobacterium nucleatum* was grown anaerobically in a nitrogen−hydrogen−carbon dioxide (85:10:5) environment at 37°C on media consisting of Trypticase (17 g), yeast extract (3 g), NaCl (5 g), K_2HPO_4 (2.5 g), glucose (2.5 g), and hemin (5 mg) per dm^3 of water (pH 7.0−7.2). When indicated, growth media were amended with the appropriate arsenicals. Because of the insolubility of $(MeAsS)_x$ in water, this compound was added in ethanol solution. Appropriate controls were run in parallel for all experiments.

Natural eco-systems

Soil was collected from three locations, namely a saltwater marsh, a mixed (deciduous/coniferous) forest, and around an exposed tree root. The compost samples were from the 'active' section of a domestic compost heap. Fresh rumen fluid was collected from the large stomach of a 12 h-starved steer. Ceca were obtained from male albino mice, eight weeks old.

RESULTS

Toxicity studies

(a) *C. humicola*

Minimum inhibitory concentrations (m.i.c.) were obtained for the three arsenic(III) compounds, arsenite, methylarsine oxide, and methylarsine sulfide for *C. humicola* as follows. To tubes containing 5 cm^3 of liquid medium was added 0.1 cm^3 of arsenic(III) solution to give a range of concentrations. The tubes were then inoculated with 0.1 cm^3 of *C. humicola* and shaken at room temperature. After 48 h the highest concentration which allowed growth and the lowest concentration which did not were recorded. Thus, the 48 h m.i.c. for arsenite was between 8 and 4.2 mmol dm^{-3}. For methylarsine oxide, the 48 h m.i.c. was between 0.8 and 0.08 mmol dm^{-3}, and for methylarsine sulfide it was less than 0.008 mmol dm^{-3}. The 72 h m.i.c. for methylarsine sulfide was between 0.880 and 0.04 mmol dm^{-3}.

In another experiment, viable counts of *C. humicola* were made after exposure to arsenic(III) compounds. After 2 h of exposure to 500 mmol dm^{-3} arsenite, 10% of *C. humicola* remained viable, whereas after 24 h less than 1% were viable. Exposure of *C. humicola* to 10 mmol dm^{-3} methylarsine oxide for 2 h and 24 h resulted in 50% and <0.1% viability,

respectively. Exposure to 1.5 mmol dm^{-3} methylarsine sulfide for 2 h and 24 h gave viability of 50% for both times.

(b) Other micro-organisms

Twenty-four-hour m.i.c. of methylarsine oxide for several micro-organisms were measured. For *E. coli* and *P. aeruginosa* the 24 h m.i.c was >50 mmol dm^{-3} for *S. sangius* it was between 10 and 20 mmol dm^{-3}, and for *B. subtilis* between 0.5 and 1 mmol dm^{-3}. The 24 h m.i.c. for *C. humicola* was between 1 and 5 mmol dm^{-3}. The discrepancy between this value and the value reported above for the 48 h m.i.c. is due to the larger inoculum used in this experiment.

Transformation of methylarsine sulfide by *C. humicola*

A garlic odor was noticed whenever *C. humicola* was grown in the presence of methylarsine sulfide, $(MeAsS)_x$. One liter of liquid media was inoculated with 10 cm^3 of *C. humicola* and grown aerobically at 25°C for 24 h. Cells were harvested, and used to inoculate 500 cm^3 of fresh media, which contained methylarsine sulfide (5 μmol dm^{-3}). After 1 h a 1.0 cm^3 head-space sample was analysed by GC and found to contain both methylarsine ($MeAsH_2$) and trimethylarsine (Me_3As) in the ratio 10:90.

