METHYL KETONE METABOLISM IN HYDROCARBON-UTILIZING MYCOBACTERIA

H. B. LUKINS[1] AND J. W. FOSTER

Department of Microbiology, The University of Texas, Austin, Texas

Received for publication 15 December 1962

ABSTRACT

LUKINS, H. B. (University of Texas, Austin) AND J. W. FOSTER. Methyl ketone metabolism in hydrocarbon-utilizing mycobacteria. J. Bacteriol. **85:** 1074–1087. 1963.—Species of *Mycobacterium* especially *M. smegmatis* 422, produced the homologous methyl ketones during the oxidation of propane, *n*-butane, *n*-pentane, or *n*-hexane. A carrier-trapping experiment demonstrated the formation of 2-undecanone, as well as 1,11-undecanedioic acid, during the oxidation of undecane-1-C^{14}. Aliphatic alkane-utilizing mycobacteria were able to grow at the expense of several aliphatic methyl ketones as sole sources of carbon. Other ketones which did not support growth were oxidized by resting bacterial suspensions. *M. smegmatis* 422 cells grown on propane or acetone were simultaneously adapted to oxidize both substrates, as well as *n*-propanol. *n*-Propanol cells were unadapted to propane or acetone. Acetone produced from propane in a medium enriched in D_2O contained a negligible quantity of D, presumably eliminating propylene as an intermediate in the oxidation. Cells grown at the expense of alkanes or methyl ketones in the presence of O_2^{18} had a higher content of O^{18} than did cells grown on terminally oxidized compounds, e.g., primary alcohols or fatty acids. An oxygenase reaction is postulated for the attack on methyl ketones. Acetol was isolated and characterized as an oxidation product of acetone by *M. smegmatis* 422. Acetol-grown cells had a higher O^{18} content than did *n*-propanol cells, and its utilization appears to involve at least one oxygenase reaction. Acetol produced from acetone in the presence of O_2^{18} was not enriched in the isotope, indicating the occurrence of exchange reactions or of oxygenation reactions at a later stage in the assimilation of acetone and acetol.

[1] Present address: Department of Biochemistry, University of Sydney, Sydney, Australia.

The most common means by which microorganisms utilize aliphatic, saturated hydrocarbons is an oxidative attack at a terminal methyl group (*for literature see* Fuhs, 1961; Foster, 1962*a*, *b*). The usual first, stable product is the homologous primary alcohol which is further converted to the corresponding fatty acid; this in turn undergoes beta-degradation of the carbon chain.

As early as 1932, methyl ketones were suspected of being involved in paraffin oxidation; *Aspergillus versicolor*, which grew at the expense of long-chain paraffins, was also able to grow at the expense of related methyl ketones (Hopkins and Chibnall, 1932). Recently, *Pseudomonas methanica* was discovered to produce methyl ketones, along with alcohols and fatty acids, during the oxidation of gaseous alkanes (Leadbetter and Foster, 1959*a*, 1960).

Methyl ketone formation is well known in mammals (Fruton and Simmonds, 1958), in bacteria (Johnson, Peterson, and Fred, 1933), and in fungi (Stärkle, 1924; Franke and Heinen, 1958). In these cases, the ketone is formed by decarboxylation of a β-keto acid and has, therefore, one less carbon atom than the precursor. On the other hand, bacterial formation of methyl ketones from alkanes is a unique alpha-oxidation, with no change in the carbon skeleton. Methyl ketones are also formed by bacterial dehydrogenation of secondary alcohols (Müller, 1931), a reaction which appears to be part of the alkane oxidation sequence (Leadbetter and Foster, 1960).

The formation of methyl ketones in the oxidation of gaseous alkanes implies that they are intermediates. Their metabolism thus automatically becomes a legitimate aspect of hydrocarbon metabolism, especially of alkyl groups. Ladd's (1956) soil diptheroid oxidized 2-undecanone at a rate better than *n*-decylaldehyde, and the possibility of an oxidation at a nonterminal carbon was

pointed out. In an abstract, Klausmeier et al. (1958) reported that a propane-utilizing mycobacterium was simultaneously adapted to the oxidation of isopropanol and propylene, which were postulated as intermediates.

Subterminal oxidation of alkyl groups is best established in the rabbit, when fed short-chain monoalkylbenzenes. For example, ethylbenzene is converted to methylphenylcarbinol (Smith, Smithies, and Williams, 1954), and *n*-propyl- and *n*-butylbenzene are oxidized at two different subterminal carbons (El Masry, Smith, and Williams, 1955).

This paper is concerned with the role of methyl ketones in the oxidation of the gaseous alkanes by species of *Mycobacterium*. Hydrocarbon utilization seems to be an intrinsic capability of mycobacteria (Lukins and Foster, Z. Allgem. Mikrobiol., *in press*), but practically nothing is known about the mechanisms they employ.

Materials and Methods

Organisms. M. smegmatis 422, which grows at the expense of several gaseous and liquid alkanes (Lukins and Foster, *in press*), was used for most of this work. When grown with continuous shaking on propane-mineral salts medium, it produces a copious, uniform suspension suitable for physiological experiments. *M. rhodochrous* 382 and the yellow, glistening *Mycobacterium* sp. "tap-water" also were extensively employed. These cultures were kindly furnished by Ruth Gordon, Institute of Microbiology, Rutgers University, New Brunswick, N. J. A number of other organisms were isolated from soils and muds, using 2-butanone or 2-undecanone as selective substrates.

The mineral salts medium contained, per liter of deionized water: $(NH_4)_2SO_4$, 1.0 g; Na_2CO_3, 0.1 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2$, 10 mg; $FeSO_4 \cdot 7H_2O$, 5 mg; $MnSO_4$, 2 mg; Cu, 50 μg (as $CuSO_4 \cdot 5H_2O$); B, 10 μg (as H_3BO_3); Zn, 70 μg (as $ZnSO_4 \cdot 7H_2O$); Mo, 10 μg (as MoO_3); pH 7.0. Water-washed agar was added when solid media were desired. Ordinarily, the liquid growth cultures were prepared in "prescription" bottles or suction flasks. Procedures for cultivation in gaseous substrates are given elsewhere (Lukins and Foster, *in press*; Kester and Foster, 1963). Washed cellular suspensions were prepared from liquid cultures, using 0.067 M phosphate buffer (pH 7) as the suspension fluid. Propane or butane was used in a 50% gas-50% air mixture, and liquid alkanes were added at 0.2% (v/v). The hydrocarbons were at least 99 moles % pure.

Chemical. Ketones were estimated photometrically by means of the colorimetric reaction with salicylaldehyde, acetone in alkaline solution (Neish, 1952), and the longer chain ketones in acidic solution (Mukherjee, 1951). Acetone and 2-butanone were distilled from the culture liquids into a solution of reagent for preparation of derivatives according to Vogel (1951). Longer chain ketones were extracted with three changes of ether. Acetol was assayed by periodate titration (Neish, 1952). Infrared-absorption spectra were obtained with a Baird instrument, and melting points with a Fisher block.

