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Biomethylation of Selenium and Tellurium: Microorganisms and Plants

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I. General Introduction

The metalloid elements have interested humans in many ways. In particular, those of the IUPAC Periodic Table Group 15 (As, Sb, Bi) and Group 16 (Se, Te) have long histories and many uses. All five of these metalloids are extensively produced and utilized by industry and in agriculture. A new and interesting application in molecular biology is that selenomethionine and telluromethionine have been used as isosteric analogues of methionine as heavy atom derivatives to facilitate structural analysis of proteins by X-ray diffraction.[1−4] In addition to their industrial/agricultural applications, there is a Janus-like situation in other interactions with humanity. On one hand, there are many medical uses including the well-known, over-the-counter preparation, Pepto-Bismol. On the other hand, these are toxic elements, and the name, arsenic, is synonymous with poison.

In one application, green-colored arsenical pigments had at one time been used extensively, even in foodstuff.[5] Wallpapers colored with arsenical pigments were very popular and as the 20th Century began, a unique type of poisoning came to light—inhabitants of areas decorated in this way suffered illness and death. An Italian physician, Bartolomeo Gosio, discovered that certain fungi produced a toxic, volatile arsenical gas when grown in the presence of inorganic arsenic. Under damp conditions, the fungi volatilized arsenic present in the wallpaper, and the so-called Gosio Gas was responsible for poisoning. In 1933, Gosio Gas was shown to be trimethylarsine.[6] One particularly active fungus then named *Penicillium brevicaule*, is now renamed as *Scopulariopsis brevicaulis*. Thus began the detailed study of the

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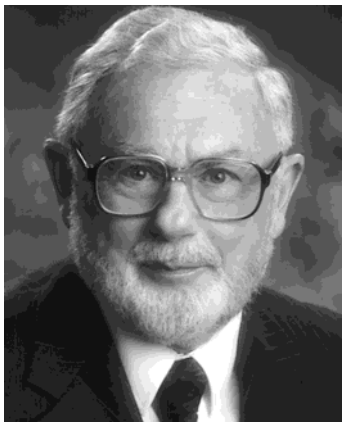
Thomas Chasteen completed a B.S. and M.S. at East Texas State University. After a 3-1/2 year stint in industry involving bench chemistry, lab coordinator, and ultimately director of research and development at a small company, he went on to a Ph.D. at the University of Colorado at Boulder under John Birks. He completed his doctorate in 1990 with work involving the determination of selenium and tellurium using fluorine-induced chemiluminescence. Now professor of chemistry at Sam Houston State University, he teaches analytical and instrumental chemistry, environmental studies, and honors courses for nonscience majors. Among others, his research interests include the determination of selenium and tellurium bioprocessed by bacteria.



Ronald Bentley received a Ph.D. degree from Imperial College, London, for work on penicillin chemistry and attempted synthesis (1943–1946). He held a Commonwealth Fund Fellowship at Columbia University's College of Physicians and Surgeons, 1946–1947, studying isotope tracer techniques and was responsible for a stable isotope tracer facility at The National Institute for Medical Research, London, 1948–1952. He joined the Faculty of the University of Pittsburgh in 1953 and was retired at age 70 in 1992 as Professor Emeritus. He held a John Simon Guggenheim Memorial Foundation Fellowship at the University of Lund, Sweden, from 1963 to 1964. His research concerned the biosynthesis of various secondary metabolites in fungi and the biosynthesis of ubiquinone and menaquinone (vitamin K_2). Other interests have included carbohydrate conformations and stereochemical implications in biology. Since retirement, he was one of six general editors for *The Oxford Dictionary of Biochemistry and Molecular Biology* (1997, revised, 2000) and has written on a variety of topics.

biomethylation of metalloid elements, a study that still continues and boasts an extensive literature. Biomethylation has considerable academic and practical implications; not the least is that biomethylation processes play important roles in the global cyclization of these elements.[7–9]

Biomethylation of the Periodic Table Group 15 metalloids by microorganisms has been reviewed recently.[10] The present article concerns the biomethylation of selenium and tellurium with the major emphasis on the conversion of inorganic forms of these metalloids to volatile materials by microorganisms and plants. One property of selenium has had a profound influence on humanity, namely, its photoconductivity. As early as 1884, a television system was devised relying on mechanical sequential scanning of an image with a selenium photocell translating the sequence of light values to corresponding electrical values. After transmission to a receiver, the sequence of light values was reproduced by a lamp. In 1926, J. L. Baird demonstrated the electrical transmission of moving pictures in half tones. It is said that in his training, Baird had devised an improved selenium cell and that this achievement led him to develop a very early form of "true" television.[11] Furthermore, selenium plays a fundamental role in the process of Xerography. It is difficult to imagine present-day life in a technology driven country with neither copying machines nor television, one technology still relying on selenium and the other profoundly influenced by selenium in its development. Few elements have had such an influence (for better or for worse!) on human lives.

II. Selenium

A. General Considerations

Compared with arsenic, selenium is much less abundant on earth, although selenium species are widely distributed; one well-known area with high selenium concentrations is the San Joaquin Valley of California, ~230 to 640 ppb Se in subsurface drainage water.[12] This element, first recognized in 1817 and named after the moon goddess, has found many uses in industry (e.g., the manufacture of ceramics and glass, in photoelectric cells and Xerography, in semiconductors, and the vulcanization of rubber), and a few in agriculture (selenium diethyldithiocarbamate is a fungicide) and medicine (e.g., selenium sulfide used as a shampoo for treatment of tinea versicolor). Moreover, selenium has a possible role as a protective agent in neurotoxicity[13] and in prevention of cancer.[14]

Several oxidation states are possible for selenium with the main oxidation numbers being −II (selenide), 0, +IV (selenite), and +VI (selenate) and the following redox processes are possible:

$$\underset{\text{selenide}}{Se^{2-}} \leftrightarrow \underset{\text{element}}{Se^{0}} \leftrightarrow \underset{\text{selenite}}{SeO_3^{2-}} \leftrightarrow \underset{\text{selenate}}{SeO_4^{2-}}$$

A significant difference from arsenic is that there are naturally occurring methylated forms in which two selenium atoms are linked together (see below).

B. Determination of Selenium

1. Introduction

Analytical methods for determination of selenium depend on whether the selenium compound is a nonpolar, volatile molecule or an ionizable, relatively polar molecule. The most commonly detected organoselenium compounds in environmental samples and as microbial products are dimethyl selenide

(CH_3SeCH_3, DMSe), dimethyl selenenyl sulfide (CH_3SeSCH_3, DMSeS), and dimethyl diselenide ($CH_3SeSeCH_3$, DMDSe).[15−20] The nomenclature selenenyl sulfide is preferred over other forms and was suggested 30 years ago by Klayman and Günther.[21] Less common are the more highly reduced and more reactive hydrogen selenide (H_2Se) and methane selenol (CH_3SeH).[22,23] Longer chain—and less volatile—mixed Se/S compounds, such as $CH_3SeSCH_2CH{=}CH_2$, have also been detected.[24,25]

In early work, Challenger and North[26] trapped DMSe gas, produced by fungi grown on bread, by reaction with Bignelli's solution (dissolved $HgCl_2$), and speciated the derivative by melting point. This method derived from their earlier work on the microbial formation of trimethylarsine.[6]

2. Use of Gas Chromatography (GC)

Volatile selenium compounds are most sensitively determined by gas chromatography (GC) since they are easily separated with capillary gas chromatographic columns. Modern organoselenium analysis with GC is exemplified by detection of DMSe using a flame ionization detector.[27] A sewage *Penicillium* strain produced DMSe when grown on a well-defined medium amended with selenate or selenite; methionine addition enhanced DMSe production. This determination was a fundamental step in determining volatile products of microbial interaction with toxic metalloids.[27]

GC with atomic emission detection[24,25,28] has allowed element specific analysis of complex mixtures of organosulfur and organoselenium compounds in garlic headspace, plant extracts, and human breath. A microwave emitter powers a plasma that destroys the eluent from a capillary column and in doing so causes emission from atomic species in the sample. Depending on the configuration of the monochromator, many atoms can be monitored simultaneously. Compound identity and structure of mixed S/Se and allyl containing compounds were confirmed by GC with mass spectrometric detection (GC-MS).[24,25,29] This work was also the first report of the production of DMSeS by a plant. More recently, DMSeS has also been found in marine samples,[15] and early reports of dimethyl selenone produced by sewage samples and therefore microbial in nature[20] were actually determinations of biogenic DMSeS.[30,31]

GC with fluorine-induced chemiluminescence detection has yielded good detection limits for selenium compounds and high selectivity over interfering compounds also present in culture headspace of facultative anaerobes and phototrophic bacteria.[31−33] In this system, molecular fluorine obtained dynamically via an electrical discharge of SF_6 is then mixed in a low-pressure reaction chamber with the gaseous output of a capillary GC column. Chemiluminescent light produced by this reaction is detected by a photomultiplier tube, and light production is proportional to analyte content over 3 orders of magnitude. To analyze headspace samples, one milliliter of gas was removed from bacterial headspace with a syringe and injected directly into a hot GC split/splitless injector of the GC. Bacterial organosulfur and organoselenium could be determined simultaneously in each chromatographic run. Organosulfur compounds (dimethyl sulfide, dimethyl disulfide, and even dimethyl trisulfide) often accompanied organoselenium production in many samples of a selenium-resistant bacterium isolated from Kesterson Reservoir in California and in work with photosynthetic bacteria amended with selenium salts. Detection limits were approximately 10 pg (10^{-12} g) per compound.[31]

GC with inductively coupled spectrometry−mass spectrometry (GC−ICP−MS) gave excellent detection of DMSe, DMSeS, and DMDSe in marine samples.[15,16] Collected water samples were purged with helium, and this gas was cryogenically trapped and then transported (frozen) for analysis. MS-based isotopic analysis allowed "correction for mass spectrometric interferences." Detection limits were in the femtomolar range (as Se) for 1 L liquid samples.

To follow the production and movement of Se in soil and amended biological systems, radioactively labeled Se (^{75}Se, a gamma emitter) in the selenite anion was used as an amendment. The radioactivity of Se-containing samples then provided Se content.[34] The presence of Se in samples from bacterial outgassing—both soils and laboratory cultures—has been determined in this way.[17,35]

3. Nonvolatile Selenium Compounds

There are several ways to detect nonvolatile selenium compounds, oxyanions, or Se in undetermined chemical forms.[36] From probably most common to least in the recent literature these methods are hydride generation atomic absorption spectrometry—HGAAS,[37−44] ICP or ICP/MS,[45,46] normal AAS,[47] LC/ion chromatography,[48,49] fluorometry,[50−53] X-ray absorption or fluorescence,[32,54−57] and neutron activation analysis.[58]

Mixtures of selenite, selenate, and selenium containing amino acids present a particularly onerous analytical task. Ion chromatographic separations using a reverse phase C_8 column with MS or ICP−MS detection have been used to determine these species simultaneously.[45] Ion exchange with postcolumn reactions and UV/Vis detection has been used to determine selenite and tellurite.[59] Selenomethionine, Se-allyl-DL-selenocysteine, *cis*-Se-1-propenyl-DL-selenocysteine, *trans*-Se-1-propenyl-DL-selenocysteine, Se-1-propyl-DL-selenocysteine, DL-selenoethionine in standards were separated is less than 17 min runs. Limits of detection were in the low parts per million range.[60]

Similarly, selenomethionine was identified at ppb levels in Indian mustard with liquid chromatography and ICP−MS detection and electrospray mass spectrometry.[61] A possible amino acid with a Se−S bridge was detected at low levels.

HGAAS can be used to determine selenite in solution. A book length review of HGAAS is an excellent resource in this field.[62] Selenium of other oxidation states must be converted to selenite (Se^{4+}) before hydride generation. Thus, elemental Se (Se^0) is oxidized to selenate, then reduced to selenite. In the hydride generation step, selenite in highly acidic aqueous solution is reduced with (almost universally)

sodium borohydride to H_2Se. Hydrogen selenide is purged from solution via a flowing inert gas into an optical cell heated either by flame or electrothermally (i.e., an electrically heated graphite furnace). The thermal decomposition of H_2Se produces the atomic Se species identical to that of normal AAS. These atoms are presented to a lamp beam of a selenium spectral line. Absorption of this light causes a measured drop in the light reaching the detector. This decrease in signal is proportional to the amount of selenium in the sample.

HGAAS in flow injection systems was used to determine Se in coastal seawaters off Brittany near Brest, France,[63] with detection limits of 21 ng L^{-1} Se(VI), 10 ng L^{-1} Se(IV), and 13 ng L^{-1} organic Se(−II). Selenite and selenate determination by alumina microcolumn separation followed by graphite furnace AAS detection showed that in natural tap water samples spiked with selenite and selenate, SeO_3^{2-} was oxidized to selenate by chlorine as a (municipally added) disinfectant and typically present at ~ 1 ppm.[49] This highlights the care that must be taken when speciating Se.

Though analytically less common recently, "normal" AAS is still used for Se determination.[64] In examining petrel nestlings' organs from birds collected in Antarctica in 1991/1992, it was found that low mercury content correlated with higher Se content.[47] Though this lends credence to theories that the presence of Se acts to detoxify Hg,[65−67] this same Se/Hg concentration relationship was not found for adult petrels. HGAAS replacement of AAS for metalloid analyses has occurred because of the greater sensitivity of HGAAS (ppb with HGAAS vs ppm working range for AAS), less chemical interference difficulties that require matching sample matrixes, and the rise in available hydride generation modules for commercial AAS instruments.

