STUDIES ON THE AEROBIC OXIDATION OF FATTY ACIDS BY BACTERIA

I. The Nature of the Enzymes, Constitutive or Adaptive[1]

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The effects of fatty acids on bacteria can be conveniently summarized as follows: (1) The acids may be toxic, causing inhibition of growth, cessation of respiration, or death of cells. (2) They may be stimulatory, allowing rapid initiation of growth, increased cell yields, or increased respiration. (3) The acids may serve as major energy-yielding substrates. A considerable body of evidence has accumulated with respect to the toxic and stimulatory effects of fatty acids. Likewise, the anaerobic metabolism of these compounds by bacteria has been thoroughly studied in a few instances, the investigations on the mechanisms of acetone and butyl alcohol formation by *Clostridium acetobutylicum* (summarized by Prescott and Dunn, 1949) and the more recent work of Barker and his group on *Clostridium kluyverii* (see, for example, Stadtman and Barker, 1949) being two examples. Concerning the aerobic oxidation of fatty acids by bacteria, little is known except that certain aerobes store considerable amounts of fat (Porter, 1946) and that some, in fact, use fatty acids as a source of carbon and energy (Dooren de Jong, 1926). To our knowledge there has been, to date, no direct and specific attempt to elucidate the mechanism of aerobic oxidation of the longer chain acids by bacteria.[3]

Studies on fatty acid oxidation in animals have indicated at least three pathways that are operative, i.e., *beta*, multiple alternate, and *omega* oxidation (Breusch, 1948; Leloir, 1946–1948; Stadie, 1945). Although it has generally been assumed that one or more of these mechanisms occur in bacteria, there is little experimental evidence to substantiate this assumption. The paucity of information may be due, in part, to certain technical difficulties encountered in work with fatty acids. In low concentration, it is difficult to analyze for the acids and for suspected intermediates that might accumulate in small amounts; toxicity and low solubility, on the other hand, put an upper limit on the concentrations that may be used.

With the introduction of the technique of simultaneous adaptation (Stanier, 1947) a possible experimental approach became available. If one assumes that bacteria exist that oxidize fatty acids through adaptive enzymes, then by adapt-

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[2] Based on data submitted in a dissertation in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] After the completion of this manuscript, an article by Randles (J. Bact., **60**, 627) appeared on this topic.

ing cells to the oxidation of a particular acid and studying the oxidative patterns on suspected intermediates, it should be possible to obtain valuable information with respect to the position of the test compounds in the oxidation pathway.

The series of investigations to be reported deal with an attempt to apply the technique of simultaneous adaptation to the elucidation of the mechanism of aerobic oxidation of fatty acids by bacteria. Required for such an investigation is an organism oxidizing fatty acids by means of adaptive enzymes. A survey of the literature failed to reveal the nature of the enzymes catalyzing aerobic oxidation of fatty acids, that is, whether they are constitutive or adaptive. It was therefore necessary to study the pattern of fatty acid oxidation by a group of different bacteria as a preliminary to the main investigation. The results of the survey follow.

EXPERIMENTAL RESULTS

Two groups of bacteria, stock cultures and enrichment isolates, were tested for ability to oxidize fatty acids. The enrichment group consisted of bacteria isolated from the soil by the enrichment culture technique, using a medium of the following composition (medium A): K_2HPO_4, 5.0 g; KH_2PO_4, 2.0 g; NH_4NO_3, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; stearic acid, 1.8 g; distilled water, 1,000 ml; pH adjusted to 7.0 with KOH. After three or four serial transfers in liquid medium, isolation was accomplished by streaking on a solid medium of the same composition but containing 1.5 per cent agar. Even though the medium contained considerable amounts of insoluble soap, no difficulty was encountered in obtaining bacteria from many different soil samples. Without exception, the isolates proved to be gram-negative rods. They were not further identified, although the presence of fluorescent pigments gave indication that most of them were members of the genus *Pseudomonas*.

The stock culture group consisted of bacteria that, with the exception of *Neisseria catarrhalis*,[4] were recent isolates from natural sources. These organisms had never been deliberately exposed to fatty acids prior to the time of the tests for fatty-acid-oxidizing ability.

Before being used for manometric studies, all organisms were transferred three or four times on medium A with 0.5 per cent glucose in place of stearic acid. In a few cases, the stock cultures failed to grow on the glucose mineral salts medium; these cultures were serially transferred on nutrient agar.

Cells were harvested from agar slants after 16 to 24 hours' growth at 37 C by washing down the slant with M/20 phosphate buffer, pH 7.0. The cells were centrifuged and resuspended in fresh buffer. This procedure was repeated twice, and the cell suspension finally obtained was adjusted to a turbidity of approximately 300 on the Klett-Summerson apparatus, using the 540-mμ filter. No attempt was made during this phase of the work to make the cell suspensions exactly comparable.

