carboxy-lyase (EC 4.1.1.7) and a heat-stable benzaldehyde dehydrogenase. Preliminary evidence, largely obtained from an examination of the enzyme complements of cells grown into the penultimate generation on various intermediates of the pathway, suggested that the three enzymes are co-ordinately induced (Kennedy & Fewson, 1968).

Confirmation of this suggestion has now been obtained by measuring the differential rates of enzyme synthesis (Monod, Pappenheimer & Cohen-Bazire, 1952) in cells growing on glutamate and (a) induced with intermediates of the mandelate pathway, (b) gratuitously induced with thiophenoxyacetate, or (c) induced with mandelate but partially repressed by subsequent intermediates of the pathway. There is a significant correlation (correlation coefficient >0.97 for any pair of enzymes) among the differential rates of synthesis of the three enzymes under all these conditions.

Further supporting evidence has come from a study of mutants unable to convert L-mandelate into benzoate. By using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as mutagen, three classes of blocked mutants have been obtained: those that lack L-mandelate dehydrogenase, those that lack benzoylformate carboxy-lyase and those that lack L-mandelate dehydrogenase, benzoylformate carboxy-lyase and the heat-stable benzaldehyde dehydrogenase. Both L-mandelate and benzoylformate induce the other two enzymes in mutants lacking benzoylformate carboxy-lyase. Benzoylformate is probably the primary inducer of this regulon because it induces benzoylformate carboxy-lyase and benzaldehyde dehydrogenase in mutants lacking L-mandelate dehydrogenase; L-mandelate on the other hand has no inductive capacity in these organisms.

There are clearly considerable differences in the enzymology and regulation of the pathways converting mandelate into benzoate in the organisms that have so far been studied. The system in bacterium N.C.I.B. 8250 is unlike that in *Pseudomonas putida* (Hegeman, 1966), *Pseudomonas aeruginosa* (Rosenberg & Hegeman, 1969) or *Aspergillus niger* (Jamaluddin, Subba Rao & Vaidyanathan, 1970), and these in turn differ from each other.

A. L. is in receipt of a Science Research Council Studentship.

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The Purification and Properties of a Bacterial Trimethylamine Dehydrogenase

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Bacterium 4B6, a non-motile Gram-negative rod isolated from river-water, is capable of growth on trimethylamine, dimethylamine or methylamine as sole source of carbon and energy; trimethylamine *N*-oxide will not support such growth. Washed suspensions of trimethylamine- and dimethylamine-grown organisms both oxidize trimethylamine and dimethylamine, but not methylamine. Cell-free extracts prepared from trimethylamine-grown organisms have been shown to contain (1) a trimethylamine dehydrogenase that uses phenazine methosulphate as primary hydrogen acceptor, (2) a glutathione-independent NAD-linked aldehyde dehydrogenase and (3) an NAD(P)H-linked dimethylamine mono-oxygenase (cf. Eady & Large, 1969). No methylamine-oxidizing activity has so far been found in cell-free extracts of trimethylamine-grown organisms, although methylamine does not accumulate during growth on trimethylamine.

The trimethylamine dehydrogenase has been purified 30-fold, and this preparation contains a single protein as judged by analytical polyacrylamide-gel electrophoresis. The enzyme has a molecular weight of about 160 000 as determined by gel filtration (Andrews, 1965). Polyacrylamide-gel electrophoresis after exposure to 1% sodium dodecyl sulphate and 1% 2-mercaptoethanol (Shapiro, Viñuela & Maizel, 1967) shows the protein to be composed of two polypeptide chains of molecular weights approx. 70 000 and 80 000. The enzyme has an absorption maximum at 445 nm that disappears on reduction with dithionite.

The purified trimethylamine dehydrogenase catalyses the conversion of trimethylamine into dimethylamine and formaldehyde. Trimethylamine and dimethylamine were determined by g.l.c. (Sze, Borke & Ottenstein, 1963); formaldehyde was determined by a colorimetric method (based on Schryver, 1910) and oxygen uptake by Warburg monometry. The stoichiometry found agrees with the following equations:

where PMS is phenazine methosulphate.