4. "Hyphenated" Methods

Increasingly, many techniques have been added together in a single method, leading to the so-called "hyphenated" instrumental methods. In one example, high performance liquid chromatography for separation, microwave irradiation to destroy selectively and aid in reduction, hydride generation to help reduce matrix interferences, and fluorescence spectrometry for sensitivity were combined.[68] As before, hydride generation requires Se as selenite in that step. The method, abbreviated HPLC−MW−HG−AFS, shows a 1.5 order of magnitude linear working curve (0.5 to 10 ppb Se) and detection limits of 0.2, 0.4, and 0.3 μg L^{-1} for selenite, selenocysteine, and selenate, respectively. This work may allow "single" analytical methods for samples containing selenium as oxyanions, Se-containing amino acids, and other organoselenium compounds. Other hyphenated metalloid detection methods include GC−MS[18,69,70] and HPLC−HG−MS[71] but have grown to include HPLC−ICP−MS,[72] GC−AED−MS,[29] GC−ICP−MS.[15,73−75]

5. Methods Using X-ray or Neutron Sources

X-ray absorption or X-ray fluorescence spectroscopy takes advantage of the slight differences in the chemical environment of metalloidal nuclei in different compounds to determine the form of the element present.[32,57,76] Using X-rays available from, for instance synchrotrons, Se-containing samples can be irradiated and the amount of X-rays absorbed or X-ray fluorescence emission detected used as a measure of the identity and rough quantification of Se present. Near-edge X-ray absorption spectroscopy probes the electronic (that is, valence and coordination) environment of Se and bulk samples. For instance, using spectra from standards, the following Se species can be differentiated in situ in biological samples: Se^0, SeO_3^{2-}, SeO_4^{2-}, DMSe, DMDSe, $(CH_3)_2SeO_2$, and various Se-containing amino acids such as selenocystine, selenocysteine, and selenomethionine.[32,56] Using near K-edge X-ray absorption spectroscopy, Van Fleet-Stalder et al.. examined harvested phototrophic bacterial cells, grown in aqueous solution amended with selenium oxyanions. There was a large percentage of Se^0 in cells from selenite-amended cultures and much less in selenate-amended samples. This confirms other observations that selenite rather than selenate is more easily microbially converted to Se^0. Also present was an ambiguous organoselenium chemical species perhaps selenomethionine or DMSe.[32] This method, therefore, can possibly be used to determine the presence of volatile selenium compounds not yet released by cells as they are generated in situ.

X-ray analysis close to the Se K-edge energies was used to image and speciate Se in a Se-concentrating plant, *Astragalus bisulcatus*. This work included pictures (100-μm resolution) showing relative content of organoselenium and selenate in plants hydroponically exposed to increasing selenate concentrations. These experiments ended with a final 5 μM selenate in the last week of 28 days of hydroponic exposure. Mature leaf tissues contained selenate but young leaves displayed deposits of organoselenium almost exclusively. It was possible that the ability to reduce selenate in Se-accumulator plants may be inducible or developmentally dependent.[77]

A sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, anaerobically reduced selenite and tellurite to the elemental forms; however, no volatile organometalloids were examined. The order of preference for Group 16 elements was Te(IV) > S(VI) < Se(IV), substantially out of order for these elements' oxyanions reduction potentials: Se(IV) > Te(IV) > S(VI). Elemental depositions in cultures were determined by X-ray diffraction X-ray absorption.[76]

Neutron activation analysis requires an intense source of neutrons: a nuclear reactor, charged particle accelerators, or radioactive neutron source—such as ^{238}Cf.[78] Se atoms bombarded with thermal neutrons (~0.04 eV) produce radioactive products with predictable decay rates. After a "cooling period," detection of the beta or gamma emissions can be used as a measure of the concentration of the irradiated nuclei. Thus, samples taken from the La Paz Lagoon—near the southern end of the Baja CA Peninsula—had Se concentrations higher than the crustal average of ~50 ppb.[79] This increase was possibly due to contributions from urban wastewater runoff or from

runoff from contributing arroyos (dry gulches or river beds). This highlights the difficulty in determining metalloidal contamination when both natural and anthropogenic sources are in close proximity.

When selenate was reduced chemically to selenite, there was an isotopic enrichment of the lighter isotopes of Se, i.e., the ratio of $^{80}Se/^{76}Se$ decreases (−5.5 ‰). This is analogous to isotopic fractionation seen in biological reduction of sulfur.[80] This process may be useful to determine sources and biogeochemical transformations of Se.

C. Nutritional Requirement for Selenium Compounds

The selenium level in plants used as foodstuffs depends on the plant species and the level of selenium in the soil. In fact, the global distribution of selenium varies widely. Some areas contain very low levels, a striking example being a soil-deficient area of Finland with a level of 0.005 ppm. At the other end of the scale, a level of 8000 ppm occurs in the Tuva area of Russia. Similarly, selenium levels vary in bodies of water, and the element may accumulate in evaporation ponds, reservoirs, and even oceanic bays. Some US waters contain as much as 400−9000 $\mu g\ L^{-1}$. The amounts of selenium in natural and environmental waters have been reviewed comprehensively.[81] Possible health effects from selenium in drinking water have been evaluated.[82] It appears that the US EPA regulation requiring no more than 50 $\mu g\ L^{-1}$ of selenium in drinking water is realistic; however, in Germany and Japan this limit is 10 $\mu g\ L^{-1}$.[83]

Both high and low soil levels of selenium pose dangers to animal and humans in terms of health. The poisoning of animals after ingestion of certain plants had been recognized for a long time. In fact, Marco Polo had described such a situation in the 13th Century in western China.[84,85] Early in the 20th Century, as the western parts of the Great Plains in the US came under settlement, stockmen and farmers were in some areas unable to graze livestock without losses; the problem was termed "alkali disease".[84] In tests with laboratory animals, feed samples were shown to contain a poison, and in 1933 the poisonous agent was identified as selenium. Although the livestock condition of "blind staggers" (a disease with acute onset, manifested by "blindness", headpressing, circling, dysphagia, and paralysis) was also said to be a result of selenosis, this conclusion has been challenged in a careful review of the historical record.[86] It is likely that many field cases of "blind staggers" were sulfate-related polioencephalomalacia and/or intoxication with plant alkaloids; the possible role of selenium is controversial.

Selenosis (selenium toxicity) has been observed in humans as well as in animals. High doses of selenium (> 900 $\mu g\ day^{-1}$) produce a toxic syndrome of dermatitis, loose hair, diseased nails, and a peripheral neuropathy.[87] However, human selenosis is rare, especially in the United States. A few cases have resulted from industrial accidents and ingestion of selenium supplements containing much more selenium than stated.[88,89] More recently, a 22-month-old child died following ingestion of "gun blue".[90] An endemic selenosis was discovered in Enshi county, Hubei province, people's Republic of China, in 1961. There was a 50% morbidity in 248 inhabitants of five villages. The most common sign of poisoning was loss of hair and nails and in areas of high incidence, lesions of the skin and nervous system. Selenium in the soil was derived by weathering of a stony coal, one sample of which contained more than 80 mg selenium g^{-1} coal. Uptake by crops was facilitated by the traditional use of lime as a fertilizer. As a result of drought and rice crop failure, the villagers were forced to eat more high-selenium vegetables and maize.[91] "Alkali disease" was observed in cattle in the area and eggs had low hatchability.

Reports have shown that rats' ability to eliminate selenium via bile was enhanced by simultaneous exposure to Group 15 metalloids. Bile content of injected selenite was increased with concomitant injection of arsenite and vice versa; however, simultaneous exposure to antimony or bismuth did not produce clear-cut trends. These excretions were accompanied by large amounts of nonprotein thiols thought to be glutathione degradation products which may provide evidence for the production of selenite-derived selenols in glutathione complexes.[92] This mechanistic hypothesis can be contrasted with results showing little increase in metallothionein-bound arsenic or selenium in rats exposed to these metalloids via injection.[93]

More recent work has shown that a mixed As/Se metabolic product is the likely means by which rabbits link the "mammalian metabolism of arsenite, selenite, and sulfur".[94] Seleno-bis(*S*-glutathionyl) arsinium ion $[(GS)_2AsSe^-]$ was detected in the bile of rabbits injected with approximately 0.6 mg kg^{-1} body weight arsenite and SeO_3^{2-}. Exposure (via injection) to either metalloid yielded some of that element in these animals' bile; however, equimolar simultaneous exposure to arsenic and selenium greatly increased the biliary elimination of this 1:1 As−Se compound. This reports goes a long way to explain the antagonism of simultaneous toxic doses of arsenic and selenium in mammals. The arsinium compound has also been synthesized and further characterized by X-ray absorption.[54]

Despite the fact that selenium is considered to be highly toxic, there is a well-established nutritional requirement for it in animals and humans at low concentrations.[85,95−100] In parts of the world where selenium concentrations are very low (e.g., China) a selenium deficiency occurs in association with Keshan disease (an endemic viral cardiomyopathy afflicting young women and children). It appears that under the conditions of selenium deficiency, avirulent Cocksackie viruses mutate into virulent forms. The selenium deficiency is a necessary but not sufficient condition to induce the disease. Dietary supplementation with selenium (50 $\mu g\ day^{-1}$) prevents but does not cure the cardiomyopathy. Further problems arise when there is an iodine deficiency in association with a selenium deficiency. Both of these conditions, as well as other factors (e.g., mycotoxins) appear to be involved in Kashin-Beck osteoarthropathy. This dis-

ease, originally investigated in Russia and now a problem in China, is characterized by atrophy, and degeneration and necrosis of cartilage leading to shortened stature.[85,99,100] Similar circumstances result in myxoedamatous endemic cretinism observed in some parts of the world.

The nutritional requirement for selenium derives from the fact that several animal and human enzymes are selenium-dependent and involve the selenium-containing amino acid analogue of cysteine, selenocysteine.[101] The same is true for microbial enzymes. Selenocysteine has assumed so much importance that it has been dubbed "the 21st proteinogenic amino acid".[102,103] Well-characterized materials containing selenocysteine include formate dehydrogenases, clostridial glycine reductases, hydrogenases of anaerobic bacteria, mammalian glutathione peroxidases, and thyroid hormone deiodinases.[101,104,105] Selenocysteine has also been identified in thioredoxin reductases, enzymes having particularly important metabolic roles with respect to cell growth and protection against oxidant damage and apoptosis.[106,107] Two other enzymes, a clostridial thiolase and β-hydroxybutyryl-CoA dehydrogenase, contain selenomethionine[104] and other proteins containing this amino acid and other selenium components are known.[101,104,108] Bacterial nicotinic acid hydroxylase and xanthine oxidase require a readily dissociable selenium compound. Moreover, selenium is also present in some bacterial tRNA species. The nucleoside, 5-methylaminomethyl-2-selenouridine, has been identified in *Escherichia coli*.[109]

Selenium metabolism has been extensively studied in animal systems,[85,95,96,105] and methylation of inorganic forms is regarded as a detoxification mechanism since the methylated forms are much less toxic than selenite or selenate.[110−113] The process proceeds through dimethyl selenide, $(CH_3)_2Se$, to the trimethylselenonium ion, $(CH_3)_3Se^+$, with *S*-adenosylmethionine (SAM) used as methyl donor. A specific methylase enzyme, *S*-adenosyl-L-methionine: thioether *S*-methyltransferase (EC 2.1.1.96) has been characterized.[114] The type reaction for this enzyme is with dimethyl sulfide, but dimethyl selenide is equally a substrate (the enzyme was formerly named as dimethyl selenide methyltransferase). Disruption of the metabolism of important trace elements such as selenium by other toxic metals and metalloids has been reviewed.[65]

It has been suggested that the proposed toxic character of the element Se is affected by the formation in vivo of highly reactive Se-containing species such as selenopersulfides or organoselenide anions. These yield superoxides and hydrogen peroxide while oxidizing thiols normally present. This catalytic process, when it exceeds the organism's ability to methylate Se metabolites, has been proposed to account for selenium's toxicity.[115] Spallholz has also proposed that this explains the antitumor activity of Se taken at dosages above those nutritionally required. A recent review of the importance of selenium's inhibition of cancer stated, "Clearly, doses above [selenium's recommended daily requirements] are needed to inhibit genetic damage and cancer".[14]

D. Selenium Reduction in Microorganisms

Many microorganisms tolerate selenium levels ranging from 5 μg L^{-1} to 2000 μg L^{-1} with transformation of this element occurring by oxidation−reduction reactions and by conversion of inorganic to organic forms especially by methylation.[116] The metabolic oxidation of elemental selenium has received almost no attention;[117] in contrast, reductive processes have been much studied. Since selenate and/or selenite reduction may play a role in biomethylation, some general work on this process will be noted. Early workers investigating microbial decomposition of selenium and tellurium salts[118−121] noted that bacterial reduction of selenium compounds tended to give reddish colors, while reduction of tellurium compounds gave dark gray materials. A review of this work is available;[122] eventually the use of selenite reduction in bacterial taxonomy was developed.[123] Selenite containing preparations are still used as enrichment media for the isolation of *Salmonella* and *Shigella* species. Milne[117] has listed bacteria, fungi, and yeasts reducing inorganic forms of selenium including work to about 1996. More recent examples are *Enterobacter cloacae* SLD1a-1, a facultative anaerobe isolated from the San Luis Drain in the San Joaquin Valley, California,[124] and *Ralstonia metallidurans* CH34 (formerly *Alcaligenes eutrophus* CH34).[50] These bacterial reductive processes have considerable potential to provide feasible and cost-effective technology for remediation purposes (see later).

The phototrophic *Rhodospirillum rubrum* reduces selenite to Se^0 particularly under anoxic phototrophic growth conditions.[125] The reduction is related to the growth kinetics, occurring only when cells reach the exponential-stationary phase transition. The cytoplasm contained particles carrying Se^0 leading to an increase in buoyant density (buoyancy). After completion of selenite reduction the buoyant density returned to that of controls. Hence, by some mechanism, Se^0 was expelled by *R. rubrum* across the plasma membrane and cell wall.

Little is known of the actual enzymology of the reductions. In *Rhodobacter sphaeroides* which shows an intrinsic, constitutive high-level resistance to selenite and selenate and to tellurite and tellurate, one mechanism involves a membrane-localized, reduced flavin adenine nucleotide dependent enzymatic reduction of the oxyanions to the metallic ground state with concomitant evolution of hydrogen gas from photoheterotrophically grown cells. In selenium-containing media, the cells become bright red.[126]

In general, it appears that denitrifying bacteria contain various nitrate reductases (both membrane-bound and periplasmic) that can utilize selenate and tellurite as electron acceptors, e.g., the enzymes of *Ra. eutropha*, *Paracoccus denitrificans*, *P. pantotrophus*.[127] The periplasmic nitrate reductase of *R. sphaeroides* f. sp. *denitrificans* IL 106 has been purified after histidine tagging. The activity of the enzyme in reducing selenate and tellurite was low, and the resistance of *R. sphaeroides* to these metalloids cannot be attributed to it.

