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ECOLOGICAL IMPLICATIONS OF METAL METABOLISM BY MICROORGANISMS

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Contents

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Arne Jernelöv and Ann-Louise Martin

"Metals are devious and will bind to just about every available site under different conditions"
(quotation from Eichhorn)

INTRODUCTION

Interaction between organisms and chemicals in their environment is generally seen from the point of view of the organisms. The purpose with this paper is to look at the contact between a group of elements (metals and metalloids) and a group of organisms (microorganisms) from the point of view of the elements. The question asked is: How do microorganisms effect metals and metalloids?

This review does thus intentionally not include the vast literature dealing with effects of metals and metalloids on organisms unless the papers also contain information on the fate of the elements in connexion with the contact.
In writing the review the authors concentrated on certain processes and certain elements, arbitarily judged to be of special interest, with the view of discussing them against a general background of microbial metabolism of metals, rather than trying to give a complete summary of the field.

This approach could not hide the fact, however, that the available knowledge in the field in general is strikingly limited and scattered. This is so much the case that it was not felt meaningful to point out where gaps in knowledge exist.

The limited available information on microbial metabolism of metals further gave rise to the question whether or not to include examples of transformation reactions demonstrated in higher organisms. In some cases this has been done with the only justification in the general argument that metabolic processes present in higher organisms as a rule also can be carried out by some microorganisms.

The quotation from Eichhorn used as a motto may be discurageing to any scholar in the field.

The binding of metals to different organic ligands may indeed seem quite capricious. This can be understood from the manyfold of possibilities that exsist with complex and chelate formation. These formations are dependent on e.g. pH, the concentration of the metal ion in the actual solution, and of the valence of the ion. Also, the accessability of suitable ligands in the organic compound may differ depending on pH, pk_a of the ligand and on structure of the compound. However, some generalizations can be made, and this paper will try to give information concerning four major types of transformations, found in the biological system.

The first type will include complex and chelate formation of metal ions with organic ligands, the binding to sulphur, nitrogen and oxygen.

The next issue to be discussed is shifts of valences of the metal as a result of the metal being involved in the metabolism of organisms.

The third type of metal interaction which is dealt with is the substituting of one metal for another. This can be found when non-essential metals are replacing essential ones, e.g. in metal-containing enzymes. The last part of the paper will be a survey of the knowledge of alkylated metals in biological systems.

Microbial degradation of organo-metal compounds will be illustrate with discussion of the decomposition of methyl mercury. Finally an attempt is made to give the criteria for prediction of microbial conversions of ecological significance.

THEORIES OF BINDING AND STERICAL FORMATIONS OF ORGANOMETALS

Ligands of low polarisability, high electronegativity, large electric charge and small size with inaccessible empty orbitals of high energy such as e.g. carboxylic groups, hydroxylic, phosphate and aminogroups (oxygen and nitrogen), preferentially complex small, highly electropositive metal-ions with a large positive charge or oxidation stage and of low polarisability with few outer electrons, not easily excited with ionic and electrostatic bonds. Examples of such metals are:

Mg^{2+}, Ca^{2+}, Mn^{2+}, Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, La^{3+}, Cr^{3+}, Co^{3+}, Fe^{3+}, Ti^{4+}, (Zn^{4+}), Sn^{4+}, As^{5+}, Cr^{6+}.

Association with oxygen rather than with nitrogen has been shown for Mn^{2+}, Fe^{3+} and Cr^{3+}. (65)

Ligands of high polarizability, low electronegativity, small negative charge and large size with accessible, low lying empty orbitals such as sulphydrylgroups preferentially form covalent- and -type bonds with large metal-ions, characterized by low electropositivity and high polarizability, small positive charge and low oxidation number, with several, easily excited outer electrons. Such metals are:
Cu^{+}, Ag^{+}, Au^{+}, Tl^{+}, Hg^{+}, Pd^{2+}, Cd^{2+}, Pt^{2+}, Hg^{2+}, CH_3Hg^{+}, Pt^{4+}, Te^{4+} and M^{o}.

As^{3+}, Hg^{2+}, Cd^{2+}, $Au^{1+;\ 3+}$, Pb^{2+}, Bi^{3+}, Co^{2+}, Ag^{+}, Sb^{3+}, V^{2-5+} are all examples of metal-ions that will strongly attack the -SH groups of essential enzymes. A rough order of affinity can be obtained from the solubility product constant of metal sulfides.
pk_s: $Hg^{2+} > Cu^{2+} > Pb^{2+} > Cd^{2+} > Zn^{2+}$
(Selenium ions oxidaze -SH groups rather than complex them. The same can be assumed for cupric ion: $4\ RS^{-} + 2Cu^{2+} \rightarrow 2RSCu + R\text{-}SS\text{-}R$)

Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Sn^{2+}, Sb^{2+}, Bi^{3+}, As^{3+} and others are of intermediate character. (68)

Under favorable conditions, the disulphide bonds of protein would be expected to react with Ag^{+} or Hg^{2+} in a manner analogous to simple disulfides, e.g. cystine. However, in the native state from pH 4 to 9, the disulfide bonds of many proteins appear to be rather resistant to chemical reaction. At concentrations of mercuric chloride in the order of 10^{-3}M, no reaction of the disulfide bonds of serum albumin was observed. (16)

Complexes formed may be linear, square planar, tetrahedral or octahedral with the coordination numbers 2, 4 or 6. The coordination number of 5 is not common, but may be found in cobalt complexes of biological molecules.

Most of the complex forming and metal binding theories mentioned above, are based on studies of reactions of metal ions with simple organic molecules or model compounds.

In the living organism, however, the metal ion will encounter large molecules, such as e.g. proteins , with intricate formations where the ligands are not free to move and cluster around the metal ion. Possible sites for binding may be buried inside a macromolecule and thus be inaccessible. Hence, a protein having very many ligand groups, may have only one or two metal ions attached to them, and occasionally a metal ion may be held by only one group.

A protein also contains positive and negative charges, e.g. $-NH_3^+$, $-S^-$ and $-CO_2^-$, that can act attracting as well as repulsing on the approaching metal ion, even though they are not directly involved in its eventual bonding.

From this background of organometallic chemistry would follow that the major metabolic products will come out of the primary affinity of the metal ion. On the other hand the combination of Pb^{2+} with a single sulfhydrylgroup might be overlooked in the presence of 100 carboxyl groups.

Examples of Metal-Complex Formations with Oxygen and Nitrogen

The metal ions Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Fe^{2+} and Mo^{2+} have been shown to form chelate complexes with the carbonylic oxygen and the nitrogen of the ajacent ring in guanosin and the riboflavin moity of FAD. (56)

Other workers found Ag^{+} to complex in a similar way, as shown for guanosine. (59)

The free base adenine was shown to react with Cu^{2+} (17), Ni^{+2} and Co^{2+}. In this case, however, the imidiazol aminogroup appears to be involved in chelate formation. (41)

Zink and lead bind to phosphate groups, which may lead to the cleavage of the phosphate bridges of polynucleotides.

