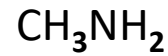
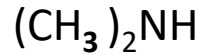
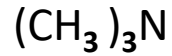
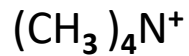


Oxidation of methylated amines



Tetramethylammonium

Trimethylamine

Dimethylamine

Methylamine

Ammonia

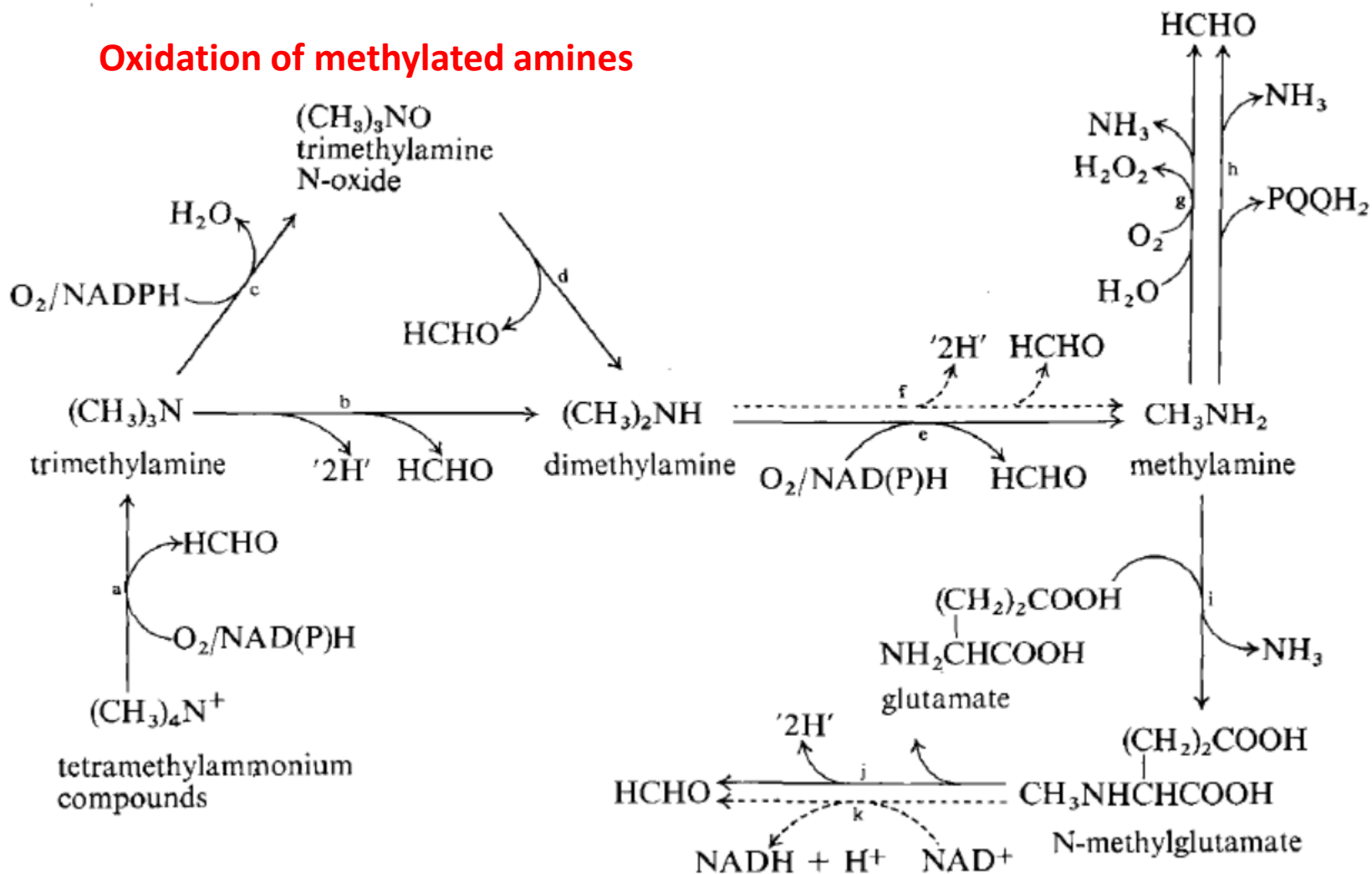
Formaldehyde

Metabolism involves demethylation which always occurs at the same time as an oxidation. One methyl group is removed at a time, the C1 unit being removed as formaldehyde.

