Utilization of various hydrocarbons by microbes is well known (Beerstecher, 1954), but little has been reported on the cellular composition of hydrocarbon-grown cells. No doubt it has been assumed that these cells differ little from those cultivated on nonhydrocarbon substrates and, in general, this assumption is probably true. But it is recognized that substrates and cultural conditions can influence not only extracellular products but also the composition of microbial cells.

In the course of studying conditions for rapid utilization of hydrocarbons by microbes we have observed a marked increase in the lipid content of nocardia grown on liquid n-alkanes. This was noted particularly in the case of n-hexadecane and n-octadecane cultures. As much as 70 per cent of the cellular material formed from these substrates can be extracted with fat solvents. This lipid consists principally of glycerides and straight-chain waxes. In contrast, nocardial cells grown on n-hexane or n-tridecane contain about the same amount of lipid as glucose-grown cells, less than 30 per cent, and no waxes.

This paper describes the conditions used for culturing a soil-isolated nocardia on liquid n-alkanes; the conversion of n-alkane to gross cellular products; and some of the factors which influence growth and cellular lipid formation.

**EXPERIMENTAL METHODS AND RESULTS**

**Soil Isolate**

The microbe employed was isolated with ethane enrichment using the soil plating procedure described previously by Davis et al. (1956). When grown on nutrient agar the microbe produces long filaments which fragment in about 6 to 8 hr to form bacillary cells about 1.5 by 3 μ. These become progressively more coccoidal. The cells are gram positive at all stages observed and are not acid-fast. When grown in a mineral salts medium with liquid n-alkanes, shorter filaments are formed which fragment into coccoidal rods 2 by 3 μ. These are also gram positive and are not acid-fast at any stage of growth. Pigmentation of growth is orange on both nutrient agar and hydrocarbon media. No aerial mycelium or sporulation has been observed. The microbe has been identified along with many other similar isolates as belonging to the genus Nocardia.

**Cultural Procedures**

The nocardia ordinarily were grown in 4-L resin kettles equipped with stainless steel baffles, a stainless steel fritted sparger, and a motor driven impeller (figure 1). These are much like those described by Lakata (1954) for studies on enzyme production. The impeller shaft rotated within a graphite impregnated Bost-bronze bearing which was mounted in a neoprene plug fitted in the center opening of the kettle top. The units were temperature controlled by water baths at 30 C (figure 2).

About 2 L of sterile mineral salts medium were added to each sterilized culture system. The following mineral salts in g per L of distilled water were employed unless otherwise stated: (NH₄)₂SO₄, 1.0; MgSO₄·7H₂O, 0.2; FeSO₄·7H₂O, 0.005; KH₂PO₄, 2.0; Na₂HPO₄, 3.0; Na₂CO₃, 0.1; CaCl₂, 0.01; MnSO₄, 0.002; and ashed yeast extract, 0.008. The initial amount of liquid

1 Manufactured by the Keystone Co., Maryville, Pennsylvania.
n-alkane added to the system varied but was usually about 8 g, making the initial concentration about 0.4 per cent. Additional alkane was added as it approached depletion in the system.

Inoculum was grown at 30 C in mineral salts-alkane medium in 250-ml bottles on a Gyrotory Shaker. The bottles had indentations in the sides to act as baffles and contained about 0.1 per cent alkane in 50 ml of medium. The inoculum added to the 4-L culture systems usually amounted to about 0.4 g, dry weight.

Cell Yields

The impellers were rotated at very high rates, as high as 1750 rpm. At this rate, for example, 8.3 g of nocardial cells were produced from 11.8 g of n-octadecane in 60 hr (70 per cent yield). At a rate of 1150 rpm, 6.4 g of cells were produced from 8.0 g of octadecane in the same length of time (80 per cent yield). Capsular slime, which sloughed off the cells without any apparent impairment to them, was not included in these cell yields. The amount of slime which separated from the cells varied from culture to culture and was dependent on speed of agitation and possibly other factors.

