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MICROBIAL ALKYLATION OF METALS

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ABSTRACT

Microbial alkylation has to date been demonstrated for a number of metals and metalloids and on physical/chemical grounds predicted to occur with a number of others (see J. Wood these proceedings). The group of metals for which the process has been demonstrated includes mercury, selenium, tellurium, arsenic and tin, and it has been postulated also for platinium and gold.

RÉSUMÉ

On a pu démontrer que l'alkylation microbienne se produisait en fait dans le cas d'un certain nombre de métaux et de métalloides et, d'après certains phénomènes physico-chimiques, qu'elle devrait être possible pour d'autres (voir J. Wood au cours de ce symposium). Le groupe d'éléments pour lesquels ce procédé a été démontré comprend le mercure, le sélénium, le tellure, l'arsenic et l'étain et, hypothétiquement, le platine et l'or entre autres.

INTRODUCTION

PROCESSES OF BIOCHEMICAL ALKYLATION

A. Methylation of Selenium

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

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

$$CH_3Se(0)O \xrightarrow{-CH_3^+} (CH_3)_2SeO_2 \xrightarrow{red} (CH_3)_2SeO \xrightarrow{red} (CH_3)_2Se.$$

The biosynthesis of volatile dimethylselenide is a major metabolic pathway for detoxifying selenite in rats for example.

This process occurs directly in the animal tissue and, thus, is not the result of an intestinal microfloral activity in the animal. Further studies showed a specific requirement for glutathion for the formation of dimethylselenide.

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 (Byard 1969; Palmer *et al.* 1969; Palmer *et al.* 1970).

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 identified in the air expired by rats given a higher dose of selenite ion (Vlašaková *et al.* 1972).

This compound is also synthesized by toxic plants, as shown for some *Astragalus* species (Virupaksha and Schrift 1965).

The less toxic compounds, which, nevertheless, 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.

B. Methylation of Tellurium

Tellurium was found to methylate in the presence of different *Penicillium* strains. The volatile metabolite was identified as dimethyltelluride (Bird and Challenger 1939).

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 (Challenger 1945; Wood 1974).

C. Methylation of Arsenic

From the early experiments with *Scropulariopsis brevicaulis* the following mechanism for methylation of arsenic was proposed:

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

Recent investigations of biologcial 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 (Cox and Alexander 1973).

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 (Braman and Foreback 1973). 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 oxygen and are oxidized to arsenious acid.

The explanation must be that the rate of biologcial formation of methy-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:

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

$$\xrightarrow{red} CH_3As(OH)_2 \rightarrow CH_3AsH(O)OH \xrightarrow{CH_2O} HO \cdot CH_2As(CH_3)OOH \rightarrow$$

$$\rightarrow (CH_3)_2AsO \cdot OH \xrightarrow[b)]{repetition} (CH_3)_3AsO \xrightarrow{red} (CH_3)_3As$$

a) arsenious acid

b) cacodylic acid

(see Fig. 1)

D. Methylation of Mercury

After the demonstration that mercury in fish predominantly was present in the form of methylmercury (Westöö 1966) it was shown that unidentified microorganisms in natural organic lake sediment could methylate mercury (Jensen and Jernelöv 1967). 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 (Jensen and Jernelöv 1969).

The mechanism of methylation has more recently been the subject for many studies, but it is still not fully understood, although several hypotheses

