FATTY ACID METABOLISM IN SERRATIA MARCESCENS

I. OXIDATION OF SATURATED FATTY ACIDS BY WHOLE CELLS

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ABSTRACT

BISHOP, D. G. (University of Sydney, Sydney, Australia), AND J. L. STILL. Fatty acid metabolism in Serratia marcescens. J. Bacteriol. 82:370-375. 1961.—A study has been made of the oxidation of saturated fatty acids containing between two and 18 carbon atoms by whole cells of the bacterium, Serratia marcescens. This organism was found to be capable of oxidizing all of the acids tested, but variations in the rate and total oxygen uptake were found. These variations were dependent on the length of the carbon chain in the substrate molecule and the pH of the reaction mixture. The concept that these variations are due to a cellular permeability barrier to the substrate is discussed.

Investigations into the fatty acid metabolism of microorganisms have not progressed as far as the corresponding studies in animals and plants. As part of a detailed study of the fatty acid metabolism of the bacterium, Serratia marcescens, this communication describes the oxidation by whole cells of saturated fatty acids containing between two and 18 carbon atoms.

A variety of effects of long chain unsaturated fatty acids on the growth of microorganisms has been reported (Nieman, 1954). Linnane and Still (1955) observed that certain long chain unsaturated fatty acids were able to stimulate markedly both the growth of S. marcescens and the production of the red pigment, prodigiosin, even to induce pigmentation at temperatures higher than normal. The mechanism of this stimulation has remained obscure since surface-active agents such as the Tweens, saturated fatty acids, and biotin were unable to produce a similar effect. Accordingly, a detailed study of all aspects of the fatty acid metabolism of this organism is in progress.

The enzymes responsible for the oxidation of fatty acids in mammalian and plant tissues have been thoroughly investigated (Green, 1954; Lnen, 1955; Stumpf and Bradbeer, 1959). However, in the case of bacteria in which a variety of effects of fatty acids on growth and metabolism has been observed, the knowledge of enzymatic processes is still comparatively undeveloped. The oxidation of short chain fatty acids by a cell-free extract of a Vibrio was described by Callely, Dagley, and Hodgson (1958), and Goldman and Gelbard (1959) have isolated from mycobacteria several enzymes of the fatty acid cycle which catalyze the oxidation of butyrate. Stadtman, Barker, and co-workers have also studied the metabolism of short chain fatty acids by the anaerobe, Clostridium kluyveri (for reviews see Stadtman and Stadtman, 1953; Barker, 1956).

A previous study of the oxidation by whole cells of S. marcescens of fatty acids containing between two and 14 carbon atoms has been reported by Silliker and Rittenberg (1951a, b). They found that fatty acid oxidation by glucose-grown cells occurred only after a lag period, whereas on caprate-grown cells oxidation began immediately, an indication that adaptive enzymes might be involved. But investigations on the effects of various inhibitors (Silliker and Rittenberg, 1952; Waltman and Rittenberg, 1954) did not conclusively confirm this.

METHODS

The strain of S. marcescens used in all of the experiments reported here was designated as 120 and was chosen for use because, when grown at 27°C on the medium suggested by Bunting (1946), it produced abundant red pigment and showed little tendency to form colorless mutants. The same strain, then designated as “C red,” was used by Linnane (1954) and proved to be very responsive to unsaturated fatty acids in the pigment-stimulation tests.

Culture media. It is generally agreed (Sullivan, 1905-06; Goldsworthy and Still, 1936; Kost,
1942) that glucose is an unsatisfactory carbon source for maximal pigment production by S. marcescens; also, in the absence of unsaturated fatty acids no pigment is formed when the organism is grown at 37 C. However, the medium selected for use (at pH 7.0) was K2HPO4 0.5%; KH2PO4 0.2%; NH4NO3 0.1%; MgSO4·7H2O, 0.05%; glucose, 0.5%; and agar, 2.0%. The use of this medium eliminated the very high basal respiration which results from growth on the glycerol-citrate medium of Bunting (1946) and allowed a comparison between strain 120 and the strain used by Silliker and Rittenberg (1951a, b). The stock cultures were maintained on agar slopes of the same medium and the inocula transferred to the medium in Roux flasks with the use of sterile distilled water. The cells were grown for 24 hr at 37 C, after which they were washed off the surface of the agar with distilled water and collected by centrifuging at 10,000 X g. The packed cells were resuspended and washed twice with distilled water on the centrifuge. The concentration of cells was then adjusted to approximately 10 mg (dry weight) per ml and the exact concentration determined by dry weight measurement.

Manometric experiments. The oxygen uptake in the presence of fatty acids was measured at 30 C by conventional Warburg techniques (Umbreit, Burns, and Stauffer, 1951). Unless otherwise stated, each cup contained phosphate buffer, 50 μmole; cell suspension, approximately 10 mg (dry weight); fatty acid, 1.0 μmole (except for acetic, propionic, butyric, and valeric acids which, when used, were added in 2.0-μmole quantities); and water to 3.0 ml. The center well contained 0.3 ml of 20% KOH. The manometers were equilibrated for 30 min, after which the substrate was poured in from the side arm.