Figure 3 shows the yield of cellular material, including the slime polymer obtained from a pound of n-octadecane. The yield totals 95 per cent on a weight conversion basis and, of course, includes material produced in a series of culture systems. The highest actual dry weight quantity of cells produced in a single system containing 2 L of medium was 48.8 g from 57.0 g of n-octadecane in 4 days, an 85 per cent yield. This amounts to 24.4 g of cells (dry weight) per L of culture.

Analyses

Following centrifugation to recover the nocardial cells, the culture liquor was tested for neutral and acidic distillable products using chromic acid treatment of the alkali liquor distillate followed by titration of the acidified liquor steam distillate with standard 0.05 N NaOH. These tests were essentially negative.

Ethyl ether or n-hexane extracts of the cell-free culture liquor were nil. The small amount of unconsumed n-alkane that sometimes remained in the cultures adhered to the cells and was recovered during cell extraction.

Chromic acid wet combustion of aliquots of the culture liquor indicated fairly large amounts of organic carbon in solution. This was due principally to the nocardial slime which was recovered from the culture liquor by precipitation with acetone.

The nocardial cells were air dried and extracted for 24 hr in a ball mill with chloroform-methanol (2:1); the extract was dried under N₂ at reduced pressure. The presence or absence of residual alkane was determined by its chromatographic separation on an activated silica gel column. The alkane was eluted with n-heptane; the more polar material remained on the column. Using the method of Blair et al. (1953) the two principal lipid components, glycerides and waxes, were separated, based on their solubilities in acetone. The material insoluble in cold acetone contained the wax fraction; tests for phosphorus and nitrogen were essentially negative, indicating the absence of phosphatides.

The insoluble fraction was purified by reprecipitation upon cooling from hot acetone and analyzed with a modified Consolidated 21-103³ mass spectrometer using the method of Meinschein and Kenny (1957). The grid form of Clerc et al. (1955), in which the mass spectral peaks are arranged according to mass number, was employed. For example, an ion of the wax (C₃₇H₇₄O₂)⁺ has the same mass number as the hydrocarbon ion (C₃₇H₇₂+19⁻) or (C₃₇H₄₁)⁺ and would be

* Manufactured by the New Brunswick Scientific Co., New Brunswick, New Jersey.

* Consolidated Engineering Corporation, Pasadena, California.
found opposite carbon number 37 in the appropriate column (−10 column) of the mass series grid. According to this method the parent peak ion of an aliphatic wax, because the wax molecule has two oxygen atoms, is indicated as a hydrocarbon ion having three more carbon atoms (the mass of these ions being the same). Therefore, the parent peak ion of octadecyl palmitate (C₁₈) appears as hydrocarbon ion (C₂₇) and the acyl R or wax acid ion (C₃₇) of this wax appears as hydrocarbon ion (C₄₆) because the wax acid ion also contains two oxygen atoms (Meinschein and Kenny, 1957). The number of carbons in the alcohol moiety (alkyl R) of a wax is indicated by the difference in carbon number between the wax and the wax acid. In the above case, the alkyl R is indicated to have 18 carbons.

The acetone-soluble fraction of the cell lipid principally contained glycerides. For example, this fraction from n-octadecane-grown cells was saponified and the glycerol determined by the method of Neish (1952). The recovered fat acids were titrated with alcoholic potassium hydroxide and the neutralization equivalent indicated fat acids consisting of 18 carbon atoms. The lead salts of these acids were extracted with ethyl ether; more than half were soluble, indicating unsaturated acids. The acetone-soluble lipid fraction of n-octadecane- and n-hexadecane-grown cells comprised about 60 per cent of the total cellular lipid. The wax fraction in each case comprised most of the other approximately 40 per cent.