In the RNA double helix the binding of metals to the nitrogene bases of the nucleotides will disrupt the hydrogene bonds which are holding the structure together. (18)

The phosphorus containing lipid phase of some marine organisms have been found to complex inorganic cat-ions such as iron, zinc and cobalt. Also selenium was found in the same fraction. As the selenium could not be extracted until both polar and non-polar solvents were used, it seems to be associated with phospholipids, though nothing else is known about the structure.

Results from feeding marine fish with different arsenic compounds show that practically all arsenic in the organism appeared in an organic form. These arseno-organicals were found to be water soluble as well as lipid soluble. Hence, also in this case the lipid soluble compounds have much in common with phospholipids, being polar, but not attacking the cellmembranes in the manner that phospholipids do. The water soluble phase consists of the glue-water, which is a collagen-containing extract. This phase is not very well characterized apart from being alcalic and having a molecular weight higher than aminoacids, but lower than proteins, in which no compounds of this kind were shown.

The biotransformations found have been established in a marine environment, but not in terrestrial systems. Thus, the transformation might be specialized, occurring only in marine environment and not applicable as a model for a generalized biosynthesis. (36, 37, 38, 39, 40)

Examples of Metals Binding to -SH Groups

One example of a very specific metabolic pathway including binding of -SH groups, is the formation of metallothioneine with cadmium, zinc, copper and mercury. This protein has a high content of cysteine but few aromatic aminoacids.

The bonds with the metals are mainly in the form of mercaptides. The protein holds 2,9 % Cd and is thus the second highest metal-containing protein known, ferritine being the first.

In metallothioneine, Cd is stored mainly in the kidneys. The protein is constantly broken down and synthesized de novo. By these transformations, very small amounts of Cd are let out in the organism. Metallothioneine is thus acting as an interacellular isolationsmechanism and as a transporter of Cd.

Apart from metallothioneine, a metalloprotein with a considerably higher molecular weight was found in the mammary gland of rat. Later, this proteine was found in certain organ systems, where it seems to act as a barrier against Cd-transports. The actual organ systems were mammaryglands and placenta along with liver and the gastro-intenstinal tract. (49, 45)

Apart from containing Cd and Zn, metallothioneine is also able to bind Hg and Cu, though not to the same extent. (50, 69)

CHANGES OF VALENCIES

Metals in biological systems are likely to take part in a number of reactions of different kinds. Reactionsinvolving changes of valency of a metal are very much dependent on factors as e.g. pH, charge of the ligands and of the metal as well as the redox-potential. In the organism these reactions may be classified into two groups. In the first one the metal is participating in redox-reactions, energetically favourable to the organism. The second kind of reaction involves an active investment of energy in transferring the metal from one stage of valence to another. This can be seen as a route of detoxification for the organism.

The state of valence that a metal-ion has when normally occuring in an organism, can be assumed to be the least toxic to the organism. The stability of the valence state of a metal ion is different in aquous solutions and in organic complexes. This may lead to a change of valence upon chelate formation. By chelation, the redoxpotential of the metal-ion is altered, and the affinity of the metal-ion for certain ligands may also be changed.

Some examples of energetically favourable reactions are e.g. Fe and Mn in redox-reactions of the respiratory chain, enzyme activation of the Krebs' cycle and glycolysis, respectively, and the chemosynthesis of Fe^{3+} from inorganic Fe^{2+} by the bacteria species Ochrobium and Siderocapsa. A bacteria, even more specialized, is Theobacillus ferro-oxidans, which is able to oxidize ferrous ion to ferric at pH 2 in mixed sulfide ores. (60)

Examples of reactions which are more ambigous in their relations to the suggested classification are described.

At least fifteen strains of bacteria have been found to oxidize arsenite in cattle dip solutions to arsenate. Specific enzymatic activity (arsenite dehydrogenase) was found in purified prepara-

tions. It was shown that cytochrom-a enhanced arsenite oxidation, suggesting that the bacteria were obtaining energy from the oxidation. Because the bacteria were grown in the presence of organic carbon, an energy yield from the oxidation could not be established. (61)

Recently, a possible way of arsenate reduction has been proposed. In microbial heterotrophic metabolism, arsenate would be able to act as an electrone acceptor, in the absence of O_2 and NO^-_3. This reaction, which has not been investigated, would be energetically more favourable compare to sulfate reduction, which does occur in similar environments. (21)

A culture of Schizosaccaromyces, supplied with tellurite-ions will carry out a reduction of the tellurite-ion which brings the metal to the elementary stage. The yield or loss of energy in the reaction is not established. (54)

Further stages of reduction of tellurite- and selenite-ions will be treated in the chapter of methylation and of selenium.

SUBSTITUTION

The displacement or substitution of one metal ion for another may occur in metal complexes, also in the living organism. This displacement is dependent on the strength of the individual ligand complexes, generally following the extended Irving-William series: $Ca^{2+} < Mg^{2+} < Mn^{2+} < Fe^{2+} < Co^{2+} << Zn^{2+} << Ni^{2+} < Cu^{2+}$ (which is independent of ligand type) and also on the concentrations of ligands and metal ions present in the system.

It is also dependent on the relative strength of the complexes involved. The effect of this may be illustrated by the following example. According to the Irving-Williams series cupric ions should replace ferrous ions in ligand complexes. However, this will not happen when the ferrous ion is complexed by an aromatic nitrogentype ligand, where the ferrous ion is abnormally stabilized, resulting in a very small difference in stability constants for this type of complexes whith cupric and ferrous ions, compared to those of almost any other complex possible in the system. (67)

For Mn, a specific metabolic pathway through the organism have been suggested where substitution with other cat-ions is not possible. (13)

Non-essential cat-ions substituting for essential cat-ions in biological systems, e.g. in metalloenzymes, have been shown. Zinc occuring in carbonic anhydrase will be replaced by administered cupric ions, with the consequent loss of activity for the enzyme.(67 In carboxypeptidases, Cd inhibits peptidase and enhances esterase activity by displacing Zn from its nitrogen- sulfur ligands. (48) Large doses (0,25 % in diet) of pure Zn-salts retarded growth of rats and caused hypocromic anemia, probably resulting from displacement of Cu and Fe. (62)

It is possible that Te may act as a metabolic antagonist to Se. (53

Ni has the ability to displace Be from alkaline phosphatase and reactivate the enzyme. (52)

The feeding of tungstate (W^{6+}) ion in large quantities has caused displacement of molubdate in the body of experimental animals. (15)

Mg^{2+} can be replaced by Mn^{2+} in the DNA scheme, with the reactions still proceeding though giving a different range of products. (68)

The displacement or substitution of one metal ion for another may or may not have a net effect on the substituting metal, e.g. pentavalent arsenate (AsO_4^{3-}) may displace phosphate ion in the glycolysis. The reaction proceed in the usual way but without the generating of high energy phosphate-type bonds, since the arsenate is immediately split off. This substitution has no net effect on the arsenate ion. Evidence for arsenic compounds not beeing incorporated in different protein fractions have been given, supporting the theory given above. (35)

BIOMETHYLATION OF INORGANIC METAL IONS

A comprehensive revue of the knowledge concerning biological methylation up to 1945 has been made, presenting different theoretical pathways, either to synthesize accessible methylgroups or to make use of methylgroups already formed. The revue also presents the finding of biomethylated cat-ions like arsenic, selenium and tellurium. Inorganic compounds of As and Se were shown to be methylated within Scropulariopsis brevicaulis, and Te-componunds were predicted to follow the same metabolic pathway in the organism.

