establish that the exchange of hydrogen atoms between water and deoxyadenosylcobalamin is an integral part of the reductase reaction the following facts are consistent with this view. Both the exchange reaction and the overall reduction of ribonucleotides have an essential requirement for dihydrolipoate. Further, deoxyribonucleotides that accelerate the reduction of specific ribonucleotides also cause much greater exchange to occur than that in the absence of nucleotides. The two reactions are catalysed by the same highly purified enzyme, and treatment of the enzyme preparation with protein reagents such as 1-fluoro-2,4-dinitrobenzene, *N*-bromosuccinimide and *N*-acetylimidazole decreases the rate of ribonucleotide reduction and of the exchange reaction to approximately the same extent. Finally, rate calculations show that the rate of the exchange reaction is comparable with that of ATP reduction under similar conditions. From these preliminary experiments it appears possible that deoxyadenosylcobalamin participates in hydrogen transfer between dihydrolipoate and the nucleotide substrate.

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Dicarboxylic Acid Catabolism by Bacteria

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A recent study of the bacterial metabolism of glutaric acid (Numa, Ishimura, Nakazawa, Okazaki & Hayaishi, 1964) prompted us to examine the routes by which bacteria degrade the higher members of the homologous series of straight-chain dicarboxylic acids. Acids of this series have been identified in cultures of bacteria utilizing *n*-paraffinic hydrocarbons (Ali Khan, Hall & Robinson, 1964; Kester & Foster, 1963) and alicyclic hydrocarbons (Colla & Treccani, 1960). Enzyme systems catalysing the ω-oxidation of fatty acids or their CoA thio esters to the corresponding dicarboxylic acids have been demonstrated in both bacteria (Kusunose, Kusunose & Coon, 1964) and mammals (Preiss & Bloch, 1964). The isolation of pimelic acid from cultures of azelaic acid-grown *Pseudomonas* sp. (Janota-Bassilik & Wright, 1964) suggested that bacteria may degrade dicarboxylic acids by a pathway involving β-oxidation, as proposed by Verkade (1938) from the results of animal-feeding experiments. Accordingly experiments to examine this possibility were undertaken.

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Two micro-organisms, *Moraxella lwoffii* (*Vibrio* O1, N.C.I.B. 8250) (Sebald & Véron, 1963) and *Pseudomonas aeruginosa* 8602 were selected for study. Cells were grown at 30° with shaking in a medium containing (g./l.): KH_2PO_4, 1·8; K_2HPO_4, 3·6; NH_4Cl, 2·0; $MgSO_4,7H_2O$, 0·40; with an appropriate dicarboxylic acid (0·05% w/v) neutralized with 5 N-NaOH as sole carbon source. Initially experiments were conducted with *M. lwoffii*. Though oxalate and malonate did not support growth, cells grew readily at the expense of succinate and the higher dicarboxylic acid homologues up to and including dodecane-1,12-dicarboxylate.