The lipid fractions of nocardial cells grown on n-hexane, n-tridecane, n-hexadecane, and n-octadecane were compared with those grown on glucose in mineral salts medium. Table 1 gives the results. The acetone-insoluble lipid fractions of cells grown on n-hexane and n-tridecane were liquid at room temperature and the latter was dark in color; whereas these fractions from n-hexadecane- and n-octadecane-grown cells were white crystalline solids with melting points of 48.5 and 51.0 °C, respectively.

**Factors Affecting Cellular Growth and Lipid Formation**

**Nitrogen sources.** An investigation of nitrogen sources was initiated for two reasons. First, it was obvious that in some experiments the nitrogen source in the form of ammonium sulfate was being depleted, thus limiting growth. Secondly, the microbial utilization of the ammonium ion left the highly acidic sulfate ion in the medium, lowering the pH and necessitating frequent neutralization with alkali.

Table 2 shows the effect of various nitrogen sources on the pH, cell yields, and lipid formed by the nocardia culture. It should be emphasized that rather slow growth occurs in bottles incubated on a mechanical shaker as compared with the rapidly agitated culture systems previously described. Residual n-octadecane was determined by silica gel chromatographic separation from the cell lipid following extraction of the cells with chloroform-methanol (2:1). The culture liquor and culture bottles were washed with n-hexane to recover the residual n-octadecane not adsorbed to the cells.

Urea appears to be the nitrogen source of choice if one is interested in utilization of substrate and maintenance of pH neutrality (table 2). The effect of urea on lipid formation is of particular interest. The 0.3 per cent urea system represents the highest concentration of nitrogen employed and the lowest percentage of cell lipid. These data (table 2) are interpreted to indicate the adverse effects of a lowered pH in the 0.3 per cent ammonium sulfate and nitrate systems, and of the limitation of nitrogen in the 0.1 per cent po-

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**TABLE 1**

*Acetone soluble and acetone insoluble fractions of nocardial lipid*

<table>
<thead>
<tr>
<th>Growth Substrate</th>
<th>Cell Lipid</th>
<th>Acetone Soluble</th>
<th>Acetone Insoluble</th>
<th>Composition of Waxes in Acetone Insoluble Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>28</td>
<td>100</td>
<td>No wax</td>
<td>No wax</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>22</td>
<td>90</td>
<td>No wax</td>
<td>No wax</td>
</tr>
<tr>
<td>n-Tridecane</td>
<td>26</td>
<td>87</td>
<td>No wax</td>
<td>No wax</td>
</tr>
<tr>
<td>n-Hexadecane</td>
<td>48</td>
<td>62</td>
<td>38</td>
<td>C₁₈ (14%) C₁₆ C₁₈ C₁₆</td>
</tr>
<tr>
<td>n-Octadecane</td>
<td>56</td>
<td>61</td>
<td>39</td>
<td>C₁₈ (86%) C₁₆ C₁₈ C₁₆</td>
</tr>
</tbody>
</table>

* Lipid extracted with chloroform-methanol, 2:1.
† Soluble in boiling acetone; contains wax, if any.