Solutions of the less soluble fatty acids were made by dissolving the fatty acid in distilled water, to which sufficient 20% KOH had been added. Fatty acids containing ten carbon atoms or less were made up as 0.01 M solutions, others as 0.002 M solutions.

RESULTS
The first series of measurements of oxygen uptake in the presence of fatty acids was performed at pH 7.0 in order to compare the behavior of strain 120 with the strain used by Silliker and Rittenberg (1951a, b). An immediate difference in the pattern of oxidation of the saturated fatty acids was noted. It was found that, of the saturated fatty acids containing between two and 18 carbon atoms tested, all were oxidized at pH 7.0 without any detectable lag period in the commencement of oxygen uptake except for margaric acid (C17) which was not oxidized at all. However, margaric acid was oxidized appreciably at pH 6.5. From the oxidation rates obtained in simultaneous experiments, the fatty acids were divided into three groups and the pattern of oxygen uptake of these groups is shown in Fig. 1, 2, and 3.

The first group (Fig. 1), containing acetic, propionic, butyric, and valeric acids, showed a decrease in oxygen uptake with increase in the number of carbon atoms in the molecule. Acetic acid was oxidized most rapidly without the lag period noted by Silliker and Rittenberg (1951a, b); these authors were also unable to detect the oxidation of propionic, butyric, and valeric acids at all.

The oxidation pattern of the nine saturated fatty acids containing between six and 14 carbon atoms was quite different (Fig. 2). Once again, there was no lag period before the commencement of oxidation but there was a stepwise increase in the initial rate and total oxygen consumption corresponding to the increase in the number of carbon atoms in the molecule. As the initial

![Oxidation of short chain fatty acids.](http://jb.asm.org/ on October 2, 2017 by guest)
rate of oxidation increased with the length of the carbon chain, it seems certain that there was no permeability barrier to the entry of the substrates into the cell. With further increase in the number of carbon atoms in the substrate molecule, a new pattern of oxidation appears (Fig. 3). As margaric acid was not oxidized at pH 7.0, the graph shown in Fig. 3 was from an experiment at pH 6.5. The oxidation patterns of palmitic and stearic acids are the same at both pH values. No pentadecanoic acid was available, but it was found that the initial rate of oxidation of palmitic acid was less than that of myristic acid. However, the rate of oxidation of palmitic acid increased above the initial rate and, after about 45 min, the total oxygen consumption overtook that of myristic acid. The oxidation of fatty acids containing 17 and 18 carbon atoms showed a greatly decreased rate and total oxygen consumption.

It is evident, then, that the properties of S. marcescens strain 120 differ somewhat from those of the strain used by Silliker and Rittenberg (1951a, b). The absence of a lag period at the commencement of oxidation indicates that for saturated fatty acids containing up to 18 carbon atoms there is a constitutive enzyme system.

Oxidation of higher fatty acids at pH values at which they exist as soaps is unusual. However, Burness and King (1956) isolated from soap an unidentified Micrococcus which could oxidize fatty acids over a pH range from 7 to 11 with the optimum at pH 9. The cells used in this investigation were, therefore, tested for their ability to oxidize fatty acids at pH 6.0, 6.5, 7.0, 7.5, and 8.0.

It was found that the majority of fatty acids were oxidized at all five pH values. However, among the longer chain acids, stearic and palmitic acids were not oxidized at pH 8.0 and margaric acid was not oxidized at a pH greater than 6.5. At pH values at which there was no oxidation of the acids, the basal respiration was also completely inhibited by the acid.

The pattern of oxidation was also altered. The initial rate of oxidation was lower at the alkaline pH's and, in some cases, a distinct lag period was observed before the maximal rate of oxygen uptake was obtained. Such a pattern is shown in Fig. 4 and 5 for the oxidation of undecanoic and myristic acids. It can be seen that as the pH increases the initial rate drops, but, in the case of myristic acid, the total oxygen uptake was greater at the more alkaline pH values over a 3-hr period.

**Fig. 2. Oxidation of medium chain fatty acids.** Oxygen uptake measured at 30 C and pH 7.0. 10 mg of cells (dry weight) per flask. 1 μmole of each substrate added. Bl—basal respiration; C4—caproic acid; C7—heptanoic acid; C9—caprylic acid; C10—lauric acid; C12—tridecanoic acid; C13—undecanoic acid; C14—myristic acid.

**Fig. 3. Oxidation of long chain fatty acids.** Oxygen uptake measured at 30 C and pH 7.0. 10 mg of cells (dry weight) per flask. 1 μmole of each substrate added. Bl—basal respiration; C16—palmitic acid; C17—stearic acid; C18—oleic acid; C19—myristic acid.
At no stage during this work has the observed oxygen uptake reached the theoretical amount for the complete oxidation of the substrate to carbon dioxide and water. This lack of complete oxidation was thought to be due to the process of oxidative assimilation. Addition of intermediates of the citric acid cycle such as succinic and malic acids had no effect on the rate or total oxygen uptake due to fatty acid. 2,4-Dinitrophenol is known to prevent oxidative assimilation (Clifton, 1937), and chloramphenicol inhibits protein synthesis (Gale and Folkes, 1953). Accordingly, the effect of these compounds on the oxidation of capric and lauric acids was examined. The results are shown in Table 1.

