then the simplest explanation for the poly(U) binding to the 60S subunit fraction is that it is nonspecific binding as indicated by its relative insensitivity to inhibition by ATA.

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Biosynthesis of Dimethylarsine by Methanobacterium*

B. C. McBride† and R. S. Wolfe‡

ABSTRACT: Cell extracts and whole cells of *Methanobacterium* strain M.o.H. reduce and methylate arsenate to dimethylarsine under anaerobic conditions. Methylcobalamin is the methyl donor of choice. Adenosine triphosphate and hydrogen are essential for the formation of dimethylarsine by cell extracts. In the pathway arsenate is reduced to arsenite which is methylated to form methylarsonic acid. Dimethylarsinic acid, which is formed by the reductive methylation of methylarsonic acid, is reduced to dimethylarsine. Selenate and tellurate also inhibit methane formation by serving as methyl traps.

The first systematic study of the biological formation of arsines was provided by Gosio (1897), who reported that a number of fungi gave off a strong garlic-like odor when they were grown in the presence of sodium arsenite. Bignelli (1900) erroneously concluded that the gas was diethylarsine. Challenger *et al.* (1933) proved that the gas was trimethylarsine, and investigated the ability of a number of arsenic derivatives to serve as prescursors in arsine synthesis (Challenger, 1945). Arsenic, selenium, and tellurium are metabolized by a number of fungi in a similar manner. Challenger and North (1934) showed that *Scopulariopsis brevicaulis* formed dimethyl selenide from sodium selenate and dimethyl telluride from sodium tellurate (Bird and Challenger, 1939). Although bacteria are known to reduce selenate to selenide and tellurate to telluride, we are not aware of references to bacterial synthesis of arsine or its alkylated derivatives. Recently Wood *et al.* (1968) described the biosynthesis of dimethylmercury by extracts of *Methanobacterium* strain M.o.H. We present evidence now that this organism can synthesize dimethylarsine from a variety of arsenic derivatives; the identifica-

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tion of dimethylarsine and some of the physiological parameters governing its biosynthesis are discussed. A preliminary report of this work has been presented (McBride and Wolfe, 1969).

Experimental Section

Materials. [^{14}C]CH_3-B_{12}[1] and CH_3-B_{12} were prepared as described previously (Wood *et al.*, 1965). Dowex 50W-X4 (200–400 mesh, H^+) was obtained from J. T. Baker Co. B_{12r} was made by anaerobic photolysis of methylcobalamin (Yamada *et al.*, 1966). Methylarsonic acid ($CH_3AsO(OH)_2$) was prepared by heating sodium arsenite and methyl iodide in dilute alcoholic solution for 6 hr at 75°, using the procedure outlined by Quick and Adams (1922). The product was shown to be free of arsenite by thin-layer electrophoresis in 4% formic acid followed by development with silver nitrate (Raiziss and Gavron, 1923). N^5-Methyltetrahydrofolate was prepared according to the procedure described by Wood *et al.* (1965). Thin-layer electrophoresis was performed on a Desaga Brinkman electrophorator. Prepoured cellulose thin-layer plates (Eastman Chromagram) were purchased from Distillation Products. All experiments with CH_3B_{12} were carried out in a semidark room.

Alkylarsine Assays. The reaction vessel and conditions used for the biosynthesis of alkylarsines were similar to those described for methane biosynthesis (Wolin *et al.*, 1963). To trap alkylarsines the reaction vessel was connected by polyethylene tubing to a glass tube which contained 2 ml of 2 M HNO_3. The trapping tube was placed in an ethanol–ice bath. A slow stream of H_2 was passed into the reaction flask and the volatile alkylarsines were swept into the nitric acid, where they were condensed and trapped by oxidation to nonvolatile acids (Raiziss and Gavron, 1923). The contents of the trap were assayed for arsenic by atomic absorption spectrometry (Perkin-Elmer Model 303) and for ^{14}C by counting in Bray's scintillation fluid.