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**TABLE 2**

*Effect of nitrogen sources on nocardial cell yields and lipid formation*

<table>
<thead>
<tr>
<th>Nitrogen Source</th>
<th>N in Compound</th>
<th>n-Octadecane Utilized</th>
<th>Dried Cell Wt</th>
<th>Conversion to Cells</th>
<th>Cell Lipid</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH₄)₂SO₄ (0.1%)</td>
<td>21</td>
<td>606</td>
<td>445</td>
<td>74</td>
<td>72</td>
<td>6.0</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ (0.3%)</td>
<td>21</td>
<td>293</td>
<td>224</td>
<td>83</td>
<td>43</td>
<td>5.0</td>
</tr>
<tr>
<td>NH₄NO₃ (0.3%)</td>
<td>35</td>
<td>293</td>
<td>207</td>
<td>70</td>
<td>46</td>
<td>5.0</td>
</tr>
<tr>
<td>KNO₃ (0.1%)</td>
<td>14</td>
<td>394</td>
<td>252</td>
<td>64</td>
<td>70</td>
<td>8.0</td>
</tr>
<tr>
<td>KNO₃ (0.3%)</td>
<td>14</td>
<td>795</td>
<td>530</td>
<td>67</td>
<td>65</td>
<td>8.1</td>
</tr>
<tr>
<td>Urea (0.1%)</td>
<td>47</td>
<td>808</td>
<td>603</td>
<td>75</td>
<td>64</td>
<td>7.0</td>
</tr>
<tr>
<td>Urea (0.3%)</td>
<td>47</td>
<td>772</td>
<td>522</td>
<td>68</td>
<td>72</td>
<td>7.2</td>
</tr>
<tr>
<td>Asparagine (0.1%)</td>
<td>21</td>
<td>512</td>
<td>369</td>
<td>72</td>
<td>78</td>
<td>6.9</td>
</tr>
<tr>
<td>Asparagine (0.3%)</td>
<td>21</td>
<td>791</td>
<td>586</td>
<td>74</td>
<td>69</td>
<td>6.0</td>
</tr>
</tbody>
</table>

* Substrate, 824 mg n-octadecane.
† Initial pH was buffered at neutrality with the 0.5 per cent phosphate salts in the regular mineral salts medium. Incubation, 144 hr at 30 °C in 100 ml medium in 250-ml bottles agitated on a gyratory shaker.
tassium nitrate system on alkane utilization. Asparagine may have been utilized also as a carbon source, but if so, it obviously did not lower cell lipid production.

Oxygen. Since no extracellular products other than carbon dioxide have been observed with nocardia growing on n-alkanes at atmospheric oxygen concentration (~20 per cent), lower oxygen concentrations were tried. Limited oxygen might conceivably result in extracellular products caused by "incomplete" oxidation of n-octadecane. Nocardia were grown for 24 hr on n-octadecane in air in the 4-L culture units and then grown an additional 24 hr at 20, 10, and 2 per cent oxygen concentration, respectively. The 10 and 2 per cent oxygen concentrations were adjusted by mixing tank oxygen with tank nitrogen under pressure and continually adding the mixtures to the culture units. Ordinary air was continued for the 20 per cent oxygen system.

Cell recoveries and product analyses were performed as described above. No extracellular products (besides capsular slime) were indicated. Table 3 gives the results. Note that the percentage of lipid formed is not a direct function of oxygen concentration; that is, the intermediate concentration of oxygen, 10 per cent, resulted in the most lipid. Growth in the 2 per cent oxygen system was limited and the percentage of lipid formed was low, only 21 per cent.

Hydrocarbon concentration. The ratio of liquid alkane to nocardial cells is important, especially in the early stages of growth. This was tested using a very large inoculum. To 2 L of mineral salts medium in a culture unit were added 24 g of n-octadecane and 1.2 g of nocardia (dry weight basis). The ratio of hydrocarbon to microbial cells was thus 20 to 1 on a weight basis. No growth occurred during an incubation period of 5 days.

In contrast, a similar 2-L system was treated as follows: the same amount of inoculum was used, but the n-octadecane was added in increments of 8 g initially, and at 24, 42, and 48 hr, respectively. Growth of the nocardia was profuse, a total of 13.8 g (dry weight) of cells being produced from about 18 g of utilized hydrocarbon. The initial ratio of hydrocarbon to nocardial cells in this case was about 7 to 1.

Presumably in the system where the ratio was 20 to 1 (no growth) the cells became embedded in the oil phase, out of sufficient contact with the required aqueous phase to allow growth. (The extraordinary affinity for oil of these nocardial cells can be observed microscopically, compared, for example, with pseudomonas or yeast cultures.) Where the ratio of hydrocarbon to nocardial cells was only 7 to 1 there were enough cells in adequate contact with the aqueous phase to initiate growth and multiplication.