Methylated amines in the marine environment: Quote from [Yin Chen](#),¹ [Nisha A. Patel](#), [Andrew Crombie](#), [James H. Scrivens](#), and [J. Colin Murrell](#)¹

Methylated amine compounds, including TMA, are ubiquitous in the environment—for example, as end products of protein putrefaction ([18](#)). In the marine environment, methylated amines are released as a result of degradation of quaternary amine osmoregulators, such as glycine betaine, which are used by marine organisms to counteract water stress ([19–21](#)). Once released into the environment, methylated amines can be used by microorganisms as a C or N source. In fact, in the oceans, methylated amines represent a significant pool of C and N, and standing concentrations up to hundreds of nanomolar and micromolar have been reported in the water column ([22, 23](#)) and sediment pore water ([24, 25](#)), respectively. In addition to being involved in biogeochemical cycles of C and N, recent studies also suggest that methylated amines have the potential to affect global climate, being precursors of aerosol formation in the upper atmosphere ([26–28](#)).

Oxidation of methylated amines



(a) Tetramethylammonium monooxygenase; (b) trimethylamine dehydrogenase (in obligate methylotrophs); (c) trimethylamine monooxygenase (in facultative methylotrophs); (d) trimethylamine TV-oxide demethylase (aldolase); (e) dimethylamine monooxygenase (all methylotrophs); (f) dimethylamine dehydrogenase (anaerobic *Hyphomicrobia*); (g) amine oxidase (in *Arthrobacter* and methazotrophic yeasts); (h) methylamine dehydrogenase; (i) N-methylglutamate synthase; (j) and (k) N-methylglutamate dehydrogenases.

Bacterial oxidation of methylated amines

Much of the earlier work on this was done by Bob Eady in the lab of Peter Large in Hull in *Pseudomonas* AM1 (now *Methylobacterium extorquens* AM1) which was first isolated on methylamine (AM1 means Airborne Methylamine 1). 1968 -

He showed that oxidation is catalysed by a dye-linked methylamine dehydrogenase, assayed in a reaction system like methanol dehydrogenase.



The prosthetic group of methylamine dehydrogenase is TTQ, tryptophan tryptophyl quinone [McIntire et al 1991]. It is covalently bound, being a modified part of the protein backbone.

Remarkable fact: the first 2 types of quinoprotein to be described were both isolated in *Methylobacterium extorquens* AM1.

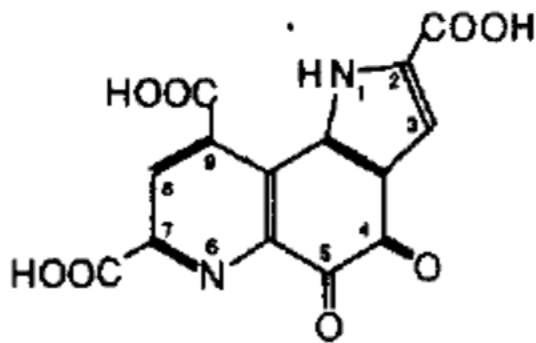
The electron acceptor for methylamine dehydrogenase was shown in 1981 by Tobari and Harada to be a blue copper protein called Amicyanin. This is a *single electron* acceptor.

The prosthetic groups of some Quinoproteins

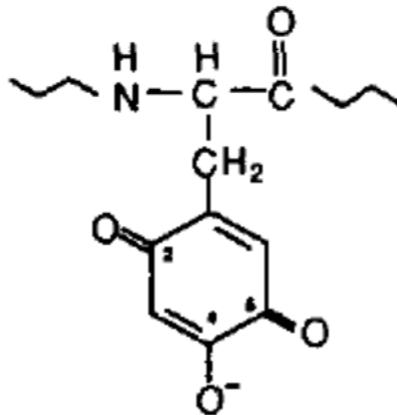
The prosthetic group of methanol dehydrogenase is PQQ, Pyrrolo-quinoline quinone [Kennard's group 1979]

The prosthetic group of methylamine dehydrogenase is TPQ, tryptophan tryptophyl quinone [McIntire et al 1991]