The isotope, ^{74}As, also was used to follow the formation of volatile alkylarsine. Extracts were incubated with [^{74}As]Na_2HAsO_4 (Amersham-Searle) and the volatile methylated ^{74}As was measured. Each sample was removed with syringe and was injected directly into a scintillation vial. A second method took advantage of the property of alkylarsines to react with the red-rubber serum stoppers which were used to seal the reaction flasks. After alkylarsine biosynthesis had ceased the reaction mixture was inactivated by heating on a steam cone. The flasks were then incubated for an additional 20 min to ensure that all the volatile dimethylarsine had adsorbed onto the rubber stopper. The serum stopper was removed from the flask, rinsed in water, cut in half, and placed in a scintillation vial together with 15 ml of Bray's scintillation fluid. This mixture was either counted directly, as the rubber stopper did not quench the high-energy β particles emitted by ^{74}As, or the stopper was removed after the ^{74}As had been leached from the rubber (1 hr). The rubber stopper technique was found to be an efficient and specific means of separating [^{14}C]dimethylarsine from [^{14}C]CH_4.

The intense garlic odor was used cautiously as a rapid qualitative assay for the formation of alkylarsines.

[1] Abbreviations used in this work that are not listed in *Biochemistry 5*, 1445 (1966): CH_3-B_{12}, methylcob(III)alamin; B_{12r}, cob(II)alamin; aquo-B_{12}, cob(III) alamin; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

TABLE I: Requirements for Dimethylarsine Synthesis by Cell-Free Extracts of *Methanobacterium* Strain M.o.H.[a]

Flask	Omissions	Volatile ^{74}As (cpm)
1	None	13,400
2	$-CH_3$-B_{12}	100
3	$-H_2$	0
4	−ATP	3,600
5	−Cell extract	0
6	None (reaction mixture boiled)	0

[a] Complete reaction mixture contained: TES buffer, pH 7.0, 200 μmoles; adenosine triphosphate, 10 μmoles; CH_3-B_{12}, 10 μmoles; crude cell extract, 32 mg of protein; [^{74}As]Na_2HAsO_4, 1 μmole (7.0 μCi/μmole); gas atmosphere, H_2; air replaced H_2 in flask 3. Flask 6 was heated for 5 min in a boiling-water bath prior to incubation; total liquid volume, 1.2 ml; temperature, 40°; reaction time, 10 min.

Culture Methods and Preparation of Cell Extracts. Cells were grown as described by Bryant *et al.* (1968). Rumen fluid, volatile fatty acids, and resazurin were omitted from the growth medium, but vitamin solution (Wolin *et al.*, 1963) was added. Cell extracts were prepared as described by Roberton and Wolfe (1969); [^{14}C]CH_4 was assayed in a scintillation counter after syringe injection of a gas sample into a vial which contained Bray's scintillation fluid, and CH_4 was assayed by gas chromatography as described previously (Wolin *et al.*, 1963).

Results

Requirements for Biosynthesis. Conditions for the biosynthesis of alkylarsine derivatives are described in Table I. In these experiments cell extracts were incubated under anaerobic conditions with [^{74}As]Na_2HAsO_4, and the formation of volatile ^{74}As compounds was measured. A methyl donor, H_2, ATP, and arsenate were required for the reaction; with the exception of arsenate these same components are required in the CH_4-synthesizing system. Results were negative if nitrogen or air replaced hydrogen. The detection of radioactive arsenic in the atmosphere of the reaction flasks was strong evidence supporting the contention that the garlic odor was attributable to alkylarsine. A total of 34 μg of arsenic was found in an alkylarsine trap linked to a reaction flask which contained arsenate and cell extract, providing additional evidence that alkylarsine was being synthesized. Volatile alkylarsenic compounds were not detected in a similar control reaction mixture which contained arsenate and boiled extract.

A typical alkylarsine-producing reaction is shown in Figure 1. Evolution of the alkylarsine began after a short lag period and ended when the methyl-donor supply was exhausted.