The nocardia in the 20 to 1 ratio system were not killed, even though agitated in the culture unit for 5 days. At the end of this time the viable count was 2.5 \( \times \) 10^8 "cells" per ml, determined by cultivation on nutrient agar. This count is quite low judging from the large inoculum, but actually large masses of cells, because of their occlusion and adsorption by the liquid alkane globules, were the foci of individual colonies.

**Gross Composition of Cells**

The large amount of lipid formed by the nocardia under certain conditions is, of course, correlated with the cellular protein and carbohydrate composition of the cells. Table 4 gives the approximate balance of lipid, protein, and carbohydrate of cells grown under various conditions. Protein was determined on the basis of Kjeldahl nitrogen determinations.

**Carbon Balance**

Efforts were made to account accurately for all the carbon utilized in the microbial oxidation of n-alkanes. Failure to obtain an accurate carbon balance was due principally to loss of carbon dioxide from the agitated culture units. Almost 90 per cent of the carbon of utilized hydrocarbon was accounted for, as shown in table 5 for two nocardia growth systems. The two systems contained different nitrogen sources at a level of 0.063 per cent available nitrogen: unit 1, ammonium sulfate and unit 2, urea. Despite a difference in the amount of octadecane utilized, the conversion of utilized carbon into cells was 60 per cent in each case and the conversion of utilized carbon into carbon dioxide was a measured 20 per cent in both cases. As

---

**TABLE 3**

*Effect of oxygen concentration on n-alkane utilization, cell yields, and cellular lipid*

<table>
<thead>
<tr>
<th>( % \text{O}_2 )</th>
<th>( \text{n-Octadecane} )</th>
<th>Cells</th>
<th>Conv. into Cells</th>
<th>Cell Lipid</th>
<th>Cell Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added</td>
<td>Residual</td>
<td>Utilized</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>10.4</td>
<td>0.29</td>
<td>10.11</td>
<td>8.52</td>
<td>84</td>
</tr>
<tr>
<td>10</td>
<td>10.4</td>
<td>0.36</td>
<td>10.04</td>
<td>6.61</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
<td>10.4</td>
<td>4.71</td>
<td>5.69</td>
<td>3.07</td>
<td>54</td>
</tr>
</tbody>
</table>

* Dry weight.

**TABLE 4**

*Approximate balance of lipid, protein, and carbohydrate in nocardia cells grown under various conditions*

<table>
<thead>
<tr>
<th>Culture</th>
<th>Lipid</th>
<th>Protein</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{n-Octadecane, } 10% \text{ O}_2 )</td>
<td>73.2</td>
<td>14.4</td>
<td>12.4</td>
</tr>
<tr>
<td>( \text{n-Octadecane, } 20% \text{ O}_2 )</td>
<td>56.0</td>
<td>20.8</td>
<td>23.2</td>
</tr>
<tr>
<td>( \text{n-Hexane, } 20% \text{ O}_2 )</td>
<td>28.0</td>
<td>46.1</td>
<td>26.0</td>
</tr>
</tbody>
</table>

* Calculated by weight difference.
mentioned above, the discrepancy in carbon recovery is probably due to loss of carbon dioxide. An even greater discrepancy was noted in earlier experiments, prior to improving the packing glands around the impeller shaft bearings of the culture units. It is assumed that a more nearly correct value for converted alkane carbon to carbon dioxide is approximately 30 per cent.

The data bear out previous indications that oxidation of n-alkanes by this nocardia culture does not yield appreciable products other than cells (including capsular slime) and carbon dioxide.

**DISCUSSION**

Rapid growth of a soil isolate belonging to the genus *Nocardiola* occurs at the expense of liquid n-alkanes such as octadecane when the culture medium is violently agitated. The agitation performs a special function because of the water insolubility of the hydrocarbon; namely, that of increasing the surface of the oily substrate available to the microbial cells. Small globules of n-alkane are formed and maintained only by constant agitation. Microscopic inspection at progressive stages of growth indicates that the nocardial cells have a strong affinity for these globules. Nonfat stains such as crystal violet and rose bengal which have affinity for the cells indicate that they are adsorbed to, but not occluded in, the oil phase.