Washed cells grown with pimelate immediately

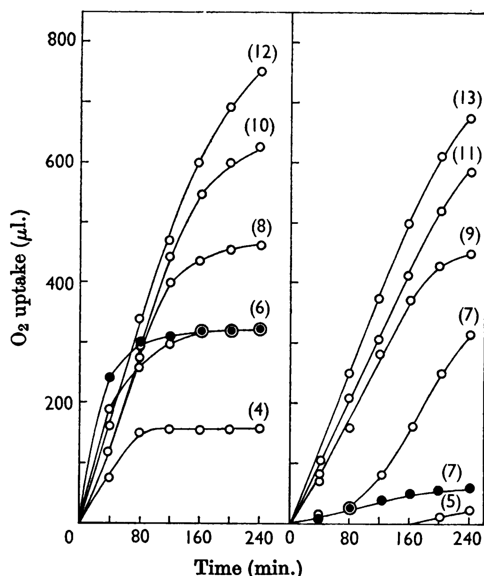

Fig. 1. Oxidation of dicarboxylic acids by cells of *M. lwoffi* grown on adipate. Reaction vessels contained, in 2·9ml.: 67μmoles of phosphate buffer, pH 7·0, 3μmoles of substrate (neutralized), 3·4mg. dry wt. of cells and, where indicated, 150μg. of chloramphenicol. Centre wells contained 0·1 ml. of 20% (w/v) KOH. Oxidation rates with the following acids in the presence (●) and the absence (○) of chloramphenicol are shown corrected for cell respiration without added substrate (16–20μl. of O_2/hr.): succinate (4), glutarate (5), adipate (6), pimelate (7), suberate (8), azelate (9), sebacate (10), nonane-1,9-dicarboxylate (11), decane-1,10-dicarboxylate (12) and undecane-1,11-dicarboxylate (13).

oxidized all dibasic acids from C_4 to C_{12} to the same extent (60–65% of theory) and at similar rates (110–140μl. of O_2/hr./mg. dry wt. of cells). Similar oxidation patterns were observed with cells grown with azelate (C_9) and brassylate (C_{13}), both 'odd-numbered' acids. Glutarate-grown cells, however, oxidized only glutarate and succinate without lag, and cells grown with succinate oxidized only succinate readily. In these latter experiments oxidation of the longer-chain dibasic acids was preceded by a lag phase indicative of induced enzyme synthesis. The results of an experiment with adipate-grown cells are shown in Fig. 1. Essentially similar results were found with cells grown with suberate (C_8), sebacate (C_{10}) and decane-1,10-dicarboxylate (C_{12}), all 'even-numbered' acids. Such cells readily oxidized all even-numbered acids and the higher odd-numbered acids (C_9, C_{11}, C_{13}), but oxidized glutarate and pimelate only after a lag period. Chloramphenicol (Parke, Davis and Co., Hounslow, Middlesex) prevented the complete oxidation of pimelate but permitted a limited oxidation (approx. 1μmole of O_2/μmole of substrate). Adipate oxidation was not inhibited by chloramphenicol. When adipate-grown cells were transferred to growth medium containing suberate, immediate growth was observed turbidimetrically, whereas a lag of 90min. preceded growth when adipate-grown cells were placed in medium containing pimelate. These results suggested that cells grown with even-numbered dibasic acids lacked the enzymes necessary for complete catabolism of pimelate and glutarate.

To isolate the product of oxidation of pimelate by chloramphenicol-inhibited cells an incubation mixture containing 150μmoles of sodium pimelate, 335μmoles of potassium phosphate buffer, pH 7·0, 350mg. dry wt. of adipate-grown cells and 7·5mg. of chloramphenicol in 150ml. of distilled water was shaken in a 1l. Erlenmeyer flask at 30° for 3hr. After the addition of 150μmoles of sodium pimelate in 15·0ml. of water, incubation was continued for a further 3hr. and the reaction terminated by centrifuging the cells and acidifying the supernatant to pH 2·0 with 5N-H_2SO_4. Reaction products were continuously extracted with diethyl ether for 24hr. and the organic phase was concentrated under reduced pressure after drying over anhydrous Na_2SO_4. Two acidic compounds, R_F 0·30 and R_F 0·43, were detected after chromatography on Whatman no. 1 paper with propan-1-ol–aq. NH_3 (sp.gr. 0·88) (3:2, v/v) by spraying with 0·03% (w/v) methyl red in borate buffer, pH 6·8 (Kalbe, 1954). In this solvent glutaric acid and pimelic acid have R_F values 0·30 and 0·42 respectively.

When the reaction products were esterified with diazomethane prepared from *N*-methyl-*N*-nitroso urea and examined by gas–liquid chromatography at 150° on a column containing 5% (w/w) butanediol succinate on Chromosorb P (Johns–Manville Co., New York, N.Y., U.S.A.) by using an Aerograph A–600C gas chromatograph (Wilkens Instruments, Walnut Creek, Calif. U.S.A.), two components were detected having retention times identical with those of dimethyl glutarate (3·8min.) and dimethyl pimelate (9·3min.).