Some Penicillum-strains were also shown to methylate selenite- and telluritions, appearing together in bread cultures. At the time, there was no evidence of bacterial methylation. (10)

More recently it was shown that methanobacteria were able to reduce and methylate arsenate to dimethylarsine under anaerobic conditions. (42) The methyl donor was found to be methylcobalamin. The biological methylation of mercury, discussed in more detail below, may be carried out by bacteria as well as fungi.

Many attempts have been made to postulate and predict the metals and metalloids in addition to those where biological formation of alkyl compounds have already been demonstrated, that may be subjact to such a metabolism.

1) The metals Hg, Sn, Pd, Pt, Au and Tl and the metalloids As, Se, Te and S have been postulated to accept methyl groups from methylcobalamine in biological systems. Those underlined have to date been demonstrated to be biologically methylated.

2) Methyl-B_{12} will not transfer methylgroups to Cd, Pb and Zn. The alkylated forms of Cd and Zn are highly unstable in aquous systems. (1, 72)

Methylation of Selenium

Already from the studies in the first part of the century a mecanism for biological methylation of selenium by fungi was proposed

$$H_2SeO_3 \rightarrow H^+ + SeO(OH)O^- \xrightarrow{CH_3^+} CH_3SeOH \xrightarrow{\text{ionization + red}}$$

$$CH_3Se(O)O^- \xrightarrow{CH_3^+} (CH_3)_2SeO_2 \xrightarrow{red} (CH_3)_2SeO \xrightarrow{red} (CH_3)_2Se.$$

Biosynthesis of volatile dimethylselenide is a major metabolic pathway for detoxifying selenite in e.g. rat.

This process occures directly in the animal tissue and, thus, is not the result of an intestinal microflora in the animal. (24) Further studies showed a specific requirement for glutathion for the formation of dimethylselenid.

This compound is known to be excreted by respiration, but it is also able to convert into trimethylselenonium ions in the organism and, in that form, it will be excreted in the urine. (9, 46, 47)

Dimethylselenide, therefore, is to be considered as an intermediary metabolite, excreted by respiration only when the rate of its formation exceeds the rate of its further methylation.

In addition to dimethylselenide, the highly toxic dimethyldiselenide has been identifyed in the air expired by rats given a higher dose of selenite ion. (63)

This compound is also synthesized by toxic plants, as shown for some Astralgus species (19, 12)

The less toxic compounds, which, though, have a very limited margin between toxic and non-toxic doses, have a reversible interconversion, where the dimethylselenide is lipid soluble and the trimethylselenonium is watersoluble.

Methylation of Tellurium

Tellurium was found to methylate in the presence of different Penicillumstrains. The volatile metabolite was identifyed as dimethyltelluride. (5)

The mechanism for methylating tellurium was proposed to follow the same metabolic pathway as for selenium and arsenic. Later investigations of the biological cycle for these elements principally follow the same theories. (10, 11, 72)

Methylation of Mercury

Very little new information on biological formation of methyl-metal-compounds was generated between the mid-fourties and the mid-sixties when the studies of due environmental behaviour of mercury started in Sweden. After the demonstration that mercury in fish predominantly was present in the form of methylmercury (66) is was shown that unidentified microorganisms in natural organic lake sediment could methylate mercury (27). The net-result of the process could be mono- or dimethylmercury and the rate of biological methylation of mercury was found to be well correlated with general microbiological activity in the sediment (28).

The mechanism of methylation has later been the subject for many studies, but it is still not fully understood, although several hypotheses have been suggested.

Non-enzymatic methylation of mercury by cell-free extract was shown of a methanorganic bacterium, with methyl cobalamine ($CH_3 - B_{12}$) as donor of methyl groups in the presence of ATP and a mild reductant. (70)

Later a more complete picture of the mechanisms involved in the methylation of mercury under both aerobic and anaerobic conditions was proposed by the same authors. (71)

Figure 1 and 2.

During 1974 it was found by different workers that mercury was methylated in a neutral water solution by a purely abiotic reaction (32, 4). The methyl donor was methyl cobalamine and the reaction was very fast and almost quantitative both under aerobic and anaerobic conditions.

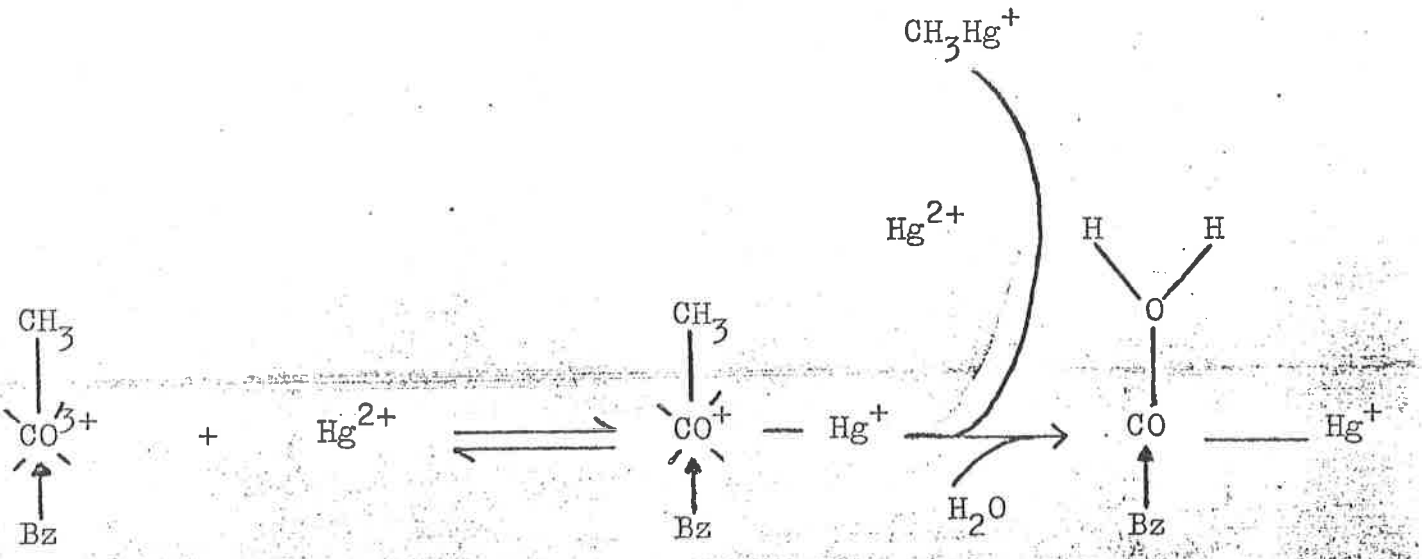
However, in several experiments it has been shown that microbial activity is a prerequisite for the synthesis of methyl mercury under natural conditions, unless other methyl metal compounds (e.g. tetramethyl lead or methyl tin species (26)) are added (3, 29).