The kinetics of the reaction were studied by using an anaerobic spectrophotometric assay in

which phenazine methosulphate is coupled to 2,6-dichlorophenol-indophenol. The pH optimum is 8.5 and the K_m for trimethylamine is about $2\mu\text{M}$. Of 42 compounds tried as substrate, the following gave an initial rate 10% or more of that with trimethylamine under the conditions tested: ethyldimethylamine, diethylmethylamine, 2-aminoethyldimethylamine, 2-hydroxyethyldimethylamine, 2-chloroethyldimethylamine and diethylamine.

The first step in the breakdown of trimethylamine by bacterium 4B6 thus appears to be the oxidative *N*-demethylation catalysed by trimethylamine dehydrogenase.

J. C. thanks the Science Research Council for a studentship.

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The Metabolism of Trimethylamine *N*-Oxide by *Bacillus* PM6

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Bacillus PM6 is an aerobic spore-forming Gram-positive rod-shaped organism isolated from soil. It is capable of growth on trimethylamine *N*-oxide, trimethylamine, dimethylamine or methylamine as sole source of carbon, nitrogen and energy. Washed suspensions of trimethylamine *N*-oxide-grown organisms rapidly take up oxygen in the presence of trimethylamine *N*-oxide, dimethylamine and methylamine, but not in the presence of trimethylamine. Such suspensions, and their cell-free extracts, convert trimethylamine *N*-oxide anaerobically into dimethylamine and formaldehyde, indicating the presence of a trimethylamine *N*-oxide demethylase. Assays of trimethylamine *N*-oxide (Bystedt, Swenne & Aas, 1959), dimethylamine (by g.l.c. and paper chromatography) and formaldehyde (Nash, 1953) yield results that agree with the stoichiometry required by the following equation:

In early experiments the reaction rate was followed by measuring formaldehyde production colorimetrically (Nash, 1953); subsequently a more sensitive and convenient assay was developed in which the trimethylamine *N*-oxide demethylase is

coupled to formaldehyde dehydrogenase (EC 1.2.1.1) (partially purified from baker's yeast) and the reaction is followed spectrophotometrically at 340nm.

The trimethylamine *N*-oxide demethylase was purified 175-fold. The purified enzyme moves as a single protein on analytical polyacrylamide-gel electrophoresis and has a molecular weight of 50000 as determined by gel filtration on Sephadex (Andrews, 1970). Treatment with 1% sodium dodecyl sulphate + 1% 2-mercaptoethanol caused no resolution of the protein as determined by polyacrylamide-gel electrophoresis (Weber & Osborn, 1969), suggesting that it consists of a single polypeptide chain; this experiment yielded a molecular weight of 37000. Ultracentrifugal analysis (sedimentation equilibrium) gave a molecular weight of 36000–47000. The pH optimum of the purified enzyme is 7.5, the K_m for trimethylamine *N*-oxide is 2.85mM and the absorption spectrum shows only a single peak at 280nm. Ferrous iron, glutathione and L-ascorbate are strongly stimulatory.

Benzyltrimethylamine *N*-oxide, chlorpromazine *N*-oxide and (+)-propoxyphene *N*-oxide were also active as substrate for the enzyme, but the maximum rates obtained were only about 5% of that obtained with trimethylamine *N*-oxide. Trimethylamine is a non-competitive inhibitor, and SKF 525-A is a competitive inhibitor.

The initial step in the metabolism of trimethylamine *N*-oxide by *Bacillus* PM6 thus appears to be a demethylation, catalysed by trimethylamine *N*-oxide demethylase, yielding dimethylamine and formaldehyde.

P. M. gratefully acknowledges the award of a Science Research Council Studentship.

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Configurational Dependencies of [^{19}F]Fluorine Chemical Shifts and Coupling Constants in Fluoromonosaccharides

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In general, chemical shifts and coupling constants for ^{19}F are of an order of magnitude greater