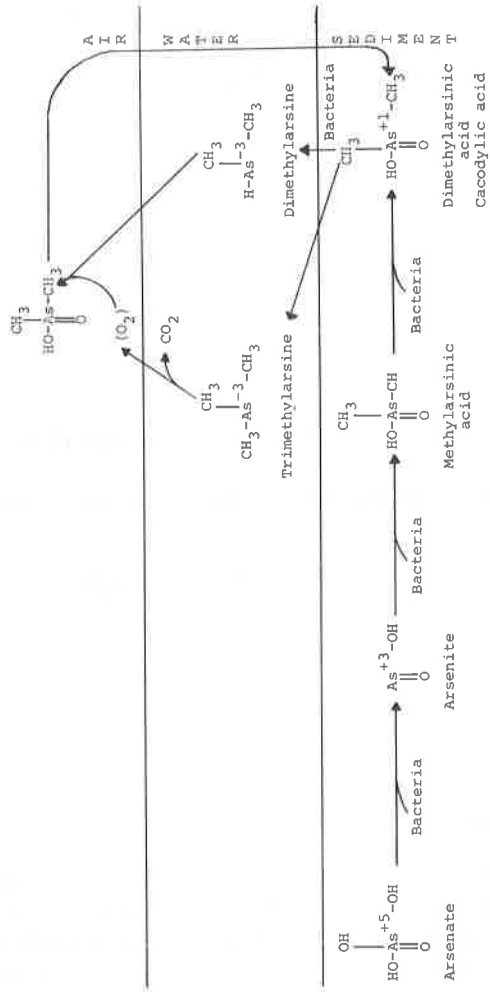

Fig. 1. The arsenic cycle (after Wood 1971).

have been suggested. Nonenzymatic 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 (Wood *et al.* 1968).

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 (Wood 1971). (Figs. 2 and 3).

During 1974 it was found by different workers that mercury was methylated in a neutral water solution by a purely abiotic reaction (Imura *et al.* 1971: Bertilsson and Neujahr 1971). The methyl donor was methyl cobalamine and the reaction was very fast and almost quantitative both under aerobic and anaerboic conditions.

However, in several experiments it has been shown that microbial activity is a prerequisite for the synthesis of methylmercury under natural conditions, unless other methyl metal compounds (e.g. tetramethyl lead or methyl tin species (Huey *et al.* 1974)) are added (Beijer *et al.* 1971).

In addition, bacteria isolated from mucuous material on the surface of fish (Jernelöv 1968), and bacteria belonging to the genus *Pseudomonas* isolated from soil (Kitamura *et al.* 1969), have been shown to be able to methylate mercury under aerobic conditions.

Methylation *in vivo* was studied in aerobic cultures of *Neurospora crassa* (Landner 1971). Mercury tolerant mutants were very effective in 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" snythesis 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

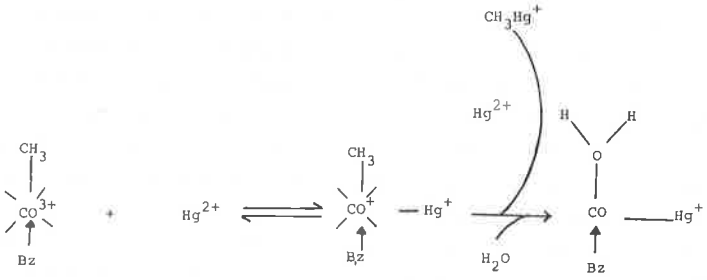

Fig. 2. Proposed methylation of mercury by a methylcorrinoid under nonenzymatic aerobic conditions (after Wood 1971).

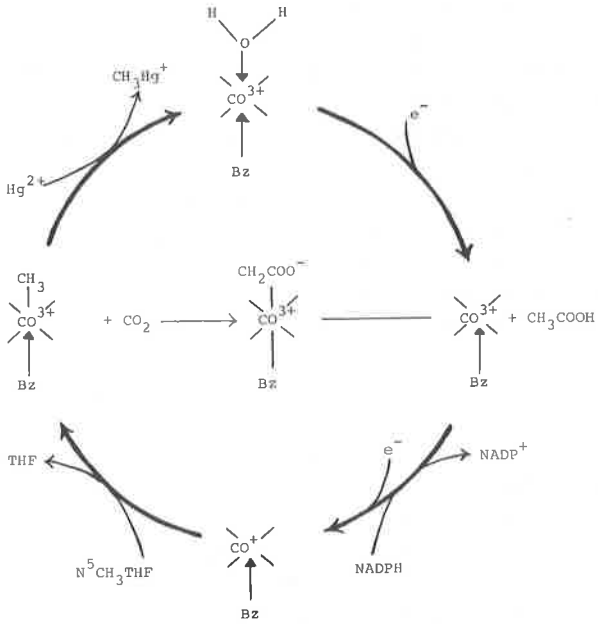

Fig. 3. Proposed mechanism of mercury methylation in methylcobalaminacetate synthetase system under anaerobic conditions (after Wood 1971).

cultures have the capacity of methylating mercury, when added as mercuric chloride (Vonk and Kaars Sijpesteijn 1973) (see Fig. 4).