Since chloramphenicol prevented the complete oxidation of pimelate and led to the accumulation of glutarate, the effect of this antibiotic on the oxidation of the C_9, C_{11} and C_{13} dibasic acids by adipate-grown cells was examined. Incubation mixtures containing these acids were prepared as described above and the reaction products examined by paper and gas–liquid chromatography. Glutarate together with smaller quantities of pimelate accumulated in each case. From these experiments it appears that adipate-grown cells contain enzymes catalysing β-oxidation of the odd-numbered dicarboxylic acids or their CoA thio esters to glutarate or glutaryl-CoA. In the manometric experiments

(Fig. 1) initial oxidation of the C_9,C_{11} and C_{13} acids presumably yields sufficient acetate and ATP for induction of the glutarate-degrading enzymes to occur more readily than with pimelate or glutarate. It may be inferred that the enzymes catalysing β-oxidation also function in the catabolism of the even-numbered dibasic acids, since cells grown with adipate, pimelate and all higher homologues of this series readily oxidized the long-chain acids irrespective of the number of carbon atoms they possess.

The route of degradation of glutarate in *M. lwoffii* has not been studied. Glutaconate is readily oxidized by cells grown with glutarate or higher odd-numbered dibasic acids, but it is not known whether glutaconyl-CoA is decarboxylated to crotonyl-CoA, as in pseudomonads (Numa *et al.* 1964), or undergoes β-oxidation to malonyl-CoA.

When preliminary manometric experiments were conducted with cells of *Ps. aeruginosa*, oxidation patterns differing from those found with *M. lwoffii* were obtained. Cells grown with adipate immediately oxidized succinate, glutarate, adipate and pimelate. Suberate, azelate and higher homologues were not readily oxidized. Moreover, chloramphenicol (50μg./ml.) was without effect on the rate and extent of oxidation of glutarate, adipate and pimelate. An alternative approach to this problem was possible, however, by using two mutants of *Ps. aeruginosa* (Skinner & Clarke, 1965) that lacked isocitrate lyase (EC 4.1.3.1), an enzyme of the glyoxylate cycle necessary for the provision of anabolites when bacteria grow at the expense of acetate (Kornberg & Krebs, 1957). The wild-type organism grew readily with all homologous members of the fatty acid series (C_2–C_{10}) and the dicarboxylic acid series (C_3–C_{10}). Mutants lacking isocitrate lyase, however, were unable to utilize even-numbered fatty acids and odd-numbered dibasic acids for growth, suggesting that the metabolism of these compounds by *Ps. aeruginosa* leads solely to the formation of acetate. These findings are consistent with a mechanism of β-oxidation for both fatty acids and dicarboxylic acids. Callely, Dagley & Hodgson (1958) established that acetate is the sole product of β-oxidation of even-numbered fatty acids in *M. lwoffii*. A similar degradation of odd-numbered dibasic acids would yield malonate or glutarate, the further metabolism of which is known to lead to the formation of CO_2 and acetyl-CoA in pseudomonads (Hayaishi, 1955; Numa *et al.* 1964). Growth of mutants with odd-numbered fatty acids and even-numbered dibasic acids occurs, presumably because of the respective formation of propionate (Callely *et al.* 1958) and succinate in addition to acetate, thus avoiding an obligatory requirement for isocitrate lyase.

Indirect evidence that adipate is converted into β-oxoadipate or β-oxoadipyl-CoA in pseudomonads has been presented by Ornston (1966). In *Ps. aeruginosa* and *Ps. multivorans* the synthesis of three enzymes involved in the catabolism of catechol and protocatechuate is induced by β-oxoadipate or its CoA thio ester. Adipate-grown cells contain significant amounts of all three enzymes. Since β-oxoadipyl-CoA is cleaved to acetyl-CoA and succinyl-CoA (Katagiri & Hayaishi, 1957) by bacterial enzymes, β-oxidation of adipate is inferred.

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