ENVIRONMENTAL BIOCHEMISTRY OF ARSENIC

Prepared Under Contract for the
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The San Joaquin Valley Drainage Program was established in mid-1984 as a cooperative effort of the U.S. Bureau of Reclamation, U.S. Fish and Wildlife Service, U.S. Geological Survey, California Department of Fish and Game, and California Department of Water Resources. The purposes of the Program are to investigate the problems associated with the drainage of irrigated agricultural lands in the San Joaquin Valley and to formulate, evaluate, and recommend alternatives for the immediate and long-term management of those problems. Consistent with these purposes, Program objectives address the following key areas: (1) Public health, (2) surface- and ground-water resources, (3) agricultural productivity, and (4) fish and wildlife resources.

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EXECUTIVE SUMMARY

Arsenic is a naturally occurring element which has many positive agricultural applications in contrast to its biological toxicity and threat to wildlife. Arsenicals are used in agriculture as feed additives, herbicides, insecticides, and cotton defoliants. The arsenic cycle involves both biotic and abiotic reactions. Various plants, invertebrates and microorganisms play a major role in the transformation and movement of arsenicals in soil and water.

Arsenic, a metalloid, is naturally present in soil, water, air and all living matter. Being in Group V A of the periodic classification it forms alloys with various metals and covalently bonds with carbon, hydrogen, oxygen and sulfur. Arsenic can occur in the +5, +3, 0, and -3 states and is subject to 8 electron reductions which is very similar to phosphorus. Because of its similarity to phosphorus and its ability to bond with sulfur, it is highly toxic. Arsenate (AsO_4^{3-}) is taken up via the phosphate transport system and is incorporated into energy transfer phosphorylation reactions. Arsenite (AsO_2^-) inactivates many enzymes by having a high affinity for thiol groups of proteins.

Arsenic, primarily in the inorganic form, is present in the earth's crust at an average of 2-5 mg kg^{-1}. The mean concentration of arsenic in shales is 13 mg kg^{-1}; igneous rocks, 1.8 mg kg^{-1}; and sandstones, 1.0 mg kg^{-1}. Sedimentary rocks range from 0.1 mg kg^{-1} to as high as 2900 mg kg^{-1} arsenic. Sulfidic ores contain arsenic in the form of arsenides of

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nickel, cobalt, copper and iron. The most frequently found ores are: arsenopyrite (FeAsS); enargite (Cu_3AsS_4); orpiment (As_2S_2); and realgar (As_4S_4). Inorganic arsenic compounds such as arsenic trioxide (As_2O_3), arsenite and arsenate are weathered from arsenic-containing rocks. This is considered the major natural source of arsenic estimated to release 45,000 metric tons/year. The arsenic compounds released as a result of weathering may be retained in soils or dissolved in water to be transported and further redistributed. The soluble arsenic forms may be adsorbed to clay particles in both soils and sediments.

Precipitation from the atmosphere and the application of agricultural products are other major sources of arsenic influx to soil. Arsenic deposited from the atmosphere is estimated to be 63,600 metric tons/year including both wet and dry deposition. Influx of arsenic by herbicides to the soil is approximately 4,560 metric tons/year and by dessicants, 12,000 metric tons/year. It is estimated that over 100,000 metric tons of arsenic/year is deposited into landfills as slag, a by-product of smelting operations, but only a small percentage of this total arsenic enters the global cycle.

Worldwide, arsenic in soil ranges from 0.1 to 40 mg kg^{-1} with a median concentration of 6 mg kg^{-1}. Arsenic in seawater averages 1.7 μg L^{-1} with a relatively homogeneous range from 1.5 to 5 μg L^{-1}. In contrast, freshwater from lakes and rivers varies widely in arsenic concentration and is dependent upon the minerals subject to transport. Freshwater arsenic concentrations range from 1 to 10 μg L^{-1} with an average of 1.7 μg L^{-1}. The recommended maximum concentration for arsenic

in irrigation water is 100 µg L^{-1} with the drinking water standard being at 50 µg L^{-1}. Arsenate is more predominant in oxygenated water while arsenite is more common under reduced anaerobic conditions. The total arsenic influx into oceans is estimated at 246,110 metric tons/year. Of this total 62,900 metric tons is dissolved arsenic, 178,900 metric tons is sediment suspended arsenic and 4,310 metric tons is from the atmosphere per year.

Atmospheric arsenic concentrations are considerably higher over land masses in comparison to oceans. The atmosphere over land in the northern hemisphere is approximately 2.8 x 10^{-3} µg arsenic m^{-3}, while over the southern hemisphere, it is estimated at 1 x 10^{-3} µg m^{-3}. Atmospheric concentrations of arsenic over oceans is dependent on the proximity to land being 6 x 10^{-4} µg m^{-3} over the North Atlantic and 1.8 x 10^{-5} µg m^{-3} over oceans in the southern hemisphere. In suburban areas, air samples have shown arsenic concentrations of 1.7 x 10^{-3} µg m^{-3} with 50% being associated with particles greater than 0.3 microns. The alkylarsenic forms comprise an average of 20% of the total atmospheric arsenic.

The total arsenic input into the atmosphere has been estimated to be 73,540 metric tons/year. Forty percent is from natural sources such as volcanism and low temperature volatilization. Sixty percent is from anthropogenic sources such as copper smelting, coal combustion, non-ferrous metal production, agricultural chemicals and agricultural burning.

The two commonly used analytical techniques for total arsenic determination are atomic absorption spectrometry (AAS) and inductively coupled argon plasma emission spectrometry (ICAP) in conjunction with hydride generation. Although these two methods are sensitive, they are not selective in determining different arsenic species. Analyses by AAS or ICAP does not allow direct quantification of arsenate in environmental samples but a method was recently developed for the direct determination of arsenate in aqueous soil extracts by single-column ion chromatography (SCIC) at trace levels.

In terrestrial plants, arsenate is preferentially taken up 3-4 times the rate of arsenite. In the presence of phosphate, arsenate uptake is inhibited while in the presence of arsenate, phosphate uptake is only slightly inhibited. There is a competitive interaction between arsenic and phosphate for the same uptake system in terrestrial plants. The mode of toxicity of arsenate is to partially block protein synthesis and interfere with protein phosphorylation but the presence of phosphate prevents this mode of action. There appears to be a higher affinity for phosphate than arsenic with a discriminate ratio of 4 to 1.

Bacterial resistance to arsenate is related to its chemical simi-larities to phosphate and occurs by two distinct mechanisms. Arsenate and phosphate are transported into and out of bacterial cells by highly specific energy-dependent membrane pumps. One method of arsenate resistance in bacteria occurs by activation of a phosphate uptake pump with a higher selectivity for phosphate which confers reduced levels of arsenate uptake. The second method of resistance is a result of an

accelerated efflux of arsenate, arsenite, and antimonate but not phosphate from the cell by a highly specific membrane-associated pump. This method of resistance for arsenate/arsenite is highly specific in preventing the export of phosphate from the cell, and is widespread among different bacterial species. Bacterial arsenic resistance is often associated with other types of heavy metal and antibiotic resistances. There is a 10-fold increase in resistance to arsenate compared to arsenite in bacteria.

Bacterial oxidation of arsenic from arsenite to arsenate is proposed to be a detoxification mechanism. This transformation is induced by arsenite, consumes oxygen and is not an energy-yielding reaction. Heterotrophic bacteria play an important role in oxidation of arsenite to arsenate.

Methylation of arsenic involves the conversion of inorganic and organic arsenic to volatile organic methylated forms such as dimethyl-arsine and trimethylarsine. Inorganic arsenic methylation is coupled to the methane biosynthetic pathway in methanogenic bacteria and may be a mechanism for arsenic detoxification. The pathway proceeds by reduction of arsenate to arsenite followed by methylation in the presence of co-enzyme M (CoM), a low molecular weight cofactor found in all methanogenic bacteria. Anaerobic biomethylation of arsenic by bacteria proceeds only to dimethylarsine, which is stable in the absence of oxygen. In anaerobic environments, dimethylarsine can react with disulfide bonds on particu-lates in soil thus reducing the concentration of soluble arsenic. In general, bacteria are more resistant to methylated arsenic compounds than inorganic arsenic species.

It has been well known as far back as the 1800s that fungi are able to transform inorganic and organic arsenic compounds into volatile methylarsines. In Germany and England there were incidences of arsenic poisoning caused by molds growing on wallpaper laced with arsenic containing pigments producing trimethylarsine, a toxic, garlic smelling gas. Since that time, several fungi have been identified as arsenic methylators. In the presence of phosphate, biomethylation of inorganic arsenic and methanearsonic acid (MAA) is inhibited.

Arsenate is the primary arsenic species in seawater at 1-2 µg L^{-1}. Marine algae partially detoxify arsenate by producing large quantities of stable non-volatile methylated arsenic compounds. This is considered to be a beneficial step not only to the primary producers, but also to the higher trophic levels, since methylated arsenic is much less toxic to marine invertebrates.

Four classes of marine phytoplankton have the ability to absorb arsenate and convert it into a reduced and methylated species. These are diatoms, coccolithophorides, dinoflagellates and green algae. The organoarsenic products most commonly excreted from algae are MAA and dimethylarsinic acid (DMA). These products are not very stable in natural waters since their production and release is balanced by bacterial removal by demethylation and oxidation of arsenite to arsenate.

Marine invertebrates and fish are part of the higher trophic levels in the food chain of aquatic environments and often retain 99% of the arsenic in the organic form upon consumption. Crustacean and mollusk tissues are generally higher in arsenic concentrations than are fish.

Arsenosugar and arsenolipids are transformed into arsenobetaine which is generally the end product that accumulates in the higher trophic levels. Degradation of arsenobetaine is required to complete the biological cycling of arsenic. Microorganisms in sediment samples degrade arsenobetaine into trimethylarsine oxide, then into DMA and finally to MAA and inorganic arsenic. Apparently these derivatives may be subject to volatilization since the arsenic concentration in these sediments often decreases.

Variability of arsenic in freshwater, ranging from 1 to 10 µg L^{-1} with some estimations as high as 64 µg L^{-1}, depends upon evaporation and condensation rates, direct contamination by herbicides or indirectly as runoff, industrial pollution and natural contamination. Arsenic concentrations as high as 70 µg L^{-1} have been reported in lakes of New Zealand as a result of hot springs rich in arsenic arising from geothermal activity. Hot springs in Yellowstone are as high as 3500 µg of arsenic L^{-1}. In the Central Valley of California, extensive irrigation has been a major factor in mobilizing and redistributing arsenic. Concentrations as high as 2400 µg L^{-1} have been reported in evaporation ponds in the Tulare Lake Basin of central California.

At least four species of freshwater green algae including Ankistrodesmus sp., Chlorella sp., Selenastrum sp., and Scenedesmus sp., methylate arsenite to MAA and DMA and all, except Scenedesmus, produce trimethylarsine oxide. Freshwater algae like marine algae synthesize lipid-soluble arsenic compounds and do not produce volatile methylarsines. Aquatic plants also synthesize similar lipid-soluble arsenic compounds.

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In humans and animals, arsenic enters the body by ingestion and inhalation and is removed by first being rapidly absorbed, then assimilated into the blood followed by removal in the kidneys and excreted in the urine. Another possible route is where inorganic arsenic is converted to methylated forms, which are less toxic and are rapidly excreted from the body.

Arsenic toxicity, characterized by decreased motor skills, nervous disorders, respiratory distress and damage to the kidneys, depends on its oxidation state. Arsenate breaks down energy metabolism by inhibiting ATP synthesis by uncoupling oxidative phosphorylation. Arsenite inactivates enzymes by linking with sulfur and reacting with thiol groups on the active site of many enzymes and proteins. For this reason arsenite is not excreted through urine as easily as arsenate making arsenite more toxic. Methylation of inorganic arsenic in animals and humans produces much less toxic organoarsenic compounds. The LD_{50} for DMA in rats ranges from 700 to 2600 mg kg^{-1} and for MAA 700 to 1800 mg kg^{-1} compared to inorganic arsenic forms such as potassium arsenite at 14 mg kg^{-1} and calcium arsenate at 20 mg kg^{-1}. The arsenic analogues of choline and betaine do not bind to thiol groups and are therefore considered nontoxic.