In addition, bacteria isolated from mucuous material on the surface of fish (30), and bacteria belonging to the genus Pseudomonas, isolated from soil (33), have been shown to be able to methylate mercury under aerobic conditions.

Methylation in vivo was studied in aerobic cultures of Neurospora crassa (34). Mercury tolerant mutants were very effectivly methylating when an excess of cysteine or homocysteine was present in the substrate. From a series of experiments it was suggested that the methylation might be an "incorrect" synthesis of methionine (which is normally formed through methylation of homocysteine). Later, it was demonstrated that at least five different defined bacterial species and three fungal species in pure aerobic cultures have the capacity of methylating mercury, when added as mercuric chloride (64).

Figure 3

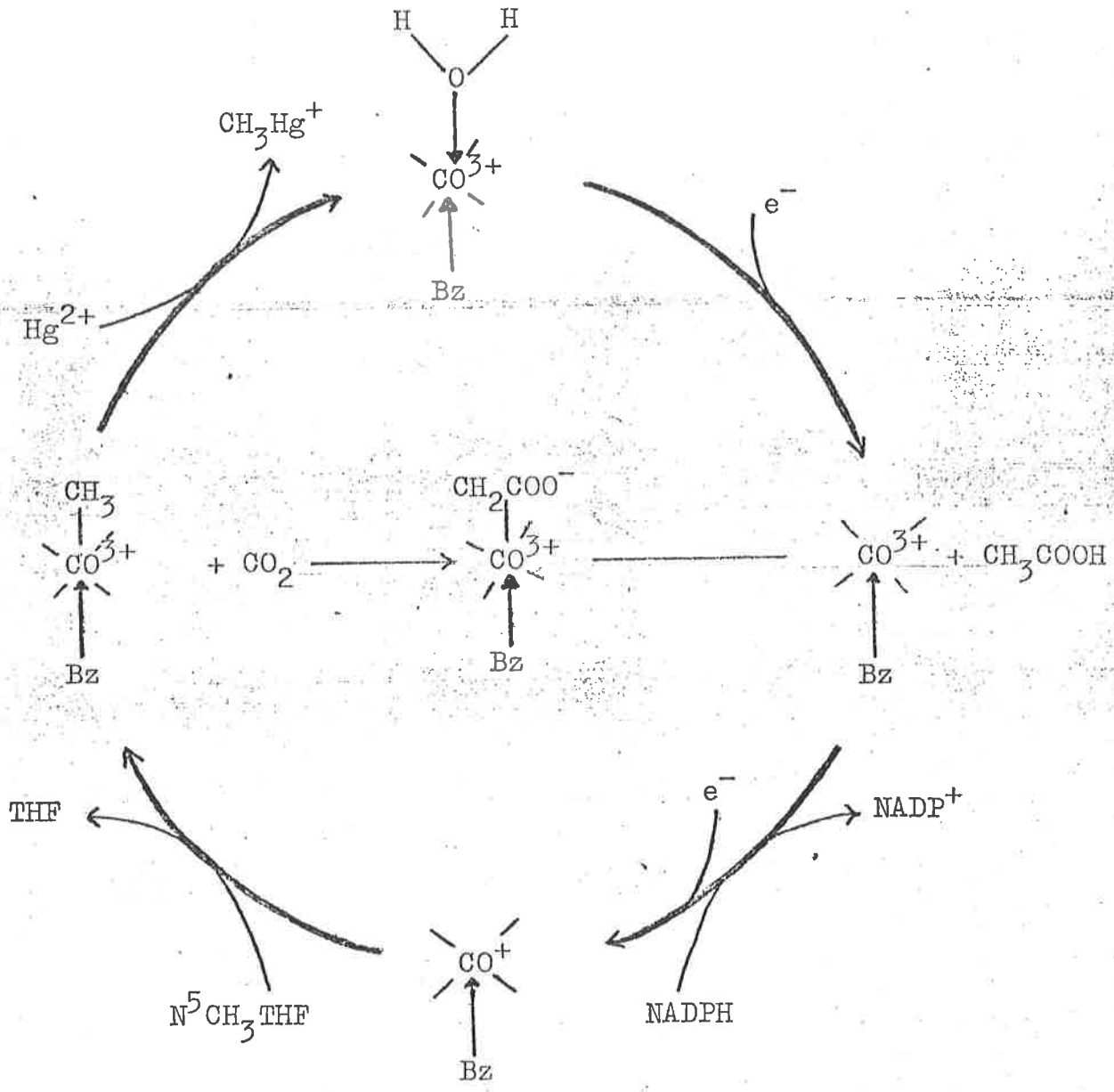
Figure 1



Proposed methylation of mercury by a methylcorrinoid under nonenzymatic aerobic conditions.

(After Wood, 1971)

