The Biosynthesis of $\Delta^9$- and $\Delta^5$-Monosaturated Fatty Acids by Bacteria

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Two different pathways for the biosynthesis of long chain monounsaturated fatty acids have been demonstrated. One of these, found in higher animals (1-3), yeast (3-6), protozoa (7), red algae, blue-green algae, and in certain bacteria (8, 9) involves the oxygen-dependent direct desaturation of long chain saturated fatty acids to the $\Delta^9$-derivatives. The other pathway proceeds anaerobically and produces $C_{16}$ and $C_{18}$-monounsaturated fatty acids by way of $\Delta^3$-unsaturated intermediates. To date, this pathway has been found only among the bacteria. The two mechanisms seem to be mutually exclusive; no organism has yet been found which can utilize both. While the oxygen-dependent pathway seems to be universal among eucaryotic organisms, both the aerobic and anaerobic mechanisms are found in the procaryotic group. The occurrence of both pathways in bacteria raised the question of whether the mode of unsaturated fatty acid biosynthesis can be correlated with morphological, physiological, and other biochemical characteristics which have been used to delineate phylogenetic relationships within this group. Thus, on the assumption that the anaerobic pathway is the more primitive, it might be argued that organisms possessing an oxygen-dependent mechanism would be more advanced than groups which possess the anaerobic pathway. Although recent findings in this laboratory suggest that the development of the oxygen-dependent pathway in procaryotic organisms does not parallel the change from anaerobic to aerobic energy metabolism, it appeared from a limited survey that the oxidative pathway occurred only in the morphologically and physiologically more advanced procaryotic forms, i.e. in the blue-green algae and in representatives of the bacterial orders. Actinomycetales (8, 9), Beggiooales, and Myxobacteriales. In the more primitive procaryotic organisms, i.e. in representatives of the orders Pseudomonadaceae and Eubacteriales, only the anaerobic pathway had been found to date (11-15). However, as is shown in the present report, the oxygen-dependent desaturation of fatty acids occurs also in these more primitive groups. Representatives of three bacterial families of the order Eubacteriales were examined for the presence of the aerobic pathway. Corynebacterium diphtheriae was studied because of close structural similarities between the complex cellular lipids in corynebacteria and mycobacteria (18) and because the latter possess the aerobic pathway (9) Micrococcus lysodeikticus, an organism that contains large amounts of branched chain fatty acids (19, 20), was also examined. After both these organisms were shown to form $\Delta^3$-unsaturated fatty acids by aerobic desaturation, Bacillus megaterium was investigated because its fatty acid spectrum is closely similar to that of $M. lysodeikticus$ (21). Again the oxygen-dependent pathway was found, but unexpectedly, $B. megaterium$ produced $\Delta^9$-hexadecenoic and octadecenoic acids rather than the more common $\Delta^9$ isomers.

EXPERIMENTAL PROCEDURE

Materials—Radiochemicals were obtained from New England Nuclear Corporation. All 1-14C fatty acids were purified before use by treatment of the methyl esters with mercuric acetate followed by silicic acid chromatography to remove any polar or unsaturated material (22). The purified fatty acids, which contained less than 0.05% radioactive unsaturated material and were more than 97% radiochemically pure as judged by gas- and silicic acid chromatography, had the following specific activities (in microcuries per mmole): stearic-1-14C, 11.7; palmitic-1-14C, 10.0; myristic-1-14C, 2.5; lauric-1-14C, 1.9; and octanoic-1-14C, 2.2. Sodium-1-14C acetate (2.8 µc per mmole) was used without preliminary purification. Activated silicic acid (Unisil, 100 to 200 mesh) was purchased from the Clarkson Chemical Company, Inc. N-Methyl N-nitroso p-toluene sulfonamide (Diazald) used for generating diazonamide (23) was purchased from the Aldrich Chemical Company.