The extent of arsine production was measured by utilizing methyl[^{74}As]arsine sulfide, $(MeAsS)_x$. Media (1 dm^3) containing methylarsine sulfide (30 μmol dm^{-3}) ($\equiv$0.1 μCi ^{74}As) was inoculated with 10 cm^3 of *C. humicola*. A glass microfiber paper presoaked in 5% mercuric chloride solution was suspended in the head-space of the culture. After three days' growth, the distribution of the arsenic label was measured. The mercuric chloride trap contained 50% of the total arsenic-74. The culture accounted for ~49.5%. There were a few counts on a mercuric chloride trap which had been affixed externally over the neck of the culture flask. The mass spectrum of the crystals which had formed on the internal mercuric chloride trap confirmed the presence of Me_3As. It is more difficult to observe $MeAsH_2$ using mass spectrometry owing to the overlapping cracking patterns of $MeAsH_2$ and Me_3As. However, GC analyses of the gases produced by heating the crystals to 100°C in a sealed vial confirmed the presence of $MeAsH_2$.

No dimethylarsine (Me_2AsH) was detected in any of these experiments.

Transformation of dimethylthioarsinites by *C. humicola*

An actively growing culture of *C. humicola* (15 cm^3) was made 5 mmol dm^{-3} in the dimethylthioarsinite derivatives of cysteine, glutathione, mercaptoethanol, and thioglycollic acid. Control flasks were prepared using $Me_2AsO(OH)$ as a substrate. The cultures were allowed to stand for 24 h in firmly stoppered flasks, after which time the head-space was analyzed for volatile arsines by using GC. In one series of experiments the average volume of trimethylarsine evolved from the $Me_2AsO(OH)$-containing culture measured 208 units (GC area), from the glutathione derivatives it was 43 units, and from the cysteine derivatives 70 units. No Me_3As was produced from the thioacetic acid or mercaptoethanol derivatives. Dimethylarsine (Me_2AsH) is always produced from cultures containing the glutathione derivative; for example, the volume was 86 units in the set of experiments just described.

Transformation of methylarsine oxide

An actively growing culture of *C. humicola* (15 cm^3) was made 5 mmol dm^{-3} in methyl[^{74}As]arsine oxide. A mercuric chloride trap was placed in the head space. Over a period of 24 h the volume of the culture was increased to 150 cm^3 by continuous addition of media. After 24 h the traps were counted, and the cells and supernatant analyzed for arsenicals. A similar procedure was used for other cultures.

Apart from unchanged methylarsine oxide, dimethylarsinate was detected in the supernatant of four cultures. The ratios of dimethylarsinic acid to methylarsine oxide for *C. humicola, V. alcalescens, L. brevis,* and *S. brevicaulis* were 0.24:1, 0.01:1, 0.11:1, and 0.12:1, respectively (ratio of counts of arsenic-74).

The mercuric chloride traps all contained small amounts of arsenic-74. The highest were *V. alcalescens* (0.2% total initial ^{74}As) *S. brevicaulis* (0.1%) and *E. coli* (0.1%).

Competitive effects of arsenic compounds on the conversion of methylarsine oxide to dimethylarsinate by *C. humicola*

Actively growing cultures of *C. humicola* (15 cm^3) were made 5 mmol dm^{-3} in ^{74}As-labeled methylarsine oxide $[(MeAsO)_x]$ and 10 mmol dm^{-3} in either arsenite, arsenate, methylarsonate, dimethyl-

arsinate, or trimethylarsine oxide, or 20 μmol dm^{-3} in methylarsine sulfide. A mercuric chloride trap was placed in the head-space of each culture. Media was added continuously to each culture over 24 h. The final volume was 105 cm^3. After 24 h the supernatant of each culture was analyzed for dimethy[^{74}As] arsinate, and the amount of volatile arsines produced from methylarsine oxide estimated by counting the mercuric chloride trap. The results are shown in Table 1.

Table 1 The metabolism of $(MeAsO)_x$ by *C. humicola* and the effect of additives[a]

Arsenical	Gas trap (cpm)	Conversion to dimethyl-[^{74}As]arsinate (%)
Control $(MeAsO)_x$	2500	19
Arsenite (10 mmol dm^{-3})	2100	14
Arsenate (10 mmol dm^{-3})	2700	19
Methylarsonic acid (10 mmol dm^{-3})	2000	16
Dimethylarsinic acid (10 mmol dm^{-3})	2500	18
Trimethylarsine oxide (10 mmol dm^{-3}	2105	14
Methylarsine sulfide (20 mmol dm^{-3})	1645	39

[a] 1 μCi $CH_3{}^{74}AsO$ (2×10^6 cpm) was added to each culture.

