very slow fumarate oxidation in the facultative group.

Correlation between rapid fumarate oxidation and the presence of a particulate malate oxidase has been found in a further range of bacteria including pseudomonads, although the malate dehydrogenase in *Pseudomonas B₂aba* has been found to be more easily detachable (Kornberg & Gotto, 1961).

The particulate malate oxidase system has been studied in the strict aerobes, especially *Sarcina lutea* and *Moraxella lwoffii*, which both contain a soluble and a particulate malate dehydrogenase. In *S. lutea* malate dehydrogenase activity was found by Sephadex column chromatography to be associated with the carotenoids and membranes (see also Erickson & Parker, 1969). Preliminary results with *M. lwoffii* show the particulate malate oxidase to be very firmly bound to the membranes and associated with the electron-transport chain as in other strict aerobes studied, e.g. *Azotobacter vinelandii* (Jurtshuk, Bednarz, Zey & Denton, 1969).

We gratefully acknowledge financial assistance from the Science Research Council.

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Oxygenation of Methane by Methane-Utilizing Bacteria

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Studies with intact cells of *Methanomonas methanooxidans* (Brown, Strawinski & McCleskey, 1964) indicate that the route of methane oxidation in this micro-organism is:

Enzymes catalysing the stepwise oxidation of methanol to carbon dioxide have been reported in cell-free preparations of *Pseudomonas methanica* (Johnson & Quayle, 1964), but little is known about the mechanism of the oxidation of methane to methanol. By analogy with higher-alkane oxidation (Peterson, Kusunose, Kusunose & Coon, 1967) the reaction might be mediated by a mono-oxygenase:

where XH_2 is a reducing agent. Another possibility

might be an oxygenase system in which two molecules of methane are oxygenated in the overall reaction:

Leadbetter & Foster (1959) obtained indirect evidence in favour of oxygenase involvement in *P. methanica* by measuring the extent of ^{18}O incorporation into intact cells. On the other hand, Whittenbury (1969) pointed out that cell-yield data obtained in his laboratory and the inhibitor studies by Brown *et al.* (1964) might not be consistent with involvement of an oxygenase. Accordingly he suggested the possibility of a hydroxylation reaction giving methanol and two electrons for energy transduction:

Direct measurements of the incorporation of ^{18}O into methanol during oxidation of methane by whole cells of *M. methanooxidans* and *P. methanica* have now been made by using a combined gas chromatograph-mass spectrometer. When incubations were carried out under mixtures of methane and ^{18}O -enriched oxygen (55.93 atom % excess of ^{18}O) in the presence of unenriched water, the proportion of ^{18}O in the methanol varied between 53.9 and 59.8 atom % excess. In control experiments with mixtures of methane-unenriched oxygen in the presence of enriched water (19.0 atom % excess of ^{18}O) there was negligible incorporation of ^{18}O into the methanol. These results show that the conversion of methane into methanol by these micro-organisms involves an oxygenase.

We thank Dr H. F. West, Dr E. Bailey and Dr W. J. Cole of Nether Edge Hospital, Sheffield, for their help and for the use of their Perkin-Elmer 270 instrument.

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Coproporphyrinogenase Activities in Extracts of *Rhodopseudomonas spheroides*

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A crude extract of *Rhodopseudomonas spheroides* catalyses the conversion of coproporphyrinogen into protoporphyrin when incubated aerobically or anaerobically (Tait, 1969). Under anaerobic conditions there is no conversion unless MgSO_4 , L-

methionine and ATP are added. *S*-Adenosyl-methionine can replace methionine, but ATP is still required. The formation of protoporphyrin is inhibited by *S*-adenosylethionine and *S*-adenosyl-homocysteine.

The aerobic reaction has an optimum at about pH 7, whereas the anaerobic reaction has an optimum at about pH 8.5. A number of compounds markedly inhibit the anaerobic reaction but have no effect on the aerobic one. 1,10-Phenanthroline (0.17 mM) inhibits by 90%, $\alpha\alpha'$ -bipyridyl (1.5 mM) by 77%, riboflavin, FMN and FAD (30 μ M) all by about 95% and 2,4-dinitrophenol (0.7 mM) by 90%.