The volatile arsine gases are very toxic to mammals because they destroy red blood cells (LD_{50} in rats; 3 mg kg^{-1}). Further studies on dimethylarsine and trimethylarsine toxicity by inhalation to test animals are still needed.

Many organisms including microorganisms, plants and invertebrates are involved in the distribution and cycling of arsenic. Arsenic can accumulate and be subject to various transformations including reduction, oxidation and methylation. The reduced form (arsenite) is considered more toxic than the oxidized species (arsenate) because it reacts with sulfhydryl groups of cysteine in proteins inactivating many enzymes.

In aquatic systems, arsenic tends to accumulate as complex organo-arsenic compounds with only a few being identified (e.g., arsenobetaine, arensocholine and dimethylarsenosoribosides). MAA and DMA are present in seawater and freshwater but appear to be degradation products of these complex organoarsenic compounds.

Arsenic is emitted into the atmosphere by high temperature processes such as coal-fired power generation plants, burning vegetation and volcanism. Inputs into the atmosphere include industrial and fossil fuel emission (780×10^8 g As yr^{-1}), mining (28×10^8 g As yr^{-1}) and continental and volcanic dust fluxes (28×10^8 g As yr^{-1}).

Natural low temperature biomethylation also releases arsenic into the atmosphere. Microorganisms including bacteria, fungi and yeast form volatile methylated derivatives of arsenic under both aerobic and anaerobic conditions. Bacteria only produce dimethylarsine while fungi synthesize trimethylarsine. Dimethylarsine is an oxidation product of trimethylarsine and both compounds are subject to demethylation by soil bacteria. It is estimated that as much as 210×10^8 g of arsenic is lost to the atmosphere in the vapor state annually from the land surface. The continental vapor flux is about eight times that of the continental dust

flux indicating that the biogenic contribution may play a significant role in cylcling of arsenic. It has not been established whether volatile arsenic can be released by plants. Further studies are needed to determine mass balances in the rate of transfer (fluxes) of arsenic in the environment.

ENVIRONMENTAL BIOCHEMISTRY OF ARSENIC

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INTRODUCTION

The San Joaquin Valley, California is one of the most productive agricultural regions in the world because of its climatic conditions. However, soils on the west side of the valley contain high amounts of soluble salts and trace elements being derived from marine sedimentary parent material of the Coastal Range. These fine textured soils contain clay lenses which impede water flow and cause shallow water tables. In areas of poor drainage, subsurface collectors remove shallow saline drainage water to evaporation ponds. Adverse effects have been reported on fish and wildlife as a result of selenium contamination at a regional evaporation pond facility, Kesterson Reservoir (Merced County), Calif.

The Coastal Range is the primary source of many potentially toxic natural trace elements in the valley drainage water. The elements of concern include selenium, arsenic, boron, chromium, mercury, and molybdenum. Recently there has been some concern with arsenic occurring in the Tulare Lake Basin constituting the southern half of the San Joaquin Valley. The Tulare Basin occupies approximately one-third of the Central Valley. Elevated concentrations of arsenic in sediments and water in the Tulare Lake Drainage District's South Basin evaporation ponds may pose a threat to the wildlife. Levels of arsenic in these

drainage evaporation ponds have been reported as high as 770, 830 and 2,400 µg L^{-1} at Pyrse, Lost Hills and the Carmel Ranch, respectively. The ubiquity of arsenic in the environment, its biological toxicity and its redistribution are factors invoking public concern. This report will review the chemistry of arsenic and methods currently employed to speciate the prevalent chemical forms in various environments. The major focus of this paper is on biological transformations of arsenic in both terrestrial and aquatic environments. Emphasis will be placed on the link of these biotic transformations to the global cycle of arsenic.

CHEMISTRY

The arsenic cycle involves both biotic and abiotic reactions. Arsenic is a naturally occurring element, present in soil, water, air and all living matter. It is a metalloid of Group V A of the periodic classification with properties which allow it to form alloys with various metals and covalent bonds with carbon, hydrogen, oxygen and sulfur (Ferguson and Gavis, 1972). The oxidation states and electron orbitals are similar between arsenic and phosphorus. Arsenic is subject to eight electron reductions and can occur in +5, +3, 0 and -3 states. The metal, As, is very rare and is only found under extreme redox potentials. Natural sediments and soil produce both nonvolatile and volatile methylated arsenic compounds. Its similarity to phosphorus and its ability to form covalent bonds with sulfur are two reasons for arsenic toxicity. Arsenate (H_3AsO_4) is an analogue of the essential mineral phosphate and is taken up via the phosphate transport system by

most organisms. Arsenate has been postulated to replace phosphate in energy transfer phosphorylation reactions. Arsenite (AsO_2^-) has a high affinity for thiol groups of proteins inactivating many enzymes.

DISTRIBUTION OF ARSENIC

Arsenic is present in the earth's crust at an average of 2-5 mg kg^{-1} and is primarily associated with igneous and sedimentary rocks in the form of inorganic arsenic compounds. The mean concentration of arsenic in shales, igneous rocks and sandstones is 13, 1.8 and 1.0 mg kg^{-1}, respectively (Onishi and Sandell, 1955; Lemmo et al., 1983). Sedimentary rocks including coal have been found to contain 0.1 to 2900 mg kg^{-1} of arsenic (Irgolic et al., 1983). It is frequently a component of sulfidic ores in the form of arsenides of nickel, cobalt, copper and iron (Irgolic et al., 1983). The most commonly found ores are: arsenopyrite (FeAsS) (most common and widespread); enargite (Cu_3AsS_4); orpiment (As_2S_3); and realgar (As_4S_4). Weathering of arsenic-containing rocks liberates arsenic in the form of inorganic compounds including arsenic trioxide, arsenite and arsenate. Microorganisms have been shown to increase the rate of arsenic release from sulfidic ores by catalyzing the oxidation of sulfide to sulfate and ferrous to ferric iron. Weathering of rock is considered the major natural source of arsenic, estimated to release 45,000 metric ton of arsenic/year (Ferguson and Gavis, 1972). Two additional major sources of arsenic influx to soil are precipitation from the atmosphere and the application of agricultural products. Wet and dry deposition of arsenic from the atmosphere is estimated to be 63,600 metric tons/year.

Table 1 shows the uses of arsenicals in agriculture including feed additives and pesticides. Many of these arsenicals do not persist in soils and are thought to be rapidly removed by volatilization and/or perhaps leaching (Morrison, 1969; Woolson, 1974). The application of herbicides and desiccants to soils is estimated to be 4,560 and 12,000 metric tons/year, respectively. Deposit of arsenic into landfills as slag resulting from smelting operations has been estimated to be over 100,000 metric tons/year but only a small percentage of the total arsenic enters the global cycle.

Worldwide, the median soil concentration is 6 mg kg^{-1} with a typical range of 0.1 to 40 mg kg^{-1} (Bowen, 1979). The weathered arsenic compounds may be retained in soils or dissolved in water to be transported and further redistributed (Wakao et al., 1988). The soluble forms may be adsorbed to clay particles in both soils and sediments. Primary components in soil which adsorb arsenic include porous sesquioxides and silico-sesquioxidic complexes. Arsenate sorption increases with increasing pH exhibiting a maximum at pH 10.5 (Goldberg and Glaubig, 1988). Sorption of arsenic to metals may be a more important mechanism in immobilization than organic matter (Huang and Liew, 1979). Desorption of arsenite is highly dependent on reduction of Fe^{3+} to Fe^{2+}.

Arsenic concentrations in seawater are relatively homogeneous ranging between 1.5 to 5 μg L^{-1} (Sanders, 1980) with an average of 1.7 μg L^{-1} (Chilvers and Peterson, 1987). In contrast, concentrations in freshwater (rivers and lakes) vary widely and are dependent on the available minerals subject to co-transport. The average arsenic concentration in freshwater is approximately 1.7 μg L^{-1} (Chilvers

Table 1. Agricultural uses of arsenic.

Arsenicals	Use
Feed Additives	
Arsanilic acid and Roxarsone (3-nitro-4-hydroxyphenyl arsonic acid)	increase rate of gain, and improve feed efficiency in chickens and swine, control swine dysentery
Carbarsone and Nitarsone (4-nitrophenyl arsonic acid)	antihistomonads in turkeys
Pesticides	
Herbicides (mono-disodium salts of methanearsonic acid)	post emergence grass herbicides
Insecticides	
Calcium arsenate	control boll weevil in cotton fields
Lead arsenate	control codling moth, plum curculio, cabbage worm, potato bug, tobacco hornworm
Cotton dessicants	
Dimethylarsinic (cacodylic) acid	cotton defoliants
Others	
Sodium arsenite	Used to preserve railroad and telephone posts, fungicide and wood preservation. Control measles and dead arm of table grapes
Lead and calcium arsenate	control acidity in grapefruit
10,10-oxybisphenoxarsine	fungus control in cotton sailcloth and vinyl films
Calcium arsenate	component in snail baits; used in fly control in poultry houses, herbicide for grass Poa annua

and Peterson) with most waters ranging from 1 to 10 µg L^{-1} (Sanders, 1980). The drinking water standard for arsenic is 50 µg L^{-1} and the recommended maximum concentration for irrigation water is 100 µg L^{-1} (Letey et al., 1986). The arsenate species is most often found in oxygenated water, while arsenite predominates under reduced conditions. Particulates of river discharge may contain arsenic concentrations of 3 to 74 mg kg^{-1} dry wt (Crecelius et al., 1975). Influx into oceans consists of 62,900 metric tons of dissolved arsenic, 178,900 metric tons of sediment-suspended arsenic, and 4,310 metric tons from the atmosphere per year. Thus the total arsenic influx into oceans is estimated at 246,110 metric tons/year. The major sources of loss from oceans is by sedimentation and sea-salt spray leaving a net increase of 136,000 metric tons of arsenic/year (Edmonds and Francesconi, 1987). No formation of arsine gas has been reported from marine environments.

Atmospheric concentrations of arsenic are considerably higher over land masses with 2.8×10^{-3} µg m^{-3} reported in the northern hemisphere and 1×10^{-3} µg m^{-3} in the southern hemisphere. The atmospheric concentration of arsenic over oceans is dependent on the proximity to land approaching 6×10^{-4} µg m^{-3} over the North Atlantic and 1.8×10^{-5} µg m^{-3} over oceans in the southern hemisphere (Chilvers and Peterson, 1987). Air samples collected in suburban areas have shown concentrations of 1.7×10^{-3} µg m^{-3} with 50% of the atmospheric arsenic associated with particles greater than 0.3 microns. On an average, 20% of the total arsenic in the atmosphere is in the alkyl-arsenic form (Johnson and Braman, 1975).

The emission of arsenic into the atmosphere is from natural and anthropogenic sources with a ratio of approximately 60 to 40 percent,

respectively. The total arsenic input into the atmosphere has been estimated to be 73,540 metric tons/year. Natural sources include low temperature volatilization and volcanism. Copper smelting and coal combustion account for 60% of the total anthropogenic source with nonferrous metal production, agricultural chemicals and agricultural burning accounting for the remaining inventory (Chilvers and Peterson, 1987).

To enter the biological cycle arsenic must be in a dissolved form. The oxidation and ionization state of arsenic is dependent on the pH and oxidation-reduction potential (pE) of the aqueous solution (Lemmo et al., 1983). Figure 1 shows the thermodynamically stable forms of arsenic in various aqueous conditions. In natural waters, arsenate is the predominant species and is in equilibrium with the reduced form, arsenite. Dissolved inorganic arsenic compounds may be assimilated by organisms and transformed into less toxic methylated derivatives or into volatile arsines. Concentrations of dimethylarsinic acid have been reported from 0.05 to 1 μg L^{-1} in interstitial waters (Crecelius, 1975).

