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* B_2aba 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 lwoffi*, 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. lwoffi* 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).

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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:

$$CH_4 \rightarrow CH_3 \cdot OH \rightarrow H \cdot CHO \rightarrow H \cdot CO_2H \rightarrow CO_2$$

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:

$$CH_4 + O_2 + XH_2 \rightarrow CH_3 \cdot OH + H_2O + X$$

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:

$$2CH_4 + O_2 \rightarrow 2CH_3 \cdot OH$$

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:

$$CH_4 + H_2O \rightarrow CH_3 \cdot OH + 2[H]$$

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.

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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-