With a crude extract there is no requirement for a hydrogen acceptor under anaerobic conditions, although nicotinamide nucleotides stimulate slightly. An extract freed from small molecules by passage through Sephadex G-25 or G-50 has no activity unless nicotinamide nucleotides are added as well as *S*-adenosylmethionine and ATP. NAD⁺ or NADH partially restore activity, and NADP⁺ or NADPH are slightly more effective; maximum activity, equal to that of crude extract, is obtained with NADH plus NADP⁺. Sephadex-treated extracts lose activity rapidly, but addition of succinate prevents this. The succinate is probably oxidized via the succinoxidase system, thus keeping the extract free of oxygen.

The enzyme responsible for the aerobic reaction seems similar to that described in liver mitochondria (Sano & Granick, 1961; Batlle, Benson & Rimington, 1965) where O₂ is the hydrogen acceptor, and no cofactors are required. The results suggest that in the anaerobic reaction a flavoprotein and iron, either as a component of the flavoprotein or as a non-haem-iron protein, are involved in the removal of hydrogen from coproporphyrinogen, and that NAD(P)⁺ is the final hydrogen acceptor. The inhibition by 2,4-dinitrophenol suggests that ATP is required for energy-linked reverse electron transfer (cf. Sewell & Aleem, 1969). *S*-Adenosyl-methionine does not seem to be metabolized during the reaction and thus it may simply activate the enzyme. Whatever its role, its involvement explains why *R. sphaeroides* excretes coproporphyrin when illuminated anaerobically under conditions of methionine deficiency caused by adding ethionine or threonine to the medium (Gibson, Neuberger & Tait, 1962).

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Multiple Activities of Penicillin Acyltransferase of *Penicillium chrysogenum*

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Natural penicillins are thought to be formed by substitution of the L-aminoadipyl group of isopenicillin N by various acyl groups (Demain, 1966). Three enzyme activities have been detected in *Penicillium chrysogenum* that could be involved in this substitution, namely: (a) penicillin acyltransferase, which catalyses isotopic exchange of ³⁵S between penicillins and 6-aminopenicillanic acid (Pruess & Johnson, 1967); (b) 6-aminopenicillanic acid acyltransferase, which synthesizes penicillins from acyl-CoA and 6-aminopenicillanic acid (Spencer, 1968; Gatenbeck & Brunsberg, 1968); (c) penicillin acylase, which hydrolyses penicillins to 6-aminopenicillanic acid and the side-chain acid (Vanderhaeghe, Claesen, Vlietinck & Parmentier, 1968). A fourth relevant activity now observed in *P. chrysogenum* 51-20F3 extracts is: (d) the hydrolysis of phenylacetyl-CoA. The optimum conditions for the assay of these activities in 0.05 M-sodium phosphate buffer containing 2 mM-dithiothreitol and 1 mM-EDTA were: (a) 2 mM-[³⁵S]penicillin G and 7.5 mM-6-aminopenicillanic acid, pH 7.8; (b) 1 mM-6-aminopenicillanic acid (K_m 5×10^{-4} M) and 7.5 mM-[¹⁴C]phenylacetyl-CoA (K_m 6.7×10^{-3} M), pH 7.4; (c) 0.25 mM-[³⁵S]penicillin G (K_m 1.67×10^{-4} M), pH 7.9; (d) 7.5 mM (not optimum)-[¹⁴]-phenylacetyl-CoA (K_m 5×10^{-3} M), pH 8.0.

When *P. chrysogenum* was transferred from a glucose to a lactose medium, the appearance of each of the four activities in mycelial extracts showed a similar pattern. Disruption of the mycelial extracts in the French press, precipitation with (NH₄)₂SO₄ and fractionation on Sephadex G-100 and DEAE-cellulose gave a 130-fold purification of each of the activities. Apart from the crude extract, in which non-specific enzymes hydrolyse the phenylacetyl-CoA, the ratios of the activities remained constant throughout the various purification stages. The fractionation patterns on DEAE-cellulose and Sephadex G-100 were identical, and on polyacrylamide-gel electrophoresis all four activities were found in the same narrow band. The pH and temperature inactivation curves were the same. Complete inhibition was given by 2 mM-N-ethylmaleimide and activities were restored by addition of excess of dithiothreitol. CoA did not enhance the rate of exchange activity (a), nor was there an absolute requirement for dithiothreitol since, after its removal by using Sephadex G-100 more than 50% of each of the activities was retained.