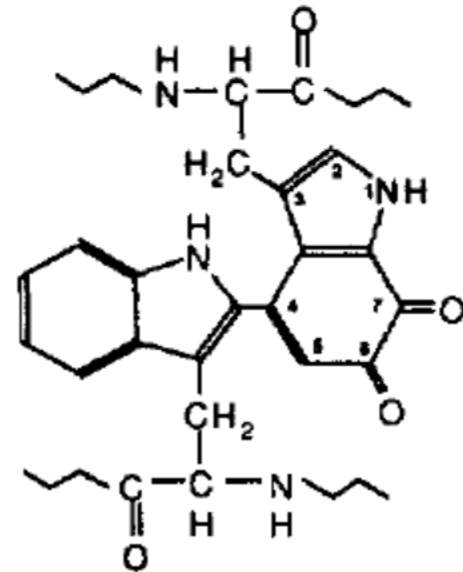
The prosthetic group of the copper containing amine oxidases is TPQ, topa-quinone [Klinman's group 1990]



PQQ



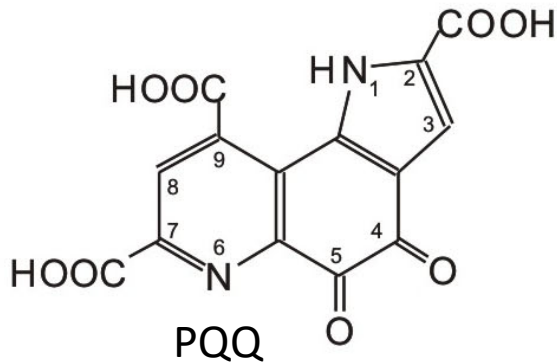
TPQ



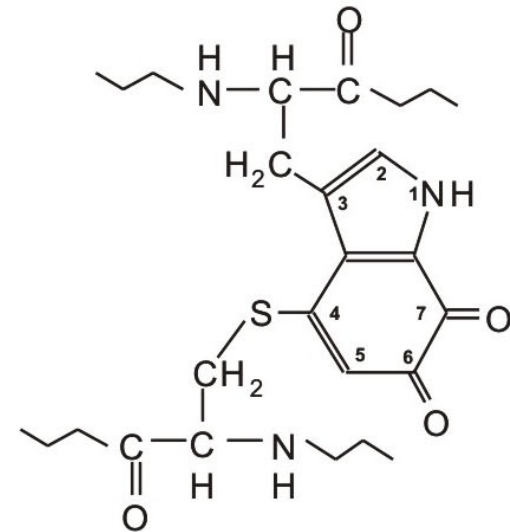
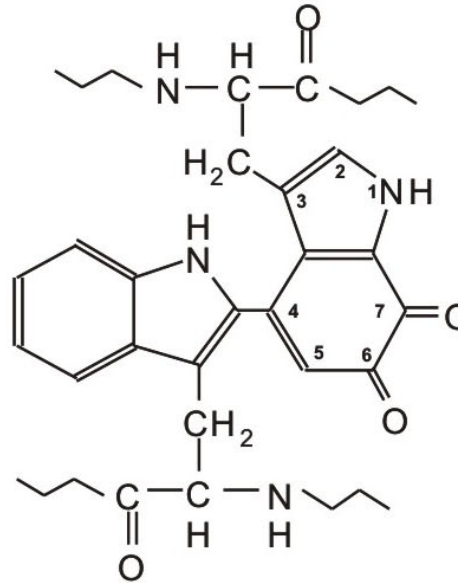
TPQ

Prosthetic groups of quinoproteins

Dehydrogenases



Not covalently bound

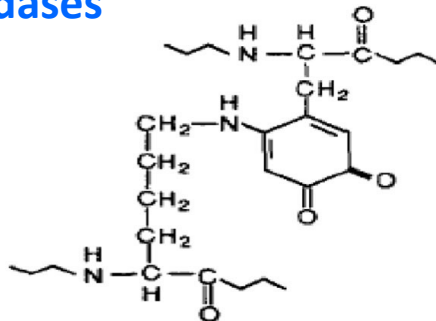
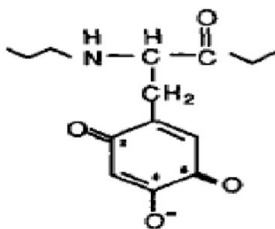


TTQ

[Derived from tryptophan]

CTQ

Oxidases



TPQ

LTQ

[derived from tyrosine]