Similarly, for the reduction of selenite to Se^0 by *E. cloacae* SLD1a-1, tentative evidence suggested that the responsible enzyme might be a nitrate or nitrite reductase of broad specificity.[124] An active process of denitrification was required for the production of selenium. Similar indications of a periplasmic nitrite reductase or component of the nitrite respiratory system were obtained for *Thauera selenatis*.[128] In other work, the selenite → selenium conversion by *Bacillus subtilis* was believed to require an inducible detoxification system rather than dissimilatory electron transport.[129]

A selenate reductase has been purified by conventional enzymological procedures and characterized. The enzyme was obtained from *T. selenatis*, an organism isolated from selenate contaminated wastewater in the San Joaquin Valley, California. This organism reduces selenate to selenite with nitrate and oxygen as alternative electron acceptors; however, the terminal reductases are different for nitrate than for selenate[12] with one located in the cytoplasmic membrane and then other in the periplasm. *T. selenatis* is relatively nonspecific with respect to electron donors; these materials include acetate, lactate, pyruvate, and other compounds. The purified enzyme is a trimer of α-, *β*-, *γ*-subunits with molecular masses of, respectively, 96, 40, and 23 kDa. Molybdenum, iron, acid-labile sulfur, and heme b are prosthetic group constituents, and the reductase is selenate-specific with chlorate, nitrate, and sulfate not being substrates.[130,131] The genes encoding the selenate reductase have been cloned and sequenced.[132]

E. Selenium Biomethylation

In the 19th Century, various investigators treated animals with selenium or tellurium compounds presumably in toxicity studies. In such experiments, strong garlic-like odors were often observed and were attributed to hydrogen selenide or hydrogen telluride.[26] However, Hofmeister suggested a different possibility. On the basis of inadequate evidence he believed that the volatile tellurium compound in dog breath was dimethyl telluride.[133] In 1902, Maassen grew Gosio's arsenic-volatilizing fungus in media containing selenium and tellurium compounds.[134] At that time, Gosio believed his volatile gas was diethylarsine and Maassen concluded that he had observed diethyl compounds. He attempted to make a distinction between animals carrying out various methylations and fungi carrying out ethylations.

In 1902, Rosenheim also described the action of "*Penicillium brevicaule*" on selenium and tellurium compounds.[135] The odor produced by growth of this fungus in the presence of selenium compounds was "of a very disagreeable character," somewhat like that of skatole (3-methylindole) or mercaptan, but from tellurium it was more garlic-like. These volatile materials were later conclusively identified as DMSe and dimethyl telluride, $(CH_3)_2Te$.[26,136]

As already noted, detailed examination of the microbial methylation of selenium, began with the aspiration of volatile compounds into Biginelli's solution ($HgCl_2$ in hydrochloric acid). Analysis of the precipitated mercuric chloride complexes indicated that the volatile compound was DMSe. As more precise methods for the analysis of headspace gases of various cultures became available, a second phase of selenium investigations began. These analytical developments have already been discussed and the impact of these methods has been analyzed.[31]

Doran and Alexander[22] found that resting cell suspensions of a soil *Corynebacterium* produced DMSe from selenate and selenite, elemental selenium, and from several seleno amino acids. Cell-free extracts produced DMSe from selenite and Se^0, the reaction being enhanced by *S*-adenosylmethionine. Moreover, resting cell suspensions of a methionine-utilizing *Pseudomonas* (*Pseudomonas A*) converted selenomethionine to DMSe.

In the previous year, sediments from Canadian lakes in areas known to be contaminated with metals (Sudbury area) produced DMSe and DMDSe when incubated in closed flasks with materials such as selenate/selenite, selenocysteine, selenourea and seleno-DL-methionine. An unidentified volatile material was also present.[137] Three bacteria (*Aeromonas sp.*, *Flavobacterium sp.*, and a possible *Pseudomonas sp.*)- and an unidentified fungus isolated from the sediments also produced the three volatile selenium compounds. These observations and those of Doran and Alexander were the first evidence for formation of DMDSe by a pure culture of a microorganism although its production in plants was known as early as 1968.[138,139]

Similarly, the activities of mixed microbial populations in sediments, sewage, soils, and waters toward selenium compounds have been investigated. When sewage sludge was incubated with sodium selenite or Se^0, production of DMSe, DMDSe, and an unknown component was observed.[20] With a selenite concentration of 1000 $\mu g\ g^{-1}$, and incubation in air, the major volatile material was DMDSe followed by the unknown and DMSe. From Se^0 (500 $\mu g\ g^{-1}$) sewage produced only DMSe. The yields of volatile selenium compounds were considerably reduced under anaerobic conditions (nitrogen atmosphere). Other investigators, using sewage sludge containing a significant level of selenium (1.5 ± 0.5 mg kg^{-1} dry weight) and with anaerobic incubation, found no volatile selenium species.[140] However, pure cultures of microbial species typically associated with sewage sludge did produce volatile selenium materials (see Table 1). Moreover, very small amounts of unidentified volatile selenium compounds were detected in landfill gases,[73] and volatile selenium derivatives were found in gases over certain hot springs in British Columbia[74] apparently as a result of microbial action.

Reamer and Zoller considered their unknown volatile compound to be dimethyl selenone, $(CH_3)_2SeO_2$.[20] And this identification of dimethyl selenone seemed of considerable significance since this compound had not previously been identified as a microbial metabolite. Moreover, Challenger's group had suggested that it was the last intermediate before the formation of DMSe (see later). At the time of Challenger's work, it had not been synthesized. However, the Reamer and Zoller identification has since been shown to be

Table 1. Biomethylation of Selenium to Volatile Products[a]

organism(s)	substrate	products			ref(s)
		DMSe	DMDSe	DMSeS	
Algae					
Chorella sp.	IV	+	+	+	141
cyanophyte-dominated Mat	IV	+	+	+	2
Bacteria					
Aeromonas sp. *VS6*	VI	+	+	+	23
Citrobacter freundii KS8	VI	+	+	+	23
Clostridium collagenovorans	IV	+	+		140
Corynebacterium sp.	VI	+	+		27
Desulfovibrio gigas	IV	+	+		140
Desulfovibrio vulgaris	IV	+	+		140
Enterobacter cloacae SLS1a-1	IV	+			17
Methanobacterium formicicum	IV	+	+		140
Methanosarcina barkeri	IV	+	+		140
Pseudomonas aeruginosa VS7	VI	+	+	+	23
Pseudomonas fluorescens K27	VI	+	+	+	23,30
Pseudomonas sp. VW1	VI	+	+	+	23
Rhodobacter sphaeroides	IV,VI, Se^0	+	+	+	32,33
Rhodocyclus tenuis	IV, VI, Se^0	+	+		33,151
Rhodospirillum rubrum S1	VI, Se^0	+	+		33,151
Fungi					
Acremonium falciforme	IV	+	+		18
Alternaria alternata	VI	+			179
Cephalosporium sp.	IV,VI	+			290
Fusarium sp.	IV,VI	+			290
Penicillium citrinum	IV	+	+		18
Penicillium sp.	IV	+			291
Penicillum sp.	IV	+			27,290
Scopulariopsis brevicaulis	IV	+			173
Humans					
	normal diet	+			292
Plants					
Allium ampeloprasum	Se amino acids	+	+	+	24
Salicornia bigelovii	−	+			170
Spartina alterniflora	−	+			215
Unidentified					
lake water	−		+		28
soils	Se-methionine	+	+		217
soils	IV	+			35
soils	−	+			230

[a] IV = selenite; VI = selenate Se^0 = elemental selenium; + = detected; − = unknown

incorrect; a sulfur atom (relative atomic mass, 32) was masquerading as two oxygen atoms (relative atomic mass, $2 \times 16 = 32$) in the mass spectrometry used to identify this unknown. The material was clearly DMSeS.[30] It is likely that the unknown described by Chau et al. much earlier was also DMSeS. In view of the chemical properties of dimethyl selenone, particularly the *melting point* of 153 °C, it is very unlikely to be detectable by gas chromatographic methods. Chasteen has analyzed the gas chromatographic and mass spectrometric data that led to the incorrect identification.[31] With pure cultures of selenium-resistant bacteria (collected at California's Kesterson and Volta reservoirs) the composition of headspace gases was determined (Table 1). The major volatile products, DMSe and DMDSe, were produced by all the organisms that were examined. Five of the six bacteria also produced some DMSeS.[30] More recently, others have also found biogenic DMSeS releases.[16,141,142]

The partitioning of DMSe between water and gaseous headspace in equilibrium (Henry's law constant, K_h) always favors the aqueous phase for this organoselenide with approximately 10 to 20 times as much dissolved in solution as is found in the headspace at normal biospheric temperatures.[143−145] There have been two efforts to measure this partition coefficient for dimethyl diselenide; however, one reported essentially the same K_h for DMDSe and DMSe[145] and the other described a degradation of DMDSe in aqueous solution before solution phase/gas-phase equilibration could be reached.[144]

Very recent work has shown that various phytoplankton in surface waters of the North Atlantic Ocean produced DMSe and DMSeS.[16] Statistical analysis showed a strong relationship between the coccolithophores, the microscopic plants that dominate the annual planktonic bloom in the North Atlantic, and both DMS and DMSe. This correlation suggested that higher populations of this plant meant increased DMSe production in the collected sample. DMSeS was also detected in these samples; however, it apparently did not significantly correlate with any planktonic species population. The authors propose that this meant that DMSeS was produced "by oxidation of methyl thiol and methylselenothiol moieties." A gas-phase reaction between organosulfides and organoselenides as a source of DMSeS had

earlier been proposed.[30] All organometalloidal volatile species in this marine study were found to be in the low picomolar range as determined in liquid samples and were analyzed by gas chromatography with inductively coupled plasma spectrometric detection.[16] DMS concentrations were thousands of times higher.

A further methylated selenium compound, methaneselenol, CH_3SeH, has been identified in a few, unidentified, selenium-resistant microorganisms isolated from reservoirs and a pond. Only small amounts of this metabolite were observed.[31] It was of interest that methylated sulfur species, dimethyl sulfide, dimethyl disulfide, and methanethiol were also produced.

Microbial biomethylation of selenium is widespread occurring with bacteria, fungi, and yeasts; algae, animals, plants, and *Tetrahymena thermophila* also biomethylate selenium or metabolize organic selenium compounds. Examples of the microbial biomethylation of inorganic selenium compounds are summarized in Table 1. It is of interest that rhizosphere bacteria enhanced the accumulation and volatilization of selenium by Indian mustard plants, *Brassica juncea L.* (see later).[61,146]

F. Selenium Bioremediation

1. Use of Microorganisms

Selenium has a very complex environmental behavior.[147,148] In view of the high levels of selenium in certain areas such as the Kesterson National Wildlife Refuge, CA,[149] the possibility that waters, soils, etc., contaminated with selenium, might be subject to bioremediation by the use of microorganisms has been investigated. One situation, the use of reduction, has already been noted. It is also clear that the use of microorganisms carrying out the methylation of selenium might have potential for bioremediation.[150,151] In this event, the selenium volatilized as methyl derivatives is dispersed to the atmosphere. DMSe does not undergo photolysis in the atmosphere but reacts with radicals (OH and NO_3) and ozone fairly quickly. The calculated lifetime in the lower troposphere is a "few hours or less".[152] The unknown degradation products may be sorbed onto submicrometer particles with a relatively long atmospheric residence time (about 1 week). Hence, the selenium can travel considerable distances and in so doing disperse, decreasing the local toxic effects by dilution.

Different soils show widely divergent rates for volatilization of inorganic selenium. Moreover, the rates are influenced by environmental factors (moisture, temperature, etc.), and the presence of a variety of organic materials.[20,153−160] A limited number of recent publications concerning bioremediation only by volatilization will be briefly discussed here; work prior to 1982 has been reviewed.[116]

A useful observation for possible bioremediation work is that selenium volatilization from pond water, primarily as DMSe, can be stimulated through specific amendments. A 2-fold increase was obtained from natural waters by addition of methionine or of *Alternaria alternata* cultures. A more impressive result was a dramatic increase in volatilization, as much as 60-fold, by addition of casein and egg albumin; gluten addition gave a 15-fold increase.[153] A model system developed for selenium volatilization from water was consistent with a role for protein as the source of methyl groups.[154] Similar stimulatory effects were observed with soil samples. In one case, addition of zein and L-methionine strongly increased volatilization, whereas DL-homocysteine had a smaller effect, thus suggesting a possible role for SAM.[161]

Cultures of an euryhaline alga (*Chlorella* sp.) from drainage pond waters contaminated with selenium produced DMSe, DMDSe, and DMSeS under aerobic conditions. At high selenium concentrations a red deposit, probably Se^0, was formed. The use of this alga for low-cost, in situ bioremediation was proposed.[141]

Laboratory cultures of cyanobacteria harvested from algal mats in the Tulare Lake Drainage District of California volatilized selenite added at different concentrations. The Se in this environment came from evaporation ponds holding agricultural drainage waters. The authors investigated detectable decrease in Se in the sequence of ponds downstream from the Se source and suggested that the elements were removed via a biological mechanism. Organometalloidal compounds DMSe, DMSeS, and DMDSe were accompanied by the microbially ubiquitous dimethyl disulfide as determined by gas chromatography−mass spectrometry. The biological precursors for volatile selenium compounds were probably methylselenomethionine and methylselenocysteine.[142]

In another case, the bacteria in a selenium-contaminated hypersaline evaporation pond (at Red Rock Ranch, CA) were of interest for bioremediation.[162] The dominant bacterial types were a previously unaffiliated group of uncultured bacteria (order, *Cytophagales*) followed by a group of cultured *γ-Proteobacteria* (related to *Halomonas* species). Isolates accumulated predominantly selenate and a "selenomethionine-like" material. Selenium was believed to be assimialted via the S assimilation pathway (see later).