Conventional manometric techniques (Umbreit *et al.*, 1949) were used. Each Warburg vessel contained 1.0 ml of cell suspension in the main well. The side

[4] Kindly furnished by Dr. C. I. Randles, Ohio State University.

arms contained 1.0 ml of 0.002 to 0.0006 M fatty acid[5] in M/20 phosphate buffer, pH 7.0. The center wells contained 0.1 ml of 10 per cent KOH for the absorption of carbon dioxide. Flasks were equilibrated for 15 minutes before the substrate was poured from the side arms into the main wells. Oxygen uptake was followed for 2 or 3 hours or until all substrate had been utilized.

TABLE 1

Oxidation of capric and pelargonic acids by resting cell suspensions of various bacteria

ORGANISM	CAPRIC ACID (C_{10}) TOTAL OXYGEN UPTAKE IN µl AFTER* MINUTES			PELARGONIC ACID (C_9) TOTAL OXYGEN UPTAKE IN µl AFTER* MINUTES		
	20	40	60	20	40	60
Pseudomonas sp. 19	41	95	128	—	—	—
Pseudomonas sp. 6	30	62	92	20	49	78
Pseudomonas sp. 13	11	37	—	15	40	67
Pseudomonas sp. 21	58	116	169	20	44	69
Pseudomonas sp. 24	54	111	170	27	57	83
Pseudomonas sp. 20	25	61	94	13	36	61
Pseudomonas sp. 9	48	103	140	—	—	—
Pseudomonas sp. 7	48	95	154	—	—	—
Pseudomonas sp. 110	55	115	176	—	—	—
Pseudomonas sp. 8	49	100	200	—	—	—
Pseudomonas sp. 11	80	165	—	—	—	—
Enrich. 3†	22	44	65	13	35	53
Enrich. 43b†	40	90	137	—	—	—
Enrich. 83b†	40	100	175	—	—	—
Enrich. 43a†	82	156	274	21	51	81
Enrich. 63†	95	200	332	29	71	95
Enrich. 83a†	40	104	175	24	48	71
E. coli 2	21	40	53	—	—	—
B. brevis	13	45	80	15	46	84
B. firmus	8	17	25	5	12	19
B. subtilis	13	29	49	19	44	71
B. megatherium 1	6	13	20	8	16	24
B. megatherium 2	14	32	51	26	45	64
N. catarrhalis	70	—	—	90	—	—
Serratia (Baker)	2	9	21	2	11	17
Serratia (Alphin)	2	7	19	3	10	22

All cultures were grown on a glucose mineral salts medium except the five *Bacillus* species and *N. catarrhalis*, which were grown on nutrient agar.

Experiments were conducted in air atmosphere with KOH (10 per cent) to absorb CO_2; pH 7.0; 30 C; —, no data.

* Figures not corrected for autorespiration.

† Enrichment isolates from soil, originally grown in stearic acid mineral salts medium.

The data summarized in table 1 show that all the organisms tested oxidized capric and pelargonic acids. In general, the rates were somewhat higher on cap-

[5] Practically all experiments to be reported were conducted at pH 7.0 or 8.0, so that much of the fatty acid was present as the salt (anion). The term fatty acid is used to denote total lipid present, both salt and free acid.

rate than on pelargonate, although with some organisms the reverse was true. There was considerable variation in activity, the gram-positive sporeformers showing the lowest oxidation rates. There was no consistent difference between the rates observed for enrichment isolates and those for other gram-negative