PQQ, pyrroloquinoline quinone

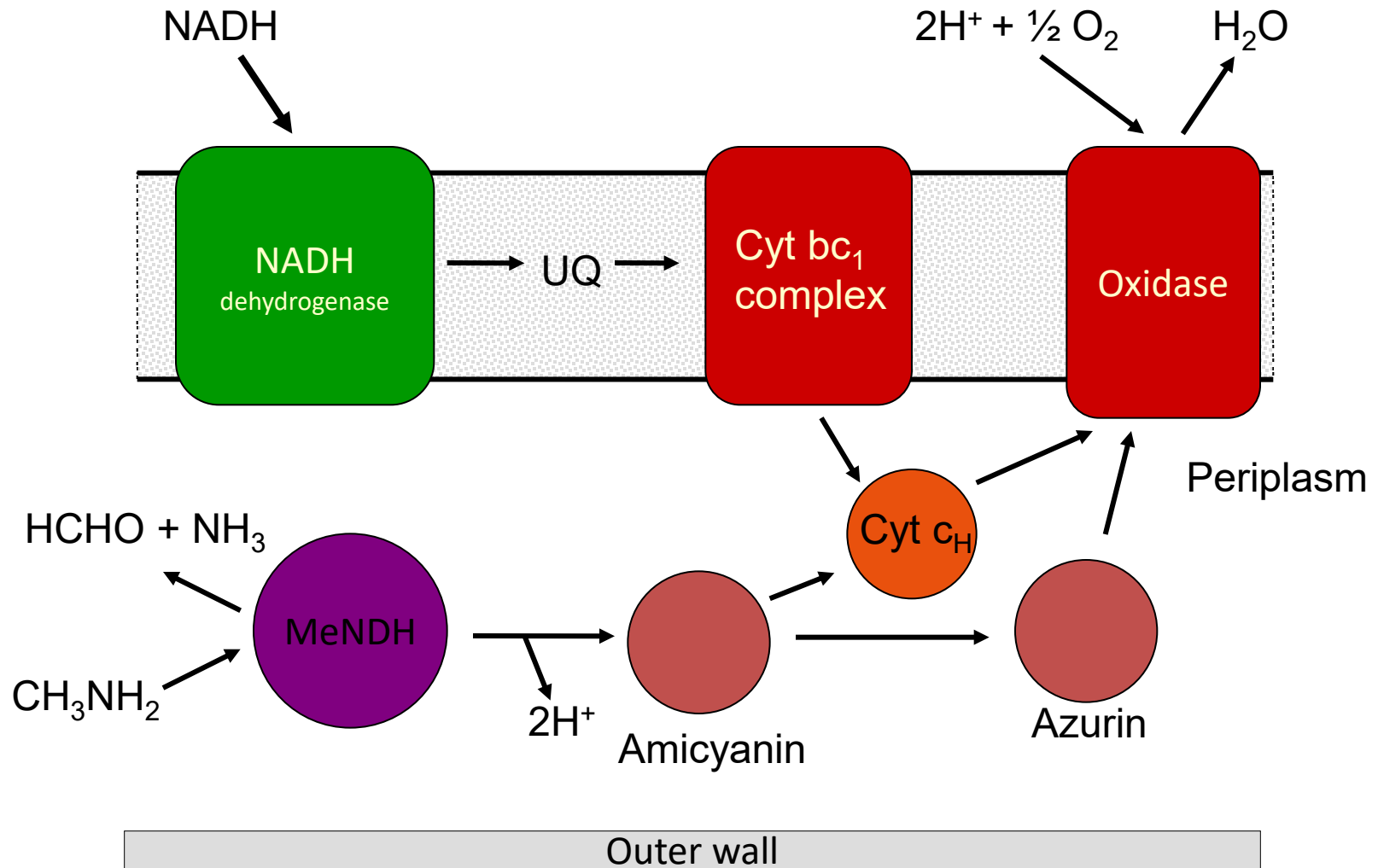
TTQ, Tryptophan tryptophylquinone

CTQ, cysteine tryptophylquinone

TPQ, Topaquinone

LTQ, Lysine tyrosylquinone

Electron transport during methylamine oxidation

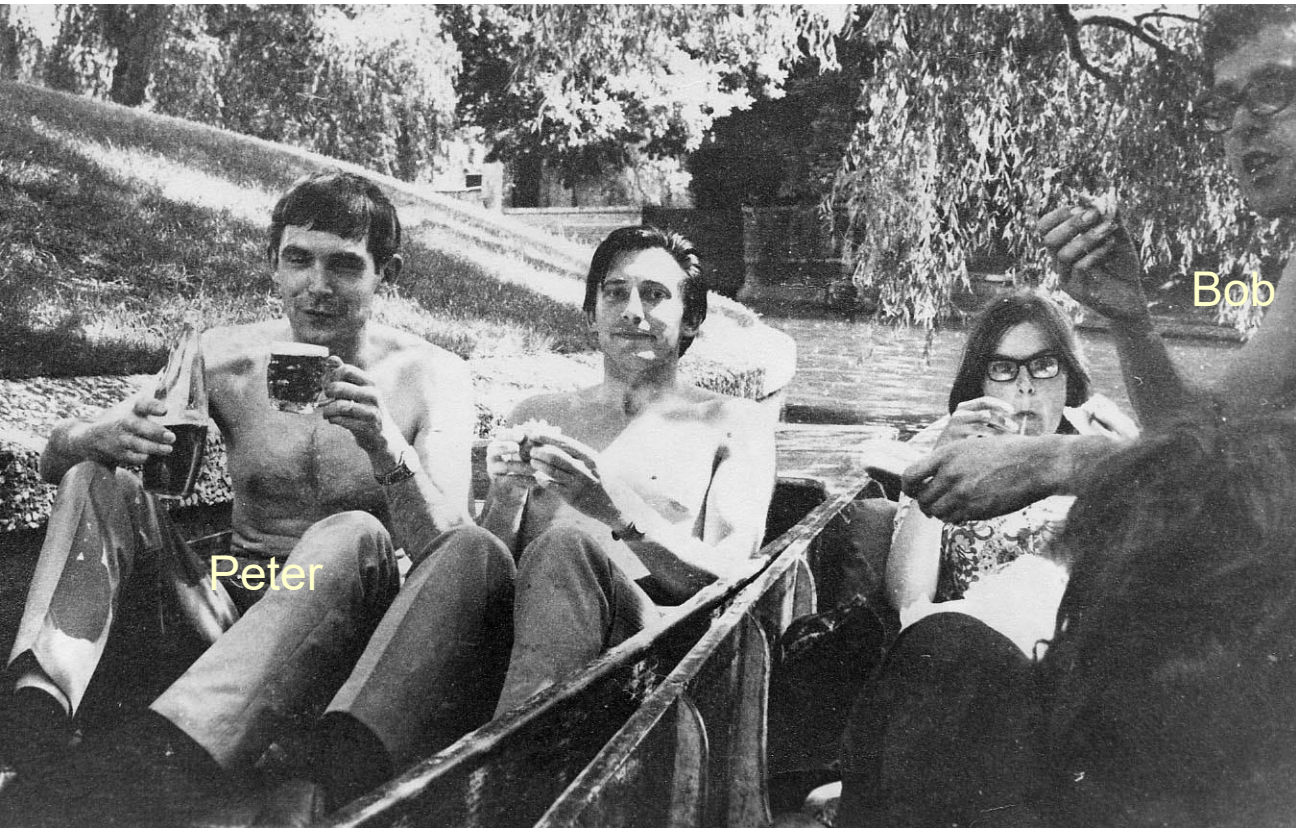


1985 - 1990 Ashley Lawton and Kevin Auton

[Amicyanin – Tobarī]

Bob Eady & Peter Large [MeNDH; 1968-1971]

Methylamine dehydrogenase [Bob Eady and Peter Large, 1968-1971]



Electron transport chain [Ashley Lawton & Kevin Auton, 1983-1989]



Ashley Lawton
President, Phyllos Inc.



Kevin Auton
Founder and CEO
of NextGen

Much of the subsequent study of the mechanism of methylamine dehydrogenase has been done by Victor Davidson and the genetics in Mary Lidstrom's group (with Mila Chistaserdova)



Other amine oxidising enzymes

Methylamine oxidase (Amine oxidase) In *Arthrobacter* P1



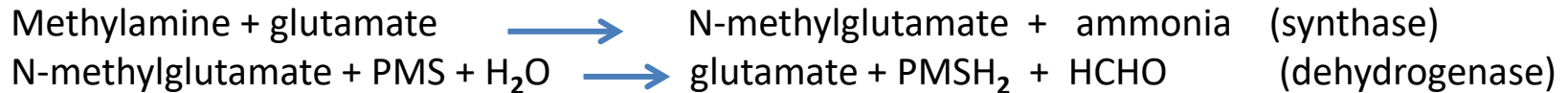
The peroxide is removed by catalase. No useable energy from this reaction.