The previously described facultative anaerobe, *E. cloacae* SLD1a-1[163] reduced selenate and selenite to Se^0 and also produced DMSe.[17] DMSe headspace production was measured by the determination of headspace gases purged with air and trapped on carbon cartridges which were later counted for the presence of radioactive ^{75}Se—added as part of the culture amendment—using a gamma counter. DMSe production was *inversely* proportional to selenite amendments for three added amounts, 1000, 100, and 10 μM. The authors suggest that this inverse relationship may be simply an effect of the toxicity of higher amounts of SeO_3^{2-} on the microbes growth. Other workers who have measured organoselenium in cells amended with selenite at two different levels (~10 and 1300 μM Se) also found a clear difference in organoselenium produced from this added selenite, with cells of the lower selenite amendment levels producing more organoselenium;[32] however, their in situ Se determination of harvested cells suggested that the organoselenium was probably present in cells as selenomethionine instead of DMSe. Dungan

and Frankenberger suggest that *if* these bioremediating bacteria produce Se^0 in the periplasmic space, they might then continue Se reduction to Se(−II) which could be methylated to form DMSe (or DMDSe or DMSeS although these authors did not detect any in the headspace) or used in formation of seleno-amino acids. Se^0 that was not further biologically reduced in this way could be "released extracellularly" and indeed both these reports describe red, elemental Se in their respective Se-amended cultures.

Dungan et al. have used a wind tunnel to measure the production of volatile Se from enclosed soils amended with (^{75}Se-labeled) selenite. The wind tunnel allowed for measurement of average air flow rate, soil and air temperature, solar radiation inside the enclosure, humidity, and permitted periodic sampling of air in the tunnel for determination of volatile Se. This innovative experiment showed that over a 135 day experiment, beginning with soil Se amendment, 84.5% of added Se was recovered. Approximately 50% of added Se was found in the top 5 cm of soil (total lysimeter depth of 40 cm deep and Se initially well mixed into the 5 cm topsoil), and 2.7% was volatilized and determined in the air flow. The remainder was found in lower soil profiles or removed as mobile dust as seen trapped in a filter. An incomplete mass balance (15% of added Se unaccounted for) suggested a loss of volatile Se in some unknown part of the apparatus.[35]

Martens and Suarez have examined the fate of DMSe and DMDSe injected into soil samples (or their headspace) in tubes containing well characterized soils.[37] While most of the added DMDSe was partitioned into the contained soil and later recovered as Se^0 and selenides, DMSe showed much less adsorption in analogous experiments. Carbon content had a strong effect on DMDSe sorption exhibiting a first-order relationship between remaining gas-phase DMDSe and added soil carbon in different samples. Soils with highest carbon content exhibited the most sorption of DMDSe at the 6 h end point. Earlier work had suggested that Se-containing amino acids, Se-cysteine, and Se-cystine were unstable in soils and aqueous solutions, respectively, and the result was formation of Se^0.[164] From a bioremediation standpoint, the authors suggest that efforts to volatilize Se in contaminated soils will necessarily require added carbon to enhance microbial methylation; however, this same process will simultaneously work to sorb DMDSe in soil profiles.

Zhang et al. have examined the fate of DMSe in soil and found that transport of injected DMSe was a function of moisture content: wet or flooded soils retained much more DMSe than dry soils.[165] Soils also retained more Se if MnO_2 as an oxidizing agent was present. Oxidized products of MnO_2 and DMSe were dimethyl selenoxide, $(CH_3)_2SeO$, and dimethyl selenone, $(CH_3)_2SeO_2$. The movement of volatile Se as DMSe in bioremediation settings may be influenced by this process. As already indicated, selenium content from dimethyl selenone was released as DMSe and DMDSe by a selenium-resistant bacterium grown anaerobically in laboratory cultures.[166] These results, coupled with the results of DMSe oxidation experiments,[165] suggest that even oxidation of reduced selenium species to more oxidized forms will not necessarily remove them from mixed cultures in bioremediating settings.

The *oxidation* of metallic (that is, elemental) metalloids to their oxyanionic forms is important from the standpoint of increased toxicity and yet at the same time increased bioavailability and therefore increased potential for biomethylation. Frankenberger and Arshad review the importance of this as well as other aspects of bioremediation of selenium-contaminated sediments and water.[110] Conversely, reduction and methylation of elemental selenium has also been reported.[33] This is described below in analogous experiments with elemental tellurium.

A numerical model has been used to describe Se volatilization from unsaturated soils.[160] Along with corresponding laboratory experiments involving soils (sandy loam at 21°C) containing 20 ppm added selenate, the results of the model compared favorably with laboratory experiments using SeO_4^{2-}-amended soils covered with unamended soil. Reduction and subsequent volatilization of Se was fast in uncovered soils: volatile Se—as DMSe—was detectable in 24 h and reached a maximum on the sixth day. Selenate-amended soil covered with unamended soil, however, showed almost no volatilization suggesting that "rapid demethylation of Se vapor appears to be the limiting step in the volatilization of Se from soil to the atmosphere".[160]

A pilot plant for remediation of selenium-containing drainage water from the San Joaquin Valley, CA, using the selenate-respiring *T. selenatis* gave a total recovery in reactor effluent of up to 96% (Se^0). The Se^0 could be removed with a precipitant-coagulant material. Approximately 11 000 L of drainage water could be treated each day.[12] Denitrification was also substantial: NO_2^- and NO_3^- exhibited a 98% reduction through the plant.

2. Selenium Phytoremediation

Bioremediation of selenium by plants (phytoremediation) relies primarily on the accumulation of nonvolatile organic selenium compounds rather than on formation of volatile materials for atmospheric dispersion. Since this review focuses on the volatile, methylated selenium compounds, other sources should be consulted for more information.[61,139,167−169]

It may be noted, however, that some attention has been paid to plant volatilization. Thus, the Se volatilization rate over 12 months for a soil−plant bioremediation system in a Se-contaminated agricultural region (the San Joaquin Valley)[170] indicated an average volatilization of 155 μg of Se m^{-2} d^{-1} under natural field conditions. This accounted for 6.5% of the Se input to the field, and the authors suggested that this could be increased by optimization of irrigation and addition of carbon sources. The movement of the volatile Se produced by this bioremediation was also extrapolated. Gas phase content of volatile Se (assumed to be mostly DMSe) was 37 times that of a unpolluted site.[171]

In a 36-ha constructed wetland designed to remove selenite for oil refinery wastewater, volatilization was

responsible for as much as 10 to 30% of the Se removed.[42] The remaining removed Se was deposited in plant tissue and immobilized in sediments. Inflows of 20−30 ppb Se were reduced to < 5 ppb in the outflow. The rates of Se volatilization in the wetland by five plant species as well as fungal mats and other nonvegetated sites were examined. Surprisingly, even open (water) channels showed significant Se volatilization as measured using experimental collection chambers which trapped gaseous Se-containing chemical species on carbon traps which were subsequently analyzed by HGAAS. This suggests significant microbial reduction and volatilization was occurring. Finally, these and other workers[172] have noted that soil moisture and depth are important in the volatilization process.

Since phytoremediation normally transfers selenium from soil etc. into plant biomass, an important component of the process is harvesting and disposal of this material. Recent work has shown that Indian mustard hydroponically grown in the presence of 3.7 ppm selenite accumulated over 90% of the growth medium's Se.[61] An intriguing possibility is that such biomass could be blended with forage for animals in selenium-deficient areas.[169] Work with the constructed wetland described immediately above showed that plant (and symbiotic wetland microbial populations) Se volatilization increased 2−4-fold when plant tops (shoots) were purposely removed.[42] In an integrated selenium remediation program—using wetlands to volatilize Se and plant biomass for enrichment of animal forage in selenium-deficient areas—one process could feed the other to the advantage of both.

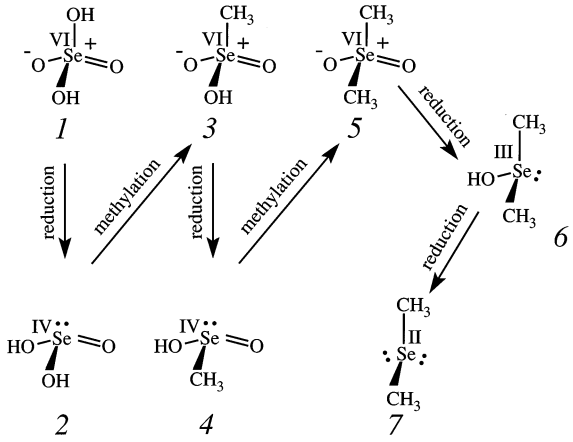
G. The Mechanism for Selenium Biomethylation

For the microbial methylation of arsenic, there is a single, generally accepted mechanism—that proposed by Challenger and his colleagues at Leeds.[173−175] The key feature is an alternation of reductive and methylating reactions, the latter involving SAM. With selenium, however, the situation is more complicated, and several possible mechanisms for biomethylation have been proposed. A further factor is that there is an extensive selenium metabolism in microorganisms and plants, some of which involves formation of volatile, methylated products.

1. The Challenger Mechanism

Early tracer experiments by the Leeds School established that the methyl group of methionine was utilized for selenium methylation by *S. brevicaulis* and *Aspergillus niger*.[176] Hence, SAM is most likely involved in these fungal methylations. While SAM is also generally assumed to be the usual methyl donor molecule, there is little direct evidence for this situation in other microorganisms. However, cell-free extracts of a *Corynebacterium* species showed a rapid formation of DMSe in the presence of SAM from either selenite or Se^0.[22,116] SAM has been established as methyl donor using enzyme extracts from mouse lung[114] and from the ciliate, *Tetrahymena thermophila*.[177] As with arsenic, there is some evidence for methylation by methyl cobalamin. Cell-free extracts

Scheme 1. Challenger Mechanism[a]

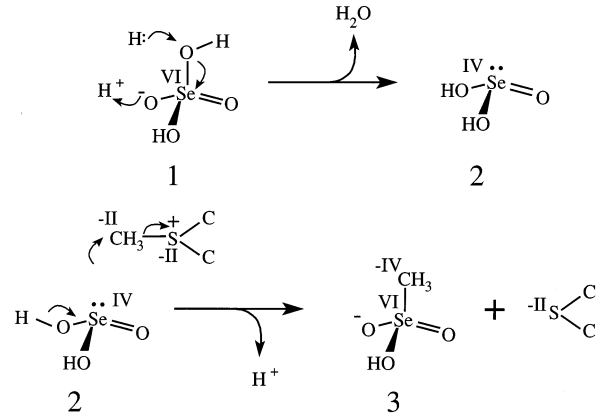


[a] Redrawn from ref 173.

of the anaerobe, *Methanobacterium bryantii*, converted [$^{14}CH_3$]cobalamin and selenate to a volatile ^{14}C-containing compound, presumed to be DMSe; in the presence of selenate, the normal production of methane by the extracts was inhibited.[178] Methyl cobalamin at 0.1 μM concentration stimulated DMSe formation by *Alternara alternata* approximately 5-fold. Higher concentrations of this methyl donor had only a small effect and direct methyl group transfer was not established by the use of isotopic tracers.[179]

By analogy with the proposed mechanism for arsenic methylation, it was suggested that a mechanism for selenium methylation from selenate involved reductive steps and the use of a methyl cation in much the same way as for arsenic methylation.[173−175] The individual steps (Scheme 1) are as follows: Selenate (**1**) → selenite (**2**) → methylselenonic acid (**3**) → methylseleninic acid (**4**) → dimethylselenone (**5**) → dimethylselenenic acid (**6**) → DMSe (**7**). In the two reduction steps to **5** the oxidation state of selenium changes from +VI to +IV. Each step leaves a lone electron pair on selenium. A basic mechanistic possibility for the conversion **1** → **2** is given in Scheme 2. Conversely, in each methylation step, an oxidation occurs as the oxidation number

Scheme 2. Subset of Challenger Mechanism Detailing a Reduction and Methylation[a]



[a] The conversion 1 → 2 diagrams a possible mechanism for reduction by a hydride ion. The conversion 2 → 3 diagrams the SAM-dependent methylation of a Se(IV) species. Abbreviations: SAM = CH_3-S-C_2; SAH = S-C_2.

increases from +IV to +VI (Scheme 1). Since the methylation is an oxidative process, and since no redox cofactor is involved, there must have been a concomitant reduction at some point. The carbon of the methyl group of SAM has oxidation number −II, but in the methylated products **3** and **5** the oxidation number of the transferred carbon is −IV (Scheme 2). The reductive component is thus accounted for. It is an interesting situation that an apparently simple methyl transferase system contains a "hidden" redox component.

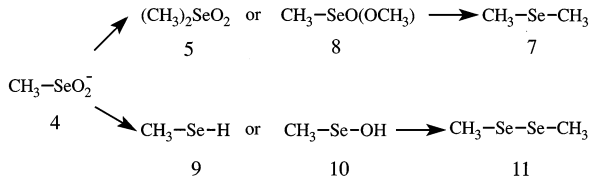
The only real evidence adduced for this mechanism was that alkane seleninic acids were methylated to methylalkyl selenides by *S. brevicaulis* and by some *Penicillium* species; the general reaction is $R-SeO_2H \rightarrow R-Se-CH_3$, where R = CH_3, C_2H_5, or C_3H_7. One possible difficulty was that another postulated intermediate, methaneseleninic acid, as its potassium salt, hydrolyzed readily to CH_3OH and H_2SeO_3.[174,180]

At the time of Challenger's work, the key component of the pathway, dimethyl selenone, (**5**) had not been obtained. Moreover, as already stated, later claims that dimethyl selenone was formed as a volatile selenium compound by microbial action on selenium compounds have been shown to be incorrect. However, chemical syntheses of dimethyl selenone are now available, and it has been shown that various phototrophic bacteria and a selenium-resistant bacterium convert dimethyl selenone to volatile products more efficiently than from inorganic sources.[33,166] For instance, the integrated peak area from chromatographic analysis of headspace samples from *Pseudomonas fluorescens* for DMSe was 2×10^7 in the presence of selenate and 1×10^{11} in the presence of dimethyl selenone, a 5000-fold increase.