In oxidized waters, the following arsenic species are stable, H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{-2}$ and AsO_4^{-3}. Arsenous acid species which exists in reduced waters include H_3AsO_3, $H_2AsO_3^-$ and $HAsO_3^{-2}$. Arsine gases are only slightly soluble in water and produced mainly in reduced waters (Ferguson and Gavis, 1972). Under most conditions, microbially produced methylated arsines are rapidly oxidized. Only a small percentage of

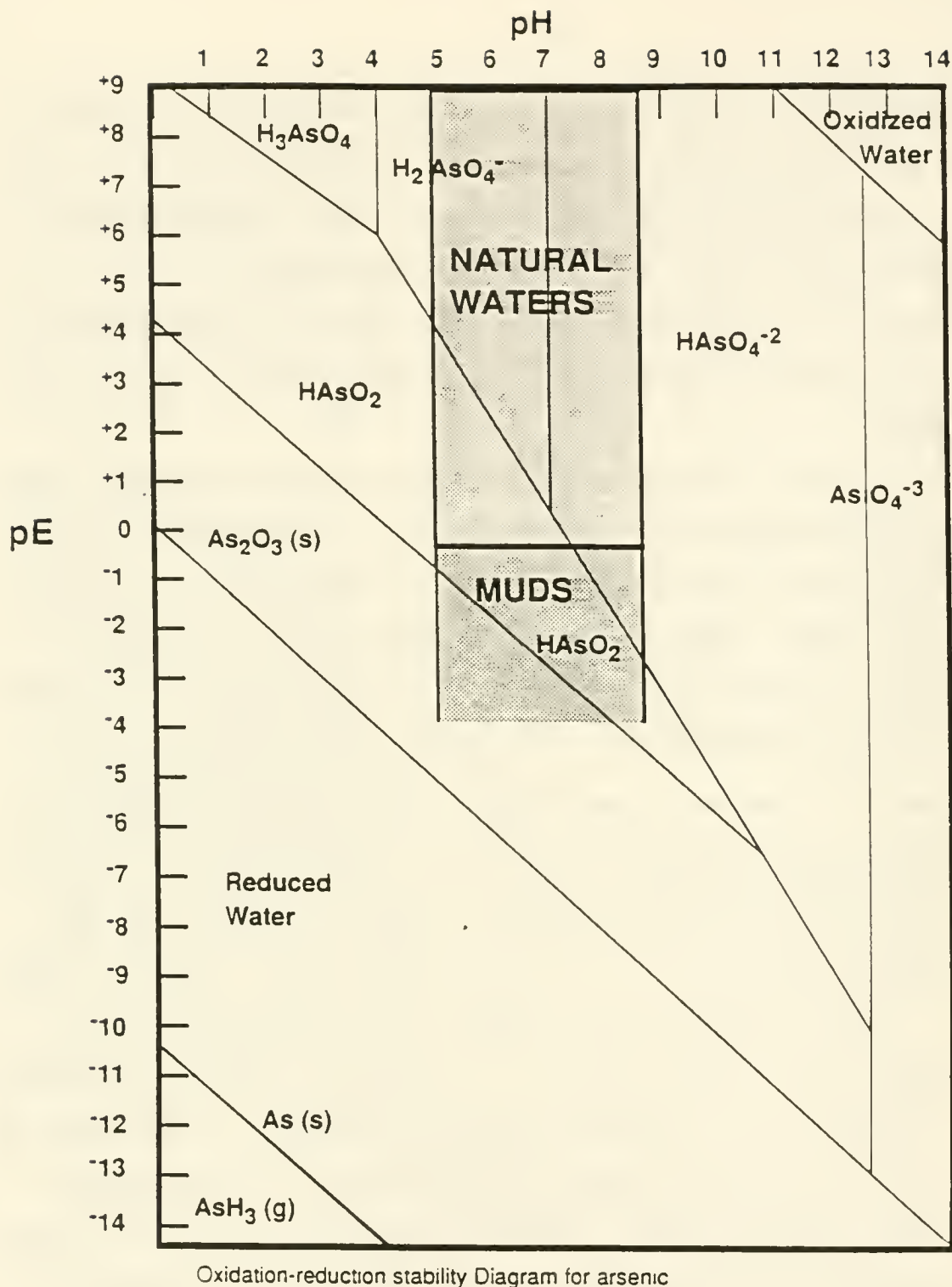Oxidation-reduction stability Diagram for arsenic

Fig. 1. Oxidation-reduction stability of arsenic.

methylarsines migrate out of their point source (Lemmo et al., 1983).
The dissolved forms of arsenic can react to form solids such as As_2O_5
and As_2O_3 (Ferguson and Gavis, 1972). Under conditions where sulfides
are stable, arsenic can interact with sulfur to form As_4S_4 (realgar)
and As_2S_3 (orpiment) which also have low solubilities and form stable
solids at pH values below 5.5 and pE values of 0.0 V.

Adsorption of arsenic onto sediments and coprecipitation with
ions of sulfur, iron and aluminum are the major mechanisms for arsenic
removal from aqueous environments. Both arsenate and arsenite co-
precipitate or adsorb onto hydrous iron oxides. Iron oxides are major
components of clays and thus removal of arsenic from aqueous solutions
is dependent on the clay content of the underlying soil or sediment.
Arsenate competes with phosphate in adsorption to clay surfaces.
Arsenite is depleted from aqueous solutions due to its strong affinity
for sulfur and adsorbs or coprecipitates with metal sulfides. Both iron
and phosphate concentrations present in waters are significant factors
in establishing the level of dissolved arsenic (Lemmo et al., 1983).
Arsenic is released from sediments if ferric iron or sulfide is converted
to ferrous iron or sulfate. However, the final concentration of arsenic
in aqueous solutions cannot be simply explained by its interaction
with iron, sulfur and phosphate but other factors such as adsorption-
desorption equilibria and the total arsenic entrapped in sediments
also plays a major role (Lemmo et al., 1983).

DETECTION OF ARSENIC IN ENVIRONMENTAL SAMPLES

The volatile methylarsines can be detected by gas chromatography (GC) with flame ionization detection. This is particularly important in speciating arsine gases such as methylarsine, dimethylarsine and trimethylarsine. Volatile species can be removed from natural waters and soil by gas stripping being collected in a cold trap and separated by GC. An element-specific detector such as a GC-arc atomic emission detector or a microwave-induced atomic emission spectrometer would be ideal in selective detection of organoarsenicals. Mass spectrometric techniques such as Fast Atom Bombardment Mass Spectrometry (FABMS) and Field Desorption MS could be used to identify organoarsenic compounds after chromatographic separation.

Many organoarsenicals are not volatile being quite polar and water soluble. Reverse phase-high performance liquid chromatography (HPLC) would be more applicable for determination of these nonvolatile organo-arsenic compounds. High selectivity could be achieved with a specific detector such as a graphite furnace atomic absorption spectrometry (AAS) with a deuterum lamp. HPLC-AAS systems have been used to detect arsenite, arsenate, methanearsonic acid, dimethylarsinic acid, phenyl-arsonic acid, arsenobetaine and arsenocholine (Craig, 1986).

The commonly used analytical techniques for total arsenic determination are atomic absorption spectrometry (AAS) (Irgolic et al., 1983; Owens and Gladney, 1976) and inductively coupled argon plasma emission spectrometry (ICAP) (Morita et al., 1981) in conjunction with hydride generation (Irgolic et al., 1983). These two methods, though sensitive

are not selective in direct determination of different species of arsenic. Quantitative data for total organoarsenicals is usually limited because of the indirect determination calculating the differences between inorganic species and total arsenic.

Analyses by AAS or ICAP do not allow direct determination of arsenate in environmental samples. Recently a method was developed for the direct determination of arsenate in aqueous soil extracts by single-column ion chromatography (SCIC) at trace levels (Mehra and Frankenberger, 1988). Separation is carried out on a low-capacity anion-exchange resin column being quantified by conductometric detection. Trace amount measurements of arsenate (detection limit, 92 µg L^{-1}) were made in the presence of other oxyanions, NO_3^-, PO_4^{3-} and SO_4^{2-}.

TERRESTRIAL PLANTS

Terrestrial plants growing on shores bordering arsenic contaminated water show relatively little arsenic content despite the fact that sediments may contain levels as high as 200 µg As g^{-1} (Reay, 1972). Furthermore resistance to arsenic can be increased by acclimating plants to successively higher concentrations of arsenic. Little bluestem, Andropogon scoparius Michx. can survive in soil containing up to 41,200 mg kg^{-1} of arsenate (Wauchope, 1982). The soil matrix plays an important role in availability of arsenic to plants. Retort oil shales have high concentrations of arsenic but plants grown on these soils show little accumulation ranging from 0.03 to 0.44 mg kg^{-1} (Kilkelly and Lindsay, 1982). Plant toxicity to arsenic is often reached prior to accumulation of levels which would be toxic to wildlife ingesting the plants.

In studies of arsenate uptake by barley, it was reported that uptake is temperature dependent increasing with increasing temperatures (Asher and Reay, 1979). Arsenate is preferentially taken up 3-4 times the rate of arsenite. The presence of phosphate inhibits the uptake of arsenate while arsenate only mildly inhibits phosphate uptake. In terrestrial plants as in most other systems, arsenate and phosphate are thought to compete for the same uptake system but there appears to be a higher affinity for phosphate (Asher and Reay, 1979). The discriminate ratio between phosphate and arsenate is approximately 4 to 1.

In corn, peas, melons and tomatoes, absorbed arsenate is rapidly reduced to arsenite. Terrestrial plants do not synthesize lipid-soluble arsenic compounds. Methylation of the arsenite occurs under phosphate deficient conditions and increases substantially when plants are also made nitrogen deficient. The methylated compounds accumulate to approximately six times greater concentration in the leaves than in the roots. Plants which show high levels of arsenic methylation are atypical and are considerably deformed due to the nutrient deficient growth conditions (Nissen and Benson, 1982). Synthesis of methylated arsenic compounds by terrestrial plants is not necessarily a detoxification mechanism.

Arsenate toxicity can be detected at levels as low as 1 mg kg^{-1} in the roots of Allium cepa, causing a significant reduction in root length while 3 mg kg^{-1} terminates root growth (Pepper et al., 1968). Inhibition of growth is reversible by removal of arsenate. The mode of toxicity of arsenate is to partially block protein synthesis and/or interfere with phosphorylation of proteins. The addition of phosphate abolishes the effects of arsenate.

MICROBIAL TRANSFORMATIONS

Bacterial Resistance

Arsenate is a biochemical analogue of phosphate and is transported by highly specific, energy-dependent membrane pumps into the cell of bacteria during assimilation of phosphate (Silver and Nakahara, 1983). In Escherichia coli, resistance to arsenic can be achieved by two distinct mechanisms: a chromosomal or plasmid encoded system. Chromosomally-encoded resistance occurs by the activation of a phosphate uptake pump with an increased selectivity for phosphate. In bacteria, two phosphate uptake systems are present, Pit (inorganic Pi transport) and Pst (phosphate specific transport). The Pit system is constitutive and does not discriminate between phosphate and arsenate having a K_m for phosphate equal to the K_i for arsenate (25 µM). During periods of phosphate starvation or arsenate toxicity the Pst system is activated and despite having an identical K_i for arsenate, the reduction in cellular arsenic is achieved by the higher affinity for phosphate. The K_m for phosphate is 0.25 µM, one hundred times greater affinity than the Pit system (Rosenberg et al., 1977). Thus the activation of the Pst system confers higher levels of arsenate resistance by virtue of reduced uptake of arsenate (Silver and Nakahara, 1983).