In a recent study it has further been shown that microbial conversion of inorganic mercury compounds into methylmercury also can take place in soil (Beckert *et al.* 1974). The anaerobic organism *Clostridium cochlearium* has been observed to have a high capacity for methylation of mercury in the presence of cysteine and vitamin B_{12} in the medium (Yamada and Tonomura 1972). 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, methyl-cobalamin 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 (Bertilsson and Neujahr 1971). Results given (Wood *et al.* 1968; Imura *et al.* 1971) do not necessarily imply that anaerobic methylation is of ecological

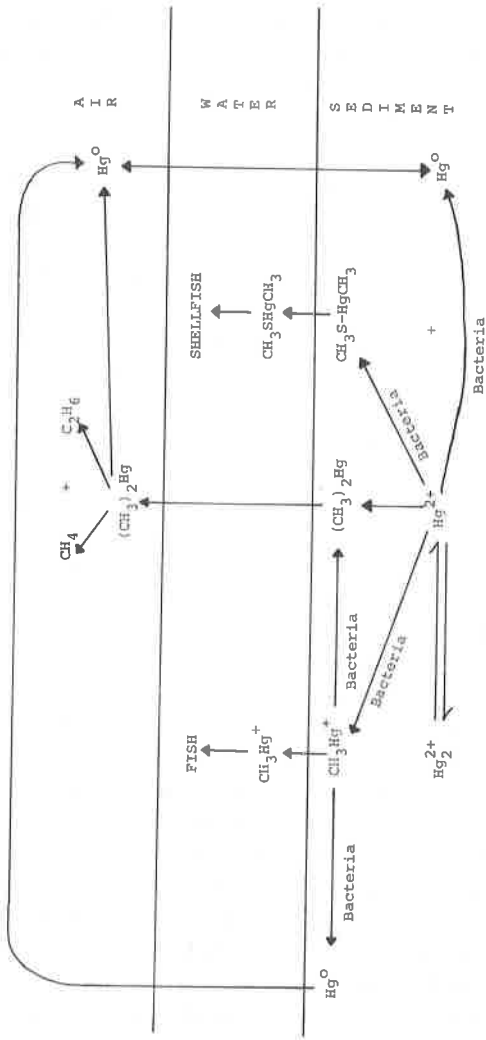

Fig. 4. The mercury cycle (after Wood 1971).

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 methylmercury in anaerobic mud has been found in reported experiments Rissanen 1974, personal communication). 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 (Fagerström and Jernelöv 1971). 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 (Gillespie 1972).

Another complicating factor in transferring laboratory results to the ecosystem field level is the existence of a demethylating capacity that was first described in 1969 (Furukawa *et al.* 1969). 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 the methylation reaction separated from the demethylation one (Bisogni and Lawrence 1973). 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. Varification 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 Monomethylmercury is the predominant product of methylation of mercury (near neutral pH values).

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 produces 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.

BIOLOGICAL DEGRADATION OF ALKYL-METAL COMPOUNDS

The biological stability of metal organic compounds vary widely. Some of them rapidly degrade 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 organomercurials by bacteria resulting in the formation of elemental mercury has been reported by several investigators. The existence of such organisms was first discovered in Japan when bacteria isolated from soil heavily contaminated with organomercurials were found to be capable of converting phenyl mercury and methyl mercury into metallic mercury and benzene and methane respectively (Tonomura *et al.* 1968).