Substrates. CH_3-B_{12}, 5-CH_3-tetrahydrofolate, serine, and CO_2 were examined for their ability to function as precursors of alkylarsines in cell-free extracts. When alkylarsine synthesis was assayed qualitatively by odor, only methylcobalamin appeared to donate methyl groups for alkylarsine synthesis. By use of more sensitive isotopic techniques in

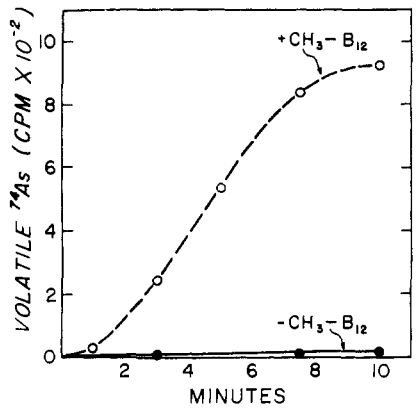


FIGURE 1: Dimethylarsine synthesis in cell extracts of *Methanobacterium* strain M.o.H. Each reaction flask contained: TES buffer, pH 7.0, 200 μmoles; adenosine triphosphate, 10 μmoles; [^{74}As]-Na_2HAsO_4, 1 μmole (7.0 μCi/μmole); where indicated CH_3-B_{12}, 10 μmoles; crude cell extract, 32 mg of protein; H_2 atmosphere; temperature, 40°; total liquid volume, 1.1 ml. The counts per minute detected ranged from 200 to 900.

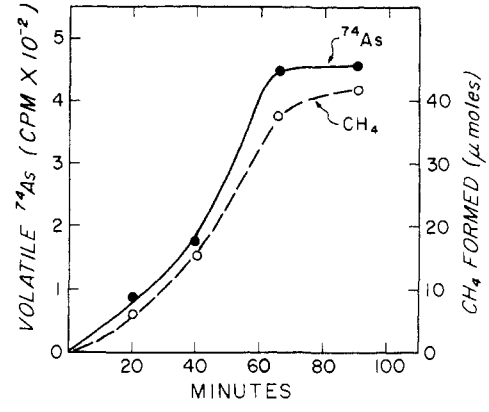


FIGURE 3: Synthesis of alkylarsine by whole cells of *Methanobacterium* strain M.o.H. Each reaction mixture contained: whole cells in their growth liquor, optical density in 18 mm tube, 0.62 at 640 nm; [^{74}As]Na_2HAsO_4, 0.005 μmole (6.2×10^6 cpm); gas atmosphere, H_2–CO_2 (80:20). Total liquid volume 1.0 ml. Temperature 40°. Volatile counts between 50 and 500 cpm were detected.

subsequent studies, it was found that CO_2 also (but not 5-CH_3-tetrahydrofolate or serine) served as a precursor for alkylarsine synthesis.

When B_{12r}, the compound found in extracts after demethylation of methylcobalamin, was added to reaction mixtures in place of methylcobalamin, arsine synthesis did not occur, proving that cobalamin derivatives were not functioning solely as reducing agents. Ethylcobalamin did not donate its alkyl group, indicating that the alkylation of arsenic is enzymic and not simply a chemical reaction.

As shown by Wolfe *et al.* (1966) arsenite inhibited CH_4 formation. However, when this occurred, B_{12r} (the normal cobamide product formed by demethylation of methylcobalamin, Wolin *et al.*, 1964) was formed, indicating that the methyl group was transferred to a nonvolatile component of the reaction mixture. To locate the methyl moiety [^{14}C]methylcobalamin was incubated with cell extract and an excess of arsenite. When the reaction was complete, as evidenced by the stoichiometric appearance of B_{12r}, the solution was extracted with hot 80% ethanol. After removal of the precipitate, the supernatant was subjected to thin-layer electrophoresis and chromatography. Radioautography was performed on the thin-layer plates. As is shown in Figure 2, the supernatant contained a radioactive compound with the electrophoretic properties of methlyarsonic acid. The radioactive compound cochromatographed with methylarsonic acid in isobutyric acid–concentrated ammonium hydroxide–water (66:1:33, v/v). These results indicate that arsenite was methylated to form methylarsonic acid, but that further reduction of the compound was blocked by an excess of arsenite. The reaction is a simple alkylation, there being no reduction as arsenic maintains a valency of +3 in both compounds. Methylarsonic acid was not reduced unless CH_3-B_{12} was present, suggesting that a second methylation must occur before the compound can be reduced to an arsine. Cacodylic acid (dimethylarsinic acid) was reduced rapidly to an alkylarsine even in the absence of a methyl donor.