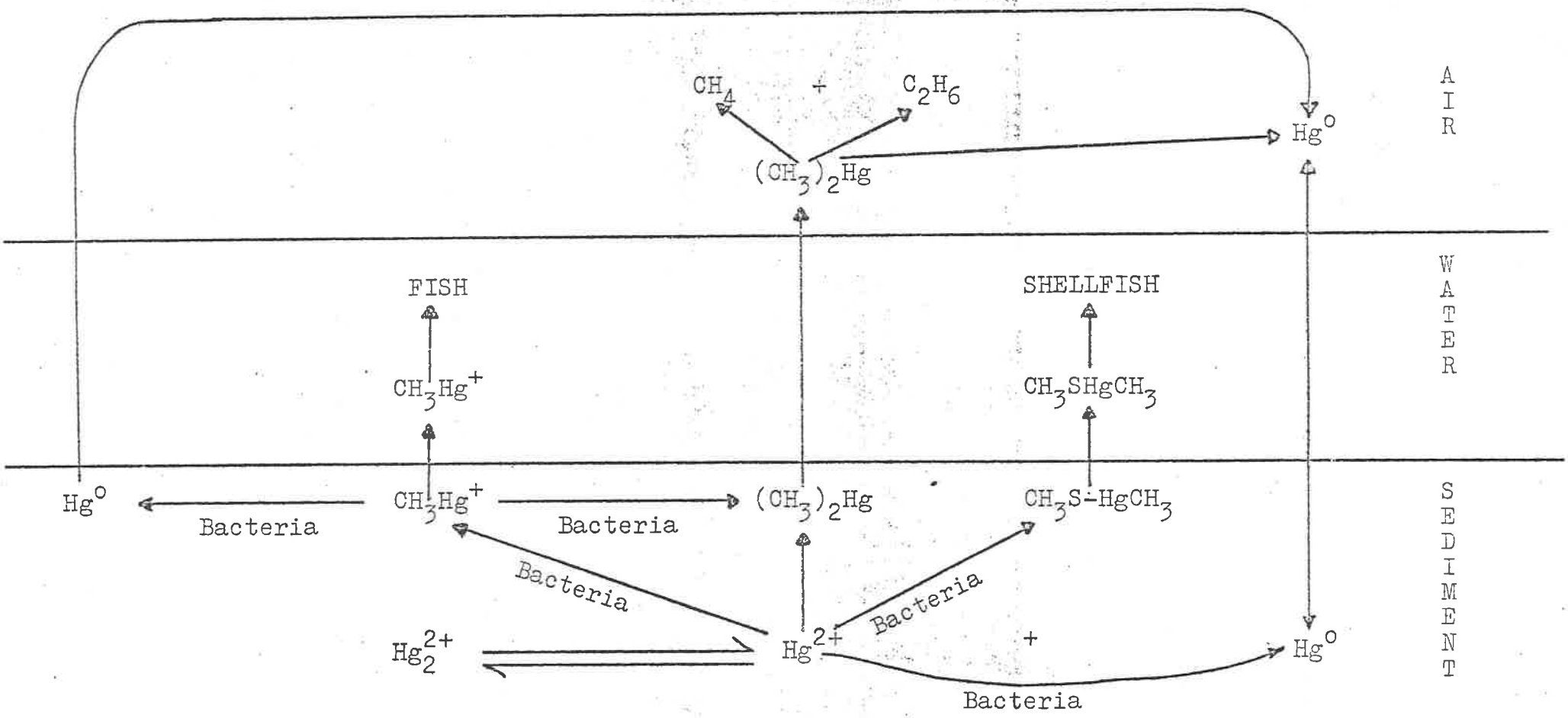
Figure 2



Proposed mechanism of mercury methylation in methylcobalamin-acetate synthetase system under anaerobic conditions.

(After Wood, 1971)

Figure 3



The mercury cycle.

(Af r Woo 1971)

In a recent study it has further been shown that microbial conversion of inorganic mercury compounds into methyl mercury also can take place in soil (2). The anaerobic organism Clostridium cochlearium has been observed to have a high capacity for methylation of mercury in the presence of cysteine and vitamine B_{12} in the medium (73). Thus, the potential for microbial methylation of mercury by fungi and bacteria has been shown to exist under aerobic as well as anaerobic conditions. It is, however, difficult to evaluate the ecological significance of these findings. For instance methylcobalamin is known to be unstable in a natural environment. It has been found that the transmethylating activity of methyl cobalamine in vitro was inhibited by cellular proteins and thiol groups (4). Results given (70, 71, 32) do not necessarily imply that anaerobic methylation is of ecological significance. Since mercury is hardly present in nature under anaerobic conditions without the simultaneous presence of hydrogen sulfide, mercuric sulfide is likely to be formed. Under these conditions mercury will be effectively prevented from being methylated. Probably due to the existence of mercuric sulfide, no formation of methyl mercury in anaerobic mud has been found in certain experiments. (51) It is true that the sulfide will be oxidized to sulfate, if aerobic conditions should be reestablished, but this oxidation is probably slow. Accordingly, it was found that the methylation rate was 100-1000 times slower in aerobic sediments with mercuric sulfide as mercury donor as compared with mercuric chloride (20). In an experiment with fish, that was allowed to accumulate mercury from sediments it was found that mercuric sulfide was very slowly mobilized as compared with mercuric chloride (25).

Another complicating factor in transferring laboratory results to the ecosystem level is the existence of a de-methylating capacity that was first described in 1969 (23).
Most experiments on rates of methylation have been performed in such a way that they do not allow for a discrimination between the two competing processes, methylation and demethylation. Accordingly, it is possible that most of the experimental results that have been interpreted as measures of gross methylation rates in fact have been net methylation rates. The kinetics of the response of such a competitive system in relation to external stimuli, such as temperature, is of course more complicated; thereby making any data interpretation more uncertain than if only methylation is presupposed to occur.

In recent experiments, an experimental design enabled the investigation of methylation reaction separated from the de-methylation one (6). The methylation was studied with regard to the effects of 1) redox potential 2) inorganic mercury concentration 3) temperature 4) microbial activity and 5) sulfide concentration.

A kinetic model was proposed, which describes the rate of methylation as a function of some variables. Verification of the model and experimental estimation of system specific model coefficients were accomplished using laboratory scale mixed culture microbial reactors. The reactors were operated under anaerobic as well as aerobic conditions at microbial specific growth rates of 1/6, 1/12 and 1/24 per day. Methylation rates were determined at 10°C, 20°C and 30°C.

The kinetic studies showed: 1) Monomethyl-mercury is the predominant product of methylation of mercury (near neutral pH) 2) The rate of methylation is higher in aerobic systems than in anaerobic systems for a given inorganic mercury concentration and microbial growth rate. 3) Higher microbial growth produce higher methylation rates under aerobic as well as anaerobic conditions. 4) Methylation rates could be hampered by the addition of sulfide to some anaerobic systems. 5) Temperature affects methylation rates in accordance with its effects on the metabolic rate of the methylating organism.

Methylation of Arsenic

From the early experiments with Scropulariopsis brevicaulis the following mecanism for methylation of arsenic was proposed

$$As(OH)_3 \rightarrow H^+ + (HO)_2AsO^- \xrightarrow{CH_3^+} CH_3AsO(OH)_2 \rightarrow H^+ + CH_3ASO(OH)O^- \xrightarrow{red}$$
$$\rightarrow CH_3As(OH)O^- \xrightarrow{CH_3^+} (CH_3)_2AsOOH \xrightarrow{\text{ionization + red}} (CH_3)_2AsO^- \xrightarrow{CH_3^+}$$
$$\rightarrow (CH_3)_3AsO \xrightarrow{red} (CH_3)_3As$$

Recent investigations of biological alkylation of arsenic have shown various microorganisms capable of this synthesis. Fungi from industrial and agricultural As-containing sludge were isolated and taxonomically described, after having carried out the biotransformation (14).

An examination of fresh water, seawater, eggshells and rocksamples have been made. Chemical analysis were made for As^{5+}, As^{3+}, methylarsonic acid and dimethylarsinic acid. The methylated compounds

were found in most biological samples, but not in limestone. (8) The finding of methyl-arsene compounds in aerobic parts of the environment is slightly surprising as alkyl-arsenes are unstable in the presence of water and oxygene and are oxidized to arsenious acid.

The explanation must be that the rate of biological formation of methyl-arsene compounds is high enough compared to the subsequent oxidation to arsenious acid to allow for detectable concentrations of the intermediate form to build up.