Experiments using cell-free extracts of *C. humicola*

Cells from a 24 h culture of *C. humicola* were harvested, washed once with phosphate-buffered saline (PBS), pH 7, and resuspended in PBS at a concentration of 1 g wet weight of cells per 2 cm^3 of buffer. Cell-free extracts were obtained by rupturing the cells in a modified Hughes press, followed by centrifuging at 10 000 g for 20 min to remove any remaining whole cells and cell debris.

When 1 cm^3 of cell-free extract was incubated with 1 μmol of NAD, 50 μmol glucose, and 1 μCi ^{74}As-labeled CH_3AsO, 17% of the methylarsine oxide was converted into dimethylarsinate as determined by P2 column chromatography. This reaction is complete in ~1 h. Omitting NAD from the reaction had no significant effect on the amount of dimethylarsinate produced, whereas omitting glucose resulted in no dimethylarsinate formation. Doubling the glucose concentration of 100 μmol had no effect. The addition of twice as much substrate (i.e. 2 μCi methylarsine oxide) resulted in approximately twice as much dimethylarsinate. If 0.05 mol dm^{-3}, pH 5, potassium succinate–succinic acid buffer replaced PBS throughout, no dimethylarsinate was produced from methylarsine oxide. However, altering the pH of PBS to 8 from 7 made no change in the amount of dimethylarsinate produced.

The stability of the cell-free extract is limited. either heating on a 100°C water bath for 10 min or standing at room temperature for 24 h resulted in a total loss of the ability to convert methylarsine oxide to dimethylarsinate. However, activity was retained for at least 24 h if the cell-free extract was kept at −20°C.

The reaction of cell-free extract with methylarsine oxide was carried out by using either methyl[^{74}As] arsine oxide and uniformly labeled [^{14}C]glucose, or [^{14}C]methyl-*S*-adenosylmethionine. No [^{14}C] dimethylarsinate was produced in either case. When methyl[^{74}As]arsine oxide is used, no [^{74}As]arsenate or [^{74}As]arsenite is detected, indicating that dimethyl[^{74}As]arsinate is not being formed by the transfer of methyl from one molecule of methylarsine oxide to another.

A cell-free extract was fractionated into two parts by filtering through an Amicon PM-10 ultrafiltration membrane. The filtrate (M. wt < 10 000) and the fraction retained (M. wt > 10 000) were assayed separately for their ability to produce dimethylarsinate from methylarsine oxide. Both fractions were inactive. Furthermore, recombination of the two fractions did not result in restored activity, presumably because of the destruction of a labile moiety in the filtering process.

Metabolism of methylarsine oxide and methylarsine sulfide

By rumen fluid

Freshly collected rumen fluid (1 dm^3) was incubated under anerobic conditions at 37°C with 1 μCi methy[^{74}As]arsine oxide for 4 h; a mercuric chloride gas trap was placed in the head-space. Subsequent analysis showed 8 000 cpm ^{74}As (0.4% total ^{74}As) on the trap, and 7% of the methylarsine oxide had been converted to dimethylarsinic acid.

Methylarsine sulfide was not converted to volatile arsines by rumen fluid.

By soil and compost

Methylarsine oxide and methylarsine sulfide were incubated for 1–2 weeks with each of three soil samples

and compost. No volatile arsines were produced from the sulfide. No metabolism of methylarsine oxide was detected following analysis of the soil or compost.

By ceca

Four mice were inoculated with 1 μCi methyl[^{74}As]arsine oxide. After 4 h they were sacrificed and the ceca analyzed for arsenic-74 compounds. Dimethylarsinate, arsenate, and methylarsine oxide were all found.