There was a linear relationship between the amount of methylcobalamin added and the amount of alkylarsine formed. When the concentration of methylcobalamin in the reaction mixture was increased from 1 to 10 μmoles in the presence of [^{74}As]Na_2HAsO_4, the volatile counts of ^{74}As increased from 2×10^3 to 18×10^3 cpm in a reaction time of 20 min.

Inhibitors. Two inhibitors of methane formation were tested for their effect on alkylarsine synthesis. The experiment was performed as described in Table I. Sodium arsenate and methylcobalamin were the only substrates tested. Methylviologen (1×10^{-3} M) stopped alkylarsine synthesis. Homocysteine, a methyl trap, completely stopped alkylarsine synthesis when added in amounts equal to methylcobalamin.

Whole Cells. The ability of whole cells to synthesize alkylarsine is shown in Figure 3. In this experiment a sample of an actively growing culture was removed anaerobically from a 12-l. fermentor and was incubated anaerobically under a

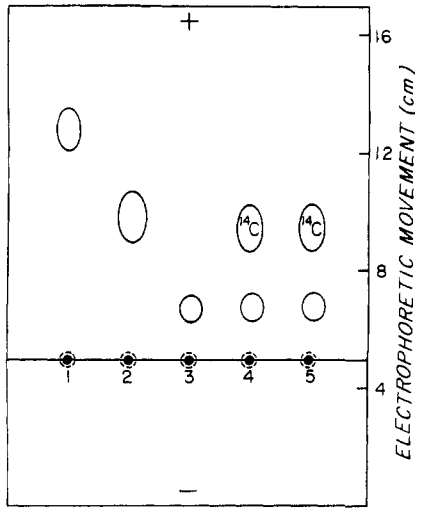


FIGURE 2: Electrophoretic identification of methylarsonic acid. Samples were spotted on Chromagram cellulose plates. Each sample of 20 μl contained (positions 1–5): (1) sodium arsenate, (2) sodium methyl arsonate, (3) sodium arsenite, (4) unknown ^{14}C compound extracted from a reaction mixture which contained cell extract, 32 mg of protein; ATP, 10 μmoles; [^{14}C]CH_3-B_{12} (0.5 μCi/μmole), 0.2 μmole; arsenite, 1 μmole; H_2 atmosphere; TES buffer at pH 7.0, 200 μmoles; (5) sodium methyl arsonate plus unknown ^{14}C compound. The compounds were electrophoresed in ammonium acetate buffer (pH 4.5) for 45 min and developed with silver nitrate. Radioactive compounds (^{14}C) were detected by radioautography.

TABLE II: Effect of Arsenate on the Products of Transmethylation.[a]

Fraction Assayed	Flask 1 +AsO_4 (cpm)	Flask 2 Control (cpm)
CH_4	34.2×10^5	38.0×10^5
Arsine trap	0.4×10^5	0.0
Extract	3.8×10^5	1.2×10^5
Extract after boiling	3.4×10^5	0.5×10^5
Total counts recovered	38.4×10^5	39.2×10^5
Total counts added as [^{14}C]CH_3-B_{12}	41.2×10^5	41.2×10^5
% cpm recovered	93	95

[a] Each reaction flask contained: TES buffer, pH 7.0, 200 μmoles; adenosine triphosphate, 10 μmoles; crude cell extract, 35 mg of protein; [^{14}C]CH_3-B_{12}, 10 μmoles (0.19 μCi/μmole); Na_2HAsO_4, where indicated, 10 μmoles; H_2 atmosphere; total liquid volume, 1.1 ml; temperature, 40°. After 20 min each reaction flask was opened and its contents transferred to a 13 × 100 mm test tube. Each tube was placed in boiling water for 5 min. Arsine was trapped in 2 N HNO_3. [^{14}C]CH_4 determinations were made in flasks not attached to an arsine trap.