However, while dimethyl selenone may well be a likely intermediate in the formation of DMSe etc., there is a complicating factor. *P. fluorescens* (and other organisms) produce dimethyl sulfide and dimethyl disulfide in addition to the volatile selenium components and these reduced sulfur species were shown to be capable of converting dimethyl selenone to DMSe and DMDSe in a chemical process. Under these conditions, however, the sulfur compounds did not reduce sodium selenate or sodium selenite. The overall process could still be regarded as a metabolic one since the formation of the reducing sulfur species is a biological process. While not definitive, these observations are generally supportive of a role for dimethyl selenone as required by the Challenger mechanism. The final step in the formation of volatile methylated compounds of selenium from selenium oxyanions may well be a combination of a direct reduction of dimethyl selenone with "metabolic" electrons and an "inorganic" reduction using electrons supplied by reduced compounds also being produced by a biological reductive process.[142,166]

Soil and sewage samples convert materials such as Se^0, selenate, selenomethionine, and selenocysteine to volatile selenium compounds, presumably by microbial action.[20,22] From Se^0, the only product was DMSe in very small amounts. Reamer and Zoller proposed a variation of the Challenger mechanism (Scheme 3) with a bifurcation at the level of methaneseleninic acid (**4**). In one direction, methylation to dimethyl selenone (**5**) or methyl methylselenite (**8**) was suggested followed by reduction to DMSe. In the other direction, a (reversible) reduction to methaneselenol (**9**) or methyl selenide (**10**) was proposed followed by methylation to DMDSe (**11**). This proposal accounted for the then supposed production of dimethyl selenone and for the effects of concentration of selenium substrates with sewage. Its present relevance is unclear.

Scheme 3. Reamer and Zoller's modified Challenger mechanism[a]



[a] From ref 20.

Another mechanistic proposal was based on the fact that with a soil *Corynebacterium* sp. the formation of DMSe from selenite was preceded by formation of Se^0.[116] Moreover, cell-free extracts of the bacterium converted both selenite and Se^0 to DMSe when SAM was present. It was proposed that Se^0 was reduced to a selenide form, H−Se−X, with the latter being converted successively to methane selenol and dimethyl selenide:

$$Se^{IV}O_3^{2-} \rightarrow Se^0 \rightarrow H-Se-X \rightarrow CH_3SeH \rightarrow (CH_3)_2Se$$

Doran offered no suggestions to account for the $Se^0 \rightarrow$ H−Se−X reaction. However, a few bacteria are said to reduce Se^0 to H_2Se,[117,181] and under anaerobic conditions, soil microflora produced H_2Se from Se^0.[22] In experiments with cell-free extracts of *Micrococcus lactilyticus* under H_2 atmosphere, it appeared that the reducing agent was dihydrogen:

$$Se^0 + H_2 \rightarrow HSe^- + H^+$$

Since the soil corynebacterium produced DMSe from selenite, an alternative explanation was possible. In rat liver and kidney cell-free systems, selenite was converted to DMSe with SAM as methyl donor and with a demonstrated role for glutathione (GSH) and GSH reductase.[182−184] In brief, a nonenzymatic reaction between GSH and selenite (or selenious acid) initiates the reaction sequences by formation of a "selenotrisulfide" derivative, G−S−Se−S−G:

$$4\,GSH + H_2SeO_3 \rightarrow G-S-Se-S-G + G-S-S-G + 3\,H_2O$$

Reduction of G−S−Se−S−G with GSH reductase yields a "selenopersulfide",

G−S−Se−H and further (anaerobic) reduction yields H_2Se:

$$G-S-Se-S-G + NADPH + H^+ \rightarrow G-S-Se-H + GSH + NADP^+$$

$$G{-}S{-}Se{-}H + NADPH + H^+ \rightarrow GSH + H{-}Se{-}H + NADP^+$$

GSH reductase was also implicated in selenite volatilization in isolated hepatocytes under aerobic conditions.[185] The oxidation number change for Se during these processes is from +IV to −II (selenite, +IV; GSSeSG, +II; GSSeH, 0; H_2Se, −II). Following formation of selenide, a SAM methyltransferase forms methaneselenol and DMSe.

2. Role of Selenoamino Acids

A difficulty with any mechanistic proposal is that selenium, unlike arsenic, has a marked tendency to form analogues of sulfur-containing amino acids as well as other selenium-containing metabolites. Hence, conversion of inorganic selenium to selenoamino acids etc., followed by subsequent metabolism, might be responsible for dimethyl selenide formation in microorganisms.[116] In the experiments with cell-free extracts of a *Corynebacterium* sp., selenocystine, selenomethionine, and some other organic selenium compounds were not converted to DMSe. At least, therefore, in this case formation of selenoamino acids is apparently ruled out.

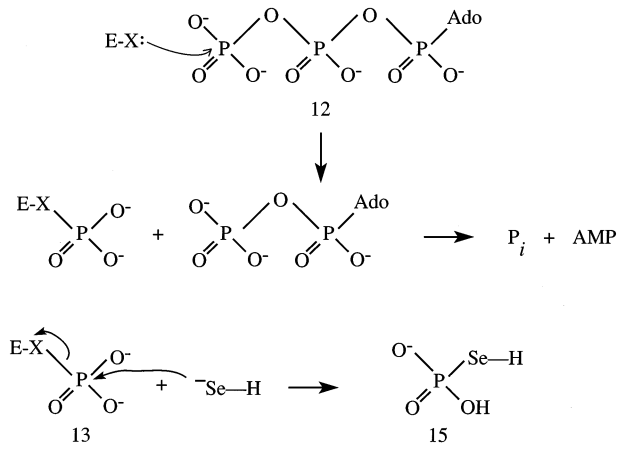
There are many pathways in bacteria, fungi, algae, plants, and animals for the conversion of inorganic selenium compounds to various selenoamino acids. Moreover, there are two mechanisms for the formation of proteins containing selenoamino acids. One is a bacterial, ribosomal pathway specifically involving selenocysteine that has been studied in bacteria with respect to genetics and molecular biology; there is an extensive literature (reviews).[101,102,105,186−188] Moreover, as already noted, selenocysteine is now regarded as a proteinogenic amino acid, "the 21st amino acid," in ribosomal protein biosynthesis.[102] It is incorporated into specific proteins by an unusual co-translational mechanism directed by the mRNA codon, UGA (normally, a terminator). In *E. coli*, a most substantial array of gene products is required for this incorporation. Only a brief summary of the very extensive literature, with emphasis on the pathway in *E. coli*, can be given (see below). The other pathway is a nonspecific incorporation of selenoamino acids into proteins in which enzymes involved in sulfur metabolism recognize selenium compounds as well. This latter possibility has been mostly studied in plants (see section "Selenium metabolism in plants") and to a lesser extent in bacteria.[189] Presumably, protein-bound selenoamino acids would, by normal "dynamic state" processes of degradation, become available as free amino acids and could be further transformed to volatile methyl derivatives. Analogously, this is also the source of volatile organosulfur compounds routinely released by many microbes.

a. The Role of Selenophosphate. Selenophosphate plays a most important role in the biosynthesis of selenocysteine;[190] it is instrumental in the conversion of a seryl-tRNA to a selenocysteyl-tRNA. Selenophosphate is synthesized by the *E. coli sel*D gene product, selenophosphate synthetase, in an ATP-requiring process;[191,192] the human enzyme has also been cloned and characterized.[193] The reaction is usually written as requiring selenide, with the formation of selenophosphate, orthophosphate and AMP in a 1:1:1 ratio:

$$ATP + \text{selenide} + H_2O \rightarrow \text{selenophosphate} + P_i + AMP$$

A nucleophilic group, X, of the enzyme (E) attacks the γ-phosphoryl group of ATP (**12**) forming a phosphoryl-enzyme intermediate (**13**) (Scheme 4). This

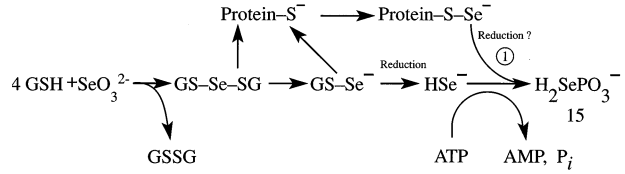
Scheme 4. Formation of Selenophosphate



intermediate reacts with selenide forming selenophosphate (**15**); the previously formed and enzyme-bound ADP (**14**) undergoes hydrolysis to P_i and AMP.[194,195] Certain amino acid residues in the enzyme protein such as Cys-17 and Lys-10 play important roles in the catalytic reaction, but it is not clear whether one of them provides the postulated nucleophilic group, X.

While free selenide is used as the selenium substrate for the in vitro study of selenophosphate synthetase the actual in vivo substrate remains unknown.[196] Experiments with the enzyme, rhodanese, in a selenium-substituted form (E-Se) showed that to some extent it could replace the high selenide concentrations normally used to assay the synthetase. Hence, a protein perselenide may be involved in a selenium delivery system.[196] The perselenide could be formed from GSSeSG and/or $GSSe^-$, and ultimately from selenite and GSH (Scheme 5). This

Scheme 5[a]



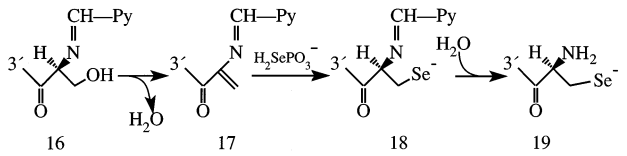
[a] Number one in circle = selenophosphate synthetase. Note that HSe^- might also be derived by reduction of Se^0. Redrawn from Figure 7 of ref 196.

process is similar to that previously discussed in animals.

b. The Role of tRNA. A unique $tRNA^{Sec}$, the *E. coli sel*C gene product, is first ligated with serine by action of seryl-tRNA synthetase. Under the influence

of selenocysteine synthase (*sel*A) the seryl residue of seryl-tRNA^{Sec} (**16**) bound to pyridoxal phosphate is converted to a selenocysteine residue by reaction with selenophosphate (Scheme 6). The selenocysteine-

Scheme 6[a]



[a] Abbreviations: 3′ terminus of tRNA^{Sec} = 3′; rest of pyridoxal phosphate structure = py.

tRNA^{Sec} (**19**) is then available for protein synthesis by way of its UCA anticodon. The process involves loss of water from the pyridoxal derivative to form (**17**) which is the actual species reacting with selenophosphate to form derivative (**18**). The latter is the pyridoxal phosphate adduct of selenocysteine- tRNA^{Sec} and is converted to (**19**). Note that while free selenocysteine is also synthesized by a different mechanism in *E. coli* and other bacteria,[189] the free amino acid cannot be ligated to the tRNA^{Sec}.

c. Decoding UGA in mRNA. The UGA codon (normally a termination unit) is recognized by the corresponding anticodon of selenocysteyl-tRNA^{Sec}. A special selenocysteine insertion sequence (SECIS) occurs in the 3′-untranslated region of the mRNA. This secondary structure is recognized by a specific translation factor, SELB (the *E. coli sel*B gene product) that directs the selenocysteyl-tRNA^{Sec} to the ribosome. The action of SELB is GTP-dependent. Seryl-tRNA^{Sec} shows little or no binding to SELB.

H. Selenium Metabolism in Plants

1. General Comments

Since a recent, comprehensive review is available[168] only a brief account of selenium metabolism in plants will be given here with the focus on formation of precursors for volatile compounds. In plants and algae, many selenoamino acids and other selenium-containing metabolites are formed from selenate/selenite. Plants are generally tolerant of selenium and some of them, termed accumulators,[168,197] assimilate inorganic selenium so well that, as already noted, their use in phytoremediation has been explored.[159,167]

Since plants frequently occur in symbiotic relationships with bacteria, one question is whether these rhizosphere bacteria play any part in selenium volatilization. In at least one case, that of pickleweed (*Salicornia bigelovii* Torr.), recent work using X-ray absorption spectroscopy for selenium speciation indicated an enhanced capacity to convert selenate to volatiles that was independent of rhizosphere microbes.[198] In other cases, the rhizosphere organisms facilitate selenate (but not selenite) uptake. However, they apparently play no role in the biosynthesis of volatile methyl compounds. Volatilization rates are higher for Indian mustard plants when supplied with selenite.[199] Selenite amended plants tended to accumulate selenomethionine and the rate of volatilization from selenate depended on the rate of its reduction to selenite.[199] In two wetland plants, salt-marsh bulrush (*Scirpus robustus*) and rabbit-foot grass (*Polypogon monspeliensis*) rhizosphere bacteria promoted accumulation of both selenium and mercury.[200] Phytoremediation of these elements in constructed wetlands may be enhanced by the rhizosphere bacteria. Unlike bacteria, plants apparently do not reduce inorganic forms of selenium to Se^0.

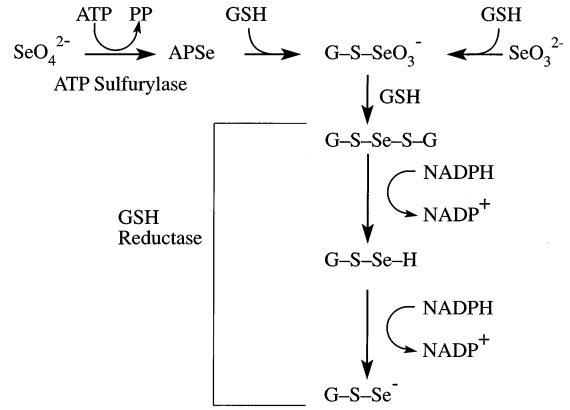
Essential selenoproteins synthesized by the ribosomal co-translational pathway are apparently not present in plants.[168] However, there is extensive, nonspecific incorporation of selenium into selenoamino acids and proteins. In general, the resemblance between sulfur and selenium leads to the conclusion that selenium is metabolized by enzymes of the sulfur assimilation pathways; the enzymes show little ability to discriminate between sulfur and selenium compounds. It appears that selenomethionine and Se-methylselenomethionine are the precursors for DMSe formation in plants.