Arsenious acid, however, may in turn again be an intermediate in the formation of methyl-arsene compounds

$$CH_2O + HAsO(\overset{a)}{OH_2}) \rightarrow HO\cdot CH_2AsH(O)OH \xrightarrow{red} CH_3AsO(OH)_2 \xrightarrow{red} CH_3As(OH)_2 \rightarrow$$

$$\rightarrow CH_3AsH(O)OH \xrightarrow{CH_2O} HO\cdot CH_2As(CH_3)OOH \rightarrow (CH_3)_2AsO\cdot \overset{b)}{OH} \xrightarrow{repetition}$$

$$\rightarrow (CH_3)_3AsO \xrightarrow{red} (CH_3)_3As$$

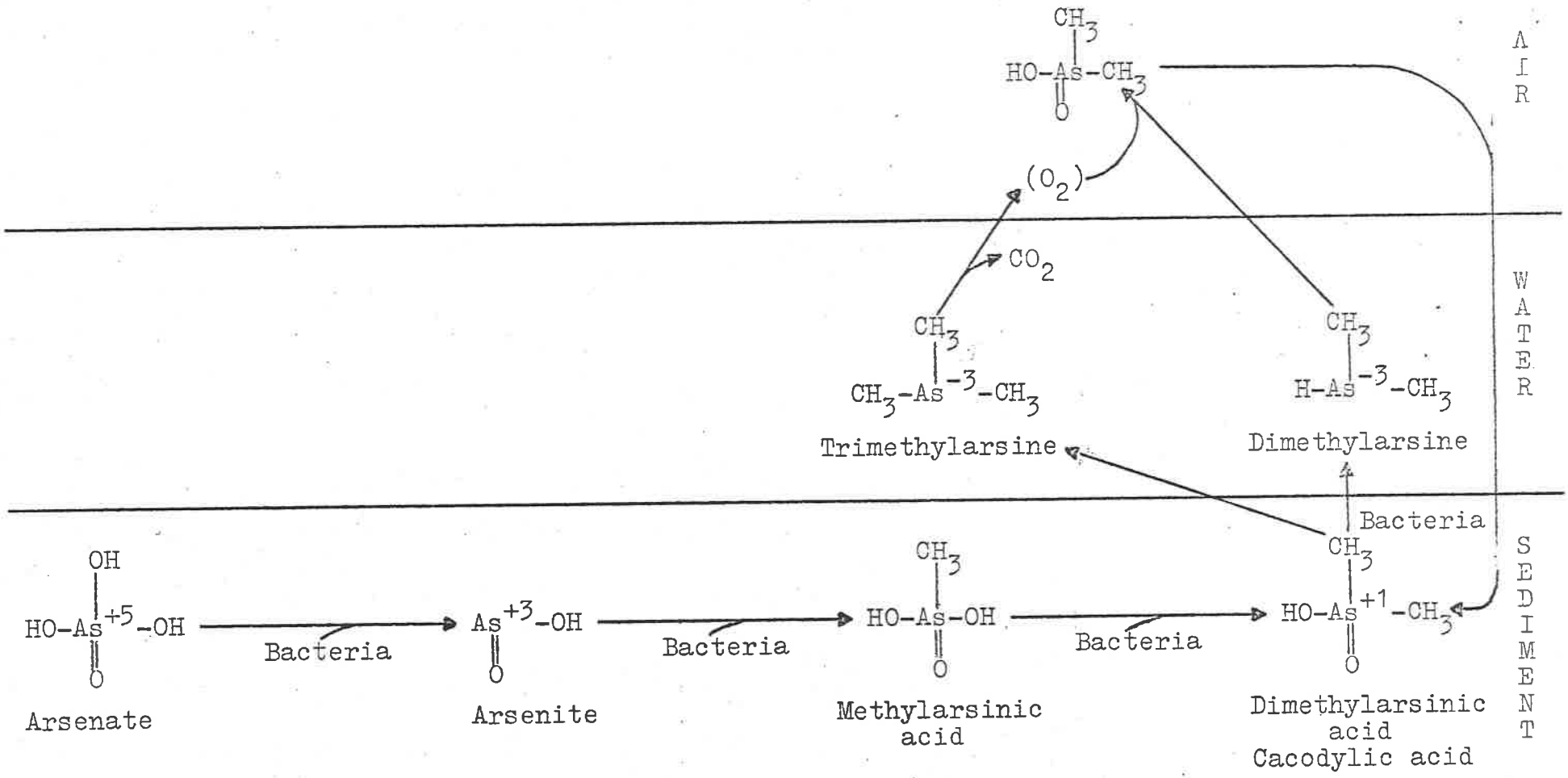
a) arsenious acid

Figure 4 b) cacodylic acid

Transalkylation

In a series of experiments with a strain of Pseudomonas, biological formation of methyl tin compounds were shown. (43)
Methyl tin compounds in a later experiment co-occurred with methylmercury in a pattern that lead the authors to suggest that the formation of methylmercury might not be a direct biological methylation but rather a transalkylation from biologically formed methyl tin compounds. (26)

Figure 4



The arsenic cycle.

(After Wood, 1971)

In experiments regarding the ability of sewage fungi to methylate different selenium compounds, Penicillumstrains were isolated from the sewage. When adding tellurium compounds ($TeCl_4$, H_2TeO_3, and H_6TeO_6), they were found to methylate as well, but only in the presence of selenium. The yield of dimethyltellurid was proportional to the input of inorganic selenium, so that no methylated tellurium compound was found until the input quotient of selenium to tellurium was about 10:1. This indicates a possible transalkylation instead of a direct biological alkylation of tellurium. (22)

DEGRADATION OF METAL-ORGANIC COMPOUNDS

The biological stability of metal organic compounds vary widely. Some of them rapidly degradate in non enzymatic processes or through the action of non-specific enzymes. Others are highly persistent and are degraded only through specific and slow biochemical processes. Short-chain alkyl mercury compounds belong to the latter group.

Decomposition of organic and inorganic mercury compounds through bacterial activity resulting in the formation of elemental mercury has been reported by several investigators.

A mercury resistant strain of Pseudomonas has been found to be capable of reductive decomposition of mercurials to metallic mercury.

The decomposition was shown to be catalysed by the enzyme systems, a NAD(P)H generating system and the metallic mercury releasing enzyme (MMR-Enz) together with a specific cytochrome-c. The MMR-Enz was found to contain FAD as a prosthetic group. The enzyme was shown to be induced in the presence of mercurials such as e.g. phenyl-mercuric acetats and mercuric chloride, whereas the cyt-c and the NAD(P)H generating system were constitutive.

A pathway for the electron flow has been proposed and compared with that of the respiratory chain: Electrons from reduced NAD(P), generated by glucose de-hydrogenase, are transferred to cyt-c via the MMR-Enz. with the mercurial becoming the terminal acceptor of eletrons in its reduction to metallic mercury. Mercury thus would be a terminal acceptor of electrons like oxygen in a respiratory chain. (57, 58)
However, it is not known whether the reduction of the mercuric ion results in a gain or loss of energy for the organism.

Anaerobic sediments treated with ionic mercury release elemental mercury (7).

Four strains of bacteria capable of converting the methyl mercury ion to methane and volatile elemental mercury have been isolated from lake sediment. (55)

Elemental mercuryvapor and benzene were products of phenylmercuric acetate degradation by selected cultures of mercury-resistant bacteria (44).

In a series of experiments incubation of a mixed bacterial population from river water, after addition of methyl mercury, led to an increase in the number of bacteria resistant to methyl mercury, some of which were capable of decomposing methyl mercury (74).

It has also been suggested that an equilibrium may be reached between the production (addition) of methyl mercury and it's degradation in mercury polluted environments. Studies by Landner and Larsson demonstrated methyl mercury degradating activity by bacteria in sediments from the Baltic Sea.(31)

In the light of these findings an earlier study of methylation of mercury in the St. Clair system on the Canada/USA border was re-evaluated and the results were demonstrated to support the idea of an equilibrium between chemically alkylated (from methyl and ethyl lead) mercury and biological degradation (29).