Methods

Maintenance and Growth of Cultures—Corynebacterium diphtheriae, strain C, s(+) tox(−) obtained from Professor A. M. Pap...
penheimer (24) and maintained in liquid culture at 30°, was grown on the medium of Mueller and Miller (25) supplemented with L-tryptophan, 0.1 g per liter, and L-glutamic acid, 0.5 g per liter. A 50% maltose solution was added to the medium to a final concentration of 2% before inoculation. Micrococcus lysodeikticus ATCC 4698 was maintained on nutrient agar slants at 4° and was grown on the defined medium of Grula, Luk, and Chu (26). Bacillus megaterium KM strain was obtained from Dr. Howard Goldfine and maintained in liquid culture at room temperature with weekly transfers. It was grown in a medium consisting of the salt mixture described by Grelet (27) plus: caseamino acids (Difco Laboratories), 5 g per liter; glucose, 5 g per liter; and sodium citrate, 0.1 g per liter. All organisms were grown in 2-liter conical flasks on a rotary shaker in either 1 liter per liter; and sodium citrate, 0.1 g per liter. All organisms were caseamino acids (Difco Laboratories), 5 g per liter; glucose, 5 g per liter; and sodium citrate, 0.1 g per liter. All organisms were grown on defined media as described. C. diphtheriae was grown for 24 hours at 30°, M. lysodeiktics for 46 hours at 30°, and B. megaterium for 37 hours at 25°. Gas chromatographic analysis was performed on the methyl esters of both the total fatty acids from each organism and, after mercuric acetate separation, on the saturated and unsaturated fractions.

Results and Discussion

Table I shows the results of gas chromatographic analysis of the fatty acids of C. diphtheriae, M. lysodeiktics, and B. megaterium. Although both the relative and absolute amounts of unsaturated fatty acids differ widely among the three organisms, the predominant unsaturated acid is hexadecenoic acid in all three cases. This is in contrast to the predominance of octadecenoic acid in most bacteria and in higher organisms. The data in Table II show that growing cultures of C. diphtheriae and M. lysodeiktics can readily desaturate added stearic or palmitic acids. The fact that label appears almost exclusively in the fatty acids with the same chain length as the added substrate indicates that the desaturation was direct and not due to breakdown of the substrate to small fragments followed by resynthesis. This conclusion is confirmed by decarboxylation of the unsaturated products (Table III) which shows that little or no randomization of label took place. The double bond in hexadecenoic and octadecenoic acids from both organisms was found by KMnO₄-KIO₅ oxidation (Table III) to be at positions 9 and 10. In all cases, 90% or more of the radioactivity in the isolated dicarboxylic acids was found to be in azelaic (C₁₄) acid. Infrared analysis of fatty acids of chain length greater than C₁₆ would not be detected when methyl elaidate and methyl stearate were used as standards.

Fatty acids of C. diphtheriae, M. lysodeiktics, and B. megaterium

All organisms were grown on defined media as described. C. diphtheriae was grown for 24 hours at 30°, M. lysodeiktics for 46 hours at 30°, and B. megaterium for 37 hours at 25°. Gas chromatographic analysis was performed on the methyl esters of both the total fatty acids from each organism and, after mercuric acetate separation, on the saturated and unsaturated fractions.

**Table I**

<table>
<thead>
<tr>
<th>Fatty acid*</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. diphtheriae</td>
</tr>
<tr>
<td>Branched, saturated</td>
<td></td>
</tr>
<tr>
<td>&lt;C₁₀</td>
<td>0.5</td>
</tr>
<tr>
<td>C₁₀</td>
<td>7</td>
</tr>
<tr>
<td>C₁₂</td>
<td>47</td>
</tr>
<tr>
<td>C₁₄</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Total saturated</td>
<td>55</td>
</tr>
<tr>
<td>Branched, monounsaturated</td>
<td></td>
</tr>
<tr>
<td>&lt;C₁₀</td>
<td>5</td>
</tr>
<tr>
<td>C₁₀</td>
<td>0.1</td>
</tr>
<tr>
<td>C₁₈</td>
<td>1</td>
</tr>
<tr>
<td>Other*</td>
<td>1</td>
</tr>
<tr>
<td>Total unsaturated*</td>
<td>45</td>
</tr>
</tbody>
</table>

* Fatty acids (mg) per g, wet weight, of cells were as follows: C. diphtheriae, 12.0; M. lysodeiktics, 4.5; B. megaterium, 3.3.

† Not detected.

Any hydroxy acid or other polar acids were removed prior to gas chromatography by silicic acid chromatography of the methyl esters. Fatty acids of chain length greater than C₁₄ would not have been detected by the gas chromatographic procedure used.