In another experiment, the ceca were removed from four untreated mice, and the cecal contents made up to 20 cm^3 with pH 5 0.05 mol dm^{-3} sodium acetate–acetic acid buffer, 1 μCi ^{74}As-labeled $(MeAsO)_x$ was added, and the preparation incubated under anaerobic conditions at 39°C for 6 h. As with the *in vivo* experiment, arsenate, dimethylarsinate, and methylarsine oxide were all present.

DISCUSSION

It is well known that the toxicity of arsenic towards a particular organism depends on the chemical form of the arsenic.[23] In general, arsenic(III) derivatives such as arsenite are more toxic than arsenic(V), such as arsenate. Organoarsenicals, especially the widely used arsenic(V) derivatives methylarsonate and dimethylarsinate, are relatively non-toxic towards micro-organisms.

The present results indicate that the arsenic(III) derivative $(MeAsO)_x$ is more toxic than arsenite to *C. humicola*. The sulfide $(MeAsS)_x$ is considerably more toxic than either arsenite or $(MeAsO)_x$. Evidently the cells are prevented from metabolizing, but are not killed, by the sulfide, because the viability of *C. humicola* is not affected by prolonged exposure to the sulfide from 2 h to 24 h.

It is often assumed that methylation of inorganic arsenic to methylarsenicals is a detoxification process. The present results indicate that the truth of this assumption may well be limited to situations where there is no possibility of accumulation of intermediates.

There are numerous reports of the production of volatile arsines Me_xAsH_{3-x}, (x = 0–3), following exposure of pure cultures of micro-organisms to arsenicals, and also following treatment of other biologically active media such as soils, sediments, and rumen fluid with arsenicals.[7,8] However, it seems that compounds with As–H bonds are more likely to be the metabolic products of bacteria; Me_3As is produced by fungi, an observation which is the basis for studies resulting in the mechanism of Scheme 1.[1,2] The present results show that Me_3As is produced by *C. humicola* from $(MeAsS)_x$, as would be expected from Scheme 1. However, some $MeAsH_2$ accompanies the Me_3As, and this is unique in our experience with *C. humicola*. What is also remarkable about this reaction is its rapidity, with 50% of the added arsenic being volatilized in three days. The conversion of other arsenicals such as arsenate or dimethylarsinate to Me_3As by *C. humicola* occurs slowly; even after 10 days only 1% is volatilized from arsenate.[24] Apart from the methylation of $(MeAsO)_x$, to be described next, the only other rapid biological process involving arsenicals noted to date is the reduction of Me_3AsO to Me_3As.[25,26]

Methylarsine oxide, another arsenic(III) derivative, is metabolized by *C. humicola*, *S. brevicaulis*, and other organisms such as *Veillonella alcalescens* not normally associated with arsenic metabolism. The principal metabolic product, again in a rapid process (24 h), is dimethylarsinate; smaller amounts of volatile arsines, not identified, can be trapped (Table 1). The isolation of dimethylarsinate is noteworthy, as it is the first time that a non-volatile methylated intermediate (shown in Scheme 1) has been identified in the growth medium of a pure culture. Previously, methylated intermediates have been isolated from cell extracts of *C. humicola*.[20]

The methylation of $(MeAsO)_x$ to $Me_2AsO(OH)$ is a result which would be predicted from Scheme 1. The absence of significant change in the ratio of unchanged $(MeAsO)_x$ to $Me_2AsO(OH)$ (Table 1) when arsenate, arsenite, methylarsonate, dimethylarsinate, and trimethylarsine oxide are present during the incubation of *C. humicola* with $(MeAsO)_x$, suggests that the transport and/or reaction is independent of these arsenicals. There is, however, a 50% increase in dimethylarsinate production when 20 μmol dm^{-3} $(MeAsS)_x$ is present. This suggests that the sulfide stimulates the ability of the cell to methylate methylarsenic(III) species; however, under the same conditions the sulfide is methylated to Me_3As, whereas the oxide is methylated only as far as $Me_3AsO(OH)$. Clearly the metabolic pathways available to these two compounds are different and the sulfide is not methylated via a $Me_2AsO(OH)$ intermediate which is released into the medium.