The failure of chloramphenicol to affect the oxygen uptake in any way suggests that no adaptive enzyme formation has taken place. 2,4-Dinitrophenol, however, at certain concentrations increased the total oxygen uptake, thereby indicating that some oxidative assimilation probably does take place.

The oxidation of the saturated $\alpha,\omega$-dicarboxylic acids containing between two and 12 carbon atoms was also tested at pH 7.0 and it was found that, with the exception of succinic acid which was oxidized slowly and without any lag period, none of the acids was oxidized. However, in this case the basal respiration was not inhibited.

Selected experiments were repeated with three other strains of *S. marcescens*, including a stable white mutant, and all results obtained were comparable to those reported for strain 120.

**DISCUSSION**

From the data presented, it is evident that the enzyme system oxidizing saturated fatty acids in *S. marcescens* is constitutive and that under normal conditions a wide variety of fatty acids can be readily attacked. There is also no evidence to indicate the pathway of oxidation. Acetic acid is oxidized rapidly with the uptake

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**TABLE 1. Effect of chloramphenicol and 2,4-dinitrophenol on fatty acid oxidation**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Final conc in reaction mixture</th>
<th>Oxygen uptake*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Capric</td>
</tr>
<tr>
<td>Nil</td>
<td></td>
<td>67</td>
</tr>
<tr>
<td>Chloramphenicol........</td>
<td>$10 \times 10^{-4}$ M</td>
<td>67</td>
</tr>
<tr>
<td>2,4-Dinitrophenol......</td>
<td>$15 \times 10^{-4}$ M</td>
<td>75</td>
</tr>
<tr>
<td>2,4-Dinitrophenol......</td>
<td>$10 \times 10^{-4}$ M</td>
<td>82</td>
</tr>
<tr>
<td>2,4-Dinitrophenol......</td>
<td>$7.5 \times 10^{-4}$ M</td>
<td>76</td>
</tr>
<tr>
<td>2,4-Dinitrophenol......</td>
<td>$5 \times 10^{-4}$ M</td>
<td>76</td>
</tr>
</tbody>
</table>

* Oxygen uptake corrected for basal respiration. Experiment carried out at pH 7.0.

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**FIG. 4. Effect of pH on the oxidation of undecanoic acid. Oxygen uptake measured at 30°C. Corrected for basal respiration. 1 µmole of undecanoic acid added. The values obtained at pH 7.0 and 7.5 were identical. 9.8 mg of cells (dry weight) per flask.**

**FIG. 5. Effect of pH on the oxidation of myristic acid. Oxygen uptake measured at 30°C. Corrected for basal respiration. 1 µmole of myristic acid added. 9.8 mg of cells (dry weight) per flask.**
of three atoms of oxygen per molecule of acetate and, although propionic, butyric, and valeric acids are not oxidized to the same extent, the concept of β-oxidation is not upset because molecules such as these may well be incorporated by whole cells directly into other metabolic pathways in which carbon skeletons of a similar dimension are required, rather than being catabolized to carbon dioxide and water.

The increase over the initial rate observed in the case of palmitic acid could well be due to a permeability barrier in the cell. The much lower rate of oxidation of longer chain acids could be due to one or all of three factors: (i) a permeability barrier to the entry of the substrate into the cell, (ii) a greatly decreased lack of affinity by the enzyme system for the substrate, or (iii) the utilization of the substrate in a reaction other than straightforward oxidation. As palmitic and stearic acids are among the most commonly occurring fatty acids in nature, it would be unlikely that factor (ii) was responsible for the observed results; rather, it would seem that a reduced permeability of the cell to stearic acid is the cause. It is hoped that studies on individual enzymes and the fatty acid composition of the cell will throw some light on these observations.

Further support for the concept of a permeability barrier is obtained from the results of experiments at alkaline pH's. Under these conditions, a distinct lag period was observed before the maximal rate of oxygen uptake was reached. In the case of the longer chain acids, palmitic and stearic, oxidation at pH 8.0 was completely inhibited, perhaps because of a damaging effect of the fatty soap on the cell membrane. On the basis of these observations, therefore, it is suggested that the lag period occurring before the commencement of maximal rate of oxygen uptake observed by Silliker and Rittenberg (1951a, b) was due to a permeability barrier to the substrate and that growth on a fatty acid as sole carbon source removed this barrier. Some preliminary observations have already been made that S. marcescens strain 120 shows different patterns of oxidation at alkaline pH values when grown on varying media not containing fatty acids.

ACKNOWLEDGMENTS
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LITERATURE CITED