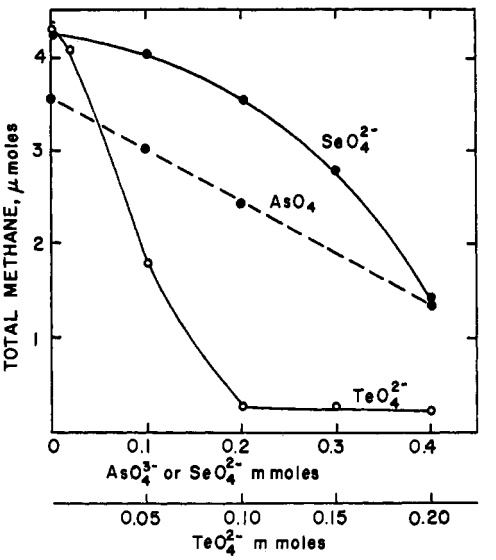


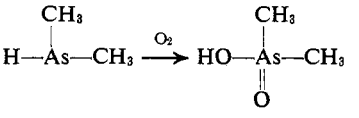
FIGURE 4: Effect of arsenate, selenate, and tellurate on CH_4 production in cell extracts of *Methanobacterium* strain M.o.H. Each reaction flask contained: TES buffer at pH 7.0, 200 μmoles; adenosine triphosphate, 10 μmoles; crude extract, 35 mg of protein; CH_3-B_{12}, 10 μmoles; sodium tellurate or sodium selenate as indicated; H_2 atmosphere; total liquid volume, 1.2 ml; temperature, 40°; reaction time, 100 min. Data for arsenate inhibition were obtained in a separate experiment. Conditions were the same except that a different crude extract 37 mg of protein and a reaction time of 60 min were used.

H_2–CO_2 (80:20) atmosphere with [^{74}As]Na_2HAsO_4. Arsine synthesis continued only as long as CH_4 was formed; there was no arsine synthesis in the absence of CO_2. Competition of arsenate with phosphate in the growth medium may account for a slower rate of alkylarsine synthesis by whole cells.

Effect of Arsenate on Transmethylation. Addition of arsenate to cell extracts reduced the amount of CH_4 formed; the inhibition increased with increasing arsenate concentration as shown in Figure 4. For unknown reasons the inhibition by a given concentration of arsenate varied from one extract preparation to another. Not enough alkylarsine was synthesized to account for the difference between the amount of CH_4 in the control and that in the arsenate-containing flasks. After CH_4 production had stopped cobalamin compounds were isolated from the reaction mixture and analyzed for unreacted methylcobalamin by spectrophotometry and photolysis. CH_3-B_{12} could not be detected, suggesting that methyl groups had been transferred to nonvolatile derivatives. To extend these observations the effect of arsenate on the disposition of methyl groups originating from [^{14}C]CH_3-B_{12} was followed (Table II). Identical reaction mixtures were prepared; one flask was connected to the arsine trap to measure incorporation of ^{14}C into alkylarsine, the other flask was kept sealed to measure [^{14}C]CH_4. The majority of the ^{14}C was found in [^{14}C]CH_4. In the presence of arsenate counts were found in the arsine trap. To ensure that the radioactive compound was not dissolved [^{14}C]CH_4, the flask contents were boiled; a significant amount of ^{14}C remained in the arsenate-treated reaction mixture. Results of chromatographic and electrophoretic analysis of the boiled reaction mixture indicated that the majority of radioactivity was present in a compound having the properties of methylarsonic acid. Enough [^{14}C]-methylarsonic acid was found to account for the missing methyl groups.