A growth rate of 0.24 g cells (dry weight) per hr per L has been achieved. Factors besides agitation which are necessary to obtain growth rates of this magnitude include adequate available nitrogen and neutral pH. Surprisingly, oxygenation of the medium which spontaneously occurs with agitation in the culture units (figure 2) was sufficient for maximum growth. No forced aeration was required, but a flow of air of approximately 150 ml per min in the 4-L culture units was used to maintain a positive pressure in the systems as a safety precaution against contamination.

One per cent liquid n-alkane in the medium constitutes a large initial concentration which in the presence of small inocula is inhibitory to growth. However, incremental or even continual addition of hydrocarbon at a slow rate circumvents this inhibitory effect.

The magnitude of the conversion of n-alkane into nocardial cells is depicted in figure 3. When cellular yields are considered purely on a weight basis, without regard to carbon values, yields are very high. As the hydrocarbon is converted into microbial cells and the water-soluble slime polymer, oxygen is incorporated, thus accounting for the high weight yield.

The polymer produced in n-octadecane cultures appears to be a carbohydrate which is fairly resistant to acid hydrolysis. Following hydrolysis the solution gives a weak test for reducing sugar. The material, carefully separated from nocardial cells by filtration, was found to contain no nitrogen (Kjeldahl analysis). It has a high molecular weight since some of it can be centrifuged out of apparent solution at 35,000 × g. The polymer dissolves in water, can be precipitated out of solution with acetone, then dried and readily redissolved in water.

The wax components of nocardial cellular lipid are particularly interesting in view of the observations of Stewart et al. (1959) and Stewart and Kallio (1959) regarding the microbial formation of such waxes. They reported similar waxes to be in the cell-free liquor of cultures of a gram negative (soil isolate) cococcus grown on n-octadecane, n-hexadecane, or n-tetradecane. They pointed out that the cococcus did not exhibit fat globules at any stage of growth. However, their data show that cell lysis was often very marked. They reported further that "the waxes produced from alkanes by bacterial action do not accumulate within the cells but appear to be excreted into the medium; thus, they do not seem to be storage products or integral parts of the bacterial cell structure" (Stewart and Kallio, 1959).

In contrast, the nocardia grown on n-alkanes produced a remarkable amount of cellular lipid including ordinary fat. One of the major cellular lipid components of nocardial cells grown on n-hexadecane or n-octadecane consists of a true wax, i.e., an ester of a long-chain fat acid and a long-chain alcohol. n-Hexadecane yielded cetyl palmitate (C_{32}) as the principal wax and n-octadecane yielded principally octadecyl stearate (C_{34}) (table 1). In each case, the n-alkane was converted into significant quantities of a wax having double the number of carbons, and each

**TABLE 5**

Products recovered from n-octadecane cultures on the basis of carbon (approx.)

<table>
<thead>
<tr>
<th>Carbon Utilized and Recovered</th>
<th>Unit 1</th>
<th>Unit 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>g carbon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-Octadecane carbon utilized</td>
<td>43.2</td>
<td>57.0</td>
</tr>
<tr>
<td>Products recovered (as carbon):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nocardial cells</td>
<td>26.00</td>
<td>34.40</td>
</tr>
<tr>
<td>Organic carbon in culture liquor*</td>
<td>3.29</td>
<td>3.51</td>
</tr>
<tr>
<td>Volatile acids (as acetic acid)</td>
<td>0.12</td>
<td>0.19</td>
</tr>
<tr>
<td>Carbon dioxide†</td>
<td>8.81</td>
<td>11.65</td>
</tr>
<tr>
<td>Total</td>
<td>38.22</td>
<td>49.75</td>
</tr>
<tr>
<td>Carbon recovery</td>
<td>88.5%</td>
<td>87.5%</td>
</tr>
</tbody>
</table>