Plasmid-determined resistance is a consequence of an accelerated efflux of arsenate from the cell. A highly specific membrane-associated pump exports arsenate, arsenite and antimonate but not phosphate from the cell (Mobley and Rosen, 1982). Plasmid-encoded resistance is distinct from the chromosomally encoded Pst system and the level of resistance

obtained from each system is additive. Molecular analysis of the plasmid encoded resistance has shown three genes primarily responsible for the export function. The genes for resistance are clustered on an R-Factor plasmid called R773 (Chen et al., 1986). ArsA and ArsB genes are involved in the export of arsenite and antimonate while ArsC is required to confer resistance to arsenate. The oxyanion pump is composed of only two proteins, a 63 kd hydrophilic ArsA protein and a 45.5 kd ArsB protein (Rosen et al., 1988). The ArsA gene has been sequenced and the deduced amino acid sequence shares homology with several adenylate-binding proteins such as nitrogenase and the β-subunit of the mitochondrial ATPase. The ArsA protein encodes two distinct adenylate-binding consensus sequences which have binding affinity for nucleotides and specifically catalyzes the hydrolysis of ATP. The binding of ATP by ArsA is independent of the presence of oxyanions, however, the rate of ATP hydrolysis is dependent on their presence and is stimulated 5-fold by the addition of arsenite and 50-fold with the addition of antimonate (Rosen et al., 1988). The ArsA protein is mainly cytosolic but a portion is found sedimented within the cell membrane and is thought to complex with ArsB. ArsB is found in the inner membrane of E. coli and has been postulated to be the portion of the pump responsible for the export of anions from the cell. The deduced amino acid sequence of ArsB reveals several regions of the protein are potentially transmembrane regions. The 16 kd ArsC polypeptide modifies the ArsA-ArsB complex allowing the pumping of arsenate. ArsC is not required for the efflux of arsenite or antimonate (Chen et al., 1986). The plasmid-encoded resistance for arsenate/arsenite is highly specific for oxyanions. It fails to protect E. coli cells from

concentrations of 0.1 mM phenylarsine oxide or 10 mM Na cacodylate (Mobley et al., 1983) but is highly selective in preventing the export of phosphate from the cell (Mobley and Rosen, 1982).

Resistance to arsenic is inducible and recently a fourth gene has been identified which regulates the arsenic resistance operon. Plasmid encoded resistance results from the activation of an anion-translocating ATPase with high selectivity for arsenate, arsenite and antimonate (Rosen et al., 1988).

Plasmid-encoded resistance for arsenate/arsenite is widespread among different bacterial species (Nakahara et al., 1977; Smith, 1978; Dabbs and Sole, 1988). Hybridization studies indicate that a number of arsenate resistant strains of Klebsiella pneumoniae and E. coli possess sequences homologous to the plasmid encoded genes. However, genes involved in arsenic resistance are not completely conserved among different bacterial strains. In Staphylococcus, three genes are also involved in conferring resistance to arsenic, however, sequence analysis indicates only ArsB, the gene encoding the transmembrane protein, shares homology with sequences of R773 (Silver and Misra, 1988). Furthermore, strains of E. coli, K. pneumoniae and K. oxytoca that are resistant to arsenic have been isolated which lack sequences homologous with ArsA, ArsB or ArsC genes. One significant feature of the epidemiology of bacterial arsenic resistance is the fact that it is often associated with other types of heavy metal and antibiotic resistances (Smith, 1978).

Bacterial Oxidation

Bacteria are approximately 10-fold more resistant to arsenate than arsenite. Bacillus and Pseudomonas species have been isolated which can oxidize arsenite to arsenate. A strain of Alcaligenes faecalis obtained from raw sewage was capable of oxidizing arsenite (Phillips and Taylor, 1976). Energy is not recovered from the reaction but rather, oxidation is proposed to be a detoxification mechanism (Osborne and Ehrlich, 1976). Alcaligenes sp. isolated from various soils can oxidize arsenite. Biochemical studies on resting cells revealed that the oxidation process is induced by arsenite. The transformation to arsenate consumes oxygen. The use of respiratory inhibitors prevented further oxidation of arsenite indicating that oxygen served as the terminal electron acceptor. In extreme environments such as acid mine waters, arsenic concentrations are as high as 2 to 13 mg L^{-1} and the major inorganic species is arsenite. Oxidation of arsenite by heterotrophic bacteria play an important role in detoxifying the envrionment catalyzing as much as 78 to 96% of the arsenite to arsenate (Wakao et al., 1988).

Bacterial Methylation

Table 2 reveals the microbial diversity of organisms which can methylate arsenic into various volatile forms. Bacterial methylation of inorganic arsenic has been studied extensively in methanogenic bacteria. Methanogenic bacteria are a morphologically diverse group consisting of coccal, bacillary and spiral forms but are unified by the production of methane as their principal metabolic end product. They are present in

Table 2. Microbial production of alkylated arsines.

Microorganism	Product formed	Conditions	Reference
Methanobacterium strain M.o.H.	Dimethylarsine	cell-free extracts, anaerobic conditions in presence of CH_3-B_{12}, ATP, H_2	McBride and Wolf, 1971
Desulfovibrio vulgaris strain 8303	volatile As derivative with strong garlic odor indicative of an arsine	cell-extracts stimulated by CH_3-B_{12}	McBride and Wolf, 1971
Penicillium brevicaule (Scopulariopsis brevicaulis)	trimethylarsine	bread crumbs spiked with As2O3 or methylarsenate or cacodylate	Challenger et al, 1933
Penicillium notatum Penicillium chrysogenum Aspergillus niger	trimethylarsine trimethylarsine trimethylarsine	sodium methylarsonate and sodium cacodylate	Bird et al., 1948
Aspergillus glaucus	trimethylarsine	bread crumbs treated with arsenous and methylarsonic acids	Bird et al., 1948
Lenzites trabea	volatile As derivative with garlic odor (probably trimethyl-arsine)	medium containing As trioxide	Merril and French, 1964

Table 2 (continued)

Microorganism	Product formed	Conditions	Reference
Candida humicola	trimethylarsine	arsenic sources: sodium arsenate methanearsonic acid dimethylarsinic acid	Cox and Alexander, 1974
Candida humicola	trimethylarsine	substrate was chromate copper arsenate (wood preservative)	Cullen et al., 1984
Gliocladium roseum Pencillium sp.	trimethylarsine	methanearsonic acid dimethylarsinic acid	Cox and Alexander, 1973a, 1973b, 1974
Aeromonas sp. Flavobacterium sp. Escherichia coli	trimethylarsine	nutrient broth, containing As(III) or methanearsonic acid or dimethylarsinic acid	Chau and Wang, 1978

large numbers in anaerobic ecosystems, such as sewage sludge, freshwater sediments and composts where organic matter is decomposing (McBride et al., 1978). It has been shown that at least one species of Methanobacterium is capable of methylating inorganic arsenic to produce volatile dimethylarsine. Arsenate, arsenite and methanearsonic acid can serve as substrates in dimethylarsine formation. Inorganic arsenic methylation is coupled to the CH_4 biosynthetic pathway and may be a widely occurring mechanism for arsenic detoxification.

Methanobacterium strain M.o.H. cell-free extracts, when incubated under anaerobic conditions with $[^{74}As]Na_2HAsO_4$, a methyl donor (methylcobalamin, CH_3CoB_{12}), H_2, and ATP, produced a volatile ^{74}As-dimethylarsine (McBride and Wolfe, 1971). The pathway involves the reduction of arsenate to arsenite with subsequent methylation by a low molecular weight cofactor Coenzyme M (CoM). CoM has been found in all methane bacteria examined and chemically is 2,2'-dithiodiethane sulfonic acid (McBride et al., 1978). Methanearsonic acid added to cell-free extracts is not reduced to methylarsine but requires an additional methylation step before reduction. However, dimethylarsinic acid is reduced to dimethylarsine even in the absence of a methyl donor (McBride and Wolfe, 1971). Whole cells of methanogenic bacteria under anaerobic conditions also produce dimethylarsine as a biomethylation end product of arsenic but not heat-treated cells indicating that this is a biotic reaction. Furthermore, samples collected from a number of different anaerobic ecosystems (anaerobic sewage digestor sludge and rumen from cattle) which produced methane also transformed arsenate into dimethylarsine. The

pathway for anaerobic biomethylation of arsenic differs from fungal methylation under aerobic conditions and is shown in Fig. 2.

Under anaerobic conditions biomethylation of arsenic proceeds only to dimethylarsine, which is stable in the absence of oxygen, but is rapidly oxidized under aerobic conditions. Dimethylarsine in anaerobic environments can react with disulfide bonds present on particulates thus reducing the concentration of soluble arsenic.

Interestingly, another study indicated that resting cell suspensions Pseudomonas and Alcaligenes incubated with either arsenite or arsenate under anaerobic conditions produced arsine but no other intermediates were found (Cheng and Focht, 1979). Aeronomonas sp. and Flavobacterium sp. isolated from lake water were capable of methylating arsenic to dimethylarsinic acid (Wong et al., 1977). Flavobacterium sp. methylated dimethylarsinic acid to trimethylarsine oxide. Methylation of arsenic is pH-dependent with the highest rates often occurring at pH 3.5 to 5.5 suggesting that arsenic mobilization from the sediments to the overlying water phase is enhanced by acidification (Baker et al., 1983). Freshwater algae also produce methanearsonic acid and dimethylarsinic acid; however, there is some question on whether biomethylation of arsenic in freshwater is a widespread common process.

Bacteria are generally more resistant to exposure of methylated arsenic compounds than to inorganic species. Achromobacter, Flavo-bacterium, Nocardia, Pseudomonas, Alcaligenes, Aeromonas and Enterobacter isolates were all capable of growing in media amended with 100 µg ml^{-1} methanearsonic acid (Shariatpanahi et al., 1981). Five of the isolates,
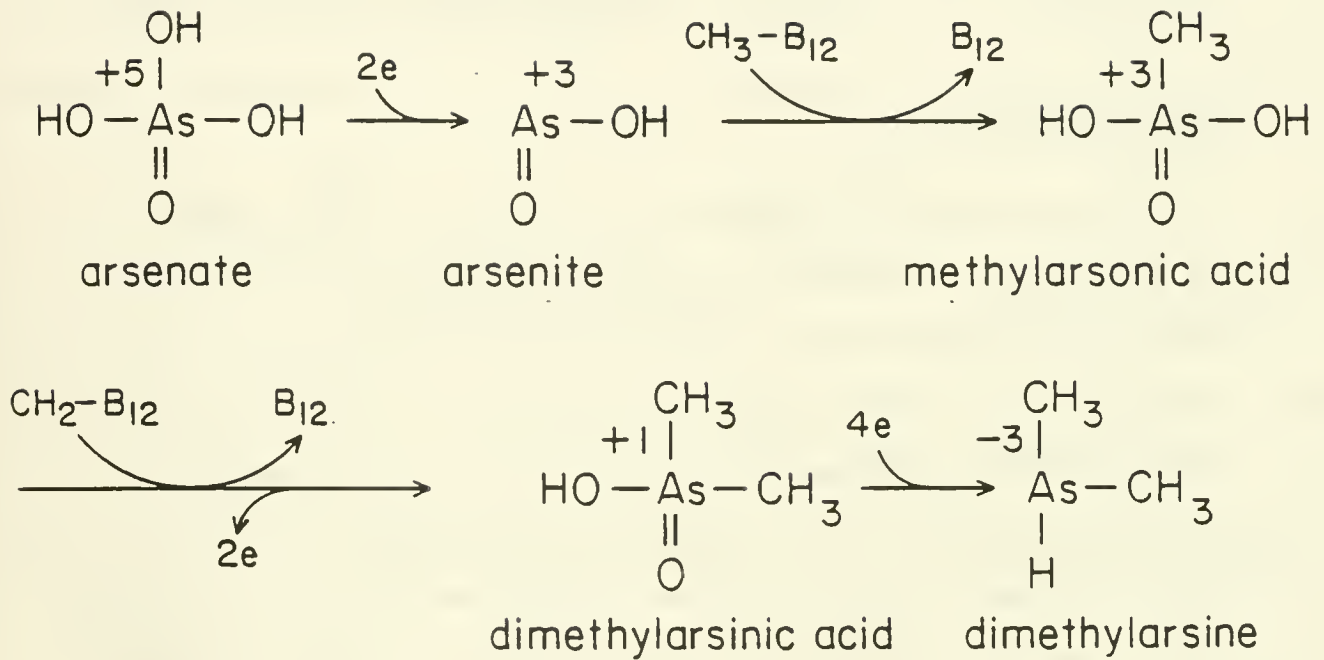
Fig. 2. Anaerobic biomethylation pathway for dimethylarsine
production by <u>Methanobacterium</u> sp. (McBride and
Wolfe, 1971).