Isotope experiments. O^{18}-incorporation experiments were conducted in 500-ml growth cultures in 4-liter suction flasks incubated with continuous shaking. The atmosphere consisted of N_2 containing 10% O_2 labeled with O^{18} prepared by electrolysis of water (5.5 to 6.5% H_2O^{18}). Immediately after gassing the culture vessel, a sample of the gas was taken for isotopic analysis. At maximal growth, the cells were harvested by centrifugation, washed with water, lyophilized, and pyrolyzed to CO_2 (Rittenberg and Ponticorvo, 1956). Analysis of the masses 44, 45, and 46 in the mass spectrometer permitted calculation of the O^{18} content as follows:

$$\% O^{18} = \frac{100 \times I_{46}}{2(I_{44} + I_{45} + I_{46})}$$

where I_{46} equals intensity of mass 46, etc. Likewise, the O^{18} content of the O_2 in the gas phase was calculated from the intensities of masses 32, 33, and 34.

Results and Discussion

Production of methyl ketones from n-alkanes. P. methanica growing on methane co-oxidizes propane and butane to acetone and 2-butanone, respectively (Leadbetter and Foster, 1959*a*). This organism cannot utilize propane or butane for growth, and it is desirable to determine whether organisms that can utilize them for growth are likewise able to produce the ketones.

Acetone from n-propane. Washed cells of *M. smegmatis* 422 from 3 liters of a 3-day culture were resuspended in mineral salts solution with

TABLE 1. *Melting points of hydrazones prepared from products of the oxidation of short-chain n-alkanes by Mycobacterium smegmatis 422*

Product source	2,4-Dinitrophenylhydrazone	*p*-Nitrophenyl hydrazone
	C	C
Propane growth culture...	123–124	—
Propane replacement culture.....	124	149
Authentic acetone.....	124	147
Mixed sample from replacement culture and authentic acetone.....	124	147
n-Butane growth culture..	108–109	122–123
Authentic 2-butanone.....	110	123
Mixture of two above.....	109–110	123
Pentane growth culture...	138	
Authentic 2-pentanone....	140	
Mixture of two above.....	138–139	

the $(NH_4)_2SO_4$ omitted, and exposed to 30% propane-70% air for 15 hr. Both the growth culture and the replacement liquids were distilled, and crystalline derivatives were prepared and identified as acetone products (Table 1). Quantitative analyses showed that the growth filtrate contained 59 μg and the replacement liquid 193 μg of acetone per ml. Similar experiments with growth and replacement cultures of *M. rhodochrous* 382 yielded the same crystalline derivatives of acetone.

During acetone and 2-butanone production by *P. methanica*, the corresponding primary alcohols and fatty acids were concomitantly produced in amounts exceeding the ketones (Leadbetter and Foster, 1960). On the other hand, *M. smegmatis*, which produced 160 mg of acetone, formed less 1 mg of neutral volatile substances calculated as *n*-propanol [presumably *n*-propanol or propionaldehyde determined as acids produced by oxidation with H_2SO_4-$K_2Cr_2O_7$ (Neish, 1952)], and the steam-volatile fatty acids totaled only 0.27 mg (as propionic acid).

2-Butanone from n-butane. Propane-grown cells of *M. smegmatis* 422 were resuspended as above and supplied with a 30% *n*-butane-70% air mixture. After incubation, distillates gave crystalline derivatives which were identified as those of 2-butanone (Table 1).

2-Pentanone from n-pentane. Propane-grown cells of *M. smegmatis* 422 resuspended as above were allowed to oxidize 1 ml of *n*-pentane for 20 hr. A salicyaldehyde estimation indicated there were 83 μg of 2-pentanone per ml of the supernatant culture liquid. The diethyl ether extract of the supernatant liquid was concentrated almost to dryness, and then taken up in 1 ml of ethanol. This solution gave a crystalline 2,4-dinitrophenylhydrazone which was identified by melting-point determinations (Table 1) and by a comparison of the infrared-absorption spectra of the bacterial and authentic 2-pentanone derivatives.

2-Hexanone from n-hexane. Propane-grown cells of *M. smegmatis* 422 were furnished with 2 ml of *n*-hexane, and after 20 hr of incubation the concentrated ether extract was analyzed in a Perkin-Elmer gas chromatograph with a 2-m column of didecylphthalate, at 120 C. Two peaks, with retention times of 6 and 7 min, were observed. They were shown to have the same retention times as authentic 2-hexanone and 2-hexanol, respectively. They could not be differentiated from the authentic specimens by cochromatography in this instrument. The ether extract contained no *n*-hexanol or hexaldehyde.

After the relative heights of the two peaks in a typical ether extract were determined, a sample of the extract was treated with CrO_3 in acetone. After the oxidation, the ether-soluble fraction was again analyzed by gas chromatography. The height of the second (2-hexanol) peak was greatly reduced in relation to the first (2-hexanone), indicating hexanol had been present and oxidized to hexanone.

Ether extracts of the culture reacted with 2,4-dinitrophenylhydrazine to give an orange precipitate that could not be separated from contaminating fatty material. Paper chromatography of the precipitated hydrazone by a "reverse-phase" technique (Seligman and Edmonds, 1955) could not distinguish it from authentic 2-hexanone-2,4-dinitrophenylhydrazone. It is concluded that 2-hexanol and 2-hexanone are oxidation products of *n*-hexane by *M. smegmatis* 422.

2-Undecanone from n-undecane. Ether extracts of growth and replacement cultures of *M. smegmatis* 422 acting on *n*-undecane contained a negligible amount of material reacting with 2,4-dinitrophenylhydrazone. Failure to detect 2-undecanone may merely be due to a rapid oxidation that prevents its accumulation. The transient production of 2-undecanone was investigated by an isotopic dilution-tapping experiment. The bacterial oxidation of *n*-undecane-1-C^{14} was con-

ducted in the presence of a pool of 2-undecanone as a carrier added at the end of or during the incubation period.

Washed, hexane-grown cells of *M. rhodochrous* 382 in buffer were distributed in 50-ml portions in 500-ml flasks equipped with special side arms containing alkali for absorption of respiratory CO_2. To each flask were added 25 mg of *n*-undecane containing the C^{14}-labeled tracer. One of the flasks received 50 mg of 2-undecanone and was incubated for 20 hr; the second flask received no further addition and was incubated for 40 hr. At the end of the incubation, the cells were killed with H_2SO_4, and 50 mg each of 2-undecanone, undecanoic acid, and 1,11-undecanedioic acid were added. The acids are also likely coproducts of long-chain alkane metabolism (Kester and Foster, 1963).

The undecanone was extracted at slightly alkaline pH and reacted with 2,4-dinitrophenylhydrazine. The hydrazone was then extracted by *n*-hexane and separated from residual labeled undecane by passage through a silicic acid column with *n*-hexane as the eluting solvent. The undecane passed directly through, but the hydrazone was retained until the polarity of the eluting solvent was increased to 70% chloroform in *n*-hexane. A paper chromatogram of the yellow eluate fraction (Seligman and Edmonds, 1955) revealed only one hydrazone spot.