Analysis of the Alkylated Arsine. The structure of the alkylarsine formed in cell extracts was indicated by two experimental procedures. In one procedure Na_2HAsO_4 was incubated with [^{14}C]methylcobalamin, and the trapped alkylarsine was analyzed for arsenic and ^{14}C. The results of two such experiments are presented in Table III. Dissolved [^{14}C]CH_4 was removed by bubbling CH_4 through the trapping solution after it had been disconnected from the reaction flask. The number of methyl groups in the trap was calculated from the specific activity of [^{14}C]CH_3-B_{12}. This value was divided

TABLE III: Identification of Dimethylarsine.[a]



Expt	CH_3 Groups (μmole)[b]	As (μmole)[c]	Ratio CH_3:As
1	0.058	0.033	1.90
2	0.096	0.053	1.81
Control	0	0	

[a] Each reaction flask contained: potassium phosphate buffer, pH 7.0, 200 μmoles, adenosine triphosphate, 10 μmoles; [^{14}C]CH_3-B_{12}, 10 μmoles (0.9 μCi/μmole); Na_2HAsO_4, 1 μmole; crude cell extract, 36.0 mg of protein; the arsines were trapped in 2 N HNO_3 and assayed for arsenic by atomic absorption spectrometry and for radioactivity by scintillation counting in Bray's solution. [b] Calculated from specific activity of [^{14}C]CH_3-B_{12} added. [c] Determined by atomic absorption spectrometry.

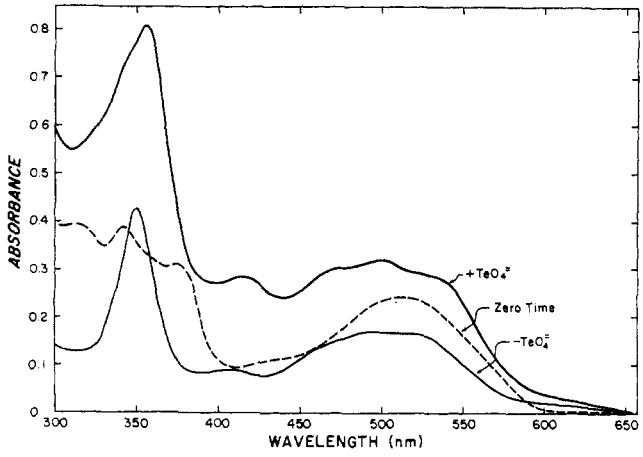


FIGURE 5: Absorption spectra of B_{12} compounds incubated with tellurate. Two reaction flasks prepared according to the protocol outlined in Figure 4 were incubated with 50 μmoles of sodium tellurate. A third reaction flask was prepared according to a similar protocol, but without sodium tellurate. The reaction was started by tipping in CH_3-B_{12}. One of the reactions, containing sodium tellurate, was stopped at zero time, the other two reactions after 30 min. All B_{12} compounds were extracted into 80% EtOH. The ethanol was removed and the B_{12} compounds were chromatographed on CM-cellulose. Compounds were eluted with water followed by 0.2 M NaCl. Each B_{12} compound eluted as a single peak and was assayed spectrophotometrically. The sample from the control reaction not containing sodium tellurate was diluted with an equal volume of water prior to determining the spectrum.

by the amount of arsenic to obtain a methyl group to arsenic ratio. The ratios obtained (1.8:1 and 1.9:1) indicate that the compound is dimethylarsine.