Adding to these processes the one of transport of methyl mercury from the sediment the hypothesis can be formulated that in a sediment, under a constant flow of methyl mercury into or out of the system, biological formation and degradation of methyl mercury will result in an equilibrium, with a constant level of methyl

mercury in the sediment.

If input or output of methyl mercury is varying, a disrupted pattern will result, where the methyl mercury level in the sediment tend to reestablish at the equilibrium level after the disturbance.

The curve in Fig. 5 could illustrate such a situation.

Figure 5

From this model it is obvious that in a situation where no methylmercury is added the importance of the demethylating activity will be dependent on whether or not the relation between methylating activity and transport activity out of the system (sediment) is such that the equilibrium is apporached.

Figure 6

In a situation of the type represented by Fig. 6 transport out of the system effectively prevents the methyl mercury concentration from building up to levels where demethylation becomes important. In this case the difference between gross- and net-methylation rates is not very important.

An alternative situation is illustrated in a schematic figure (Fig. 7).

Figure 7

Figure 5

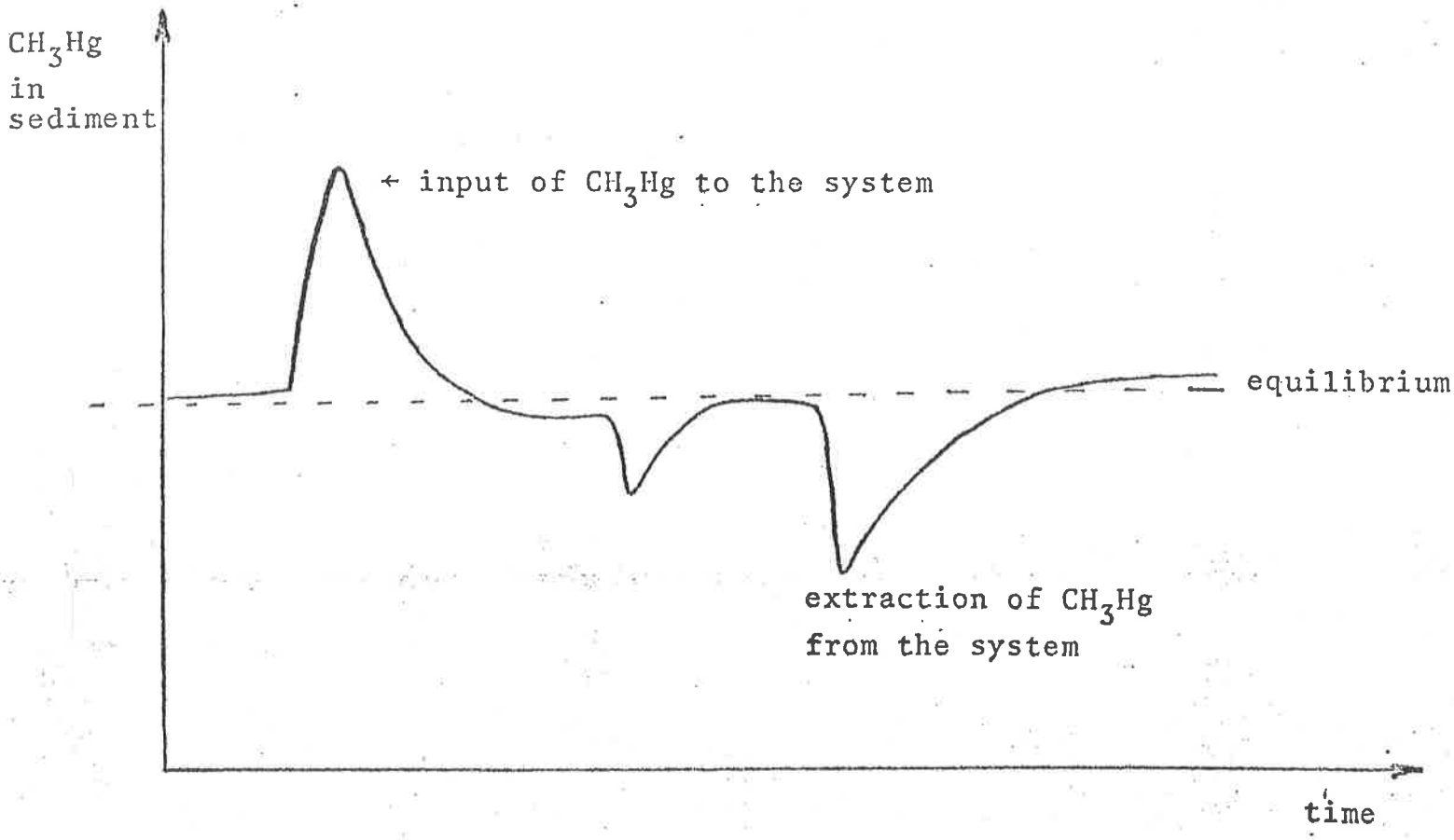

CH_3Hg in sediment
← input of CH_3Hg to the system
equilibrium
extraction of CH_3Hg from the system
time

