oxidation of a range of compounds in the environment.

The only substrates for MMO of significance *vis à vis* any selective value for broad-specificity enzymes are those which on subsequent metabolism yield acetate. There are two reasons for this. First, these compounds (long-chain alkanes, long-chain alkenes, phenylalkanes, carotenoids) are present in very substantial quantities in the environment; they are major components of biomass. Second, acetate once formed can act as a carbon source for methanotrophs and in the case of type II species which possess an intact tricarboxylic acid cycle, acetate can be oxidized to CO₂ yielding reducing power and ATP. It is well authenticated that acetate is extensively metabolized by type II species and leads to appreciable increases in growth yields when included in the medium³. Phenylalkanes are partially degraded by *Methylosinus trichosporium* to short-chain alkanolic acids and alkanes are oxidized to CO₂. The most likely mechanism for this is β -oxidation, the product of which is acetate. Further evidence for β -oxidation comes from the finding that C-odd phenylalkanes yield only C-odd phenylalkanoic acids and C-even phenylalkanes yield mainly C-even phenylalkanoic acids⁴. The example of 1-phenylheptane metabolism was given in our Table 1.

(2) There is accumulating evidence that methanotrophs possess other broad-specificity enzymes including secondary alcohol and aldehyde dehydrogenases^{5,6} that facilitate the further oxidation of products of MMO activity. Some of these enzymes, such as the secondary alcohol dehydrogenase discovered by the Exxon group, have no known function in C-1 metabolism.

(3) The environmental significance of reactions observed in our experiments using high cell densities is questioned. Of course, it is always difficult to extrapolate from laboratory experiments to the environment. It is not necessary to use such high cell densities to detect products, but clearly at very low cell densities product detection is difficult for technical reasons. We have used high cell densities to obtain in a short time sufficient material from small-scale incubations for both detection and chemical identification. In addition, because some of these substances are toxic at high concentrations, appropriate ratios of organisms to test compounds are necessary.

(4) We agree that the formation of exospores or cysts has great survival value. Would it not be advantageous, however, to be able to survive in a vegetative state using ancillary carbon and energy sources while your competitors are encysting?

(5) The fact that methanotrophs do not grow on acetate alone is not proof that acetate can act only as a carbon source. Acetate substantially increases yields of type II organisms on methane, suggesting

that NAD(P)H and/or ATP are generated from this compound. It seems likely that type II methanotrophs have retained an intact tricarboxylic acid cycle to facilitate acetate oxidation. The reasons for the inability to grow on acetate may well be found in the mechanisms of regulation of intermediary metabolism.

There are many examples of microbial co-oxidations in which ATP or NADH are generated from substances which are not growth substrates. Methanotrophs will not grow on formate but generate NADH (and possibly ATP) from this intermediate.

We suggest that more experimental work should be done to test our hypothesis that lack of specificity of MMO leads to useful supplementary metabolism and this is in progress in our laboratory.

Note added in proof: Dalton and colleagues have previously subscribed to the view that in the presence of methane small amounts of co-oxidizable compounds may well contribute significantly to the growth of methanotrophs and not just be merely oxidized without benefit to the organism^{7,8}.

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Cycloheximide inhibits DNA synthesis in SV40-transformed cells

IDE *ET AL.*¹ reported that DNA synthesis in an SV40-transformed human cell line (SV80) is not inhibited by the protein synthesis inhibitor cycloheximide. This is an apparent exception to the repeated observation that inhibitors of protein synthesis are effective inhibitors of DNA and RNA (mostly ribosomal) synthesis^{2,3}. Using various other cell types, both neoplastic and normal, we have found^{4,5} that the inhibitory effect of these drugs is also exerted on several reactions in both the salvage and *de novo* pathways of nucleotide biosynthesis, such as the formation of CTP from uridine and aspartate and the formation of GTP from hypoxanthine, adenosine, deoxyadenosine and formate.

CTP and GTP are not only required for RNA synthesis but are also intermediates in the formation of the deoxynucleoside triphosphate precursors, dCTP, dTTP and dGTP. Various lines of evidence emphasize the importance of maintaining normal pool sizes of these precursors for RNA and DNA synthesis^{6,7}, particularly for the latter, as the level of the deoxynucleoside triphosphates is very small in eukaryotic cells.

The SV40-transformed system could be pertinent to the question of whether DNA synthesis inhibition depends solely on the synthesis of some unknown protein(s) of short half life, in some way necessary for the polymerization reaction, or additionally on the levels of newly synthesized precursors. Thus, we had hoped to use the transformed cells to determine whether, in conditions in which DNA synthesis is not affected, the reactions leading to the biosynthesis of the precursors are similarly unaffected. Unfortunately, we are unable to confirm the observations of Ide *et al.* In our study, after addition of cycloheximide or puromycin in both the human SV80 line that Ide *et al.* used, and in an SV40-transformed mouse line (BALB/c SVA31), DNA synthesis was inhibited to the same extent as in the untransformed cell (BALB/c A31). In addition, all the effects on the nucleotide pool constituents that we observed with other cell types were seen with the SV40-transformed lines.

There is no apparent explanation for the differences between our results and those of Ide *et al.*, who suggest that the persistence in DNA synthesis in transformed cells is associated with the T-antigen content of SV40-transformed lines. T-antigen assays of our stocks showed that the BALB/c SVA31 and the SV80 lines are strongly positive whereas the untransformed BALB/c A31 is negative. We conclude that SV40-transformed cells are not exceptions to the statement⁸ that "inhibition of protein synthesis by any mechanism results in almost immediate interruption of DNA replication."

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