*Chromic acid wet combustion of cell-free culture liquor; carbon principally represented by polysaccharide slime which could be considered a cellular component.
†Absorbed in alkali, precipitated and weighed as BaCO₃; some carbon dioxide was very probably lost around impeller shaft bearing of culture units. Carbon dioxide-free air flow was used to carry microbially produced carbon dioxide to alkali scrubbers.
moiety of the long-chain ester had the same number of carbons as the original substrate. As Stewart and Kallio (1959) suggest, this indicates very strongly that the long-chain alkanes, n-hexadecane and n-octadecane, were oxidized at a terminal carbon atom, producing acids and alcohols that were esterified to form the respective waxes. Some wax was formed in each case which had two less than double the number of carbons in the alkane; in each case, the two fewer carbons occurred in the acyl R (table 1).

n-Tridecane was specifically tested to determine whether a wax with 26 carbons was formed in the lipid of nocardial cells. The C_{12} n-alkane did not yield a measurable amount of such a wax, nor significant amounts of any long-chain ester (table 1). This is particularly interesting since the nocardia grew very readily on the odd-numbered carbon chain alkane.

The data of table 1 indicate that the nocardia employed does not ordinarily accumulate large quantities of wax in the cellular lipid. But given certain long-chain n-alkanes, the microbes will divert a significantly large amount of oxidized substrate into intracellular wax products. Why a C_{16} or a C_{18} alkane yields a long-chain ester (wax), but a C_{13} or C_{6} alkane does not may be explained by the fact that C_{16} and C_{18} fatty acids are very common in lipid metabolism, e.g., palmitic (C_{16}), stearic (C_{18}), and oleic (C_{18}). Hence, an alkane substrate that would readily yield such products might be expected to yield more cellular lipid.

The microbe obviously synthesizes a variety of compounds from n-alkanes including protein, carbohydrates, and fats. The most likely point to start obtaining energy and carbon for these syntheses is the terminal carbon of the n-alkane molecule, followed by β-oxidation of the carbon chain and utilization of the released energy and C_{5} fragments for the biosynthetic reactions requisite to cellular growth. Baptist and Coon (1959) recently reported the bacterial enzymatic oxidation of radioactive octane to octanoic acid; and Leadbetter and Foster (1959) reported the bacterial oxidation of ethane to acetic acid. Webley et al. (1956) have good evidence for β-oxidation by nocardia of alkyl substituents of cyclic hydrocarbons; and Thijssen and Van Der Linden (1958) presented data supporting the proposition that alkane oxidation proceeds by oxidation of a terminal methyl group, leading to corresponding alcohols, aldehydes, and fatty acids, as indicated by multiple adaptation studies employing a pseudomonas. Their data further support the β-oxidation mechanism as a means of oxidizing intermediate fat acids.

The data presented here are consistent with these views. The rapid utilization of n-alkanes such as n-hexadecane and n-octadecane presumably results in a pile-up of terminally oxidized intermediates which esterify as waxes as a diversionary process to further oxidation. The function of the waxes other than as cellular storage products is unknown, but they are probably not the common intermediates of n-alkane oxidation.

**SUMMARY**

The rapid utilization of liquid n-alkanes such as n-octadecane by a soil isolate of the genus *Nocardia* is described. Under optimal conditions a 95 per cent conversion of alkane into nocardial cells and a slime polymer was obtained.

The nocardial cells cultivated on n-hexadecane or n-octadecane contained a large amount of lipid, consisting of about 60 per cent glycerides and 40 per cent waxes. No waxes were formed by cells grown on n-tridecane, n-hexane, or glucose.

Factors which influenced growth and lipid formation were nitrogen compounds, oxygen concentration, and hydrocarbon concentration.

The microbial oxidation of n-alkanes and its relation to cellular biosyntheses, including the cellular lipid waxes, are discussed.

**REFERENCES**


DAVIS, J. B., CHASE, H. H., AND RAYMOND, R. L. 1956 *Myco