In contrast with the result from $(MeAsS)_x$, the

dimethylthioarsinites (Me_2AsSR) are not metabolized rapidly by *C. humicola*. No arsine is evolved from cultures containing the thioacetate and mercaptoethanol derivatives. Trimethylarsine is evolved from the glutathione and cysteine derivatives, but less than from $Me_2AsO(OH)$ under the same conditions. Dimethylarsine is invariably produced from the glutathione derivatives, sometimes in greater amounts than Me_3As.

Because the thioarsenites are readily oxidized to $Me_2AsO(OH)$ in aqueous solution in the absence of micro-organisms,[6] it is difficult to eliminate this chemical process as the source of the arsenical which is subsequently biomethylated. However, the production of high levels of Me_2AsH from the glutathione derivative does indicate some direct biological action. This is the first observation of Me_2AsH as a metabolite from a mold culture. Bacteria can reduce $Me_2AsO(OH)$ to Me_2AsH,[7] and *Methanobacterium* MoH probably produces this arsine from arsenate.[27,28] Perhaps this reduction by *C. humicola* [and that of $(MeAsS)_x$] can be achieved by using NADH:

$$Me_2AsSGlut \xrightarrow{NADH} Me_2AsH + SGlut^-$$

Cell-free extracts prepared from *C. humicola*, like the whole organism, covert methylarsine oxide to dimethylarsinate. The conversion of 17% found by using the standard conditions is evidently due to depletion of endogenous resources rather than the attainment of equilibrium, because if the extract is first incubated with $(Me^{75}AsO)_x$ for 3 h and then $(Me^{74}AsO)_x$ for 1 h, only 0.05% of dimethy[^{74}As]arsinate is produced. The loss of activity on heating the extracts confirms the enzymatic basis for these reactions.

Preculturing *C. humicola* in the presence of 5 mmol dm^{-3} methionine or 1 mmol dm^{-3} methylarsine oxide prior to preparing the cell-free extract has little effect on the ability of the extract to metabolize $(MeAsO)_x$. In other systems pre-incubation of *C. humicola* with arsenicals has led to more rapid metabolism of arsenic-containing substrates.[20,24] The added methionine could increase the cells' internal concentration of *S*-adenosylmethionine (SAM) and might be expected to enhance any methylation process. In this connection the addition of SAM (10^{-3} mol dm^{-3}) to the extract does *not* effect the amount of dimethylarsinate produced from methylarsine oxide. This is a surprising result in view of high methyl incorporations previously found.[3] The addition of other methylating factors — dihydrofolate (10^{-2} mol dm^{-3}), tetrahydrofolate (10^{-2} mol dm^{-3}), choline (1.5×10^{-2} mol dm^{-3}) — also has no effect, as do the methyl scavenger homocysteine (10^{-2} mol dm^{-3}), the methionine antagonist ethionine (1.5×10^{-2} mol dm^{-3}), and the electron-transfer inhibitors azide (10^{-2} mol dm^{-3}) and cyanide (10^{-2} mol dm^{-3}).

Certain natural eco-systems were found to metabolize methylarsine oxide. Rumen fluid converted 7% of the substrate into dimethylarsinate within 4 h, and volatised 0.4% of the substrate. Previous results from this laboratory have shown rumen fluid to be capable of metabolizing arsenate into a moiety which sticks to red rubber, and trimethylarsine oxide is reduced to Me_3As by rumen fluid.[26,28]

Preparations of the contents of mouse ceca methylate methylarsine oxide to dimethylarsinate, and demethylate it to arsenate. Ceca from mice dosed with the oxide contain the same compounds. It is probable that the microflora present in the ceca are responsible for these transformations. Rolands and Davies report[29,30] that preparations of rat ceca incubated *in vitro* reduce arsenate to arsenite and methylate arsenate; however, processes localized in the gut do not seem to contribute significantly to the overall biotransformation of arsenic *in vivo*.[31]