Its prosthetic group is probably TPQ as in other copper-containing amine oxidases.

Systems involving methylated amino acids

In 'non-pigmented pseudomonads': *Ps. aminovorans*; *Pseudomonas* MA, & *Hyphomicrobium*

Two step system



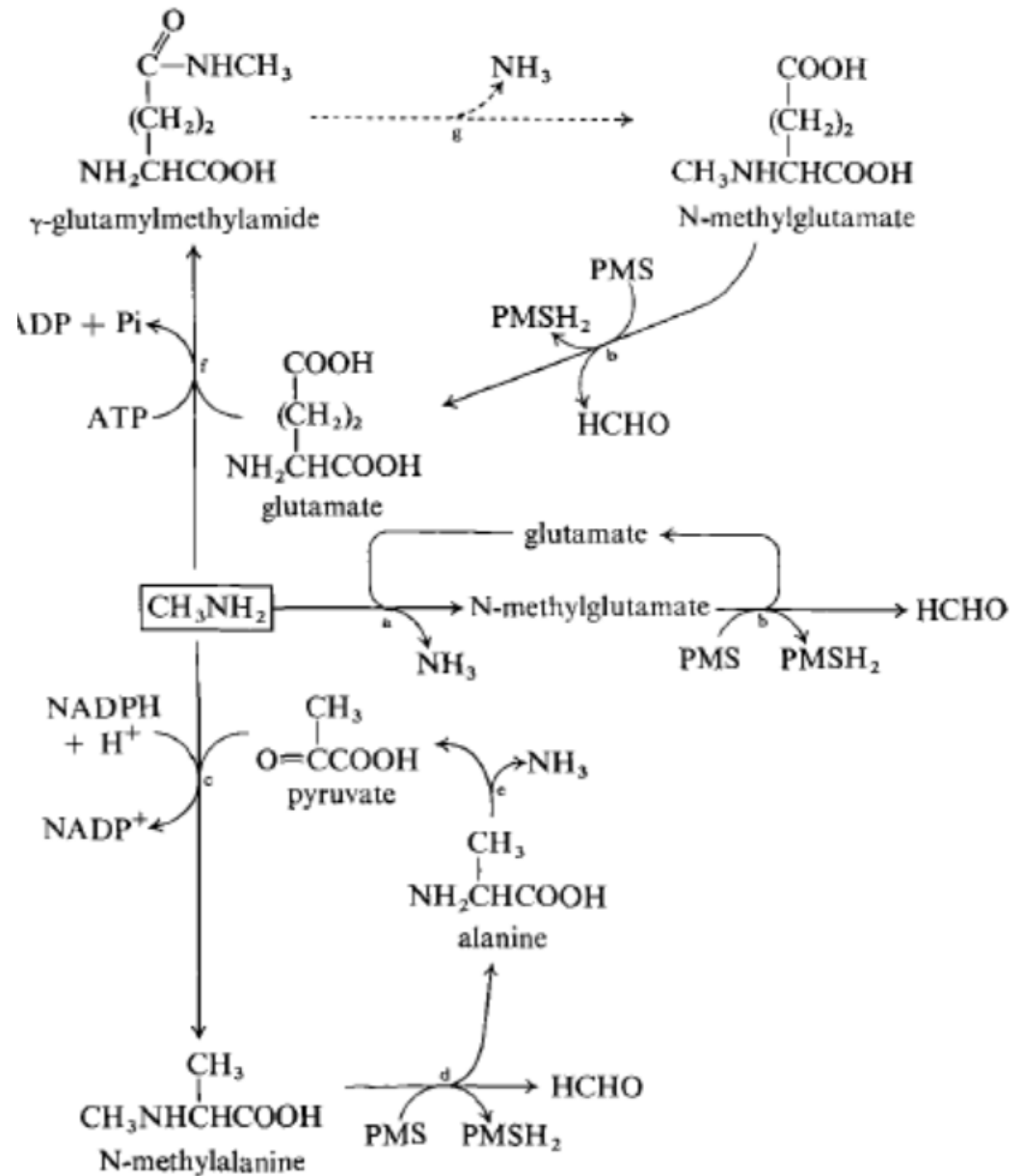
The dehydrogenase is usually a flavoprotein that interacts like other flavoproteins with the electron transport chain at the level of ubiquinone and cytochrome b.