In contrast to bacteria, plants form a reactive and somewhat unstable selenium donor compound, adenosine 5′-phosphoselenate (APSe), by use of ATP sulfurylase, EC 2.7.7.4.[201]

$$ATP + SeO_4^{2-} \rightarrow P{-}P + {}^{-}O{-}Se(O_2){-}O{-}P(O_2^{-}){-}Ado$$

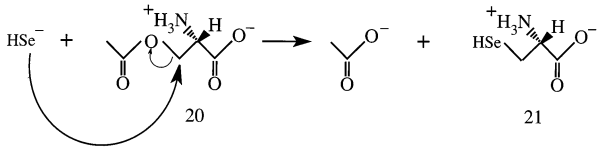
In a series of reactions (both enzymatic and nonenzymatic) involving GSH and GSH reductase, a GSH-conjugated selenide is formed; selenite can also enter this sequence (Scheme 7) (compare the similar

Scheme 7. Formation of GSH-Conjugated Selenide



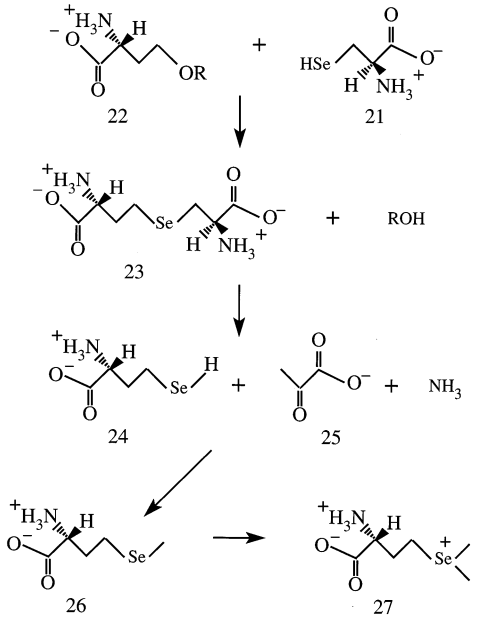
reaction sequence presented earlier). The GSH-conjugated selenide, or selenide ion itself, reacts with *O*-acetylserine (**20**) under the influence of cysteine synthase, EC 4.2.99.8, hence leading to selenocysteine (**21**) (Scheme 8).

Scheme 8. Formation of Selenocysteine



In turn, selenocysteine (**21**) after reaction with a homoserine derivative (e.g., succinyl- or phosphoryl-homoserine, **22**), can be transformed to selenocystathione (**23**), selenohomocysteine (**24**) (also formed are pyruvate (**25**) and ammonia), selenomethionine (**26**) and *Se*-methylselenomethionine (**27**) using the known reactions of the sulfur pathway (Scheme 9):

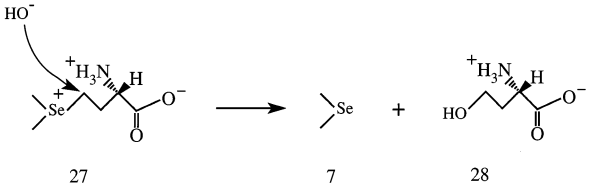
Scheme 9. Formation of Se-Methylselenomethionine



Note that compound (**27**) usually described as *Se*-methylselenomethionine is actually a selenonium compound with a dimethylseleno structure and with the selenium carrying a positive charge.

In early experiments, leaves of cabbage (a nonaccumulator of selenium) were shown to convert *Se*-methylselenomethionine (**27**) to dimethyl selenide (**7**) (Scheme 10). An isolated enzyme fraction also produced homoserine (**28**) and was apparently identical to *S*-methylmethionine sulfonium salt hydrolase (adenosylmethionine hydrolase) EC 3.3.1.2.[202,203] This enzyme is also known to form dimethyl sulfide and homoserine in onion seedlings.[204] An alternative possibility was suggested for an accumulator species forming DMDSe.[205] *Se*-methylseleno-cysteine might form a corresponding selenoxide, with hydrolysis of the latter forming DMDSe. Although there are chemical analogies for this pathway, it has apparently not been demonstrated with enzymes.

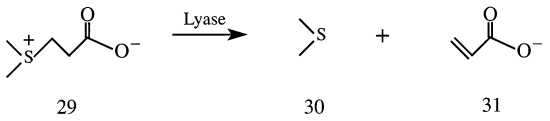
Scheme 10. Conversion of Se-Methylselenomethionine to DMSe



2. A Possible Role for Dimethylselenoniopropionate

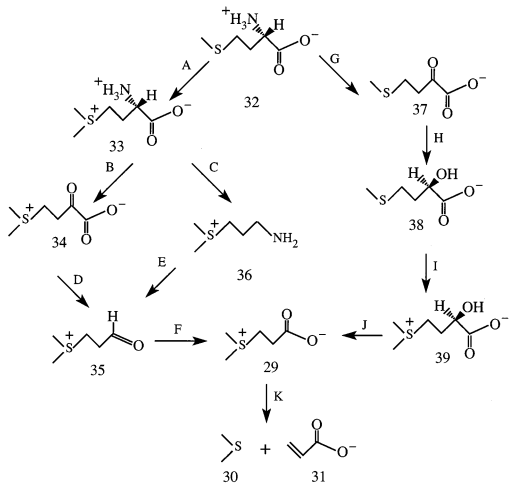
a. Plants. Yet one more possible pathway for dimethyl selenide formation in plants and possibly microorganisms comes from extensive studies of the formation of dimethyl sulfide in certain plants and marine algae. Dimethylsulfoniopropionate (**29**) is the critical precursor for dimethyl sulfide in this pathway.[206] It is cleaved by the action of a lyase enzyme with the formation of dimethyl sulfide (**30**) and acrylic acid (**31**) (Scheme 11). The responsible enzyme is usually termed dimethylsulfoniopropionate lyase but was earlier referred to as dimethylpropiothetin dethiomethylase, EC 4.4.1.3.

Scheme 11. Conversion of Dimethylsulfoniopropionate to DMS



Dimethylsulfoniopropionate (**29**) is formed from methionine (**32**) by a variety of different pathways (Scheme 12). Only a brief account of them will be given here. In plants, the first step is methylation to form *S*-methyl-L-methionine (**33**). The enzyme, SAM: L-methionine *S*-methyltransferase, EC 2.1.1.12, was purified to homogenity (620-fold) from leaves of *Wollastonia biflora*.[207] It was apparently a tetramer of 115-kDa subunits. The methylated product (**33**) is converted to dimethylsulfoniopropionaldehyde, DMSP-ald (**35**), by mechanisms not fully understood. The

Scheme 12[a]



[a] Letters refer to enzymes as follows (where available, EC numbers are provided): A, methionine *S*-methyltransferase, EC 2.1.1.12 (adenosyl-L-methionine: L-methionine *S*-methyltransferase); B, transaminase or transaminase/decarboxylase; C, *S*-methyl-L-methionine decarboxylase; D, decarboxylase or transaminase/decarboxylase; E, Dimethylsulfoniopropionate-amine dehydrogenase (?) or aminotransferase (?); F, betaine aldehyde dehydrogenase, EC 1.2.1.8; G, methionine aminotransferase (also low activiity from a nonspecific L-amino acid oxidase); H, 4-methylthio-2-oxobutyrate reductase; I, 4-methylthio-2-hydroxybutyrate methyltransferase; J, oxidative decarboxylation (?); K, dimethylsulfoniopropionate-lyase, EC 4.4.1.3 (dimethylpropiothetin dethiomethylase). The pathway via **37**− **39** occurs in algae (ref 211), that via **36** in *Spartina alterniflora* (ref 210) and that via **34** in *Wollastonia biflora* (ref 208). The unstable compound **34** may be channeled through a special transaminase/decarboxylase complex, or may exist as a bound intermediate of a single enzyme catalyzing an unusual transamination/decarboxylation.

unstable 4-dimethylsulfonio-2-oxobutyrate (**34**) may be an intermediate formed by transamination and decarboxylation possibly by the same enzyme or by a transaminas/decarboxylase complex.[208] The DMSP-ald (**35**) is oxidized to DMSP (**29**) by betaine-aldehyde dehydrogenase, EC 1.2.1.8.[209]

A different route to DMSP-ald is followed by *Spartina alterniflora* where *S*-methyl-L-methionine (**33**) undergoes decarboxylation to 3-dimethylsulfoniopropylamine (**36**) (Scheme 12).[210] There are various possible mechanisms by which **36** might be converted to DMSP-ald (**35**) including an amine oxidase, amino transferase or dehydrogenase enzyme activity.

In marine chlorophyte algae a very different process occurs (Scheme 12). By action of a methionine aminotransferase, methionine loses the NH_2 group to form 4-methylthio-2-oxobutyrate (**37**), and the latter is reduced to the corresponding hydroxybutyrate (**38**). Following methylation at the sulfur atom to form 4-dimethylsulfonio-2-hydroxybutyrate (**39**), there is likely an oxidative decarboxylation to DMSO (**29**). The biologically active hydroxybutyrate structures **38** and **39** were D enantiomers.[211]

To what extent can selenium analogues replace the sulfur structures in these pathways? There is some evidence for a dimethylselenoniopropionate (DMSeP) pathway in some plants and microorganisms.[212]

Selenomethionine →
Se-Methylselenomethionine → DMSeP → DMSe

It is known that plants supplied with selenomethionine produce volatile selenium compounds more rapidly than those treated with inorganic selenium.[167] Moreover, methylation of selenomethionine to *Se*-methylselenomethionine can be carried out by SAM: L-methionine *S*-methyltransferase (EC 2.1.1.12) from *Wollastonia biflora*.[207] It may also be noted that enzymes transferring methyl groups to selenocysteine have been identified in plants; the methyl donor is *S*-methylmethionine.[213,214] Further evidence is that DMSeP has been identified in *Sp. alterniflora* (salt marsh cordgrass)[215] The DMSeP level is highest in plants grown in full-strength seawater with a high selenium concentration (50 mg L^{-1}). The expected precursor, *Se*-methylselenomethionine, was also tentatively identified in this plant. Low levels of DMSeP have also been identified in shoots of Indian mustard plants.[216] Moreover, when various possible precursors of volatile selenium compounds were supplied to such plants, the rate of volatilization was highest with DMSeP:

DMSeP > selenomethionine >
selenite > selenate

b. Microorganisms. There is also some evidence for a DMSeP pathway in some microorganisms. DMSeP and *Se*-methylselenomethionine were found in soil samples after "spiking" with selenomethionine. These materials were not the stable forms of organic selenium in the soil; they were rapidly converted to DMSe and DMDSe, probably as follows:[217]

Selenomethionine → Se-methylselenomethionine → DMDSe

↓

Dimethylselenoniopropionate → DMSe

These transformations are, presumably, to be attributed to the actions of soil microorganisms suggesting that a microbial pathway through DMSeP is possible.

Support for such a possibility comes from experiments with bacterial enzymes from two species of marine bacteria, *Alcaligenes* sp., strain M3A (isolated from estaurine surface sediments) and *P. doudoroffii* (isolated from seawater). These bacteria contain dimethylsulfoniopropionate lyase, decomposing DMSP to dimethyl sulfide and acrylic acid.[218−222] Moreover, the DMSP lyases from both organisms (induced by DMSP) utilized the selenium analogue in the same way:

$$\text{DMSeP} \rightarrow \text{DMSe} + \text{acrylic acid}$$

In *Alcaligenes faecalis* M3A and in strain LFR of α-*Proteobacteria*, the acrylic acid is further metabolized to β-hydroxypropionate.[222,223] Hence, supposing that microorganisms do produce dimethylselenoniopropionate, the use of this precursor to form volatile selenium compounds is possible.

Less convincing support for the pathway is provided by work with a euryhaline alga (*Chlorella* sp.) isolated from a Se-laden evaporation pond. When cultures were amended with selenium compounds, this alga was active in the production of DMSe, DMDSe, and DMSeS and, as well, putative selenonium precursors. There was little accumulation of selenomethionine. By analogy with observations on the formation of dimethyl sulfide, it was believed that DMSeP could have been present as well as *Se*-methylselenomethionine. Only tentative experimental evidence for the presence of these compounds was obtained;[141] however, others have reported these same selenium compounds as well as dimethyl selenoxide in soils.[217]

In other work, a filamentous cyanophyte-dominated mat was collected from evaporation basins of the Tulare Lake Drainage District where selenium levels had shown a decreasing trend. This "attenuation" of waterborne selenium was most likely due to biological processes. The cultured organisms produced volatile selenium compounds as well as dimethyl sulfide. Evidence was obtained for the presence of *Se*-methylselenomethionine and/or trimethylselenonium ion. However, DMSeP did not appear to be the precursor for the selenium volatiles, although *Se*-methylselenomethionine was.[142] In this case, it is possible that hydrolysis of *Se*-methylselenomethionine by adenosylmethionine hydrolase (see earlier) may have been involved.

III. Tellurium

A. General Considerations

The discovery of the element, tellurium, is attributed to a Transylvanian mining inspector in 1782.

However, the name, tellurium (from Latin, *tellus*, earth) was not assigned by Klaproth until 1798. He apparently contrasted tellurium with uranium, the latter name deriving from the Greek word for heaven. Although not a particularly abundant element, tellurium does occur widely on earth. There is no single application for tellurium requiring large amounts. In fact, its proposed use as a gasoline antiknock agent (via diethyl telluride) was apparently sidelined in the early 1920s because of *low availability*; a further factor was the obnoxious garlic odor of this proposed additive.[224] The experimental records of the investigator (Thomas Midgley) still stank 17 years after the work was terminated. Tellurium is used in various alloys and glasses, and bismuth and lead tellurides are semiconductors used in thermoelectric devices. An interesting use of tellurium is in the structural analysis of proteins by X-ray diffraction (see earlier).