The carrier acids were reisolated by acid-ether extraction and purified with a silicic acid partition column (Marvel and Rands, 1950) using water as the stationary phase. The monoic acid was eluted first with benzene, and the dioic acid next with benzene containing 3% chloroform. Chemical purity of the isolated acids was verified by paper chromatography of the ammonium salts; maximal amounts were spotted to reveal any minor contaminant (solvent: 1 ml of 18 N NH_4OH in 99 ml of 95% ethanol). Table 2 shows that the carriers which were added at the end of the incubation period had radioactivity only in the two acid fractions, indicating that the organism oxidized *n*-undecane to *n*-undecanoic and 1,11-undecanedioic acids; the higher specific radioactivity of the alkanedioic acid may indicate that this metabolic product was accumulated in greater quantities. On the other hand, the absence of appreciable radioactivity in the 2-undecanone-2,4-dinitrophenylhydrazone points to the failure of the organism to accumulate the ketone product under normal incubation conditions.

A different situation was found in the flask in which the added carriers were present during the entire incubation period. A small but significant radioactivity was associated with the hydrazone which had been purified successively by silicic acid column chromatography and paper chromatography. Owing to the rapid oxidation of the carrier undecanone by the organism, insufficient hydrazone was recovered to allow recrystallization. However, repeated paper chromatography of the hydrazone in two different solvent systems failed to separate the radioactivity from the yellow hydrazone spot. It may be concluded that, although 2-undecanone was not accumulated as a product of undecane oxidation by *M. rhodochrous* 382, it was, however, trapped in small quantities as a transient intermediate.

Utilization of ketones by alkane-oxidizing bacteria. Formation of methyl ketones implies a function as intermediates in the oxidation or assimilation, or both, of the homologous hydrocarbon substrates. Also implied is the utilization of methyl ketones as growth substrates for organisms capable of utilizing alkanes, e.g., especially the mycobacteria. Tested under the same conditions as the alkane substrates, ketone-producing strains of *Mycobacterium* utilized five different ketones as exclusive carbon sources for growth. *M. smegmatis* 422, *M. rhodochrous* 382, and *M. fortuitum* 389 each grew on acetone, 2-butanone, 2-pentanone, 2-tridecanone or 2-octadecanone. In comparable tests, none of the organisms grew on 3-pentanone, 2,4-pentadione, 2-hexanone, 2-heptanone, 3-heptanone, 4-heptanone, 2-octanone, or 2-undecanone. In general, the short-chain ketones supported more rapid and abundant growth than the long-chain ketones, a relation opposite to that observed for alkane utilization. The inability of the strains tested to utilize the methyl ketones of intermediate chain length is reminiscent of the situation encountered with the corresponding aliphatic hydrocarbons (Lukins and Foster, *in press*). There was no obvious, direct correlation between the capacities to grow on a particular *n*-alkane and on its corresponding methyl ketone, since *M. rhodochrous* 382 grew at the expense of the C_6, C_7, and C_8 *n*-alkanes but not the homologous ketones. This conclusion is subject to the reservation that the ketones may be toxic at the levels tested (0.2%, v/v).

The results with the pentanones are note-

TABLE 2. *Distribution of radioactivity in oxidation products of n-undecane-1-C^{14}*

Fraction counted	Specific radioactivity (counts per min per mg)
Undecanoic acid*	40
1,11-Undecanedioic acid*	590
2-Undecanone*	5
2-Undecanone-2,4-dinitrophenylhydrazone†	54

* Carrier 2-undecanone, undecanoic, and 1,11-undecanedioic acid added at end of incubation period.

† Carrier 2-undecanone added at beginning of incubation period.

TABLE 3. *Growth on hydrocarbons of organisms isolated on methyl ketones**

Isolation substrate	Bacterial strain	Incubation	Growth substrate				
			Propane	Butane	Heptane	Undecane	Tridecane
		days					
2-Undecanone	UB_4	7	−	−	−	−	−
		14	−	−	−	++	++
	UC_5	7	−	−	−	−	−
		14	−	−	−	+	+++
	UC_1	7	−	−	−	−	−
		14	−	−	−	+++	+
	UB_6	7	−	−	−	−	+
		14	−	−	−	+++	++
Methyl ethyl ketone	MB_5	7	−	−	−	+	++
		14	−	−	−	+++	+++
	MB_2	7	−	−	−	−	++
		14	++	−	−	−	+++
	MA_0	7	++	−	−	+++	+++
		14	+++	−	−	+++	+++
	MC_5	7	+	−	−	−	+++
		14	++	−	−	++	+++

* Symbols: +++, abundant growth; ++, moderate growth; +, slight growth; −, no growth.

worthy. Whereas 2-pentanone was used by all three organisms, shift of the carbonyl group to the 3-position or introduction of a second carbonyl group rendered these compounds useless as growth substrates.

Utilization of alkanes by ketone-oxidizing bacteria. A number of bacteria were isolated from soils by conventional enrichment and selection procedures employing methyl ketones as carbon sources. All eight organisms tested grew on the long-chain hydrocarbons (C_{11}, C_{13}), but only those which had been isolated on a short-chain ketone (i.e., 2-butanone) were able to grow on the short-chain hydrocarbons (Table 3). A similar relation between isolation substrate and substrate specificity for growth has been encountered with hydrocarbon-utilizing mycobacteria (Lukins and Foster, *in press*). The essential point, however, is that organisms isolated for their ability to grow on ketones likewise attack hydrocarbons.

Oxidation of "non-growth" ketones. The limited range of ketones utilizable as growth substrates by the hydrocarbon-oxidizing mycobacteria does not offer very strong support for the idea that methyl ketones are intermediates in the oxidation of alkanes. But, as found previously for "non-growth" hydrocarbons (Leadbetter and Foster, 1960; Lukins and Foster, *in press*), methyl ketones not supporting growth of particular organisms were nevertheless oxidized by cellular suspensions of those organisms in Warburg respirometers. It is obvious that all of the ketones listed in Table 4 were oxidized vigorously, although, as described above, only five supported growth. The position and number of carbonyl groups and the nature of the alkyl or cyclic carbon structures had no particular adverse effect on the oxidation of any of the compounds.

Simultaneous adaptation to methyl ketones and alkanes. Use was made of the adaptive nature of

TABLE 4. *Oxidation of ketones by organisms known to grow at the expense of hydrocarbons*

Substrate (1 μliter)	Oxygen uptake in 30 min	
	M smegmatis 422	OS15*
	μliters	*μliters*
None	13	14
Acetone	241	325
2-Butanone	196	266
2-Pentanone	180	
3-Pentanone	176	
2,4-Pentandione	92	
2-Hexanone	217	183
3-Heptanone	98	
4-Heptanone	103	
2-Octanone	116	78
2-Undecanone	89	52
2-Tridecanone	109	78
Acetophenone	52	

* A *Corynebacterium* sp. isolated from propane enrichment cultures.

hydrocarbon oxidation to characterize further a putative intermediary role of methyl ketones. Cells adapted to the oxidation of a particular alkane should be coincidentally capable of oxidizing the corresponding methyl ketones (Stanier, 1947). Conversely, cells not adapted to alkane oxidation should be unadapted to methyl ketones, or at least less than fully adapted.