A large number of resistant bacterial strains capable of hydrolysing and reducing mercury compounds have been found since, some of which have been investigated more thoroughly (Bongers and Khattak 1972; Billen *et al.* 1974; Spangler *et al.* 1973; Nelson *et al.* 1973).

Two distinct groups of organisms have been found: those resistant to organomercurials as well as mercury (II), and those resistant to mercuric ion only. The cleavage of the mercury-carbon bond in organic mercury compounds and the reduction of mercury (II) have invariably been found to be catalyzed by enzyme systems either inducible or constituitive; one or more to catalyze the cleavage of the mercury-carbon bond in different organic compounds and one to catalyze the reduction of the mercuric ion formed in the first reaction or encountered in the environment. In some cases the work on purifying and characterizing the different enzymes has progressed very far (Furukawa and Tonomura 1972; Tezuka and Tonomura 1976; Schottel and Silver 1976; Izaki *et al.* 1974).

Thus in one case two different hydrolases with different physical properties have been isolated from a bacteria, however the differences in

enzymatic properties are not yet fully understood (Tezuka and Tonomura 1976). In another case at least two hydrolases have been found and purified in a mutant of *Escherichia coli*, one active against methyl mercury the other against phenyl mercury (Schottel and Silver 1976).

Where the genetics for the resistance has been worked out it is always found to be associated with plasmids (Summers and Lewis 1973; Schottel *et al.* 1974; Silver *et al.* 1975).

It has also been suggested that an equilibrium may be reached between the production (addition) of methylmercury and its degradation in mercury polluted environments. Studies by Landner and Larsson demonstrated methylmercury degradation activity by bacteria in sediments from the Baltic Sea (Jernelöv *et al.* 1974).

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.

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

If input or output of methylmercury varies, a disrupted pattern will result, whereby the methylmercury level in the sediment will tend to re-establish at the equilibrium level after the disturbance.

The curve in Fig. 5 could illustrate such a situation. From this model it is obvious that in a situation where no methylmercury is added the importance of the demethylating activity will be dependent upon whether or not the relationship between methylating activity and transport activity out of the system (sediment) is such that the equilibrium is approached.

In a situation of the type represented by Fig. 6, transport out of the system effectively prevents the methylmercury 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).

This shows that the transport activity of methylmercury out of the system is low and the demethylating activity is important as a 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

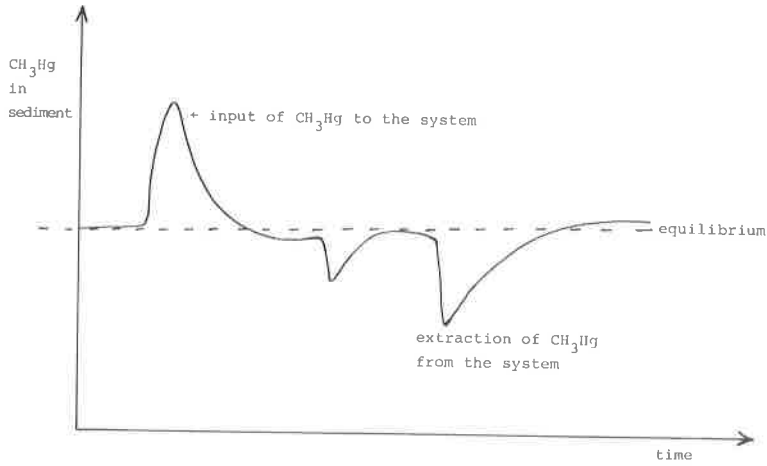

Fig. 5.