Achromobacter, Flavobacterium, Nocardia, Pseudomonas, and Alcaligenes possessed a demethylating enzyme which released $^{14}CO_2$ when ^{14}C-methane-arsonic acid was added as a substrate. Demethylating activity has also been reported in two isolates of Actinomycetes (Von Endt et al., 1968).

Fungal Methylation

It is well established that fungi are able to transform inorganic and organic arsenic compounds into volatile methylarsines. The volatil-ized arsenic dissipates from the cells effectively reducing the arsenic concentration the fungus is exposed to. The importance of fungal meta-bolism of arsenic dates back to the early 1800's where a number of poisoning incidents in Germany and England were caused by a volatile methylarsine gas. The victims lived in musty rooms with a characteristic garlic-like odor. Trimethylarsine was identified as the toxic compound (Challenger, 1945). Molds growing on wallpaper decorated with arsenical pigments (Scheele's green and Schweinfürter green) produced the toxic trimethylarsine gas. Since then, several species of fungi have been identified that are able to volatilize arsenic (Cox and Alexander, 1973a). The fungus, Penicillium brevicaule (Scopulariopsis brevicaulis) produces trimethylarsine when grown on bread crumbs containing either methanearsonic acid or dimethylarsinic acid. A biochemical pathway for trimethylarsine production has been proposed by Challenger (1945) (Fig. 3).

In recent studies, three different fungal species Candida humicola, Gliocladium roseum and Penicillium sp. were capable of converting

$$H_2As^VO_4^- \xrightarrow[\text{reduction}]{2e^- \quad O^{2-}} \quad \overset{..}{As}^{III}O(OH)^- \xrightarrow[\text{methylation}]{CH_3^+ \quad H^+} \quad CH_3-\overset{\overset{\textstyle OH}{|}}{\underset{\underset{\textstyle O^-}{|}}{As}}^V=O \longrightarrow$$

arsenate reduction arsenite methylation methanearsonic acid

$$\xrightarrow{2e^- \quad O^{2-}} \quad CH_3-\overset{\overset{\textstyle O^-}{|}}{As}^{III}=O \xrightarrow{CH_3^+ \quad H^+} \quad CH_3-\overset{\overset{\textstyle CH_3}{|}}{\underset{\underset{\textstyle O^-}{|}}{As}}^V=O \longrightarrow$$

methanearsonous anion dimethylarsinic acid

$$\xrightarrow{2e^- \quad O^{2-}} \quad CH_3-\overset{\overset{\textstyle CH_3}{|}}{As}^{III}-O^- \xrightarrow{CH_3^+ \quad H^+} \quad CH_3-\overset{\overset{\textstyle CH_3}{|}}{\underset{\underset{\textstyle CH_3}{|}}{As}}^V=O \longrightarrow$$

dimethylarsinous anion trimethylarsine oxide

$$\xrightarrow{2e^- \quad O^{2-}} \quad :\overset{\overset{\textstyle CH_3}{|}}{\underset{\underset{\textstyle CH_3}{|}}{As}}^{III}-CH_3$$
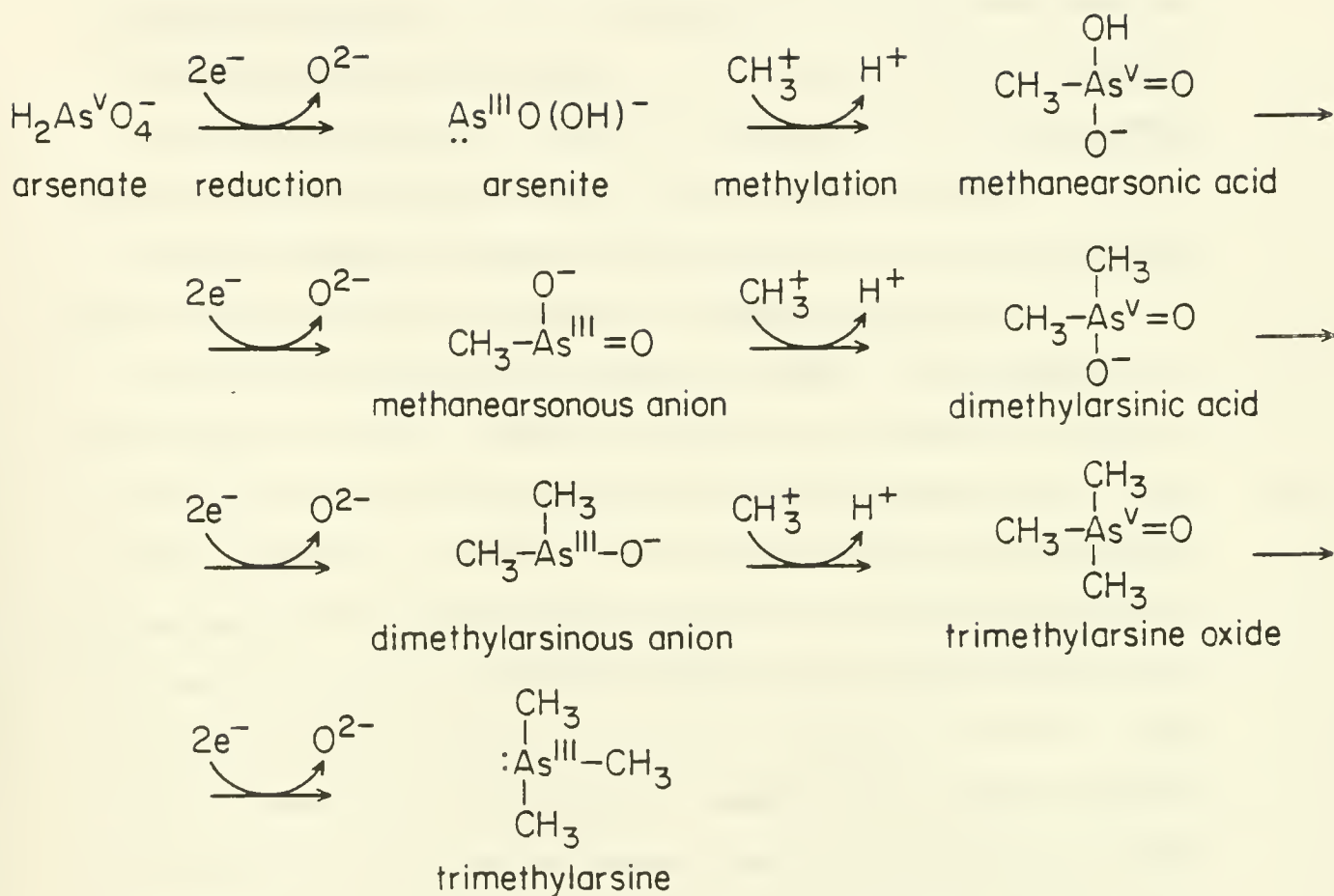
trimethylarsine

Fig. 3. Fungal methylation pathway for the formation of
trimethylarsine (Craig, 1986 [modified after
Challenger, 1945]).

methanearsonic acid and dimethylarsinic acid to trimethylarsine (Cox and Alexander, 1973a). In addition, C. humicola used arsenate and arsenite as substrates to produce trimethylarsine. Cell-free homgenates of C. humicola transformed arsenate into arsenite, methanearsonic acid and dimethylarsinic acid (Cullen et al., 1979). Although methylation of inorganic arsenic and methanearsonic acid is inhibited by the presence of phosphate, the rate of trimethylarsine formation from dimethylarsinic acid is increased in the presence of phosphate (Cox and Alexander, 1973b).

Methylation of arsenic is thought to occur via transfer of the carbonium ion from S-adenosylmethionine (SAM) to arsenic. Incubation of cells with an antagonist of methionine inhibits production of arsines thus supporting the role of methionine as a methyl donor (Cullen et al., 1977). The addition of either methanearsonic acid or dimethylarsinic acid to cell-free-extracts yields trimethylarsine oxide (Cullen et al., 1979). Further reduction of trimethylarsine oxide to trimethylarsine requires the presence of intact cells (Pickett et al., 1981). Various arsenic thiols (cysteine, glutathionine and lipioc acid) are thought to be involved in the reduction step of trimethylarsine oxide to trimethyl-arsine (Cullen et al., 1984a; 1984b). The final reduction step is inhibited by several electron transport inhibitors and uncouplers of oxidative phosphorylation (Zingaro and Bottino, 1983; Pickett et al., 1981). Preincubation of cells with trimethylarsine oxide increases the rate of conversion to trimethylarsine suggesting an inducible system (Pickett et al., 1981). In addition, the rate of transformation of arsenate to trimethylarsine is increased by preconditioning the cells with dimethylarsinic acid (Zingaro and Bottino, 1983). The compounds

isolated during the reduction of arsenate by C. humicola is consistent with the intermediates reported in the pathway for methylation of arsenic as proposed by Challenger (1945).

Soil fungal species may play a major role in the transformation and movement of arsenic chemicals used in agriculture. The methylation of arylarsonic acids is important because of their wide use as food supplements for swine, turkeys and poultry. Candida humicola is capable of methylating benzenearsonic acid to produce volatile dimethylphenylarsine (Cullen et al., 1983). In addition, methylphenylarsinic acid and dimethylphenylarsine oxide are also reduced by C. humicola to dimethylphenylarsine. Arsanilic acid, which contains an amino group at the para position of phenylarsonic acid was not converted to a volatile arsine but it has been reported that soils treated with arsanilic acid can lose its arsenic component.

The adaptiveness of C. humicola in methylating arsenic is evident by the fact that dilute solutions of the highly effective wood preserving fungicide, chromated copper arsenate (CCA), is depleted of arsenic through volatilization (Cullen et al., 1984). It has also been demonstrated that a variety of soils have the potential to produce alkylarsines (Woolson, 1977). Soils amended with inorganic and methylated arsenic herbicides produce dimethylarsine and trimethylarsine (Woolson et al., 1973; Baker et al., 1983; Hassler et al., 1984; Woolson, 1977). The organisms responsible for volatilization are from diverse environments suggesting that a number of different species have the capacity to produce alkylarsines. Mixed communities of microorganisms in soil produced dimethylarsine and trimethylarsine in headspace trapped in bell jars

over soil and lawn treated with methylarsenicals (Braman and Foreback, 1973).

Arsenic biomethylation is also widespread among higher organisms and has been demonstrated in human urine, bird eggshells, cows, dogs, rats, mice, rabbits, freshwater fish (trout), and terrestrial plants (tomatoes). Higher plants such as pine, corn, melons (honeydew) and pea reduce arsenate to arsenite but do not produce organoarsenic compounds (Nissen and Benson, 1982). Among the animals, there is some question on whether the intestinal bacteria are responsible for arsenic methylation.

AQUATIC TRANSFORMATIONS

Marine Environments

Marine algae. In seawater, arsenate is the predominant arsenic species and is present at approximately 1-2 μg L^{-1} (ppb) (Andreae, 1979). In highly productive environments, the phosphate concentration can be depleted to levels below the concentration of arsenate (Benson et al., 1981). Arsenate is assimilated due to its similarity to phosphate by marine phytoplankton (Sanders, 1979) as evident with uptake studies in both bacteria (Silver and Misra, 1988) and marine yeast (Button, 1973). However, phosphate and arsenate uptake may be independent processes in marine phytoplankton suggesting a non-competitive absorption mechanism (Andreae and Klumpp, 1979; Klumpp, 1980). Regardless of the mechanism of arsenate uptake, primary producers must adapt to the accumulation of cellular arsenate. Arsenate is partially detoxified by production of large quantities of stable methylated arsenic compounds. These methylated compounds do not interfere with phosphate esterification.

Approximately 15 to 20% of the total soluble arsenic in marine biota systems are reduced and methylated during uptake (Sanders and Windom, 1980). The seasonal increase and decrease of arsenic in marine waters is consistent with the fluctuation in biological activity (Howard et al., 1982). The formation of methylated arsenic compounds is considered as a detoxification step beneficial not only to the primary producers, but also to higher trophic levels, since these compounds are much less toxic to marine invertebrates (Sanders, 1979).