B. Determination of Tellurium

Almost all of the analytical methods for Se determination can be applied to Te determination as well. Atomic absorption spectrometry,[47,225] inductively coupled plasma spectrometry,[45,46,226,227] and chromatographic methods have all found applications with Te oxyanions,[48,49,59] volatiles,[70,228] Te-containing amino acids,[229] and undefined/unspeciated environmental samples containing organotellurium.[230]

ICP−MS determination of Te in groundwater[226] and Te oxyanions determined by HPLC with post-column complexation and UV/Vis detection have been used in an analogous way to Se analysis to address complex or interfering matrixes.[59] Ion chromatography with ICP−MS detection has also been used for TeO_3^{2-} and TeO_4^{2-} determination,[225] and these same workers determined dimethyl telluride (DMTe) via purging water samples followed by analysis via graphite furnace atomic absorption spectrometry.

DMSe and DMTe were determined in hot springs in excellent field work by Hirner et al.[74] DMTe was present at very low part per trillion concentrations (tens or hundreds of ng of Te/kg of solution). This can be contrasted with anthropogenic sources—sewage, polluted soils, and landfill gases—in which these same researchers found DMTe at much higher concentrations.[73,75,230] This report from DMTe detection in pristine settings is apparently the first determination of this organometalloid in the (unpolluted, geothermal) environment. Hot spring gases were collected by various means and analyzed by gas chromatography with inductively coupled plasma/mass spectrometry.[74]

Very recently, DMTe formed by a Te-resistant facultative anaerobe was identified by GC-MS and its production was followed hourly using fluorine-induced chemiluminescence[70] in a manner similar to the method for volatile selenium compounds (see above). Biologically produced Te^0 has been determined by these same workers using HGAAS. A mass balance showed that for 0.1 mM tellurite-amended bacterial culture taken into stationary phase, an average of 34% of added Te was recovered as Te^0 or in/on collected cells.[70]

C. Nutritional and Medical Considerations for Tellurium Compounds

Tellurium is generally regarded as a toxic metalloid; its biological effects have been reviewed.[231] Elemental tellurium is less toxic than the soluble oxyanions but can be persistent. Treatment of patients with syphilis by injection of Te^0 suspensions led to a garlic odor in breath two years after the end of the treatments (other examples of "garlic breath" are noted later). Nonobstructive hydrocephalus was present in new-born rats if the mother received Te^0 in the diet during gestation. Te^0 levels of from 500 to 3500 ppm were used.[232] In other work, the diets of developing rats with diets containing 1.1% Te^0 led to a highly synchronous primary demyelination of peripheral nerves. This effect decreased after 7 days and remyelination then began (even with the continued dietary presence of Te^0). A metabolite of Te^0 (described as tellurite, Te^{4+}) was formed and was an inhibitor of squalene epoxidase, an important enzyme in cholesterol biosynthesis.[233] This model is well suited for examination of metabolic alterations accompanying primary demyelination and remyelination.

Early evaluation of tellurium toxicity showed that tellurite was more toxic to most organisms than tellurate.[234] This conclusion has been largely borne out in more recent bacterial studies even though tellurite salts are less water soluble than those of tellurate.[70] With a metalloid-resistant bacterium, and using specific growth rates as a measure of relative toxicity, tellurite was more toxic than tellurate, and mixed Te amendments (TeO_3^{2-}/TeO_4^{2-}) showed a synergistic toxic effect.

In rats, a comparison of selenate, selenite, and tellurite found selenite substantially the most toxic. Selenite mortality for male rats was 50% in 58 days at a daily dosage of 50 ppm Se as compared to over 800 days for either selenate or tellurite augmentation.[235] Earlier, tellurium was found to be most heavily concentrated in the livers of rats dosed with tellurite. Half-lives in various organs ranged from about 8 days in the lungs to 20 days in the spleen. Estimates of 600 days for Te half-lives in bone were reported but with a very large standard error of estimate.[236] Most recently, treatment of rats with 0.1 and 0.4 mg/kg sodium tellurite resulted in impairment of learning and spatial memory.[237]

There have been limited medical uses for potassium and sodium tellurate. The 1899 Merck Manual indicates that the potassium salt was "antihidrotic" (i.e., anhidrotic, a material hindering the secretion of sweat) and was used for "night sweats of phthisis".[238] In addition, the sodium salt was antiseptic and antipyretic and was also used to treat gastric ulcerations, rheumatism, and typhoid fever. Potassium tellurate is still listed in the 12th edition, 1996, of the Merck Index as an anhidrotic.

More recently, the possible pharmaceutical properties of organo tellurium compounds have been investigated. In 1987, the compound, ammonium trichloro(dioxoethylene-*O*,*O*'-)tellurate, coded as AS-101, was shown to have immunomodulating properties and when administered to mice mediated antitumor ef-

fects.[239] Several diaryl tellurides (R−Te−R where R is usually a benzene ring with various substituents) show antioxidative properties in cell systems and may have potential for antioxidant therapy.[240] In fact, rat kidney tissues were protected against oxidative stress at diaryl telluride compound concentrations of 1 μM. Tertiary-butyl hydroperoxide-induced cell damage could also be mitigated by these materials and this was attributed to the "peroxide-decomposing, glutathione peroxidase-like capacity of these compounds".[240]

Inorganic tellurite interacts with hepatocellular selenoproteins, particularly with the selenium-dependent glutathione peroxidase providing one possible explanation for tellurium toxicity.[241] Similarly, organo tellurium compounds inhibit the selenocysteine-containing flavoenzyme, thioredoxin reductase.[242] Since thioredoxin is responsible for the growth and transformed phenotype of some human cancer cells, thioredoxin reductase may be a possible target for anticancer drug development. Some diaryl tellurides did inhibit growth of cancer cells (e.g., human MCF-7 breast cancer, HT-29 colon cancer, Colo320 colon cancer) and some antitumor activity against human breast cancer xenografts was observed in mice. However, the lack of solubility of the tellurium compounds was a major difficulty.[243] In other work, tellurapyrylium dyes inhibited cytochrome *c* oxidase upon irradiation of isolated mitochondrial suspensions, probably via formation of singlet oxygen. These chalcogenapyrylium materials may have potential for photodynamic therapy of tumors.[244]

Large organotellurides (substituted telluro phenyl ring systems etc.) have an apoptotic and cytotoxic effect on human promyelocytic cells.[245] Like analogous selenium compounds, this finding may lead to their use as chemopreventive agents. Induction of "programmed cell death" (apoptosis) was time- and dose-dependent. Two hypotheses for this process were proposed.

Fränzle and Markert[246] have recently hypothesized, using a model called the Biological System of the Elements (BSE), that tellurium may ultimately be found to be an essential nutrient. Using relationships between physiological functions, evolutionary response, and elemental biological uptakes these workers suggest that BSE relationships such as increasing molecular complexity and "increasing breadth or diversity of function in (biogenic) organic compounds" can be used to forecast possible biological functions that have so far not been observed. Analogously, selenium was, of course, well-known as a toxic element before its essentiality was determined.

In this connection, it is of interest that tellurium-resistant fungi, when grown for 7 days without a sulfur source but in the presence of 0.2% sodium tellurite, formed Te-containing proteins and amino acids. Provisional evidence for the presence of tellurocysteine, tellurocystine, and telluromethionine in protein hydrolyzates was obtained.[229] A telluroamino acid (possibly telluromethionine or Te-methyltellurocysteine) was also observed when *Saccharomyces cerevisiae* was grown in the presence of Na_2TeO_3.[247] These materials obviously run parallel to the sulfur and selenium amino acid analogues. Later work to incorporate telluromethionine in recombinant proteins was more clearly successful for studies by X-ray analysis; telluromethionine was found to be very subject to oxidation.[1] Proteins with methionine replaced by telluromethionine crystallized isomorphously.

D. Tellurium Reduction in Microorganisms

Methylation processes for group 15 elements (especially for arsenic) and for selenium generally require reductive steps as well as the transfer of methyl groups. Moreover, toxic metalloid resistance through biological reductive abilities has been proposed as one of the fundamental means of resistance; that is, many organisms that have the ability to grow in the presence of toxic metalloid salts reduce them to elemental, insoluble forms or reduce and methylate these metalloids producing volatile forms which are less soluble and leave solution.[248,249] Since the conversion of tellurite to Te^0 appears to be involved in some cases of bacterial resistance to tellurium, the subject of reduction has received much attention and will be discussed briefly here. Most recently, these investigations have involved extensive genetic studies, and at least five unrelated determinants for tellurite resistance (Te^R) have been described, some being chromosomal, others involving plasmids. Details of this genetic work are beyond the scope of this review. However, there is a brief, general review[250] and for the reader's convenience some recent articles concerning various bacteria are listed here: *E. coli*;[103,251,252] *P. aeruginosa* (and *Agrobacterium tumefaciens* and *Erwinia carotovora*);[253,254] *B. stearothermophilus*;[255] photosynthetic bacteria.[256]

Gosio had followed his work on arsenic volatilization[10] by studying the bacterial reduction of salts of selenium (see earlier) and of tellurium,[119,257] a topic that had already received attention.[120,121,134] With tellurium salts, bacteria usually produced as a reduction product, a gray or black coloration or precipitate. Gosio made an interesting application of the bacterial reduction of tellurium compounds by developing a method to determine the "visible sterility" of preparations of sera, culture media, etc.[119] A small volume of a tellurite solution was added, for instance, to a serum preparation. If the preparation contained live bacteria, a distinct gray to black coloration or precipitate developed on standing. The method could not have been totally reliable since the reducing ability of various bacteria was variable (see later), and the presence of bacterial spores would have been a problem. However, at a time when sterilization procedures were somewhat primitive, it was a useful test. American workers also recommended the use of potassium tellurite as an indicator of microbial life[258] and the differentiation between living and dead bacteria and "other low forms of organisms" was noted in a 1915 text of bacteriological methods.[259] Another early observation was that for *Mucor* strains, the (+) races of Absidia Blakesleeana (*sic*) and Circinella spinosa (*sic*) tended to have a greater reducing ability than did the (−) races.[260]

Beginning early in the 20th Century, it began to be realized that although tellurite inhibited many

microorganisms,[258,259] its action on bacteria was, in general, highly variable. In fact, tellurite-containing media were used for isolation of diphtheria bacteria, and in 1932, Fleming compared the antibacterial activities of penicillin and tellurite.[261] Organisms described as insensitive to tellurite included streptococci, staphylococci "and the diphtheroid and *acidophilus* group of bacilli." Fleming observed that penicillin and tellurite would "divide bacteria into two groups almost as definitely as Gram's stain." In nearly all cases, a penicillin-sensitive bacterium was tellurite-insensitive and vice versa.

Culture media containing tellurite have important applications in diagnostic bacteriology. In this way, *Mycobacterium avium-intracellulare* can be distinguished from other nonchromogenic, slower growing mycobacterial species[262] and more recently a potassium tellurite medium was used as a rapid (3−5 day) test for the antibiotic susceptibility of *M. avium* complex clinical isolates. The minimum inhibitory concentrations for clarithromycin determined in this way agreed well with those determined by the much slower microdilution method.[263] Tellurite tolerance is positive for *Streptococcus faecalis* and negative for *S. faecium*. Using a blood-tellurite agar, tellurium-resistant organisms show a heavy growth of jet-black colonies.[264] The mechanism and resistance of various staphylococci is not well understood. However, complex tellurite-containing media have been used to suppress coagulase-negative organisms in detection of methicillin-resistant strains of *Staphylococcus aureus*.[265] It is of interest that a selective medium for the troublesome *E. coli* O157 has also been developed.[250] Tellurite-amended media were useful in the isolation of soil populations of *Agrobacterium* biovars 1 and 2. A K_2TeO_3 concentration of 60 ppm favored growth of agrobacteria and at the same time inhibited development of other bacteria.[254]

Some gram-positive organisms show an intrinsic low-level resistance to TeO_3^{2-}, and some gram-negative organisms have a resistance associated with the presence of plasmids.[266,267] Despite much investigation of tellurite resistance the enzymatic mechanism(s) for the phenomenon remain unclear. In photosynthetic purple, non-sulfur bacteria *R. sphaeroides* (class *Proteobacteria*) there is a constitutive, high-level tellurite resistance to tellurite, selenite and several other rare earth oxides and oxyanions when grown chemoheterotrophically or photoheterotrophically.[126,249] Under photosynthetic growth conditions tellurite reduction required an intact CO_2 fixation pathway and a functional photosynthetic electron transport chain. Under aerobic growth conditions, functional cytochromes bc_1 and c_2 were required. The organism contained a tellurite-dependent $FADH_2$ oxidation activity, and the process may have involved two stages. First the $FADH_2$ step producing a nontoxic "+II valence" intermediate followed by a second two electron reduction to Te^0.[126] Two loci of the *R. sphaeroides* genome confer high-level tellurite resistance for this phototroph.[268] Two of the five genes comprising these loci (*trgA* and *trgB*) confer TeO_3^{2-} resistance through the production of what appear to be membrane-associated proteins, and disruption of a gene immediately downstream from the *trgB* gene yields a reduction in tellurite resistance. Disruption of a third gene, *telA*, found 115 kb from the *trgAB* region, resulted in a significant decrease in TeO_3^{2-} resistance. These workers suggest that *R. sphaeroides* TeO_3^{2-} resistance is complex and so far uncharacterized.

The periplasmic nitrate reductase of *R. sphaeroides* f. sp. *denitrificans*, purified after histidine tagging, had an observable but low activity in reducing tellurite (see also work described earlier on selenate reduction). This enzyme contained a molybdenum-requiring subunit (91 kDa) and a diheme cytochrome *c* (17 kDa). In addition both periplasmic and membrane-bound nitrate reductases of *Ralstonia eutropha*, *Paracoccus denitrificans* and *P. pantotrophus* were also able to utilize selenate and tellurite as electron acceptors.[127]

Work with extracts of *E. coli* indicated that the nitrate reductase activity was responsible for the basal tellurite resistance of this organism.[269] These extract activities had the same R_f values (in gel separations) as two known nitrate reductases and the authors suggested that these reductases convey resistance to metalloidal oxyanions such as tellurite and selenate. In addition, a further tellurite reductase activity, expressed under anaerobic conditions, was present in a strain devoid of nitrate reductase activity. In *E. coli*, tellurite could not be used as a terminal electron acceptor, and therefore these authors suggest that the resistance is connected to reducing ability not "bioenergetically linked".[269] Other work in both wild-type and plasmid-mediated *E. coli* indicated a possible role for RSH and thiol biochemistry in tellurite resistance with specific thiol: tellurium interactions occurring at key target sites.[248]

Cell-free extracts catalyzing the NADH-dependent reduction of tellurite to elemental tellurium were obtained from *Thermus thermophilus* and a substantial enzyme purification was achieved.[270] The most active preparation, designated Fraction 2a, was a macroaggregate of at least two different polypeptide chains (53 and 55 kDa); NADPH could replace NADH.