Figure 6

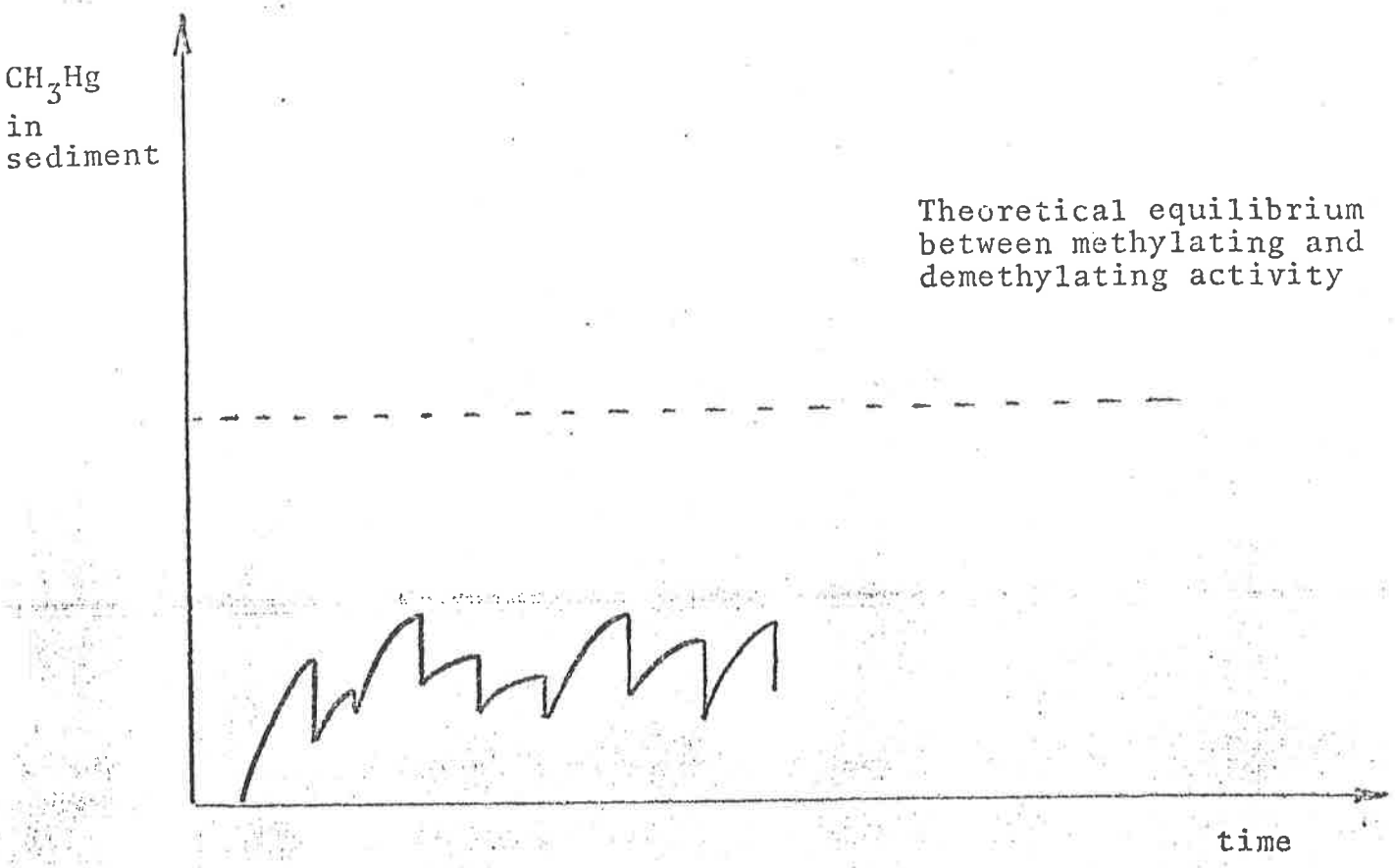
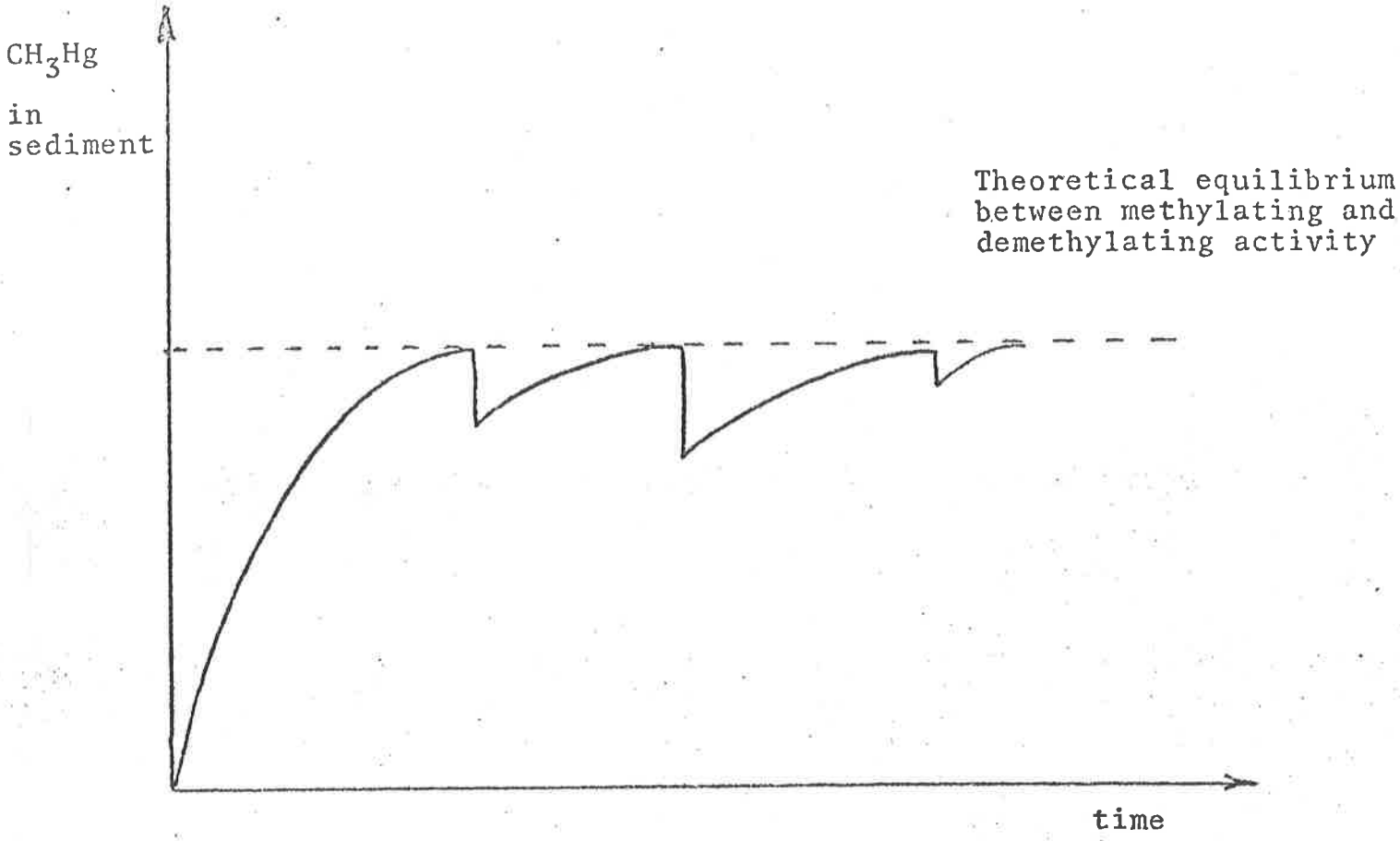

CH_3Hg
in
sediment
Theoretical equilibrium
between methylating and
demethylating activity
time

Figure 7


CH_3Hg
in
sediment
Theoretical equilibrium
between methylating and
demethylating activity
time

This shows that the transport activity of methyl mercury out of the system is low and the demethylating activity is important as regulator of the methylmercury concentration in the system (sediment).

In this case the difference between gross- and net-methylation rate is very large.
From the schematic figures it can be noted that the concentration of methyl mercury in the system (sediment) is low when the amount transported out of it is large and vice versa.

Thus, in the example a negative correlation exists between methyl mercury concentration in the system (sediment) and the amount of methylated mercury released from it.

CRITERIA FOR PREDICTION OF MICROBIAL CONVERSION OF ECOLOGICAL SIGNIFICANCE

Based on general knowledge of chemical properties of metals and metalloids and scattered experience of biological formation of organo-metal compounds, some criteria can be given for the changes in properties of the metabolized metal compared to its original form which may be of ecological significance.

Changes in properties

1) Tendency for complex formation
2) Relative and absolute water and lipid solubility
3) Volatility
4) Valence state

It is obvious from the criteria above that conversion leading to formation of alkyl metal compounds fulfill most of the criteria for changes of ecological significance, that is, the threat of the altered metal and not the action of its original form on the microorganisms involved. The knowledge of today indicates that this 'secondary' poisoning will be a vital environmental problem which should be studied with great concern.

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