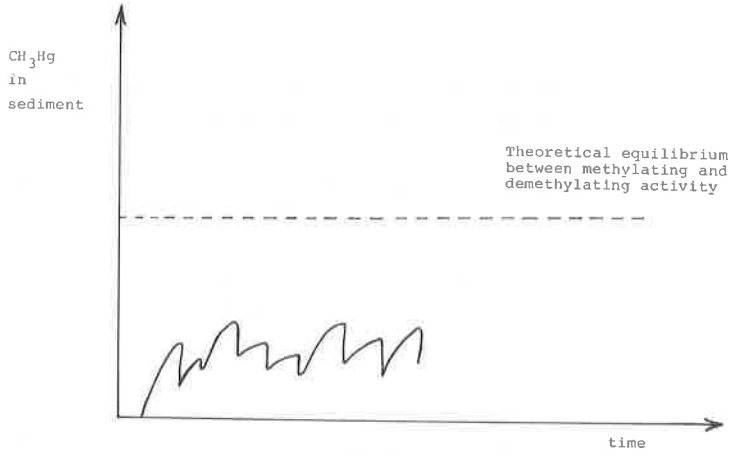

Fig. 6.

of methylmercury 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 methylmercury concentration in the system (sediment) and the amount of methylated mercury released from it.

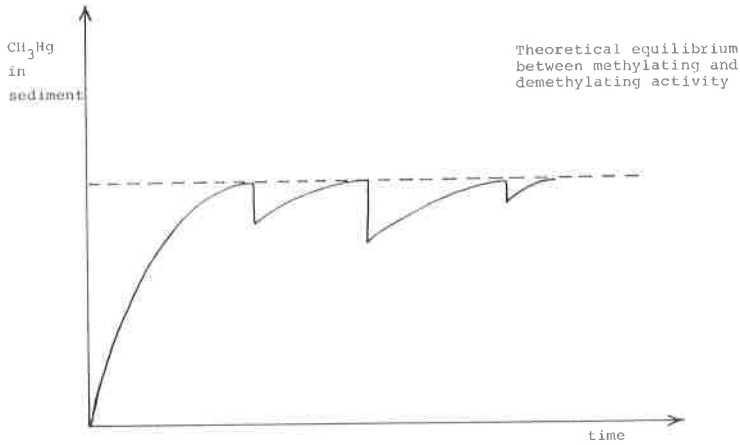

Fig. 7.

ECOLOGICAL IMPORTANCE OF BIOLOGICAL FORMATION OF ALKYL METAL COMPOUNDS

Naturally in order to be ecologically important the alkylated compounds must not only be formed but also persist sufficiently long enough to reach biologically active concentrations. Using methylmercury as an example, and assuming that the biological alkylation occurs in sediment or on suspended particles in water, Fig. 8 illustrates some properties that regulate the ecological importance of the alkylation process. The scheme can easily be modified to illustrate volatile compounds as well.

The principles are:

1 that a sort of biologically achieved equilibria may exist between the alkylated and non-nonalkylated forms of the metal (e.g. metalloid).

2 that transport processes in and out of the site of formation contributes to the net rate of formation or degradation by disturbing the "equilibria."

The properties of the alkylated compound in relation to that of the parent species must be such that the transport arrow out of the "alkyl metal box" is important.

Naturally, properties affecting persistency in other surroundings as well as those affecting tendencies for bioaccumulation and biomagnification will be

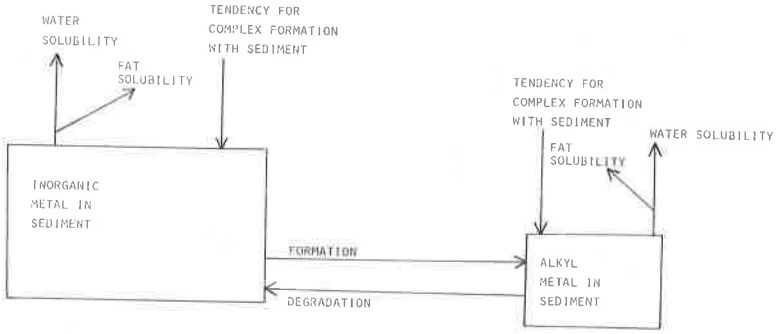

Fig. 8. Formation and degradation of alkylated metal compound in sediment, Size of boxes illustrate concentrations (amounts); arrows processes that effect the concentrations in sediment.

important for the further fate and effect. Those discussed here are only necessary first step prerequisites to re-order an alkylated compound ecologicallly important.

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