* No polyunsaturated fatty acids were detected.
Desaturation of $^{14}$C fatty acids by growing cultures of *C. diphtheriae* and *M. lysodeikticus*

*C. diphtheriae*: Two flasks, each containing 500 ml of defined medium and 0.5 $\mu$ mole of either stearic-$^{14}$C or palmitic-$^{14}$C, were each inoculated with 2-ml starter cultures, and incubated in air for 24 hours at 30°.

*M. lysodeikticus*: Two flasks, each containing 1 liter of defined medium and either 1.2 $\mu$mole of stearic-$^{14}$C or 0.5 $\mu$mole of palmitic-$^{14}$C were inoculated with 25-ml starter cultures, and incubated in air for 46 hours at 30°.

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**Table II**

<table>
<thead>
<tr>
<th>Organism</th>
<th>1-14C substrate</th>
<th>Amount incorporated</th>
<th>Desaturation</th>
<th>Distribution of 14C in fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\mu$mole</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td><em>C. diphtheriae</em></td>
<td></td>
<td>200</td>
<td>61</td>
<td>0</td>
</tr>
<tr>
<td>Stearic</td>
<td></td>
<td>180</td>
<td>45</td>
<td>54</td>
</tr>
<tr>
<td>Palmitic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. lysodeikticus</em></td>
<td></td>
<td>213</td>
<td>48</td>
<td>1</td>
</tr>
<tr>
<td>Stearic</td>
<td></td>
<td>215</td>
<td>70</td>
<td>22</td>
</tr>
<tr>
<td>Palmitic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Based on the radioactivity recovered in the fatty acids obtained by saponification of the cells.

† The chain lengths of the $C_{18}$ and $C_{14}$ acids were confirmed by hydrogenation and recrystallization as described.

**Table III**

Decarboxylation and oxidative cleavage of unsaturated fatty acids from *C. diphtheriae* and *M. lysodeikticus* grown in presence of 1-14C fatty acids

Portions of unsaturated fatty acids, isolated by the mercuric acetate procedure from organisms grown on 1-14C fatty acids (see Table II), were subjected to decarboxylation or to cleavage at the double bonds with KMnO$_4$-KIO$_4$.

<table>
<thead>
<tr>
<th>Organism</th>
<th>1-14C substrate</th>
<th>Major unsaturated product</th>
<th>14C in carboxyl carbon</th>
<th>Distribution of 14C in dicarboxylic acids from KMnO$_4$-KIO$_4$ cleavage</th>
<th>Branched %</th>
<th>$\alpha$-Saturated</th>
<th>$\alpha$-Unsaturated</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. diphtheriae</em></td>
<td>Stearic</td>
<td>Octadecenoic (99)</td>
<td>95.0</td>
<td>1</td>
<td>97</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Palmitic</td>
<td>Hexadecenoic (97)</td>
<td>91.3</td>
<td>1</td>
<td>97</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. lysodeikticus</em></td>
<td>Stearic</td>
<td>Octadecenoic (90)</td>
<td>98.5</td>
<td>2</td>
<td>00</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Palmitic</td>
<td>Hexadecenoic (99)</td>
<td>98.4</td>
<td>3</td>
<td>95</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Decarboxylation of the substrates indicated that 99.8% of the 14C was in the carboxyl carbon.

† The numbers in parentheses indicate the percentage of the 14C of the unsaturated fraction found in the major product (see also Table II).

‡ This activity was broadly distributed between the $C_{18}$ to $C_{12}$ region on the gas chromatography column.

of hexadecenoic acid samples isolated by the mercuric acetate procedure and gas chromatography indicated a trans content of less than 2%. The structure of the C$_{18}$ monounsaturated acid formed from palmitic-14C in *C. diphtheriae* and *M. lysodeikticus* is therefore cis-9-hexadecenoic (palmitoleic) acid. The available samples of the 9-octadecenoic acids were insufficient for infrared analysis. Resting cell experiments with *C. diphtheriae* and *M. lysodeikticus* (Table IV) indicate that oxygen is required for the desaturation reaction in both organisms.

*B. megaterium* is also capable of converting palmitic and stearic acids directly to monounsaturated derivatives but in this organism the extent of the conversion, at least for palmitic acid, depends critically on the temperature prevailing during growth. At 30°, only 4% of the incorporated palmitic was converted to hexadecenoic acid. This value was raised to 51% at 25° and to 80% at 23°. From the data in Table V, it seems unlikely that the increase in the amount of substrate incorporated is responsible for the more effective desaturation at the lower temperatures.