(There are some reports of NAD-linked dehydrogenases in some *Pseudomonas* and *Hyphomicrobia* Netrusov)

The next slide shows some alternative two-part systems

Some alternative two-part systems

Alternative systems for production of N-methylglutamate are in *Pseudomonas* MS and *Hyphomicrobium* but they may not be involved in growth. Kung & Wagner, Loginova & Trotsenko, Meiberg & Harder



Oxidation of tetramethylammonium salts to trimethylamine and formaldehyde

In Organism 5H2. Hampton & Zatman 1973. A non-pigmented Gram-negative, non-motile facultative methylotroph. No energy is available from this reaction

Mono-oxygenase



Oxidation of trimethylamine to dimethylamine and formaldehyde

In obligate methylotrophs; organism W3A1, & 4B6 (like *Methylophilus*), and *Hyphomicrobium* Colby & Zatman 1973, 1974

Trimethylamine dehydrogenase

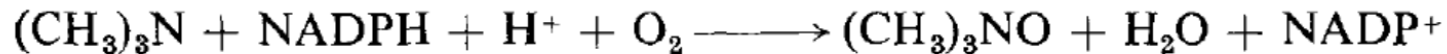


The enzyme is an unusual flavoprotein, interacting with the electron transport chain by way of a second flavoprotein, cytochrome b etc. So provides energy

Indirect route for trimethylamine oxidation

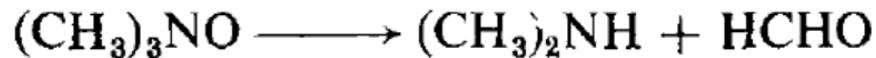
A trimethylamine mono-oxygenase produces the N-oxide which is then demethylated to formaldehyde plus dimethylamine. During growth on trimethylamine. In *Pseudomonas aminovorans* (a typical *Pseudomonas* sp.) and *Bacillus* PM6. Boulton & Large; Myers and Zatman.

a) Trimethylamine mono-oxygenase. No energy available



b) Trimethylamine N-oxide demethylase No energy available

It is the 2nd enzyme for oxidation of trimethylamine and also the 1st enzyme for growth of *Bacillus* PM6 on trimethylamine N-oxide



The oxidation of dimethylamine to methylamine and formaldehyde

There are two types of enzyme; the same one operates when dimethylamine is a growth substrate as when it is an intermediate in the oxidation of higher methylated amines.

Dimethylamine mono-oxygenase No energy is available from this reaction
In *Pseudomonas aminovorans* (a typical pseudomonad). Bob Eady and Peter Large.
Also in *Hyphomicrobium* in aerobic conditions