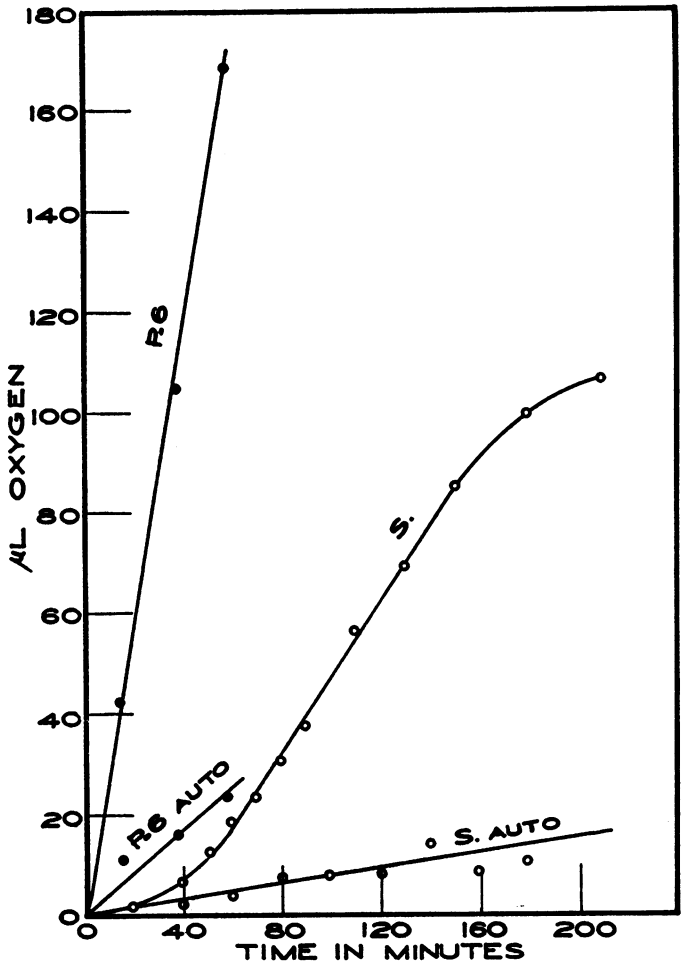

Figure 1. Oxidation of capric acid by glucose-grown cells of *Pseudomonas aeruginosa* (P.6) and *Serratia marcescens* Alphin (S.). M/20 phosphate buffer, pH 7.0, 30 C, air atmosphere with 0.1 ml 10 per cent KOH in center well, 0.6 μM capric acid per flask.

bacteria. *Bacillus megatherium*, strain 1, and *Bacillus firmus* showed very low rates, only slightly above the rates for cells in the absence of the substrate. This may be indicative of a slight stimulation of endogenous metabolism rather than actual utilization of the substrate.

With the exception of the two strains of *Serratia marcescens* and *Bacillus brevis*, all strains consumed oxygen immediately and at nearly the maximum rate when

exposed to fatty acid. With reference to table 1, it can be seen that the oxygen uptakes at the end of 40 and 60 minutes are essentially two and three times the uptakes during the initial 20-minute observation period. Figure 1 shows graph-

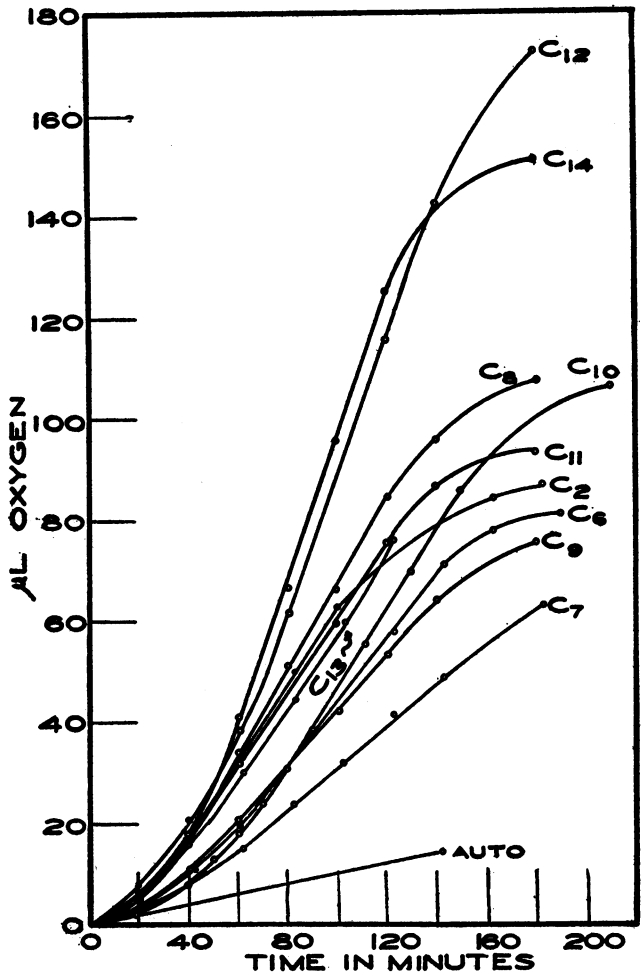

Figure 2. Oxidation of various fatty acids by glucose-grown cells of *Serratia marcescens* Alphin. M/20 phosphate buffer, pH 7.0, 30 C, air atmosphere with 0.1 ml 10 per cent KOH in center well. Substrates: acetic acid (C_2) and propionic acid (C_3) 2 µM; butyric acid (C_4), valeric acid (C_5), and caproic acid (C_8), pelargonic acid (C_9), capric acid (C_{10}), undecylic acid (C_{11}), lauric acid (C_{12}), tridecylic acid (C_{13}), and myristic acid (C_{14}), 0.6 µM. The C_4 and C_5 curves coincided with the autorespiratory one.

ically the oxidative pattern for a strain of *Pseudomonas*; this curve is typical of the majority of organisms tested against the nine- and ten-carbon acids. The immediate oxygen uptake at nearly the maximum rate suggests that in these organisms the enzymes involved are constitutive, especially since most of the

cultures had never previously been exposed to fatty acids under experimental conditions.