Experiments with two organisms (Table 5) demonstrated the following points. (i) Cells grown on glycerol did not oxidize propane or butane, or else oxidized them at a very low rate. The figures for propane and butane represent manometric uptake of O_2 plus alkane; thus, the actual O_2 consumption was less than indicated. (ii) Cells not adapted to hydrocarbon oxidation (i.e., glycerol-grown) were likewise de-adapted to ketone or secondary alcohol oxidation. (iii) Cells grown on propane and also adapted to butane rapidly oxidized the ketones and the secondary alcohols. (iv) Cells grown on either the hydrocarbon or nonhydrocarbon substrates rapidly oxidized the primary alcohols, aldehydes, and fatty acids.

It may be concluded that growth of cells on propane greatly enhanced the oxidation rate of the alpha-substituted substrates. By contrast, the oxidation rate of the primary alcohols seemed to be little affected. These results argue for an intermediate function of secondary alcohols and methyl ketones in gaseous alkane oxidation.

To obtain a closer comparison between the oxidative capacities of cells for the primary and secondary substituted intermediates, cells of *M. smegmatis* 422 were grown on propane, acetone, or *n*-propanol and subsequently tested for their ability to attack each of those three substrates. The relationship (Fig. 1) between the methyl ketone and the hydrocarbon is clear. Propane- and acetone-grown cells, but not propanol-grown cells, oxidized propane and acetone. Thus, not only were propane-grown cells simultaneously adapted to acetone, but acetone-grown cells were back-adapted to propane.

Further work underway should decide whether the back-adaptation to propane is to be attributed to (i) inductive formation of a "propane oxygenase" by acetone as an analogue inducer, (ii) attack of propane by the "acetone oxygenase," (iii) inductive formation of "propane oxygenase" by propane produced from acetone, or (iv) some other mechanism.

Simultaneous oxidation and chain length. Table 6 shows that alkanes and methyl ketones with increasing chain length were oxidized by propane-grown cells at decreasing rates. By comparison, the glycerol-grown cells oxidized only the C_{11} and C_{13} alkanes and the C_5-C_{13} methyl ketones. A clear simultaneous adaptation pattern was thus restricted to the C_3 and C_4 substrates, the oxidation of the longer chain ketones (C_5 to C_{13}) being a low-level constitutive property of *M. smegmatis* 422.

TABLE 5. *Simultaneous adaptation to oxidation of various substrates by mycobacteria grown on a gaseous hydrocarbon or a nonhydrocarbon substrate*

Substrate*	Relative oxidation rates†			
	M. smegmatis 422 grown on		*M. rhodochrous* 382 grown on	
	Glycerol	Propane	Glycerol	Propane
Glycerol	100	—	100	73
Propane	2	100	11	100
n-Propanol	64	244	216	264
iso-Propanol	12	210	27	176
Acetone	2	188	0	134
Propionaldehyde	92	189	—	—
Na propionate	78	201	—	—
n-Butane	12	100	23	97
n-Butanol	68	158	254	258
sec-Butanol	22	138	47	240
2-Butanone	5	160	0	124
Butyraldehyde	60	73	—	—
Na butyrate	63	44	—	—

* Amount of substrate per Warburg vessel, 10 μmoles. Propane and *n*-butane supplied as a 50% hydrocarbon-50% air mixture.

† Relative rates of oxidation after 1 hr, based on rate of oxidation of growth substrate taken as 100.

The results of several experiments on simultaneous adaptation in relation to chain length may be summarized as follows. (i) There was a direct relation between the oxidation of short-chain alkanes and short-chain methyl ketones. (ii) The oxidation of long-chain methyl ketones did not depend on hydrocarbon-adapted cells; a shift to constitutive oxidation of methyl ketones took place in connection with 2-hexanone and longer chains, but constitutive oxidation of alkanes was not observed. (iii) Cells grown on long-chain alkanes did not readily oxidize short-chain methyl ketones. Two implications of this

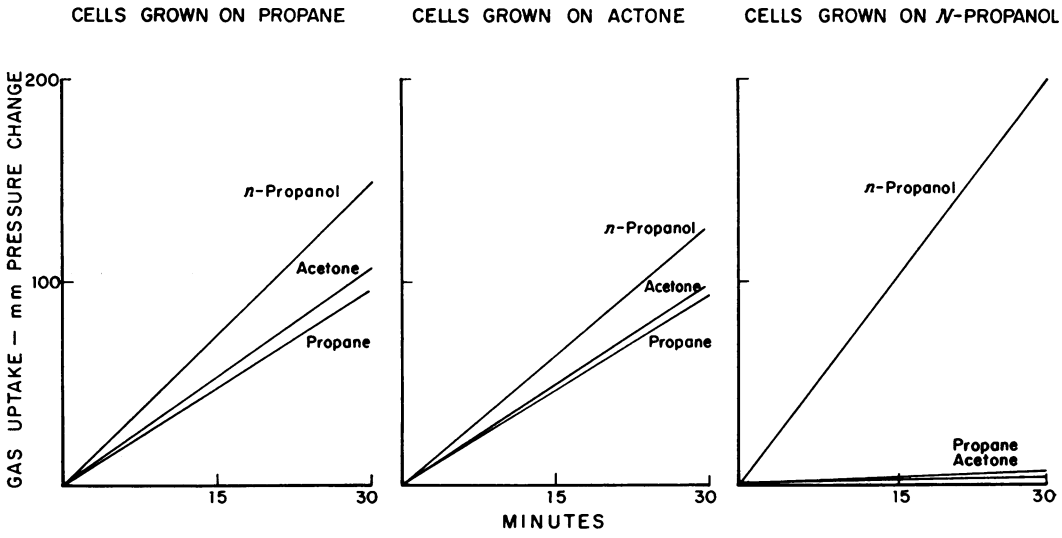

FIG. 1. *Oxidation of C_3 compounds by cells of Mycobacterium smegmatis 422 grown on the different compounds.*

TABLE 6. *Oxidation of n-alkanes and methyl ketones by Mycobacterium smegmatis 422 grown on propane or glycerol*

Substrate	Relative oxidation rates*	
	Cells grown on	
	Propane	Glycerol
Glycerol	87	100
Propane	100	4
n-Butane	152	7
n-Pentane	58	2
n-Hexane	54	0
n-Octane	46	4
n-Undecane	13	18
n-Tridecane	12	19
Acetone	221	0
2-Butanone	200	6
2-Pentanone	193	15
2-Hexanone	157	24
2-Octanone	167	48
2-Undecanone	63	53
2-Tridecanone	127	53

* Relative rates of oxidation after 1 hr, based on rate of oxidation of growth substrate taken as 100.

result are possible: cells grown on the long-chain hydrocarbons are preferentially adapted to a terminal carbon oxidation pathway, and the enzymatic machinery for degradation of long chains is distinct from that for short chains.

Oxidation pathways of gaseous n-alkanes. Having been implicated in the oxidation of certain alkanes, the methyl ketone pathway itself became of interest. At the moment, there is no information as to the relative proportions of the alkane oxidized via the terminal versus the ketone pathway. Neither can one say whether all organisms utilize both pathways as does *P. methanica* (Leadbetter and Foster, 1960), and whether the proportion is the same for different alkanes oxidized by any one organism. Obviously, ketone formation is not obligatory in alkane oxidation, since methane and ethane are utilized by a great many organisms. It appears that the production of methyl ketones from the oxidation of gaseous alkanes is of common occurrence, as shown by studies in this laboratory in which a number of soil bacteria, fungi, and actinomycetes have yielded one or more of the short-chain methyl ketones from the corresponding alkanes (Kester, Lowery, and Black, *personal communication*).