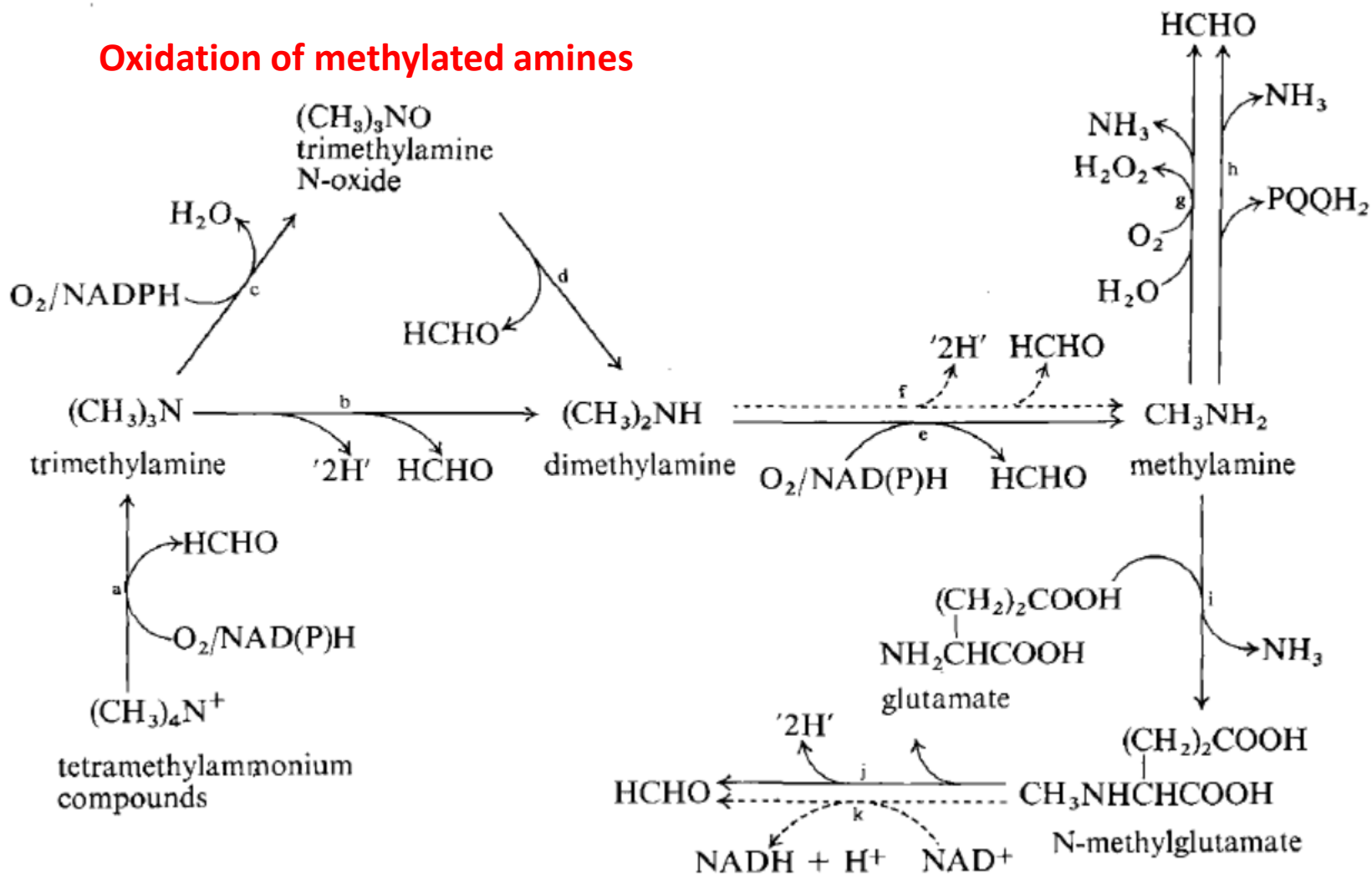


Dimethylamine dehydrogenase Energy is available. Meiberg and Harder 1978.
In *Hyphomicrobium* during anaerobic growth with nitrate as electron acceptor.



Similar to trimethylamine dehydrogenase. An unusual flavoprotein that interacts with electron transport chain before cytochrome b.

Oxidation of methylated amines



(a) Tetramethylammonium monooxygenase; (b) trimethylamine dehydrogenase (in obligate methylotrophs); (c) trimethylamine monooxygenase (in facultative methylotrophs); (d) trimethylamine TV-oxide demethylase (aldolase); (e) dimethylamine monooxygenase (all methylotrophs); (f) dimethylamine dehydrogenase (anaerobic *Hyphomicrobia*); (g) amine oxidase (in *Arthrobacter* and methazotrophic yeasts); (h) methylamine dehydrogenase; (i) N-methylglutamate synthase; (j) and (k) N-methylglutamate dehydrogenases.

Distribution of routes for oxidation of methylated amines

Trimethylamine

NAD-independent TMN dehydrogenase: Obligate methylotrophs (eg *Methylophilus*) and *Hyphomicrobium*

Mono-oxygenase system: All other methylotrophs including *Bacillus*

Dimethylamine

Mono-oxygenase: All aerobic methylotrophs including *Hyphomicrobium* growing aerobically

Dimethylamine dehydrogenase: *Hyphomicrobium* growing anaerobically

Methylamine

There are three types of system.

Methylamine dehydrogenase (MD); Methylamine oxidase (MO); N-methylglutamate-linked (NMG)

Most obligate methylotrophs	MD
Facultative autotrophs	MD
Pink facultative methylotrophs	MD or NAD-dependent or NAD-independent NMG dehydrogenase
Non-pigmented pseudomonads	NAD-independent NMG dehydrogenase
<i>Hyphomicrobia</i>	NAD-dependent or NAD-independent NMG dehydrogenase
<i>Arthrobacter</i> and yeasts	MO

NOTE: Some bacteria may have more than one system but this has rarely been tested.

There is no correlation between the system for methylamine oxidation and assimilation pathway