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REFERENCES

1. Challenger, F *Chem. Rev.*, 1945, 36: 315
2. Challenger, F *Adv. Enzymol.* 1951, 12: 429
3. Cullen W R, Froese, C L, Lui, A, McBride, B C, Patmore, D J and Reimer, M *J. Organomet. Chem.*, 1977, 139: 61
4. Challenger, F, Lisle, D B and Dransfield, P B *J. Chem. Soc.*, 1954, 1760
5. Cullen, W R, McBride, B C and Reglinski, J *J. Inorg. Biochem.* 1984, 21: 45
6. Cullen, W R, McBride, B C and Reglinski, J *J. Inorg. Biochem.* 1984, 21: 179
7. Cullen, W R and Reimer, K J *Chem. Rev.*, (in press)
8. Andrae, M O In: *Organometallic Compounds in the Environment*, Craig, P J (ed) Wiley, New York, 1986
9. Braman, R S, Justen, L L and Foreback, C C *Anal. Chem.*, 1972, 44: 2195
10. Cullen, W R *Adv. Organomet. Chem.* 1966, 4: 145

11. German Patent 1079886, 14 April, 1960; *Chem. Abs.*, 1961, 55: 19121i
12. Sheridan, J E, Whitehead, J D and Spiers, A G *NZ J. Exp. Agr.*, 1973, 1: 127
13. Michail, S H, Elarosi, H, Abd-El-Rehim, M A and Shoda, W T *Phytopathologia Mediterranea*, 1975, 14: 138
14. Agrawal, S C, Khare, M N and Agrawal, P S *Indian Phytopathol.*, 1976, 29: 90
15. Tomar, K S *Indian Phytopathol.*, 1974, 27: 364
16. Hocking, D and Jaffer, A A *Commonwealth Forestry Rev.*, 1969, 48: 355
17. Singh, R S and Joshi, M M *Pesticides (Bombay)*, 1969, 3: 19
18. Asher, C J and Reay, P F *Anal. Biochem.*, 1977, 78: 557
19. Cullen, W R, Erdman, A E, McBride, B C and Pickett, A W *J. Microbiol. Methods*, 1983, 1: 297
20. Cullen, W R, McBride, B C and Pickett, A W *Can. J. Microbiol.*, 1979, 25: 1201
21. Cox, D P and Alexander, M *Bull. Environ. Contam. Toxicol.* 1973, 9: 84
22. Cox, D P and Alexander, M *Appl. Microbiol.*, 1973, 25: 408
23. Fowler, B A (ed.), *Topics in Environmental Health*, Vol. 6: *Biological and Environmental Effects of Arsenic*, Elsevier, Amsterdam, 1983
24. Cullen, W R, McBride, B C and Reimer, M *Bull. Environ. Contam. Toxicol.*, 1979, 21: 157
25. Pickett, A W, McBride, B C, Cullen, W R and Manji, H *Can J. Microbiol.*, 1981, 27: 773
26. Pickett, A W, McBride, B C and Cullen, W R *Appl. Organomet. Chem.*, 1988, 2: 479
27. McBride, B C and Wolfe, R S *Biochemistry*, 1971, 10: 4312
28. McBride, B C, Merilees, H, Cullen, W R and Pickett, A W In: *Organometals and Organometalloids-Occurrence and Fate in the Environment*, Brinckman, F E and Bellama, J M (eds) ACS Symp. Ser. No 88, 1978, p 94
29. Rowland, I R and Davies, M J *J. Appl. Toxicol.*, 1981, 1: 278
30. Rowland, I R and Davies, M J *J. Appl. Toxicol.*, 1982, 2: 294
31. Vahter, M and Gustafsson, B In: *Spurenelem. -Symp.: Arsen, 3rd*, Anke, M, Schneider, J-J and Brueckner, C (eds), 1980, p 123