The initial uptake of oxygen by the glucose-grown *S. marcescens* and *B. brevis* cells was low. In later work, when cell suspensions were washed three times instead of twice, the initial oxygen uptake was even lower. The rate steadily increased until a maximum was reached at the end of 20 to 40 minutes; this rate was then maintained until the substrate was almost exhausted. The pattern of caprate oxidation by *Serratia* is in sharp contrast to that of *Pseudomonas* (figure 1). Glucose-grown *Serratia* cells show an initial lag period characteristic of sub-

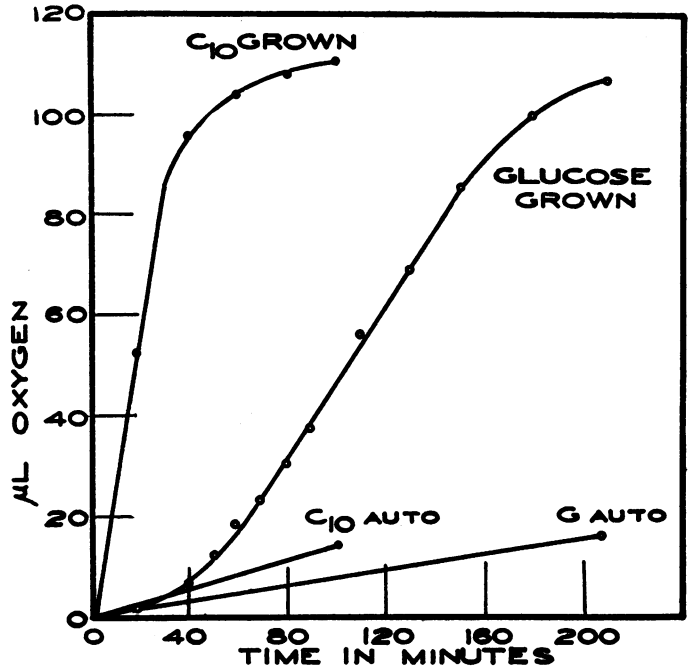

Figure 3. Oxidation of capric acid by glucose-grown (G) and caprate-grown (C_{10}) cells of *Serratia marcescens* Alphin. M/20 phosphate buffer, pH 7.0, 30 C, air atmosphere with 0.1 ml 10 per cent KOH in center well, 0.6 μM caprate per flask.

strate oxidation by adaptive enzymes. Similar oxidative patterns were noted with all fatty acids oxidized by the glucose-grown *Serratia* strains (figure 2). The lag periods varied somewhat with the different acids, from 20 minutes with acetic and heptylic to 36 minutes with capric.

It must be emphasized that not all fatty acids were oxidized. The two *Serratia* strains were apparently unable to oxidize propionic (C_3), butyric (C_4), and valeric (C_5) acids. The *Serratia* strains were also tested for ability to oxidize the C_2, C_3 C_4, C_6, C_7, and C_{10} saturated dicarboxylic acids. Of these substrates, only succinate was oxidized, and that slowly and without the lag period observed in the oxidation of monobasic acids.

With the exception of acetate and heptylate, all fatty acids oxidized by cell

suspensions of *Serratia* could also serve as a sole carbon source for the growth of these organisms. Cultures of *Serratia* were grown on a medium containing capric acid as the sole carbon and energy source. The cells harvested from this fatty acid medium showed no lag period when tested for their ability to oxidize capric acid in a manometric experiment (figure 3). Similarly, *Serratia* cells grown on any fatty acid that serves as a carbon and energy source oxidized that particular acid without a lag period.

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DISCUSSION AND SUMMARY

The data indicate that the ability to oxidize fatty acids is not an uncommon property of aerobic bacteria. All strains tested showed some ability to oxidize the nine- and ten-carbon acids. In addition it was shown that (with the exception of the sporeforming rods and *Neisseria catarrhalis*, which were not tested) all the organisms grew well on a medium with fatty acids as the sole carbon source.

The enzymes concerned with fatty acid oxidation are apparently constitutive in most cases, since cells not previously exposed to these substrates show a high level of metabolism when placed in contact with fatty acids.

The lag periods obtained with the two strains of *Serratia marcescens* and with *Bacillus brevis* are suggestive of attack through adaptive enzymes. This conclusion is further strengthened by the observed elimination of the lag period in the oxidation of a particular fatty acid as a result of the growth of *S. marcescens* on a medium with that fatty acid as the carbon source. These organisms are thus possible tools for further studies on the mechanism of fatty acid oxidation, using the technique of simultaneous adaptation.

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