Propylene as a possible intermediate in the oxidation of propane. In the case of an alkane substrate, the first problem is the nature of the primary attack; specifically, whether it involves a direct reaction with O_2 or an initial dehydrogenation. Klausmeier et al. (1958) postulated an intermediary role for propylene in the oxidation of propane; this view was based on simultaneous adaptation experiments. In our work, propane-grown cells of species of *Mycobacterium* also oxidized propylene, although this olefin would not support growth of any of the mycobacteria tested.

Since ethylene has been eliminated as an intermediate in the oxidation of ethane by *P. methanica* (Leadbetter and Foster, 1960) and heptene-1 is said to be an intermediate in heptane oxidation by *P. aeruginosa* (Chouteau, Azoulay, and Senez, 1962), information of a more direct nature is needed regarding a possible role for propylene in propane metabolism.

It might be expected that, under the conditions in which acetone is produced from propane, the ketone would likewise be formed from propylene. However, none could be detected in suspensions of *M. smegmatis* cells furnished propylene.

Further exploration of the olefin route was timely, especially in organisms with a predilection for hydrocarbons. Propane, unlike ethane, has a nonmethyl, alpha-carbon atom which conceivably could be required for the postulated dehydrogenation reaction. A deuterium-labeling experiment similar to that done by Leadbetter and Foster (1960) was therefore performed.

The conversion of propane to acetone by *M. smegmatis* 422 was carried out in a medium containing 36.4% D_2O. After 16 hr of incubation, the neutral supernatant liquid was distilled until one-tenth of the volume had been collected. The distillate was redistilled and the first three drops collected and introduced into the mass spectrometer. A control on the chemical exchange between H of the acetone and D of the medium was also run and analyzed under the same conditions.

If propylene is an intermediate, the following reactions could be expected in the presence of D_2O and H_2O:

The proportions of the two species of acetone can be obtained from the intensities of the peaks at mass 58 ($CH_3\overset{\overset{O}{\|}}{C}CH_3$) and mass 59 ($CH_3\overset{\overset{O}{\|}}{C}CH_2D$). In pure commercial acetone, the "normal" content of mass 59 was 3.88% (Table 7); in the control, this increased to 5.62%, indicating that some chemical exchange with water had taken place. The bacterial acetone had an increase of only 3.14% over the exchange control. Had the acetone been produced through propylene by the reactions described above, 36.4% should have contained D. Thus, the postulated reactions cannot account for almost nine-tenths

$$\left(100 - \frac{3.14}{(36.4 - 5.62)} = 89.8\,\%\right)$$

of the acetone produced, and no evidence is provided that propylene is an intermediary metabolite in the bacterial conversion of propane to acetone. The small difference in D content between the bacterial and the chemical control products may possibly have been caused by slow enzymatic reaction(s).

Incorporation of O_2 with alkanes. Oxygen-18 incorporation has been shown to occur during the bacterial utilization of alkanes (Stewart et al., 1959; Leadbetter and Foster, 1959*b*; Kester and Foster, 1963). Unfortunately, under the conditions of their formation and isolation, oxygen exchange between water and the oxidized pro-

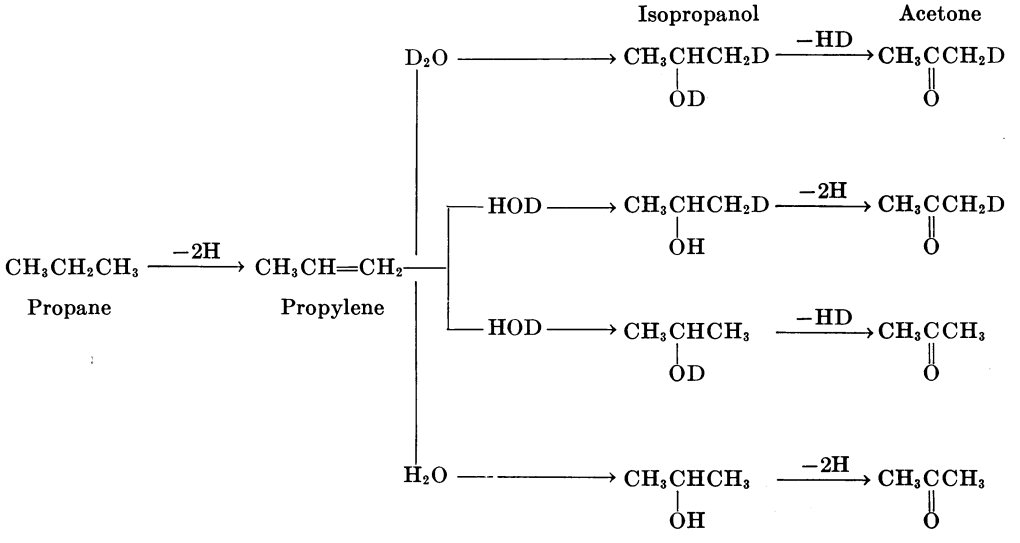

TABLE 7. *Mass spectrometric data on incorporation of deuterium in acetone*

Material analyzed	I_{58}*	I_{59}	$\frac{100 \times I_{59}}{I_{58} + I_{59}}$
			%
Pure acetone	1,013	40.8	3.88
Acetone in D_2O-exchange control	615	36.6	5.62
Acetone from bacterial oxidation of propane	705	67.5	8.76

* Intensity of peak of mass to charge ratio equals 58.

TABLE 8. *Oxygen-18 content of cells grown on hydrocarbons and nonhydrocarbon substrates*

Organism	Substrate	O^{18} in O_2	O^{18} in cell material	Cell material oxygen derived from O_2
		atom %	*atom % excess*	%
Mycobacterium smegmatis 422	Propane	5.0	0.142, 0.161	2.84, 3.22
	n-Butane	5.0	0.049, 0.049	0.98, 0.98
	Glucose	5.0	0.032	0.64
	Glycerol	5.0	0.31	0.62
Mycobacterium sp. "tap-water"	*n*-Pentane	5.0	0.080	1.60
	Glucose	5.0	0.016	0.32

ducts of short-chain alkanes precluded incorporation studies of the kind done so conclusively by Stewart et al. (1959). Hayaishi's (1957) principle of O_2^{18} incorporation in bacterial cells growing on different substrates enabled demonstration that a larger incorporation of atmospheric oxygen occurs in bacteria utilizing hydrocarbons as compared with nonhydrocarbon substrates (Leadbetter and Foster, 1959*b*). Experiments of this kind (Table 8) show that the two species of *Mycobacterium* tested also exhibit this characteristic, presumably by oxygenation of the hydrocarbon molecule.

Incorporation of O_2 with methyl ketones. Information as to the stage of O_2 incorporation was obtained by taking advantage of the excellent growth made by these bacteria at the expense of several of the intermediates in the oxidation of propane and butane. Table 9 reveals a distinct dichotomy in the response to primary- and secondary-substituted compounds, similar to that already observed in the simultaneous adaptation experiments (Fig. 1). In the C_3 series, the alkane, methyl ketone, and secondary alcohol cells clearly had more O^{18} than the *n*-propanol and propionate cells; substantially the same was true of the C_4 series.