The ability to transform arsenate to arsenite and subsequently to methylated arsenic compounds is present in four classes of marine phytoplankton: diatoms, coccolithophorids, dinoflagellates and green algae (Prasinophyceae). Arsenate is absorbed and converted to arsenite, methanearsonic acid and dimethylarsinic acid before being secreted from the cells (Andreae and Klumpp, 1979). A portion of the arsenate, however, is retained within the cell and metabolized further into complex organic compounds. The metabolism of arsenic is species dependent but in general, diatoms, dinoflagellates and green algae convert 30-50% of the retained arsenic into lipid-soluble compounds while coccolithophorids incorporate less than 1% (Andreae and Klumpp, 1979).

The chemical form of arsenic in marine waters influences the cellular concentration and metabolism of arsenic. Enrichment of media with high levels of arsenate increased the organic arsenic pool in Skeletonema costatum (Andreae and Klumpp, 1979). Arsenite invoked increased levels of arsenic in both the organic and inorganic cellular fraction. The uptake of arsenate was four times the rate of arsenite in Fucus spiralis (Klumpp, 1980).

Enrichment of marine phytoplankton cultures with dimethylarsinic acid does not increase the cellular arsenic concentration or change the inorganic/organic arsenic ratio (Sanders and Windom, 1980). Furthermore, the addition of dimethylarsinic acid does not affect diatom productivity (Sanders, 1979). Thus it has been suggested that the conversion of arsenate to methanearsonic acid or dimethylarsinic acid may possibly be a detoxification mechanism where the metabolism of arsenic into lipid-soluble compounds may in fact be an adaptation by these marine organisms to compensate for limited nitrate availability. The periodic table indicates that nitrogen and arsenic, both being from group V A, have similar chemical properties. It is known that arsenic can replace nitrogen in choline used to build arsonium phosphatides which functions efficiently as structural lipids (Wrench and Addison, 1981).

The wide variation in total arsenic among macroalgae species is often a reflection of the difference in the size of the organic arsenic pool. The arsenic concentration in three main classes of macroalgae ranged between 0.4 to 32 μg g^{-1} dry weight (Sanders, 1979). The distribution of arsenic in the organic pools were as follows: Phaeophyceae (brown algae), 78%; Rhodophyceae (red algae), 57%; and Chlorophyceae (green algae), 53%. The percentages of inorganic arsenic varied widely between species, both within and among groups but the actual concentrations were within a narrow range of 0.63 to 2.46 μg g^{-1}. Arsenate assimilation by microalgae is most likely a function of phosphate uptake. Species belonging to the Class Phaeophyceae contain high levels of phosphate and are thought to have high rates of organoarsenic metabolism to compensate for the high levels of internal arsenate.

The chemical structure of several water soluble organoarsenic compounds (dimethylarsenosugars) have been identified from the brown kelp, Ecklonia radiata (Edmonds and Francesconi, 1981; 1983). Figure 4 illustrates the pathway for the biosynthesis of arsenosugars in marine algae. The total cellular arsenic concentration can range as high as 10 µg g^{-1} fresh weight with water-soluble compounds accounting for approximately 81% of the total arsenic. In contrast, a related brown algae, Fucus spiralis contained a single lipid compound which accounted for 60% of the total cellular arsenic. Arsenolipid anabolism proceeds through water-soluble organic intermediates from arsenate (Fig. 4). The conversion of the water-soluble intermediates to lipid-soluble compounds is dependent on respiration rather than photosynthesis. Blockage of respiration leads to the accumulation of a water-soluble organoarsenics. Chromatographed cell extracts of Skeletonema revealed 12 distinct water-soluble organoarsenic compounds (Andreae and Klumpp, 1979) but it has not been determined if the metabolic pathway is related to that reported in E. radiata (Klumpp and Peterson, 1981).

The tolerance of marine algae to high concentrations of arsenic is exemplified by Tetraselmis chuii (a green flagellate, Chlorophyta). T. chuii can be acclimated to survive in arsenic concentrations as high as 1 g L^{-1} (1,000 ppm) of arsenate by synthesizing arsenolipids with an arsenocholine moiety (Bottino et al., 1978a). Highly resistant strains are physiologically altered and transfer of these strains to arsenate free-media often results in cell death (Bottino et al., 1978b). The synthesis and accumulation of organoarsenic compounds within the cell is proportional to the external concentration of arsenic in the surrounding
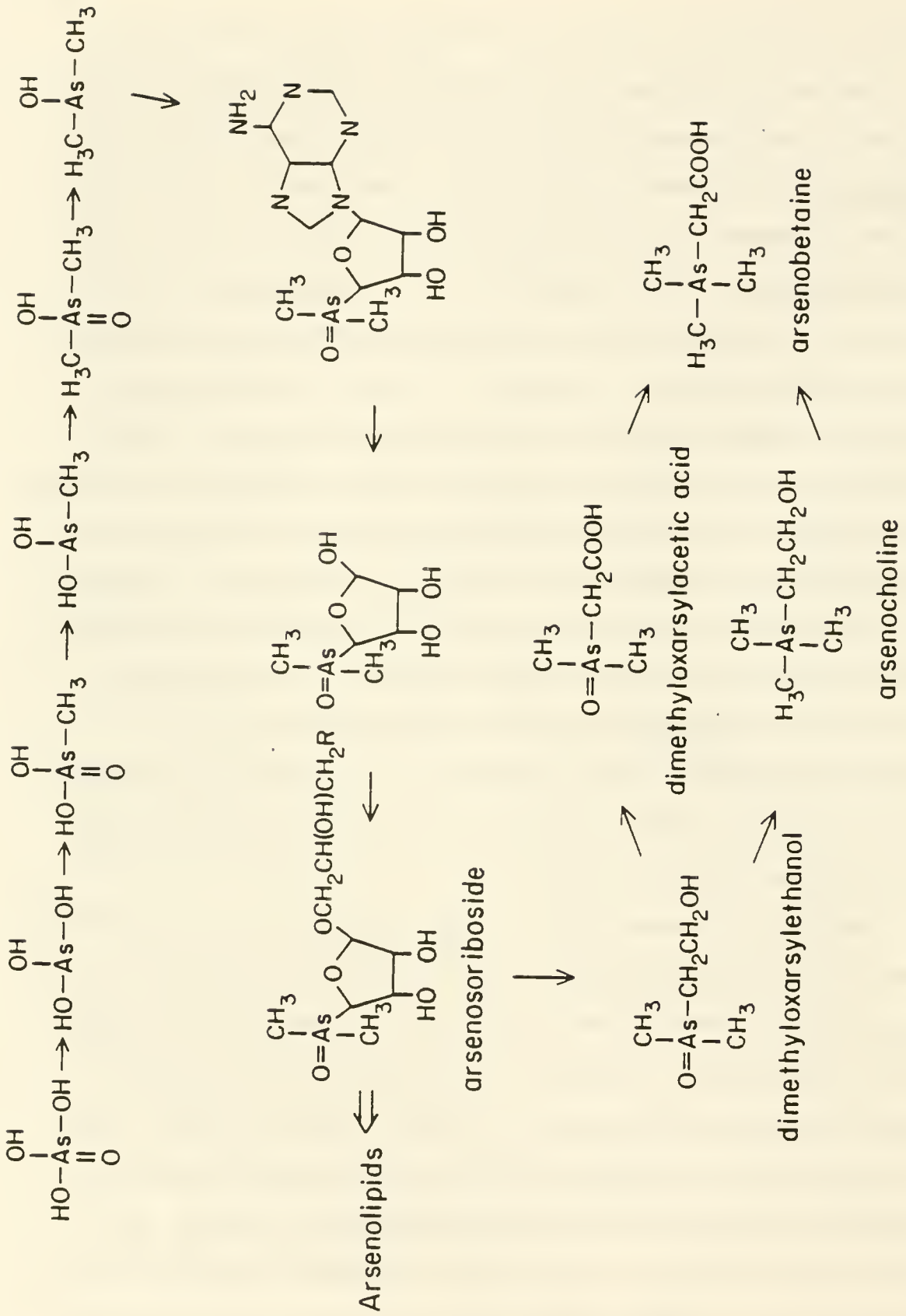
Fig. 4. Pathway for biosynthesis of arsenobetaine in marine algae.

environment. High concentrations of arsenic are often found in marine algae collected along an increasing arsenic gradient at a sea-estuary interface (Klumpp and Peterson, 1979).

Among the organoarsenic compounds synthesized from inorganic arsenic in the aquatic environment, methanearsonic acid and dimethylarsinic acid are products commonly excreted, but make up a very small fraction of the organoarsenic pool in algae. They appear to be intermediates in the synthesis of other organoarsenic compounds, including arsenolipids. Although methylarsenic acids are the major forms of organic arsenic excreted by algae, they are not very stable in natural waters. Production and release is apparently balanced by bacterial removal such as demethylation and subsequent oxidation of arsenite to arsenate. It has been estimated that the production rate and removal rate of dimethyl-arsinate are the same in seawater at about 1 ng dm^{-3} day^{-1} (Sanders, 1979).

Marine invertebrates and fish. Fish and invertebrates are part of the higher trophic levels in the food web of marine environments. The primary producing phytoplankton and macroalgae can accumulate arsenic and transform inorganic forms into complex organic molecules that may be converted into water-soluble or lipid-soluble arsenic compounds. Consumption of primary producers by a higher trophic level metabolizes the organoarsenic compounds into other forms. Fish and marine invertebrates retain 99% of the arsenic in the organic form. The concentration of inorganic arsenic rarely exceed 1 mg kg^{-1} in their tissues (Maher, 1983). Generally, arsenic concentrations are higher in crustacean and mollusk tissues compared to fish (LeBlanc and Jackson, 1973; Maher, 1983).

Chemical structures have been determined among several of the predominate water-soluble arsenic compounds in invertebrates and fish. Giant clams of the genera Tridacna and Hippopus concentrate arsenic in their kidneys to levels greater than 1000 mg kg^{-1} dry weight (Benson and Summons, 1981). The arsenic is obtained from symbiotic unicellular algae, zooxanthellae, which transforms arsenate in seawater to organic compounds that are passed to the host clam. In Tridacna maxima kidney tissue, two organoarsenic compounds were isolated and identified as trimethylarsoniumlactate and O-glycerophosphoryl-trimethylarsoniumlactate accounting for the majority of the cellular arsenic (Benson and Summons, 1981). The most predominant organoarsenic species, however, was not identified but was also thought to be a lactate derivative. The synthesis of arsenolipids are proposed as an adaptation by the clams to reduce the cellular arsenic concentration. The arsenolipids protrude from the gill membranes into the surrounding marine waters and become accessible to bacteria which oxidize the lipids and release the arsenic moiety as dimethylarsinic acid (Benson and Nissen, 1982). Dimethylarsinic acid and minor amounts of methanearsonic acid are also present in clam tissue (Benson and Summons, 1981).

The unequivocal identification of the water-soluble lactate compounds in clam kidney tissue is subject to dispute (Edmonds and Francesconi, 1987). Edmonds and Francesconi (1982) isolated and determined the X-ray crystal structure of two water-soluble arsenic compounds present in the kidneys of Tridacna maxima. These two compounds accounted for 80% of the total arsenic in the kidneys and were identical to the dimethylarsenosugars isolated from Ecklonia radiate (Edmonds and

Francesconi, 1981). Arsenosugar synthesis is not unique to E. radiata
and their presence in clams was thought to be due to the arsenosugar
synthesis by the symbiotic algae with passage to the host accumulating in
the kidneys. However, current evidence suggests that the arsenosugars
were misidentified as arsoniumlactate compounds by Benson and Summons
(1981) and thus any reference in the literature to arsoniumlactate com-
pounds must be carefully evaluated.