An inverse relationship between growth temperature and unsaturated fatty acid synthesis has been observed and studied in detail in *Escherichia coli* by Marr and Ingraham (33) and in the yeast *Torulopsis utilis* by Meyer and Bloch (34). Other examples of this rather general phenomenon include the reports by Howell and Collins (35), Gaughan (36), and Frenkel and Hopf (37).

The results presented in Table VI show that *B. megaterium* does not desaturate acids shorter than palmitate. No trace of activity could be detected in unsaturated fatty acids shorter than C$_{14}$ regardless of the chain length of the 1-14C-acid added. Both myristic and lauric acids appear to be chain elongated to palmitic acid before desaturation takes place, but the elongation process apparently does not continue beyond palmitate. On the other hand, the desaturation system acts on stearic and palmitic acids but not on lower homologues.

The fact that added stearic acid is readily desaturated by *C. diphtheriae* and *M. lysodeikticus* as well as by *B. megaterium* but that in normal cells octadecenoic acid is a minor component suggests that the accumulation of hexadecenoic acid in these organisms is due to the inefficiency of the elongation system beyond the C$_{18}$ stage.

Decarboxylation of unsaturated fatty acid samples obtained from *B. megaterium* grown in the presence of 1-14C-fatty acids (Table VII) shows that there was no significant randomization of label. Cleavage of the octadecenoic and hexadecenoic acid samples by KMnO$_4$-KIO$_4$ (Table VII) gave unexpectedly radioactive glutaric acid in every case. Thus, the double bond is located at positions 5 and 6 in both the C$_{18}$ and C$_{16}$ monounsaturated acids of *B. megaterium*. This conclusion was confirmed by the results of oxidative ozonolysis which showed that the major monoacrylylic fragments obtained from the C$_{18}$ and C$_{16}$ acids were tridecanoic and undecanoic acids, respectively. For infrared analysis, the methyl esters of the unsaturated fatty

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**Table IV**

Analysis of 9-octadecenoic acids formed from palmitic-14C in *C. diphtheriae* and *M. lysodeikticus* indicate that oxygen is required for the desaturation reaction in both organisms.

*C. diphtheriae*: Two flasks, each containing 500 ml of defined medium and 0.5 $\mu$mole of either stearic-14C or palmitic-14C, were each inoculated with 2-ml starter cultures, and incubated in air for 24 hours at 30°.

*M. lysodeikticus*: Two flasks, each containing 1 liter of defined medium and either 1.2 $\mu$mole of stearic-14C or 0.5 $\mu$mole of palmitic-14C were inoculated with 25-ml starter cultures, and incubated in air for 46 hours at 30°.
Resting cell experiments with C. diphtheriae and M. lysodeikticus (O2 requirement for desaturation)

C. diphtheriae was grown for 24 hours at 30°. Cells were harvested and washed by centrifugation, and then 200 mg of cells were added to each of three flasks containing 12.5 mmole of stearic-1-14C acid and 25 ml of 1% maltose in 0.05 M potassium phosphate buffer, pH 7.10. One flask was incubated in air for 8 hours, one under helium for 8 hours, and one was incubated first under helium for 8 hours and then in air for 8 hours. All incubations were at 30° with shaking.

M. lysodeikticus was grown for 42 hours at 30°, then harvested and washed. Cells were added to each of three flasks (100 mg of cells per flask) containing 1% glucose in 0.05 M potassium phosphate buffer, pH 7.10, and 12.5 mmole of palmitic-1-14C acid. Incubation at 30° was for 6 hours in air or under N2, or for 6 hours under N2 followed by 6 hours in air.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Fatty acid incorporated</th>
<th>Desaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmole</td>
<td>%</td>
</tr>
<tr>
<td>C. diphtheriae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>4.7</td>
<td>17.8</td>
</tr>
<tr>
<td>Helium</td>
<td>3.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Helium; air</td>
<td>4.2</td>
<td>1.2</td>
</tr>
<tr>
<td>M. lysodeikticus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>1.9</td>
<td>12.4</td>
</tr>
<tr>
<td>N2</td>
<td>4.7</td>
<td>1.3</td>
</tr>
<tr>
<td>N2; air</td>
<td>2.2</td>
<td>8.7</td>
</tr>
</tbody>
</table>

a Flasks, fitted with stopcocks, were alternately evacuated and filled with the inert gas three times before incubation.

b See Footnote a, Table II.

c This low value may be attributed to the fact that C. diphtheriae does not remain viable for long under anaerobic conditions.