The low O^{18} content of the cells grown on primary alcohols and fatty acids would indicate that the oxygenation which takes place with the alkanes does so at a stage prior to their conversion to alcohols and fatty acids. Alcohols are regarded as the first stable, terminally oxidized intermediates in alkane oxidation (Foster, 1962*a*, *b*), and the data in Table 9 are in agreement with the proposal (Stewart et al., 1959) that O_2 fixation is the first reaction in alkane oxidation.

These conclusions are predicated, of course, on the belief that the pathways by which primary alcohols and acids are oxidized as intermediates in alkane metabolism are the same as when they are furnished as substrates in the absence of alkanes. Some inconsistencies with this rationale were evident: for example, the noteworthy differ-

TABLE 9. *Oxygen-18 content of cells of Mycobacterium smegmatis 422 grown on methyl ketones and primary and secondary alcohols*

Substrate	O^{18} in O_2	O^{18} in cell material	Cell material oxygen derived from O_2
	atom %	*atom % excess*	%
Propane	5.0	0.142, 0.161	2.84, 3.22
Acetone	5.0	0.181, 0.109	3.62, 2.18
		0.161	3.22
iso-Propanol	5.0	0.159	3.18
n-Propanol	5.0, 5.8	0.039, 0.037	0.78, 0.64
Propionate (Na)	5.0	0.003	0.06
n-Butane	5.0	0.049, 0.049	0.98, 0.98
2-Butanone	5.0	0.059	1.18
sec-Butanol	5.0	0.013, 0.021	0.26, 0.42
n-Butanol	5.0	0.004	0.08
Butyrate (Na)	5.0	0.010	0.20
2-Pentanone	5.0	0.025	0.50

ence between the *n*-propanol and propionate cells. If this difference proves real, our assumptions regarding the utilization of these two substrates may be premature.

Another major finding in this experiment was the high O^{18} content of cells grown on methyl ketones and secondary alcohols. Presumably, the latter are dehydrogenated to the methyl ketones. In both the C_3 and C_4 series the incorporation equaled that in the corresponding alkane cells. Thus, it appears that O_2 fixation is also essential for the utilization of methyl ketones. The rather low O^{18} incorporation obtained with *sec*-butanol in relation to 2-butanone is difficult to explain; this alcohol was the poorest growth substrate of all the compounds tested.

As seen in Tables 8 and 9, *M. smegmatis* 422 behaved like other bacteria (Leadbetter and Foster, 1959*b*; Foster, 1962*a*) in the progressive decrease in O_2 incorporation as the chain length of the alkane increases; this apparently is a general phenomenon with alkanes. This situation may be indicative of a dilution of the incorporated O^{18} by O^{16} of water during the conversion of the longer chain substrates to simpler intermediates for general cellular biosynthesis.

The unexpected incorporation of O_2 during growth on ketones and secondary alcohols defeats the attempt of this experiment to pinpoint the oxygenation at a particular stage. However, by analogy with the terminal carbon oxidation (Stewart et al., 1959; Kester and Foster, 1963) derivation of ketonic O from O_2 seems likely, although the rapid exchange rate (Hamilton and Westheimer, 1959) under the experimental conditions used here precludes direct proof by the sotopic composition of the acetone.

O_2 incorporation in the utilization of methyl ketones is surprising, since it might be argued that an alkyl chain already oxygenated would not require additional oxygenative attack, but precedence is abundantly available with cyclic compounds (Hayaishi, 1962). Instead, the new question arises as to the stage at which O_2 is fixed during methyl ketone utilization. The one structural feature common to alkanes and methyl ketones, namely, the terminal methyl groups, suggests that the initial attack on the ketones, like that of alkanes, is also a reaction with O_2. A general principle could then be envisaged: the primary attack on saturated compounds whose terminal carbons are methyl groups is an oxygenation of one of the methyl groups.

Oxidation of acetone. At least three pathways are known for the oxidation of acetone in biological systems. Most pertinent to this work are the reports of its conversion to 1,2-propanediol and lactate in animal and plant tissues (Rudney, 1954) and the subsequent dehydrogenation of 1,2-propanediol-1-phosphate to acetol phosphate (Sellinger and Miller, 1959). In microbial systems, there is evidence for a C_2-C_1 split of acetone, presumably after an initial oxidation (Goepfert, 1941), and, on the basis of isotopic dilution experiments, Levine and Krampitz (1952) concluded that acetone was oxidized to acetol, thence to acetaldehyde and a C_1 product.

As noted, no 3-carbon products of acetone oxidation have been isolated from microbial systems; this was undertaken with *M. smegmatis* 422, which produces and utilizes acetone. Cells grown on 0.2% acetone-mineral salts medium and suspended in 0.067 M phosphate buffer (pH 7.0) were allowed to oxidize acetone (2.5%, v/v) aerobically with continuous shaking. Flasks containing cells but no acetone, or acetone but no cells, were shaken in parallel; these tested negatively. Figure 2 shows the time course of the bacterial production of a periodate-reacting substance (recorded as acetol). Acetone concentrations of 2% or more gave maximal production, and 0.5% or less gave negligible amounts of periodate-reacting material. A negative anthrone test ruled out the presence of carbohydrate material. Production of the periodate-reacting substance was dependent on the cell concentration, and negligible amounts were produced from 2-butanone, 2-pentanone, *n*-propanol, or isopropanol.

Isolation and characterization of acetol. The periodate-reacting material was shown to be propan-α-ol-β-one (acetol; CH_3COCH_2OH) by direct isolation and by analysis of the split products after periodate oxidation. Acetone growth culture filtrates were concentrated in vacuo and extracted with diethyl ether. Injection of the concentrated extract into a vapor phase chromatograph revealed a major single peak having the same retention time as an authentic sample of purified acetol. The bacterial material was collected as it emerged from the vapor phase column and was analyzed in a mass spectrometer. The mass spectrum was identical with that of acetol prepared in the same manner.

A concentrated filtrate was reacted with semicarbazide, and the melting point of the recrys-

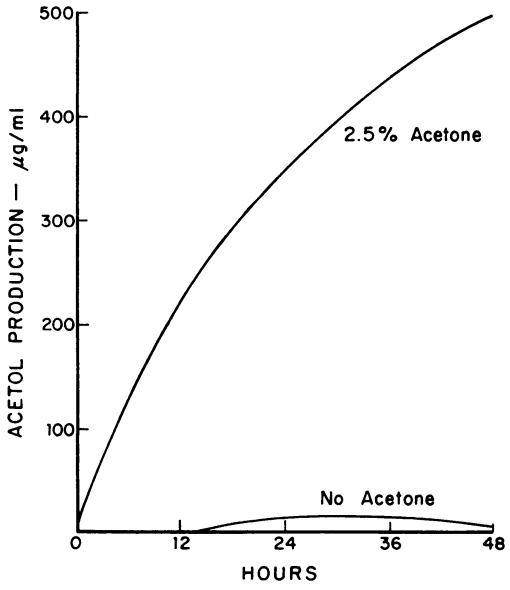

FIG. 2. *Formation of acetol by a suspension of Mycobacterium smegmatis 422 oxidizing acetone.*

TABLE 10. *Melting points of acetol derivatives*

Derivative	Melting point
	C
Semicarbazone from culture filtrate (1)	192–193
Semicarbazone of authentic acetol (2)	189
Mixed, (1) and (2)	187–188
Literature melting point for acetol semicarbazone*	196
2,4-Dinitrophenylhydrazine of product isolated from periodate oxidation mixture	162–164
Literature melting point for formaldehyde-2,4-dinitrophenylhydrazone	166
Dimedone alkylidene of product isolated from periodate oxidation mixture	187–188
Literature melting point of formaldehyde dimedone alkylidene	189

* Vogel (1951).

tallized derivative indicated the compound was acetol (Table 10). After treatment with 0.1 N periodic acid, the characteristic oxidation products of acetol, namely, acetic acid and formaldehyde, were isolated and identified: the latter by distillation and preparation of crystalline derivatives (Table 10), the former by paper chromatography of the steam-volatile fraction. Finally, the infrared-absorption spectrum of the acetol-semicarbazone was shown to be identical with that prepared from authentic acetol.

