In this paper we will withdraw from the complex world of mixed cultures and multiple substrates that characterize polluted ecosystems and treatment facilities to discuss a simple model system involving petri dishes, vapor phase pure substrates, and genetically defined bacterial strains. The laboratory situation is no less "real" than any other and has many important lessons to teach us if we are ever to be in a position to design pollutant-degrading organisms with the same level of sophistication now employed to produce medically important polypeptides. One of the major points to be made is that even a hydrocarbon oxidation system chosen for maximum simplicity reveals a genetic complexity that is beyond our current understanding.

Many hydrocarbon oxidation pathways begin with the stepwise oxidation of a methyl group to a carboxylic acid moiety. Figure 1 shows examples of gaseous, aliphatic, and aromatic hydrocarbon degradations that start in this manner. Because it is biochemically and technically the easiest to study, we chose the n-alkane oxidation pathway in Pseudomonas putida for genetic analysis. All the substrates and intermediates were readily available, and Coon's group had established conditions for biochemical characterization of the oxidizing activities (1,10,11,12). The pathway consists of the following reactions: 1) an initial hydroxylation catalyzed by a multicomponent mixed-function oxygenase that requires molecular oxygen and NADH cofactor; 2) an alcohol dehydrogenation; 3) an aldehyde dehydrogenation; and 4) β-oxidation of the resulting fatty acid to produce the substrates of intermediary metabolism.

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One of the important characteristics which n-alkanes share with many environmental pollutants is their hydrophobicity. This consideration led us to assume that the hydrophobic region of the bacterial cell, namely the membrane, would play a very important role in the physiology of alkane oxidation, and we have managed to obtain evidence in favor of this assumption. Figure 2 is a schematic cartoon that was published some years ago (3) of how we envision the first three steps in n-alkane oxidation. It shows the entry of substrate into the cytoplasmic membrane of P. putida by an unknown process which we believe depends largely on the tendency of alkane molecules to partition from an aqueous phase into a lipid phase. Once in the cytoplasmic membrane bi-layer, the substrate is hydroxylated to the primary alcohol by an elaborate enzyme complex, alkane hydroxylase, with three components. These are the membrane protein oxidase and two soluble proteins, rubredoxin and rubredoxin reductase, which must be somehow associated with the oxidase for the reaction to occur. Then the primary aliphatic alcohol product of hydroxylation is dehydrogenated by a membrane activity to an aliphatic aldehyde. We know little about the subsequent aldehyde dehydrogenation step in P. putida, but there is evidence in P. aeruginosa for inducible membrane aldehyde dehydrogenases that produce the fatty acids (G. Brandon, personal communication). We hypothesize that these products are then taken into the cytoplasm and subjected to β-oxidation to yield acetyl-CoA (and propionyl-CoA if there is an odd number of carbons in the alkane chain).