The temperature varied between 25 and 27° during growth. However, since the organisms in all flasks were grown simultaneously on the same shaker, the results should be strictly comparable.

Effect of temperature on desaturation of palmitic and stearic acids by B. megaterium

Flasks containing 1 liter of defined medium and either 200 mmole (Experiments 1 and 2) or 400 mmole (Experiments 3 to 6) of the 1-14C-fatty acid were inoculated with 5 ml of starter culture, and the organisms were grown under the conditions shown.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Initial pH</th>
<th>Temperature</th>
<th>Time</th>
<th>14C substrate</th>
<th>Amount incorporated</th>
<th>Desaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>°C</td>
<td>hours</td>
<td></td>
<td>mmole</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>30</td>
<td>36</td>
<td>Palmitic</td>
<td>94</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>30</td>
<td>30</td>
<td>Palmitic</td>
<td>94</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>30</td>
<td>36</td>
<td>Palmitic</td>
<td>56</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>25</td>
<td>36</td>
<td>Palmitic</td>
<td>48</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>23</td>
<td>40</td>
<td>Palmitic</td>
<td>62</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>25</td>
<td>36</td>
<td>Stearic</td>
<td>46</td>
<td>22</td>
</tr>
</tbody>
</table>

a See Footnote a, Table II.

b More than 95% of the radioactivity in the unsaturated fatty acids was recovered in hexadecenoic acid (Experiments 1 to 5) or octadecenoic acid (Experiment 6). All the radioactivity in the saturated fatty acid fractions obtained by the mercuric acetate procedure was recovered in the fatty acid corresponding to the added 1-14C substrate.

demonstrate an absolute oxygen requirement for the formation of Δ4 acids. More than 80% of the incorporated palmitic-1-14C acid is desaturated when cells are incubated in air, but there is essentially no conversion in an atmosphere of helium. In cells, which had been incubated in helium for 10 hours and then reincubated in air, desaturation of incorporated palmitic acid proceeded readily. The data in Table VIII also show that nitrate, added

Growth of B. megaterium in presence of 1-14C-fatty acids

Flasks containing 1 liter of defined medium and the 1-14C-fatty acid were each inoculated with 5 ml of starter culture, and the organisms were grown at ~25° for 37 hours.

<table>
<thead>
<tr>
<th>14C substrate</th>
<th>Amount added</th>
<th>Amount incorporated</th>
<th>Incorporated 14C in unsaturated fatty acids</th>
<th>n-Saturated</th>
<th>n-Unsaturated</th>
<th>Branched chain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmole</td>
<td>%</td>
<td>&lt;C14</td>
<td>&lt;C14</td>
<td>&lt;C14</td>
<td>&lt;C14</td>
</tr>
<tr>
<td>Stearic</td>
<td>0.60</td>
<td>0.20</td>
<td>3</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Palmitic</td>
<td>1.00</td>
<td>0.29</td>
<td>46</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Myristic</td>
<td>4.20</td>
<td>0.86</td>
<td>25</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Lauric</td>
<td>6.20</td>
<td>0.55</td>
<td>23</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Octanoic</td>
<td>6.20</td>
<td>0.09</td>
<td>&lt;1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Acetic</td>
<td>6.20</td>
<td>0.18</td>
<td>3</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

a The temperature varied between 25 and 27° during growth. However, since the organisms in all flasks were grown simultaneously on the same shaker, the results should be strictly comparable.

b See Footnote a, Table II.

c The chain lengths were confirmed by hydrogenation and rechromatography.
TABLE VII
Decarboxylation and oxidative cleavage of unsaturated fatty acids from B. megaterium grown in presence of 1-14C-fatty acids

Portions of unsaturated fatty acids isolated by the mercuric acetate procedure from B. megaterium grown on 1-14C-fatty acids (see Table VI) were decarboxylated or cleaved at the double bond with KMnO4-KIO4.*