Tellurite reducing activities were isolated from *B. stearothermophilus* V.[271] Two hydroxyapatite fractions, H1 and H2, were obtained, both being NADH-dependent. H1 was apparently a macroaggregate of three proteins (60, 41, 37.5 kDa) while H2 was a dimer with 60 kDa subunits. It could not be determined whether the H1 and H2 60 kDa subunits were identical in the two fractions. This organism, grown at sublethal K_2TeO_3 concentrations produced a strong garlic smell, so in addition to reduction, methylation might provide another detoxification mechanism. Genetic evidence indicates that the cysteine synthase gene (*cysK*) of *B. stearothermophilus* V, when cloned into *E. coli*, mediates tellurite resistance in that organism.[255]

An inducible, high-level tellurite resistance activity in the haloalkaphilic archaeon, *Natronococcus occultus*, was studied in cell-free extracts but was not purified.[272] The reductase activity required NADH.

In several gram-negative bacteria, the reduction of tellurite involved the respiratory chain and terminal oxidase inhibitors inhibited tellurite reduction. The organisms investigated were strains of *P. aeruginosa*, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *E. coli* DH5α, and *E. coli* GD-102bd^{2+}.[253] In general, the rate of tellurite reduction was largely dependent on the rate of electron transfer. In this work, the culture medium did not contain nitrate so tellurite reduction by nitrate reductase was ruled out.

In summary, it appears that a variety of processes, often reductive, are responsible for tellurite resistance. Apparently, there is no common mechanism. In this connection, mention should be made of instructive work with a chromium-resistant microbe—a strain of *P. fluorescens*. In this case, the presence of chromium reductase did not necessarily correlate with chromate resistance.[273−275] Moreover, while seven species of obligately photosynthetic bacteria showed high-level resistance to tellurite and accumulated metallic Te (obviously though intracellular reduction and deposition), high-level resistance without reductive ability was uncovered in two other phototrophs.[276]

E. Tellurium Biomethylation

In examining the toxicity of tellurium, it had been noted in 1824 by C. O. Gmelin that when a rabbit, poisoned with telluric acid, was dissected, a garlic odor was present.[277] In early studies the dissection of a rabbit poisoned with telluric acid was accompanied by a garlic odor[277] and sodium tellurate administered subcutaneously to a dog gave garlic-odored breath.[133] While hydrogen telluride might have been the odorant,[26] dimethyl telluride was suggested on the basis of inadequate evidence. Similarly, an unpleasant garlic odor was observed on oral administration of potassium tellurite to humans[136,174] When stomach disorders were treated with bismuth carbonate, the patients also had bad breath ("bismuth breath") due to contamination of the bismuth with tellurium.[136,279,280]

A garlic-odored volatile formed when *S. brevicaulis* was grown in the presence of tellurium was finally identified as DMTe.[26,136] It is of interest that DMTe has been claimed to have "the most abominable odour of all organometallics".[278] Much later, a *Penicillium* strain isolated from sewage was also shown to produce DMTe (identified by gas chromatography and mass spectrometry).[27] Surprisingly, DMTe was only produced when both tellurium and selenium compounds were present in the culture medium. It was suggested that some step in the biosynthesis of the methylated metabolites was induced by selenium but not tellurium. The alternative possibility of transmethylation cannot be ruled out on the evidence available.[70]

The first report of a volatile tellurium compound produced by a bacterium is probably that of King and Davis.[258] In work on the use of tellurite as an indicator of microbial life, these authors noted that with *Bact. tuberculosis (sic)* "several of the tubes had a garlic-like odor analogous to the arsines, and which in all probability were hydrogenated tellurium compounds or 'tellurines'." Their structural suggestion was almost certainly incorrect, but this observation is of historical interest. In more recent times, the age-old tentative olfactory identification of DMTe was described for *B. thermophilus* V (see earlier) and *P. aeruginosa* ML4262.[253] One surprising observation with photosynthetic, metalloid-resistant bacteria was that monocultures of three phototrophic strains (*Rhodocyclus tenuis*, *Rhodospirillum rubrum* S1, and *Rhodospirillum rubrum* G9) produced DMTe in culture headspace when amended with Te^0.[33] This solid was added to test tube cultures photoheterotrophically grown for 6 or 7 days before sampling. This is the only report of reduction and methylation of solid, elemental tellurium by microorganisms. Many of the same organisms in this research also reduced and methylated metallic selenium to yield DMSe. Table 2 details organisms involved in biomethylation of tellurium. Except for the production of dimethyl ditelluride from a few fungi, DMTe is universally the volatile biomethylated product (see below). In a few older literature reports only a stench or garlic odor was reported, but it can reasonably be assumed to be a positive test for DMTe.

More definitive work on bacterial production of DMTe has been reported recently.[70] The organism used was *P. fluorescens* K27, a facultative anaerobe, isolated from the Kesterson reservoir in the central valley of California.[281] Headspace production of DMTe by this organism was dependent on the amount and oxidation state of added tellurium oxyanions. Black, elemental Te^0 was produced by tellurium-amended bacterial cultures but not by sterile controls. After 92 h incubation under anaerobic conditions, 34% of added tellurium was found in or on the harvested cells. Tellurate/tellurite mixed amendments (total Te, 2 mM) were synergistically toxic, yielding less overall biomass in stationary phase as compared to either TeO_4^{2-} or TeO_3^{2-} amended cultures. Mixed amendment cultures also produced very little DMTe over the time courses longer than 24 h.

While DMTe production is common, there is apparently only a single instance of the documented formation of dimethyl ditelluride (DMDTe). This metabolite was discovered in the aerobic headspace of the fungus, *Acremonium falciforme*, grown in a liquid medium containing 5 μM tellurite.[18] DMTe and DMDTe were seen in two time course snapshots (24 and 48 h after inoculation) of headspace gases and these compounds' identities confirmed by GC-MS. The same organism produced volatile DMSe and DMDSe in separate experiments; however, the more oxidized Se volatile, DMDSe, did not appear at the 48 h sampling. This suggests that the diselenide—and analogously ditelluride—were not created by oxygen-based oxidation of the selenide or telluride in these fungal headspaces. Instead, biological production was more probable.

There has been almost no investigation of the actual enzymatic mechanism for the biomethylation of tellurium. Indeed, to the best of our knowledge, there is no experimental evidence for the widely held assumption that *S*-adenosylmethionine, SAM, is the

Table 2. Biomethylation of Tellurium to Volatile Products[a]

organism(s)	substrate	products		ref(s)
		DMTe	DMDTe	
Bacteria				
Bacillus stearothermophilus V	IV	odor		271
Bact. tuberculosis (i.e., *Mycobacterium tuberculosis*)	IV	odor		258
Escherichia coli	Te-methionine	odor		1
Pseudomonas aeruginosa	IV	odor		293
Pseudomonas aeruginosa ML4262	IV	odor		253
Pseudomonas aeruginosa PU21	IV	odor		293
Pseudomonas fluorescens K27	IV, VI	+		70
Rhodospirillum rubrum G9	VI, Te^0	+		33
Rhodospirillum rubrum S1	VI, Te^0	+		33
Rhodobacter capsulatus	VI	+		33
Rhodocyclus tenuis	VI, Te^0	+		33
Clostridium collagenovorans	IV	+		140
Desulfovibrio gigas	IV	+		140
Methanobacterium formicicum	IV	+		140
Fungi				
Acremonium falciforme	IV	+	+	18
Candida humicola (*Cryptococcus humiculus*)	VI	odor		294
Penicillium chrysogenum	IV	+		136
Penicillium citrinum	IV	+	+	18
Penicillium sp. (probably *notatum*)	IV	+		136
Penicillum sp.	IV, VI	+		27
Scopulariopsis brevicaulis	IV	+		136
Humans				
	IV	odor		295
	IV	odor		279
Rats				
	IV, VI	+		234
	IV	+		236
	IV	+		231
	Te^0	+		296
Unidentified				
sewage	−	+		75
sewage	−	+		73
soils	−	+		230

[a] IV = tellurite; VI = tellurate; Te^0 = elemental tellurium; + = detected; − = unknown

methyl group donor.[282] If the methylation mechanism is similar to that for selenium, with alternating reductions followed by methyl group transfer from SAM, the pathway outlined in Scheme 1 would be most likely.

While there have been tentative suggestions that biomethylation might have a role in bacterial resistance to tellurium (see earlier), there is no real experimental evidence, apart from frustrating and tantalizing observations that SAM, and possibly a methylase activity, may be involved in tellurite resistance. In *E. coli*, the chromosomal determinant, Te^R, consists of two genes, *tehA* and *tehB*. The gene product, TehB, is a protein of 197 amino acids that displays an amino acid sequence similar to those of many SAM-dependent methyl transferases (other than those for nucleic acid methylation). Such proteins have three shared motifs; evidence was obtained that in TehB motifs I and II were involved in binding SAM.[283] Hence, it appeared that TehB had a methyltransferase activity and a likely product would have been DMTe. While a SAM-dependent loss of tellurite was observed in incubations of TehB preparations, it was not possible to detect DMTe in the headspace of appropriate *E. coli* cultures using fluorine-induced chemiluminescence as a detection method (Van Fleet-Stalder and Chasteen, unpublished observations). It appears likely that tellurium methylation does occur but leads to so-far unidentified and nonvolatile products.

In a second case, the protein product of the *P. syringae tpm* gene (the Te^R deteminant), is a SAM-dependent methyltransferase with the ability to methylate 6-mercaptopurine (*tpm* = bacterial thiopurine methyltransferase gene). It was suggested that tellurite might undergo methylation to form DMTe and hence by further metabolism to produce a nonvolatile trimethyltelluronium ionic species; however, no evidence for DMTe detection was reported.[284]

F. Tellurium Bioremediation

1. Use of Microorganisms

It is possible that microorganisms can be used for the bioremediation of materials containing tellurium. As just noted, the fungus, *Acremonium falciforme*, in bioremediation settings would be likely to produce both dimethyl telluride and dimethyl ditelluride. Hence, this organism might be used to remove Te via volatilization.

Instead of tellurium bioremediation via volatilization, Te removal from a Te-contaminated waste stream might be accomplished via bioreduction to black, elemental Te^0 (no other tellurium allotropes have been reported from biological interaction). So-

called promiscuous plasmids were used to distribute tellurite-resistance determinants (Te^R) to different strains of *E. coli*. Seven of the 8 plasmids had the ability to convert tellurite (added to the culture at a concentration of 50 ppm) to black Te^0 that was distributed "along the cell membranes." It was suggested that such strains had "the potential of being exploited for the bioremediation of the oxyanion TeO_3^{2-} from liquid waste." Cell harvesting and charring could be used to recover Te from a bioremediation system based on the use of Te^R determinants.[285]

In a similar vein, Yurkov et al.[276] reported obligate anaerobic microbial reduction of tellurite to intracellularly deposited Te^0. The conversion rates for some microbes and carbon sources studied were termed "very large"; however, no quantitative rates or amounts were reported. This can be contrasted with quantified microbial (ATCC 700304 − a marine *Rhodobacter* sp.) reduction of selenite (1.1 mM initial) conversion to red elemental Se with a 99% conversion within 5 days.[286] Interestingly, the red amorphous Se, formed initially, changed to the black Se allotrope in the presence of iron.

Novel strains of purple nonsulfur bacteria, with high levels of tellurium resistance, were isolated from marine environments, and typically accumulated Te^0; two strains removed up to 94% of added tellurite.[286,287] It was suggested that these strains might have a role in prevention and bioremediation of metal pollution in the oceans. Similarly, *R. sphaeroides* 2.4.1 has the potential for bioremediation and detoxification of contaminated waters.[268] The resistance determinants from this organism might be used to construct recombinant soil bacteria and plants, a process that, if feasible, would greatly increase the potential for bioremediation.

2. Tellurium Phytoremediation

Work involving the bioconcentration of tellurium by plants is sparse. A single extant literature report of Te content in plants is available[288] and describes the distribution of Te in trees, shrubs, and flowering plants in Ely mining district of White Pine County, Nevada and from western Colorado. An increase in soil Te content did increase plant accumulation, most highly in flowers. Known selenium-accumulator plants evaluated were also apparently enriched in Te; however, no plant contained more that 1 ppm Te. Selenium-accumulators, like *Brassica* sp. can bioconcentrate hundreds of even thousands of times more Se than this.[289]

IV. Conclusions

The similarity of selenium and tellurium encompasses not only their names and positions on the periodic table but to a large degree their chemistry and biochemistry. Instrumental methods for their determination in the environment are similar; proposed remediation methods will also run parallel. And like selenium 40 years ago, it is possible that the toxic element tellurium will ultimately be found to be an essential element.

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VI. Note Added in Proof

Although our review focuses on work with microorganisms and plants, important work on the role of methylated selenium compounds in human cancer chemoprevention has been extended by recent publications. The monomethylated material, methylselenol, plays a crucial role and a number of materials provide excellent experimental tools for in vivo metabolic generation of this material. They include: methylselenocysteine, methylseleninic acid, γ-glutamyl-Se-methylselenocysteine, Se-allylselenocysteine, Se-propylselenocysteine. See especially refs 297−299 and papers cited therein. Also relevant is a new book, not available to us during manuscript preparation (ref 300). Chapter 25 "Selenium in Biology and Human Health: Controversies and Perspectives" may be especially of interest in regards to selenium and human health.

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