These results were substantiated by a double-labeling experiment in which [^{74}As]Na_2HAsO_4 and [^{14}C]methylcobalamin were substrates. The results of two such experiments are shown in Table IV. Alkylarsine was separated from contaminating [^{14}C]CH_4 by trapping in a rubber serum stopper

TABLE IV: Identification of Dimethylarsine.[a]

Expt	Compound	Volatile cpm	μmole of CH_3 Groups or As	Ratio ^{14}C:^{74}As
1	^{74}As[b]	28.0×10^3	0.0036	1.9
	^{14}C[c]	6.7×10^3	0.0071	
2	^{74}As[b]	16.7×10^3	0.0020	2.1
	^{14}C[c]	4.26×10^3	0.0044	

[a] Each reaction flask contained: potassium phosphate buffer, pH 7.0, 200 μmoles; adenosine triphosphate, 10 μmoles; crude cell extract, 35 mg protein; [^{74}]Na_2HAsO_4, 1 μmole (7.87×10^6 cpm/μmole) [^{14}C]CH_3-B_{12}, 10 μmoles; (9.48×10^5 cpm/μmole); H_2, atmosphere; total liquid volume, 1.2 ml; reaction time 10 min; temperature, 40°. Arsine was trapped in the serum stopper, serum stoppers were washed, placed in Bray's scintillation fluid for 1 hr, and then removed. The scintillation fluid was counted simultaneously on two channels in a Mark III scintillation counter (Nuclear-Chicago). [b] Specificity activity of [^{74}As]AsO_4 = 7.87×10^6 cpm/μmole. [c] Specific activity of [^{14}C]CH_3-B_{12} = 9.48×10^5 cpm/μmole.

TABLE V: Methyl Trapping and Inhibition of Methane Formation by Selenate and Tellurate.[a]

Expt	Exptl Condn: Flask	Exptl Condn: Omissions	Volatile ^{14}C (cpm)	CH_4 (μmoles)
1	1	None	38.0×10^4	6.5
	2	−ATP	4.6×10^4	0.5
	3	$-H_2$	0.0	0.0
	4	−Enzymes	0.0	0.0
2 (+SeO_4)	5	None	9.5×10^4	0.0
	6	−ATP	2.5×10^4	0.0
	7	$-H_2$	0.0	0.0
	8	−Enzyme	0.0	0.0
3 (+TeO_4)	9	None	11.7×10^4	0.0
	10	−ATP	2.2×10^4	0.0
	11	$-H_2$	0.0	0.0
	12	−Enzyme	0.0	0.0

[a] Each reaction flask contained: TES buffer, pH 7.0, 200 μmoles; ATP, where indicated, 10 μmoles; sodium selenate, where indicated, 200 μmoles; sodium tellurate, where indicated, 100 μmoles; [^{14}C]CH_3-B_{12}, 0.03 μCi/μmole, 6 μmoles; crude cell extract, 32 mg of protein; gas atmosphere, H_2; where indicated, H_2 was replaced with air; total volume, 1.6 ml; temperature, 40°. ^{14}C was measured by injecting a gas sample from each reaction vessel into a scintillation vial; reaction time, 40 min.

as described previously. The specific activity of the substrates was used to calculate the micromoles of arsenic as well as methyl groups. The ratios of methyl groups to arsenic (2.1:1 and 1.9:1) suggest that the garlic-smelling compound is dimethylarsine.

Arsine from Desulfovibrio vulgaris. Cell extracts of *D. vulgaris* strain 8303 which were incubated with isotopic sodium arsenate produced a volatile arsenic derivative. The compound had a strong garlic odor indicative of an arsine, but it was not characterized further.. The reaction occurred in the absence of exogenous methyl donors but was greatly stimulated by the addition of methylcobalamin. Reaction mixures were similar to those used with *Methanobacterium;* both H_2 and lactate served as electron donors.