<table>
<thead>
<tr>
<th>14C substrate</th>
<th>Major unsaturated product</th>
<th>14C in</th>
<th>Distribution of 14C in dicarboxylic acids from KMnO4-KIO4 cleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>carboxyl</td>
<td>&lt;Cs</td>
</tr>
<tr>
<td>Stearic</td>
<td>Octadecenoic</td>
<td>&lt;60</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Palmitic</td>
<td>Hexadecenoic</td>
<td>90</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Myristic</td>
<td>Hexadecenoic</td>
<td>2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Lauric</td>
<td>Hexadecenoic</td>
<td>&lt;70</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

* The unsaturated acids were also cleaved by oxidative ozonolysis. In all cases, 70% or more of the radioactivity was recovered in the C1 dicarboxylic acid. The remaining 14C was found chiefly in succinic acid with smaller amounts in oxalic, malonic, and adipic acids. The dicarboxylic acids other than Cs were probably artifacts arising from rearrangement of the ozonide (37).

† See Footnote b, Table III.

‡ Not decarboxylated.

TABLE VIII
Resting cell experiments with B. megaterium (O2 requirement for desaturation)

Cells were grown at 24° for 24 hours. Washed cells (400 mg) were then added to each of six flasks containing 10 mmoles of palmitic-1-14C acid and 25 ml of either resting medium (1% glucose) in 0.05 M potassium phosphate buffer, pH 7.20) or resting medium plus 0.1 M KNO3. Two flasks were incubated in air for 10 hours. Four were incubated under helium for 10 hours, and two of these were then reincubated in air for 10 hours. All flasks were incubated at 23.5° with shaking.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Atmosphere</th>
<th>Amount incorporated</th>
<th>Desaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mmoles</td>
<td>%</td>
</tr>
<tr>
<td>Resting</td>
<td>Air</td>
<td>5.4</td>
<td>83</td>
</tr>
<tr>
<td>Resting + KNO3</td>
<td>Air</td>
<td>4.9</td>
<td>90</td>
</tr>
<tr>
<td>Resting</td>
<td>Helium</td>
<td>7.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Resting + KNO3</td>
<td>Helium</td>
<td>8.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Resting</td>
<td>Helium; air</td>
<td>2.1</td>
<td>73</td>
</tr>
<tr>
<td>Resting + KNO3</td>
<td>Helium; air</td>
<td>0.7</td>
<td>86</td>
</tr>
</tbody>
</table>

* See Footnote a, Table II.

form unsaturated fatty acids anaerobically. The taxonomic significance of these findings is obscure. It would be difficult to invoke morphological or physiological characteristics for differentiating between one evolutionary line leading to M. lysodeikticus or other bacteria that have the oxygen-dependent mechanism, and another line leading to bacteria which synthesize unsaturated fatty acids anaerobically. The bacilli and the clostridia are usually classified, chiefly on morphological grounds, as related genera within the family Bacillaceae. Yet, in clostridia, the pathway for unsaturated fatty acid synthesis is anaerobic (12), whereas B. megaterium has the oxygen-dependent desaturation mechanism. In the latter case, Δ5-unsaturated fatty acids are produced, and this anomaly suggests independent origins for aerobic desaturation in bacilli and in those organisms that produce Δ5-acids. The more important question is, however, whether the development of the aerobic Δ5 desaturation mechanism was a unique event in evolution or whether it arose independently a number of times. Until this point can be answered, the validity of using the biosynthetic mechanism for unsaturated fatty acid formation as a phylogenetic marker for classifying bacteria is in question.

SUMMARY
Cornebacterium diphtheriae and Micrococcus lysodeikticus desaturate stearic and palmitic acids to the cis-Δ5 derivatives. Resting cell experiments indicate that the desaturation reaction is oxygen-dependent.

Growing and resting cells of Bacillus megaterium KM convert stearic and palmitic acids to the previously unknown cis-5-octadecenoic and cis-5-hexadecenoic acids, respectively. Myristic and laurie acids are not desaturated by B. megaterium but instead are chain-elongated to palmitic acid, which is then desaturated to the Δ5 derivative. Oxygen appears to be an absolute requirement for these desaturation reactions also.

The conversion of exogenous palmitic acid to cis-5-hexadecenoic acid in growing cultures of B. megaterium is temperature-dependent, increasing from negligible values in cultures grown at 30° to almost complete desaturation at 23°.

REFERENCES
The Biosynthesis of $\Delta^9$- and $\Delta^5$-Monosaturated Fatty Acids by Bacteria
Armand J. Fulco, Ronald Levy and Konrad Bloch


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