Evidence has also been obtained for small yields of a periodate-reacting material produced during bacterial oxidation of 2-butanone. This material has not been isolated or identified, but has the following characteristics. (i) It accumulates steadily over a 40-hr incubation period; a typical culture produced 0.004 meq per ml. Control suspensions without 2-butanone, or 2-butanone without cells, had no titration value. The periodate reaction is indicative of a hydroxy or carbonyl substitution adjacent to the carbonyl group of the substrate. Substitution at carbon 3 was eliminated, since a sensitive color test (Neish, 1952) for the likely products, namely, acetoin or diacetyl, was negative. (ii) After periodate oxidation, formaldehyde was detected by the chromotropic assay (Neish, 1952). No formaldehyde was present in the culture filtrates before reaction with periodic acid. The identity of the neutral, volatile product formed by periodate oxidation of the culture filtrate was substantiated as formaldehyde by isolation and identification of the dimedone derivative. This formaldehyde is indicative of the presence of a terminal $-CH_2OH$ group. On the basis of these fragmentary data, and by analogy with acetol formation from acetone, the product could be 1-hydroxy-2-butanone (butan-α-ol-β-one), but further evidence is needed.

This isolation of acetol verifies the belief of Levine and Krampitz (1952) that this compound is an intermediate in acetone oxidation. Microbial production of acetol is also known to take place by the dehydrogenation of 1,2-propanediol (Walti, 1934; Goepfert, 1941; Stainer and Fratkin, 1944).

Acetol metabolism and O_2 incorporation. Contrary to expectation, in an experiment similar to those recorded in Tables 8 and 9, the cell material obtained from acetol contained 2.33% of oxygen derived from atmospheric O_2. This compared with 2.88 and 0.64% for acetone- and *n*-propanol-grown cells, respectively. The similarity of the acetol and acetone cells would suggest that O_2 incorporation during acetone oxidation occurred at a stage subsequent to acetol, assuming that all of the acetone was metabolized via acetol. However, O_2 fixation in the conversion of acetone

to acetol is not excluded, since the O_2 incorporation concerned with acetol oxidation could mask an earlier fixation of O_2. A possibility for O_2 incorporation during acetol utilization may lie in the diterminal oxidation (oxygenation) of acetone analogous to that which occurs with long-chain alkanes (Kester and Foster, 1963). *M. smegmatis* 422 has been shown to carry out a diterminal oxidation of undecane (see Table 2), and conceivably all or part of the acetol might be converted to dihydroxyacetone (HOH_2CCOCH_2OH) enroute to degradation and assimilation.

An attempt was made to demonstrate O^{18} incorporation in the acetol produced from acetone by *M. smegmatis* 422. The acetol was isolated and purified by collecting the single acetol peak as it emerged from the gas chromatograph column; it was analyzed directly in the mass spectrometer. The mass spectrogram of acetol includes two major peaks having mass-to-charge ratios of 31 and 43, corresponding to the ions $-CH_2OH$ and CH_3CO-, respectively. An oxygen atom incorporated in acetol during its formation from acetone would be located in the terminal $-CH_2OH$ group, and hence the ratio of masses 31 ($-CH_2OH$) and 33 ($-CH_2O^{18}H$) is an index of the O^{18} content. Table 11 shows that the bacterial acetol had only 10.3% of the O^{18} theoretically expected from an oxygenation reaction [(0.6/5.8) × 100], and that chemical exchange could not account for the essentially negative outcome of the bacterial experiment.

These inconclusive data could be explained by a fixation of O_2 during the pathway subsequent to acetol. This would account for the equal incorporation of O_2 in acetone- and acetol-grown cells, again assuming all of the acetone is oxidized via acetol; it would also account for the absence of appreciable O^{18} labeling in the acetol produced from acetone. Although oxygenation of a terminal methyl carbon of acetone is analogous to alkane oxygenation and therefore an obvious possibility, alternative oxidative pathways have to be considered, for example, the propanediol pathway (Rudney, 1954):

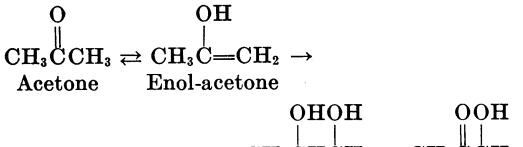

TABLE 11. *Mass spectrometric data on acetol produced from acetone in the presence of* O_2^{18}

Acetol sample	Initial atoms per cent O^{18} in O_2	$\frac{I_{33}^*}{I_{31}+I_{33}} \times 100$
Acetol produced from acetone by *M. smegmatis* 422 in the presence of O_2^{18}	5.8	0.60
Acetol incubated in the presence of O_2^{18}	5.8	6.53
Acetol incubated in H_2O containing 5.88 atoms per cent O^{18}	—	0.62

* I_{33} = intensity of peak mass to charge ratio 33, etc.

This pathway was excluded in the diptheroid studied by Levine and Krampitz (1952). As for the incorporation of O^{18} in cells during acetone and acetol utilization, oxygenases are known (Hayaishi and Sutton, 1957; Itada et al., 1961) for simple aliphatic compounds, e.g., lactate, to which acetone and acetol might be converted (Rudney, 1954). Thus, O^{18} incorporation may well occur at a later stage in acetone or acetol oxidation. Interestingly, the lactic oxygenase has been found in species of *Mycobacterium* (Sutton, 1957; Yamamura, Kusunose, and Kusunose, 1952). Studies underway are expected to help clarify this problem.

ACKNOWLEDGMENT

This work was supported in part by grants from the National Science Foundation; the National Institutes of Health, U.S. Public Health Service; and the Office of Naval Research. A few of the salient points and data described in detail in this paper have been included without experimental details in concurrent review articles (Foster, 1962*a*, *b*).

LITERATURE CITED

CHOUTEAU, J., E. AZOULAY, AND J.-C. SENEZ. 1962. Anaerobic formation of *n*-hept-1-ene from *n*-heptane by resting cells of *Pseudomonas aeruginosa*. Nature **194**:576–578.

EL MASRY, A. M., J. N. SMITH, AND R. T. WILLIAMS. 1955. The α, w- and (w-1)-oxidation of *n*-propyl and *n*-butyl benzenes in rabbits. Determination of hippuric acid. Biochem. J. **61**:i.