Methylation of Selenium and Tellurium. Sodium tellurate and sodium selenate inhibited the reductive demethylation of methylcobalamin to CH_4. The effect of increasing concentrations of these compounds on the total amount of CH_4 formed is shown in Figure 4. Both tellurate and selenate were rapidly reduced by *Methanobacterium* in the absence of a methyl donor as evidenced by odor and the characteristic color change of the reduced compounds. The decreased synthesis of CH_4 was not simply an inhibition of the demethylation, for there was no methylcobalamin remaining at the end of reaction (Figure 5). The cobalamin compounds were extracted from the reaction mixtures prior to spectral determinations. At zero time the typical methylcobalamin spectrum was obtained; after a 30-min reaction time during which B_{12r} was formed but no CH_4 was produced, the extracted cobalamin yielded a typical aquo-B_{12} spectrum, indicating that B_{12r} was oxidized to aquo-B_{12} during extraction. The spectrum has similarities to diaquo cobinamide, and it is possible that in the presence of tellurate

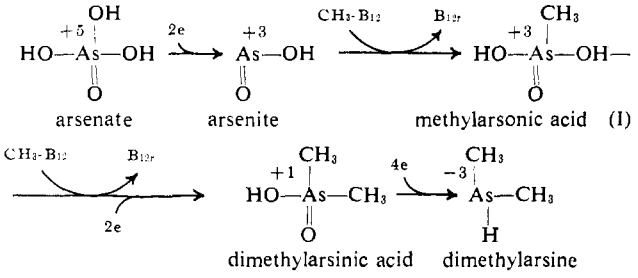
the lower axial ligand is displaced (J. M. Wood, personal communication).

Evidence that the methyl groups were diverted to the synthesis of methylated selenide and telluride is presented in Table V. In the absence of either compound [^{14}C]CH_3-cobalamin was stoichiometrically reduced to [^{14}C]CH_4 and B_{12r} as previously shown (Wolin *et al.*, 1964); in the presence of the compounds [^{14}C]CH_4 was not produced; however, approximately 25% of the ^{14}C was present in the atmosphere of the reaction vessel.

Discussion

The initial chance observation of alkylarsine synthesis was made when a strong, nauseating garlic-like odor was detected in reaction mixtures which contained cell extract, hydrogen, ATP, arsenate, and methylcobalamin. Since both methylcobalamin and arsenate were found to be required for synthesis of the volatile compound, it appeared that this compound was probably a methylated arsine; methylcobalamin proved to be the substrate of choice. Although the reaction is easily followed by odor, use of the isotopes, ^{14}C and ^{74}As has enabled us to work with small reaction mixtures and to elucidate the chemistry of alkylarsine synthesis.

Evidence indicates that arsenate is reductively methylated to dimethylarsine in *Methanobacterium* as shown in reaction I. Arsenate (arsenic valency, +5) is first reduced to arsenite



(arsenic valency, +3). Arsenite is then methylated to form methylarsonic acid. This compound was found in reaction mixtures when extracts were incubated with either arsenate or arsenite and CH_3-B_{12}. Methylarsonic acid is reduced and methylated to form dimethylarsinic acid (arsenic valency, +1). The acid is then reduced to form dimethylarsine (arsenic −3). All intermediates have been shown to be converted to dimethylarsine by cell extracts. This pathway is based on studies in which CH_3-B_{12} was used as a methyl donor; it is postulated that methylation by the product of CO_2 reduction follows the same scheme. However, the methods used to identify the alkylarsine would not distinguish dimethylarsine from equimolar quantities of mono- and trimethylarsine derivatives. Attempts to obtain evidence for mono- or trimethylarsine synthesis gave negative results. Manipulation of the concentration of the methyl donor had no effect on the ratio of methyl groups to arsenic in the arsine product.

Pollution hazards exist when arsenic and its derivatives are introduced into an environment where anaerobic organisms are growing. Arsenic pesticides (sodium methyl arsenate and calcium and lead arsenate) may be applied over large areas and eventually find their way into anaerobic aquatic or terrestrial environments where they can be converted to arsine derivatives. The small amounts of arsenic in high phosphate detergents might even be significant in the anaerobic sewage sludge digester. Biological methylation of metals is not limited to arsenic; selenium and tellurium are readily methylated by *Methanobacterium*. Wood *et al.* (1968) have reported that extracts of *Methanobacterium* strain M.o.H. methylate mercury. It is possible that other metals such as lead may be methylated. The importance of these organisms in converting toxic molecules to more toxic derivatives should not be underestimated.

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