Arsenobetaine has been isolated and purified from the Australian
rock lobster, Panulirus longipes (Edmonds et al., 1977; Cannon et al.,
1981), and the dusky shark, Carcharhinus obscurus (Kurosawa et al., 1980;
Cannon et al., 1981). In flounder, sole, lemon sole, dab, and shrimp,
the major arsenic species was recovered as arsenobetaine (Luten et al.,
1983). The concentrations of arsenic ranged from 0.45 to 31.4 $\mu g \ g^{-1}$ dry
weight. In shark, arsenobetaine is the predominant arsenic species in
both muscle and liver tissues (Kurosawa et al., 1980). In the muscle,
80% of arsenic was detected in the aqueous fraction, 10% in the residue
and almost none in the lipid fraction. In contrast, in the liver, 40% of
the total arsenic was found in the aqueous fraction while 45% in the
lipid fraction. In shrimp, arsenobetaine constitutes two-thirds of the
arsenic pool while the remainder has tentatively been identified as
arsenocholine (Norin et al., 1983; Norin and Christakopoulos, 1982).
However, other investigators have been unable to confirm the presence of
arsenocholine in other shrimp species (Luten et al., 1983; Shiomi et al.,
1984).

It is unclear how arsenosugars and arsenolipids are transformed into
arsenobetaine within the higher trophic levels of the marine environment.

The American lobster, Homarus americanus, retains arsenic in its muscle
tissue as arsenobetaine (Edmonds and Francesconi, 1981) but is unable to
synthesize arsenobetaine from organoarsenic compounds obtained from
ingested algae (Cooney and Benson, 1980). The generation of arsenobetaine
from arsenosugars requires cleavage at the C_3-C_4 bond of the sugar residue
and the subsequent oxidation of the C_4 carbon. Furthermore, reduction
and methylation of the arsenic atom must occur to form the quaternary
(tetraalkylated) arsonium compound of arsenobetaine (Edmonds and
Francesconi, 1987). Dimethylarsinoylethanol (see Fig. 4 for chemical
structure), a product of anaerobic bacterial decomposition of arseno-
sugars in Ecklonia, has been proposed as an intermediate in the formation
of arsenobetaine (Edmonds et al., 1982). However, the process in which
dimethylarsinoylethanol is transformed into arsenobetaine is unclear.
There is no evidence that suggests bacteria have the potential to carry
out the necessary quaternary methylation (Edmonds and Francesconi, 1987).
Thus the pathway involved in the synthesis of arsenobetaine remains
largely undefined.

The synthesis of organoarsenic compounds in marine environments is
principally by primary producers. Although all of the intermediates of
metabolism have not been identified, arsenobetaine is thought to be the
end-product which accumulates in marine animals (Norin and Christakopoulos,
1982). There is evidence, however, that suggests some marine animals are
able to synthesize organoarsenic compounds directly from arsenate. The
macroalgae feeding snail, Littorina littoralis, converts arsenate in
seawater, into a single water-soluble compound identified as WS-0.66,
which is distinct from arsenobetaine and arsenocholine (Klumpp and

Peterson, 1981). Interestingly, WS-0.66 is also the major arsenic com-
pound accumulated by snails which feed on the macroalgae, Fucus spiralis.
The macroalgae store arsenic predominantly as a single lipid-soluble com-
pound which is presumed to be metabolized by the snails and stored as
WS-0.66. Nucella lapillus, a predatory snail, also produces and stores
arsenic as WS-0.66 (Klumpp and Peterson, 1981). Like Littorina
littoralis, Nucella lapillus is able to convert arsenate obtained from
seawater or various organoarsenic compounds derived from its food sources
into WS-0.66. In both snail species, lipid-soluble compounds can be
recovered but are usually less than 10% of the total arsenic retained.

Arsenobetaine is the end product which generally accumulates in the
higher trophic levels (Norin and Christakopoulos, 1982) and its degrada-
tion is required to complete the biological cycling of arsenic. Microbial
degradation of arsenobetaine occurs in coastal water sediments. Sediment
samples amended with arsenobetaine contain organisms which are capable of
degrading arsenobetaine into trimethylarsine oxide (Kaise et al., 1985).
Trimethylarsine oxide is further broken down into dimethylarsinic acid
and finally to methanearsonic acid and inorganic arsenic. The breakdown
derivatives are apparently volatilized by microorganisms since the con-
centration of arsenic in the culture media steadily decreased. However,
it was demonstrated that arsenobetaine as a sole carbon source could not
support the growth of pure cultures responsible for degradation and thus
cometabolism may be involved (Hanaoka et al., 1987).

FRESHWATER ENVIRONMENTS

Freshwater bodies are more variable than the marine environment in the concentration of arsenic ranging between 1 to 10 µg L^{-1} (Sanders, 1980). Other estimations are as high as 64 µg L^{-1} (Schraufnagel, 1983). Arsenic concentrations in freshwater fluctuates depending on evaporation and condensation rates. Other factors which contribute to variations are contamination by arsenic herbicides directly or as runoff, industrial pollution and natural contamination. In lakes of New Zealand of the Taupo-Wairakei region, the concentration of arsenic has been reported to reach concentrations of 70 µg L^{-1} as a result of arsenic-rich hot springs arising from geothermal activity (Reay, 1972). Irrigation of farmland in the Central Valley, California is a significant factor in mobilizing and redistributing arsenic. In the Tulare Lake Drainage District, Kings County, California, arsenic concentrations in evaporation ponds have been reported as high as 2400 µg L^{-1}. Yellowstone hot springs are as high as 3,500 µg of arsenic L^{-1} (Stauffer and Thompson, 1984).

The resistance to arsenate among freshwater algae is highly species dependent. Five taxonomically divergent algae, Chlamydomonas reinhardtii, Melosira granulata, Ochromonas vallesiaca, Anabaena variabilis and Cryptomonas erosa showed a wide range in susceptibility to arsenate (Planas and Healey, 1978). At arsenate concentrations of 75 µg L^{-1}, the growth rates of M. granulata and O. vallesiaca were depressed by approximately 20 to 40%. Inhibition in growth by C. reinhardtii was not evident until the concentration of arsenate was 750 µg L^{-1} and A. variabilis and C. erosa both were unaffected by

concentrations as high as 7,500 µg L^{-1}. These differential resistance levels may play a significant role in species succession.

Arsenic is metabolized into various methylated forms by freshwater algae. Arsenite is methylated by at least four freshwater species of green algae, including Ankistrodesmus sp., Chlorella sp., Selenastrum sp., and Scenedesmus sp. (Baker et al., 1983). All four species methylated arsenite when present in media at 5,000 µg L^{-1}, approximately the same level of arsenite used to control aquatic plants in lakes (Hood and Associ, 1985). The levels of recovered methylated arsenic species was quite high on a per gram dry weight basis. Each of these organisms transformed arsenite to methanearsonic acid and dimethylarsinic acid and all, except Scenedesmus, produced detectable levels of trimethylarsine oxide. Unlike fungi, volatile methylarsines were not produced (Baker et al., 1983), but instead, limnetic (freshwater) algae like marine algae synthesize lipid-soluble arsenic compounds. Freshwater algae grown in media amended with 1 to 3 µg L^{-1} of arsenate synthesized lipid-soluble arsenic compounds to levels approximately equal to marine algae (Lunde, 1972; Lunde, 1973).

Aquatic plants also have the biosynthetic machinery to synthesize arsenolipids, with as much as 50 to 80% of the metabolized arsenic converted into a lipid-soluble form. The chemical structures of these compounds have not been determined but share chemical characteristics similar to those synthesized by the marine algae (Benson et al., 1981; Benson and Nissen, 1982). The aquatic dicot, Ceratophyllum demersum, can accumulate arsenic concentrations up to 650 µg g^{-1} dry weight when isolated from arsenic enriched waters (Reay, 1972). Other aquatic plants

isolated from New Zealand's Taupo-Wairakei area have been found to accumulate high concentrations of arsenic. Aquatic plants isolated from these enriched waters have considerably higher concentrations of arsenic than the identical species isolated from noncontaminated lakes (Fish, 1963; Reay, 1972).

Arsenate given orally to fresh water brown trout (Salmo trutta) is converted to an organic form mediated by the intestinal microflora and then rapidly absorbed (Penrose, 1975). Arsenate injected intramuscularly is initally detected in the blood as inorganic arsenic but is slowly converted to an organic form. Both inorganic and organic arsenic compounds accumulate in the liver and are secreted with the bile into the lumen. Inorganic arsenic is thought to be methylated by the intestinal microflora in the gastrointestinal tract and then preferentially reabsorbed into the body of the fish. Two organic arsenic compounds were detected in trout and were clearly distinct from arsenobetaine. There is no clear evidence that fish are capable of directly converting inorganic arsenic into methylated species.

MAMMALIAN METABOLISM

In humans and animals, arsenic enters the body mainly by ingestion, inhalation and sorption and is rapidly excreted or metabolized into less toxic methylated organic compounds. Extensive studies have been made on the metabolism of arsenic compounds with rats (Coulson, 1935). Rats fed arsenic trioxide accumulated 55-65 times more arsenic than control rats, whereas those fed equivalent amounts of shrimp-derived arsenic (now known as arsenobetaine), retained only 2-4 times the amount of the controls.

Approximately 80% of the ingested arsenic trioxide was retained in the body compared to less than 2% of the fed shrimp-arsenic. The shrimp-arsenic was almost exclusively excreted in the urine. In contrast to rats, monkeys fed fish-arsenic (also known as arsenobetaine), excreted 57 to 84% of the ingested arsenic in the urine within 4 days, with little found in the feces. However, inorganic arsenic trioxide was excreted much more rapidly than fish-arsenic; also exclusively in the urine (Peoples, 1983). The difference in metabolism of arsenic between the two animals has been explained by the fact that rats accumulate arsenic in their red blood cells accounting for as much as 90% of all the arsenic stored (Zingara and Bottino, 1983).

In mice, nearly 100% of the ingested arsenite and arsenate are absorbed in the gastrointestinal tract during the initial stages of exposure (Zingaro and Bottino, 1983). The retention of arsenic in the body is approximatey equal for both valence states at low doses (0.4 mg kg^{-1}), but at higher doses (4.0 mg kg^{-1}), the retention time of arsenite exceeds that of arsenate. The metabolism of arsenite and arsenate differ with arsenite ingestion leading to significantly higher concentrations of arsenic in the liver and bile. However, both inorganic compounds are eventually converted into dimethylarsinic acid.

Cows and dogs fed either arsenite or arsenate excreted 50% of the arsenic in the inorganic form while the remainder was metabolized and excreted as methylarsenic compounds. It was concluded that these animals rapidly synthesize organoarsenics and the methylation reaction was not dependent on intestinal microflora (Lakso and Peoples, 1975). Humans also convert inorganic arsenic into methylated species forming dimethylarsinic

acid and methanearsonic acid. In one study, approximately 25% of ingested arsenate was excreted in the urine within 24 hr and within 5 days the total reached 58% (Tam et al., 1979). Of the excreted arsenic, 70% had been converted into methylated forms. Dimethylarsinic acid was the predominant species in urine after the initial 24 hr period and continued to increase in proportion relative to other arsenic compounds. In another study, 200 µg of arsenate in well water was imbibed and arsenate levels in the urine increased significantly up to 10 hr of exposure at which time dimethylarsinic acid became the predominate form of arsenic (Crecelius, 1977).

Consumption of wine containing an equivalent of 50 µg of arsenite and 13 µg of arsenate led to the excretion of inorganic arsenic in urine reaching a maximum after 5-10 hr (Crecelius, 1987). The concentrations of dimethylarsinic acid and methanearsonic acid steadily increased but did not reach a maximum until 40 hr after consumption. Dimethylarsinic acid accounted for 50% of the total arsenic ingested.

Inorganic arsenic administered as arsenic trioxide (As^{+3}) was given to an individual as an oral dose of 700 µg (Yamauchi and Yamamura, 1979). After 12 hr, 40% of the total arsenic ingested was excreted in the urine and 70% had been excreted within 72 hr. The percentage of each arsenic species excreted during the 72 hr period was 21.7% As^{+3}, 4.9% As^{+5}; 19.1% methanearsonic acid and 19.6% dimethylarsinic acid. As^{+3} was rapidly excreted within 12 hr of ingestion and methylated arsenic compounds reached a maximum at 12 hr, staying relatively constant for an additional 36 hr after which levels begin to decrease.