FOSTER, J. W. 1962*a*. Bacterial oxidation of hydrocarbons, p. 241–271. *In* O. Hayaishi [ed.], Oxygenases. Academic Press, Inc., New York.

FOSTER, J. W. 1962*b*. Hydrocarbons as substrates for microorganisms. Antonie van Leeuwenhoek J. Microbiol. Serol. **28**: 241–274.

FRANKE, W., AND W. HEINEN. 1958. Zur kenntnis des Fettsaureabbaus durch Schimmelpilze. I. Über die Methylketonbildung der Schimmelpilze. Arch. Mikrobiol. **31**:50–59.

FRUTON, J. S., AND S. SIMMONDS. 1958. General biochemistry, 2nd ed., p. 605–606. John Wiley & Sons, Inc., New York.

FUHS, G. W. 1961. Der Mikrobielle Abbau von Kohlenwasserstoffen. Arch. Mikrobiol. **39**: 374–422.

GOEPFERT, G. J. 1941. Studies on the mechanism of dehydrogenation by *Fusarium lini* Bolley. XIX. Dehydrogenation of higher primary and secondary alcohols. J. Biol. Chem. **140**:525–534.

HAMILTON, G. A., AND F. H. WESTHEIMER. 1959. On the mechanism of the enzymatic decarboxylation of acetoacetate. J. Am. Chem. Soc. **81**:6332–6333.

HAYAISHI, O. 1957. Incorporation of atmospheric oxygen into the cell constituents of a growing culture of *Pseudomonas*. J. Am. Chem. Soc. **79**:5576–5577.

HAYAISHI, O. 1962. Oxygenases. Academic Press, Inc., New York.

HAYAISHI, O., AND W. B. SUTTON. 1957. Enzymatic oxygen fixation into acetate concomitant with the enzymatic decarboxylation of L-lactate. J. Am. Chem. Soc. **79**:4809–4810.

HOPKINS, S. J., AND A. C. CHIBNALL. 1932. Growth of *Aspergillus flavus* on higher paraffins. Biochem. J. **26**:133–142.

ITADA, N., A. ICHIHARA, T. MAKITA, O. HAYAISHI, M. SUDA, AND M. SASAKI. 1961. L-Lysine oxidase, a new oxygenase. J. Biochem. (Tokyo) **50**:118–121.

JOHNSON, M. J., W. H. PETERSON, AND E. B. FRED. 1933. Intermediary compounds in the acetone-butyl alcohol fermentation. J. Biol. Chem. **101**:145–157.

KESTER, A. S., AND J. W. FOSTER. 1963. Diterminal oxidation of long-chain alkanes by bacteria. J. Bacteriol. **85**:859–869.

KLAUSMEIER, R. E., L. R. BROWN, E. N. BENES, AND R. J. STRAWINSKI. 1958. Propane metabolism by a *Mycobacterium*. Bacteriol. Proc., p. 123.

LADD, J. N. 1956. The oxidation of hydrocarbons by soil bacteria. I. Morphological and biochemical properties of a soil diphtheroid utilizing hydrocarbons. Australian J. Biol. Sci. **9**:92–104.

LEADBETTER, E. R., AND J. W. FOSTER. 1959*a*. Oxidation products formed from gaseous alkanes by the bacterium *Pseudomonas methanica*. Arch. Biochem. Biophys. **82**:491–492.

LEADBETTER, E. R., AND J. W. FOSTER. 1959*b*. Incorporation of molecular oxygen in bacterial cells utilizing hydrocarbons for growth. Nature **184**:1428–1429.

LEADBETTER, E. R., AND J. W. FOSTER. 1960. Bacterial oxidation of gaseous alkanes. Arch. Mikrobiol. **35**:92–104.

LEVINE, S., AND L. O. KRAMPITZ. 1952. The oxidation of acetone by a soil diphtheroid. J. Bacteriol. **64**:645–650.

MARVEL, C. S., AND R. D. RANDS. 1950. Separation of organic acids. J. Am. Chem. Soc. **72**:2642–2646.

MUKHERJEE, S. 1951. Studies on degradation of fats by microorganisms. I. Preliminary investigations on enzyme systems involved in spoilage of fats. Arch. Biochem. Biophys. **33**: 364–376.

MÜLLER, D. 1931. Untersuchungen über Oxydasen in getotenen Essigbakterien. I. Biochem. Z. **238**:253–267.

NEISH, A. C. 1952. Analytical methods for bacterial fermentations. Report no. 46-8-3. Prairie Regional Laboratory, Saskatoon, Canada.

RITTENBERG, D., AND L. PONTICORVO. 1956. A method for the determination of the O^{18} concentration of oxygen of organic compounds. Intern. J. Appl. Radiation Isotopes **1**: 208–214.

RUDNEY, H. 1954. Propanediol phosphate as a possible intermediate in the metabolism of acetone. J. Biol. Chem. **210**:361–371.

SELIGMAN, R. B., AND M. D. EDMONDS. 1955. The separation of 2,4-dinitrophenylhydrazones by paper chromatography. Chem. Ind. (London), p. 1406–1407.

SELLINGER, O. Z., AND O. N. MILLER. 1959. The metabolism of acetol-phosphate. II. 1,2-Propanediol-1-phosphate dehydrogenase. J. Biol. Chem. **234**:1641–1646.

SMITH, J. N., R. N. SMITHIES, AND R. T. WILLIAMS. 1954. Studies in detoxication. 56. The metabolism of alkyl benzenes. Stereochemical aspects of the biological hydroxylation of ethylbenzene to methylphenylcarbinol. Biochem. J. **56**:320–324.

STANIER, R. Y. 1947. Simultaneous adaptation: a new technique for the study of metabolic pathways. J. Bacteriol. **54**:339–348.

STANIER, R. Y., AND S. B. FRATKIN. 1944. Studies on the bacterial oxidation of 2,3-butanediol and related compounds. Can. J. Res. Sect. B **22**:140–153.

STÄRKLE, M. 1924. Die Methylketone im oxyda-

tiven Abbau einiger Triglyceride (bzw. Fettsäuren) durch Schimmelpilze unter Berüchsichtigung der besonderen Ranzidität des Kokosfettes. Biochem. Z. **151**:371–415.

STEWART, J. E., R. E. KALLIO, D. P. STEVENSON, A. C. JONES, AND D. O. SCHISSLER. 1959. Bacterial hydrocarbon oxidation. I. Oxidation of *n*-hexadecane by a gram-negative coccus. J. Bacteriol. **78**:441–448.

SUTTON, W. B. 1957. Mechanism of action and crystallization of lactic oxidative decarboxylase from *Mycobacterium phlei*. J. Biol. Chem. **226**:395–405.

VOGEL, A. E. 1951. A text-book of practical organic chemistry including qualitative organic analysis. Longmans, Green & Co., Ltd., London.

WALTI, A. 1934. Action of *Aspergillus niger* on normal 1,2-diols. J. Am. Chem. Soc. **56**:2723–2726.

YAMAMURA, Y., M. KUSUNOSE, AND E. KUSONOSE. 1952. Lactic acid oxidases of *Mycobacterium tuberculosis avium*. J. Biochem. (Tokyo) **39**: 227–238.