Seafood contains high concentrations of organic arsenic either as complex arsenosugars and arsenolipids or as arsenbetaine. Humans fed shrimp, fish, or crab meat containing arsenic rapidly excrete organo-arsenic compounds in their urine. Over 75% of ingested fish-arsenic is excreted within 8-9 days and less than 1% can be accounted for in feces (Tam et al., 1982). Arsenobetaine has been identified in urine of humans fed lobster (Cannon et al., 1981). Ingestion of organoarsenic compounds from marine animals are rapidly absorbed and circulated into the blood stream where they are passed to the kidneys and excreted in the urine largely unchanged.

Ingested methanearsonic acid and dimethylarsinic acid are more rapidly excreted from the body than arsenite. Dimethylarsinic acid is recovered unchanged and no evidence suggests conversion to inorganic arsenic. The majority of methanearsonic acid is also excreted unchanged but approximately 13% is converted into dimethylarsinic acid (Zingaro and Bottino, 1983). In rats, 248 min was required for absorption of 50% of the ingested dimethylarsinic acid by the gastrointestinal tract while only 2.2 min was required for the same level of absorption through the lungs. Dimethylarsinic acid like inorganic arsenic has a high affinity for rat erythrocytes and the concentration in all tissues decrease rapidly except in the blood where the half-life of arsenic parallels the half-life of the erythrocytes (Stevens et al., 1977).

Inhalation of arsenic trioxide, As_2O_3, with smelter dust results in the absorption of arsenic into the body and its biotransformation into organic derivatives. Smith et al. (1977) reported that human urine samples contained 50 to 70% of arsenic as dimethylarsinic acid, 20% as

methanearsonic acid and the remainder as inorganic arsenite and arsenate
after exposure to arsenic trioxide. Dimethylarsinic acid levels in the
urine respond to small changes in airborne concentrations of arsenic
trioxide. Urine concentrations of dimethylarsinic acid have been
proposed as an indicator for monitoring airborne exposure to arsenic
trioxide.

In animals, inorganic arsenic is apparently removed by two
processes. The first is the rapid absorption then assimilation into
the blood followed by removal in the kidneys and passage from the body
in the urine. The second process is much slower and involves the detoxi-
fication of the inorganic compounds by the conversion to methylated forms
(Crecelius, 1977). The methylated arsenic compounds are less toxic to
animals and, in addition, the methylated forms are rapidly excreted from
the body (Zingaro and Bottino, 1983).

TOXICITY OF ARSENIC

The toxicity of arsenic depends on the valency state. Arsenate
inhibits ATP synthesis by uncoupling oxidative phosphorylation leading
to the breakdown of energy metabolism (Craig, 1986). Arsenate may also
replace phosphate in substituted monosaccharides such as glucose-6-
phosphate yielding glucose-6-arsenate. Under standard conditions,
arsenite is more toxic than arsenate, to aquatic organisms. Arsenite
reacts with thiol groups present on active sites of many enzymes and
tissue proteins such as keratin in skin, nails and hair (Schroeder and
Balassa, 1966; Knowles and Benson, 1983). It covalently links to sulfur

atoms inactivating enzymes. In mammalian systems, arsenite has a longer half life than other arsenic species. Common symptoms of toxicity include chronic intoxication with decreased motor coordination, nervous disorders, respiratory distress and damage to kidneys and respiratory tract.

The toxicity of arsenic to aquatic plants and invertebrates is a function of pH and usually decreases with increasing pH reflecting a change in oxidation states. In addition, phosphate loading introduced to a culture medium produces an antagonistic effect on the toxicity of arsenic to aquatic plants. This is thought to be a result of competition of phosphate with arsenate for uptake.

The toxicity of arsenic has been suggested to be the result of arsenate reduction to arsenite. Arsenate is excreted through urine more readily than arsenite because of its poor affinity for thiol groups. Methylation of inorganic arsenic in vivo has been reported in animals and humans with organoarsenic compounds being much less toxic than the inorganic forms. The LD_{50} for dimethylarsinic (cacodylic) acid in rats range from 700 to 2600 mg kg^{-1} compared to methanearsonic acid (700 to 1800 mg kg^{-1}), potassium arsenite (14 mg kg^{-1}) and calcium arsenate (20 mg kg^{-1}) (Craig, 1986). The arsenic analogues of choline and betaine are considered nontoxic and can be fed in high amounts (percent level) to animals. They do not have the ability to bind to thiol groups and are resistant to conversion to the more toxic forms. Organoarsenic compounds which accumulate in seafoods (fish, crustaceans, aquatic plants) are rapidly excreted in unchanged forms by animals and humans.

The volatile arsine gases (LD_{50} in rats; 3 mg kg^{-1}) appear to be highly toxic to mammals inducing lysis of red blood cells. Although arsenic hydride (AsH_3) is extremely toxic, it is very unstable, and not typically found in nature. Arsines normally undergo alkylation and arylation reactions before being released into the environment. Further tests are needed to determine the toxicity of dimethylarsine and trimethylarsine with inhalation studies to test animals.

STABILITY OF ORGANOARSENIC COMPOUNDS

Both methanearsonic acid and dimethylarsinic acid are somewhat persistent in the environment. These compounds can be taken up by plants and not metabolized to any significant extent (Hiltbold, 1975). In mammals, methanearsonic acid may be methylated to dimethylarsinic acid, but direct demethylation of the latter has not been demonstrated (Stevens et al., 1977). Consumption of seafood (lobster, shrimp, crabmeat, flounder) containing organoarsenic compounds is excreted directly through the human body unchanged in chemical form. Uptake and digestion of organoarsenic compounds by waterfowl and wildlife near and within the agricultural evaporation ponds in the Tulare Lake Basin would also not be expected to be much of a threat.

Demethylation of organoarsenic compounds in both natural waters and soil has been demonstrated and is apparently widespread among many bacteria. Achromobacter sp., Flavobacterium sp., Nocardia sp., Pseudomonas sp., and Alicaligenes sp., isolated from soil and sediment demethylate methanearsonic acid at a rate of 3-5% per 48 h (Shariatpanahi

et al., 1981). Methanearsonic acid is transformed to arsenate and carbon dioxide.

GLOBAL CYCLE OF ARSENIC

The global cycle of arsenic is illustrated in Fig. 5. Many organisms including microorganisms, plants and invertebrates are involved in the distribution and cycling of this element. Arsenic can accumulate and be subject to various transformations including reduction, oxidation and methylation. The reduced form (arsenite) is considered more toxic than the oxidized species (arsenate) because it reacts with sulfhydryl groups of cysteine in proteins inactivating many enzymes.

In aquatic systems, arsenic tends to accumulate as complex organo-arsenic compounds with only a few being identified (e.g., arsenobetaine, arsenochloine and dimethylarsenosoribosides). Methanearsonic acid and dimethylarsinic acid are present in seawater and freshwater but appear to be degradation products of these complex organoarsenic compounds.

Arsenic is emitted into the atmosphere by high temperature processes such as coal-fired power generation plants, burning vegetation and volcanism. Inputs into the atmosphere include industrial and fossil fuel emission (780×10^8 g arsenic yr^{-1}), mining (28×10^8 g arsenic yr^{-1}) and continental and volcanic dust fluxes (28×10^8 g arsenic yr^{-1}) (Mackenzie et al., 1979).

Natural low temperature biomethylation also releases arsenic into the atmosphere. Microorganisms including bacteria, fungi and yeast form volatile methylated derivatives of arsenic under both aerobic and
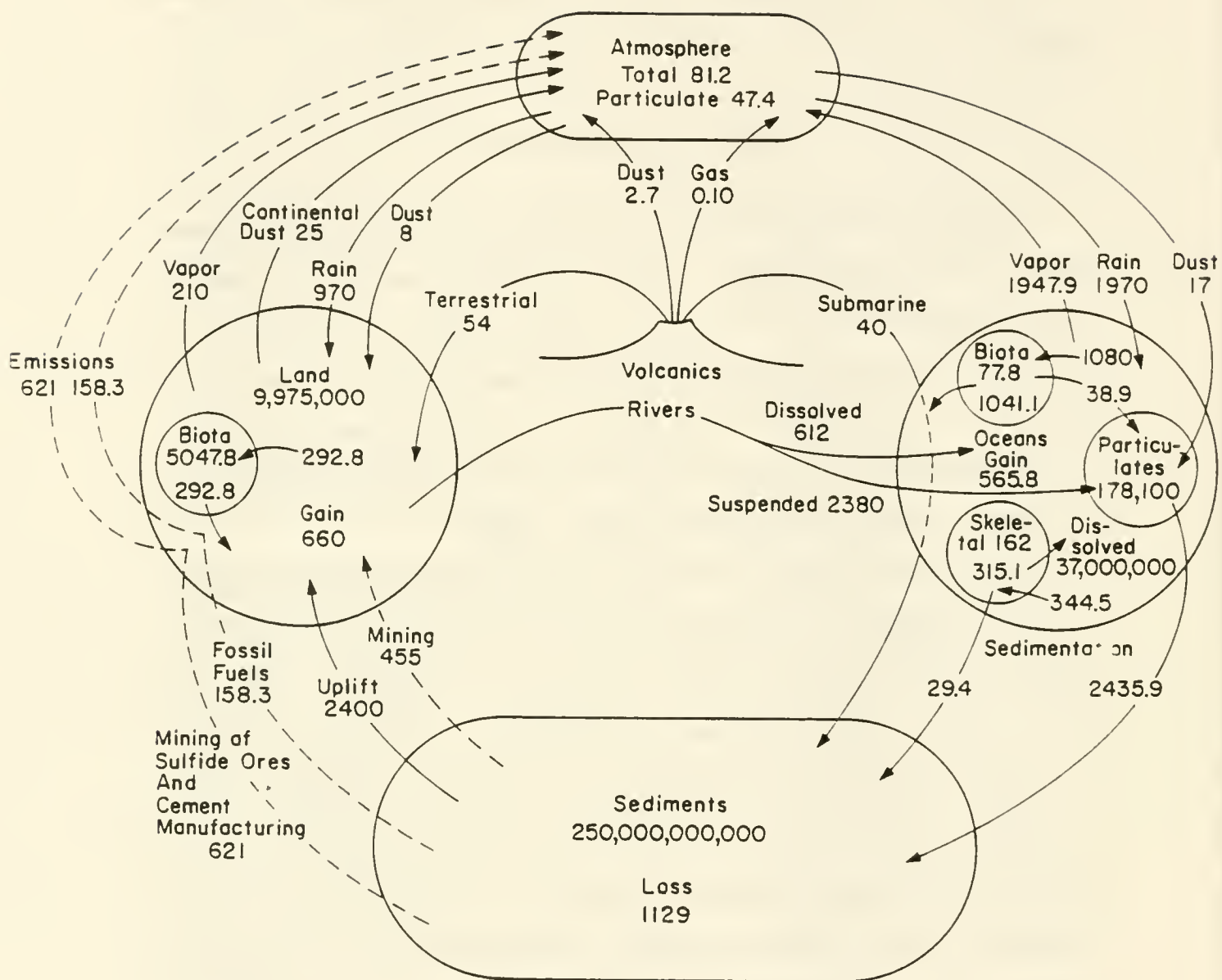
Fig. 5.   Global biogeochemical cycle of arsenic. Reservoir masses and
fluxes are expressed in units of 10^8 and 10^8 g/yr, respectively
(Mackenzie et al., 1979).

anaerobic conditions. Bacteria only produce dimethylarsine while fungi synthesize trimethylarsine. Dimethylarsine is an oxidation product of trimethylarsine and both compounds are subject to demethylation by soil bacteria. It is estimated that as much as 210×10^8 g of arsenic is lost to the atmosphere in the vapor state annually from the land surface (Mackenzie et al., 1979). The continental vapor flux is about eight times that of the continental dust flux indicating that the biogenic contribution may play a significant role in cycling of arsenic. It has not been established whether volatile arsenic can be relased by plants as appears to be the case for selenium.

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