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Lipid Metabolism During Bacterial Growth, Sporulation, and Germination: Differential Synthesis of Individual Branched- and Normal-Chain Fatty Acids During Spore Germination and Outgrowth of *Bacillus thuringiensis*

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The biosynthesis of individual branched- and normal-chain fatty acids during *Bacillus thuringiensis* spore germination and outgrowth was studied by comparing pulsed and continuous labeling of these fatty acids with [U-^{14}C]acetate. The relative specific activity of each fatty acid varies with time as the cell progresses through outgrowth. However, fatty acid synthesis does occur in two distinct phases. Upon germination, acetate is incorporated only into the iso-isomers i-C_{13}, i-C_{14}, and i-C_{16}; no normal or anteiso synthesis occurs. Subsequent to T_{30}, the full complement of branched- and normal-chain homologues is formed and there is a dramatic enhancement in the overall rate of fatty acid synthesis. Significantly, this rate increase coincides with a marked shift from the synthesis of short-chain to long-chain fatty acids. These findings illustrate a dichotomy in synthesis that may result from initial fatty acid formation by preexisting spore fatty acid biosynthetic enzymes in the absence of de novo protein synthesis. Elucidation of the timing and kinetics of individual fatty acid formation provides a biochemical profile of activities directly related to membrane differentiation and cellular development.

Bacterial spore germination and outgrowth is one of several important prokaryotic developmental systems. This process involves a variety of biochemical reactions, including differential fatty acid and membrane synthesis (4, 13). In the case of *Bacillus thuringiensis* the spore emerges from dormancy rapidly enough to undergo first cell division within 2 h. Consequently, a study of membrane synthesis is especially relevant because current hypotheses regarding deoxyribonucleic acid replication and segregation involve membrane synthesis and deoxyribonucleic acid attachment to that membrane (10, 17, 18).

Gram-positive bacteria such as *B. thuringiensis* have 90 to 95% of their fatty acids present in the spore and cytoplasmic membranes (1, 3). This feature makes fatty acid synthesis a suitable marker for examining cellular membrane differentiation. The major fatty acid components in the genus *Bacillus* are the branched-chain fatty acids, and they constitute as much as 80 to 90% of the total fatty acids in *B. thuringiensis* (2, 12). However, little biochemical information is available on the biosynthesis of these fatty acids as they relate to membrane differentiation during spore germination and outgrowth. Previously, we used [U-^{14}C]acetate and [2-^{3}H]glycerol to monitor the kinetics of lipid synthesis during outgrowth (14). Acetate was incorporated rapidly and extensively into trichloroacetic acid-precipitable material starting at T_{20} (20 min after heat-shocking). Much of this incorporation constituted fatty acid synthesis. In the present work, we studied the biosynthesis of individual branched- and normal-chain fatty acids in *B. thuringiensis* by comparing pulsed and continuous labeling of these fatty acids with radioactive acetate during spore germination and outgrowth.

MATERIALS AND METHODS

Organism and cultural conditions. Stock cultures of *B. thuringiensis* NRRL B-4027 were maintained on MD (2) agar slants. Spores for fatty acid analyses were obtained by growing the organism in a modified GYS medium (22). The harvesting and cleaning of

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spores for lipid studies has been described previously (14). Spores were germinated by heat shocking for 30 min at 80 C in the modified GYS medium amended with L-alanine (100 μg/ml), adenosine (500 μg/ml), and ethylenediaminetetraacetic acid (25 μg/ml). Germination was monitored by phase-contrast microscopy and by following changes in optical density at 600 nm.

Pulse and continuous labeling. [U-^{14}C]acetate (2.5 μCi/20 μg per ml) was used to follow synthesis of individual fatty acids in heat-activated spores (1.68 mg/ml [dry weight]) germinated in the GYS medium. Flasks were inoculated with spores at T_0, incubated at 28 C, and aerated by rotary agitation at 200 rpm. For continuous labeling of fatty acids, the [U-^{14}C]acetate was also added at T_0, and 25-ml samples were taken at 30-min intervals. For pulse labeling, radioactive acetate was added at T_{30}, T_{60}, and T_{90} to a separate series of identical flasks. Samples (25 ml) were taken 30 min after [U-^{14}C]acetate addition. All samples were immediately chilled, centrifuged at 4 C, and washed with 25 ml of 0.02 M phosphate buffer (pH 7.4).

Methyl ester preparation and analysis. Chloroform–methanol-extractable lipids were subjected to methanolysis as described by Bulla et al. (2). Methyl esters of the fatty acids were separated and determined on polar columns with an F&M model 810 dual column chromatograph equipped with hydrogen flame detectors. Operating parameters were: injection detector ports, 235 C; carrier gas, helium; sample size, 1 or 2 μl; range and attenuation, $10^2 \times 2$.

Dual stainless-steel columns (20 feet by 1/8 inch [ca. 609.6 by 0.3 cm]) packed with ethylene glycol adipate (7%) on high-performance Chromosorb G (AW-DMCS), 80 to 100 mesh (Hewlett-Packard, Skokie, Ill.), were operated isothermally at 200 C. Carrier gas flow rate was adjusted to 12 to 15 ml/min. Standards of branched-chain fatty acid methyl esters were obtained from Anspec Co. Two admixtures (BC-L and BC-1) contained the following acids: i(iso)-C_{14}, n(normal)-C_{14}, a(anteiso)-C_{15}, n-C_{15}, i-C_{16}, n-C_{16}, a-C_{17}, i-C_{18}, n-C_{18}, a-C_{19}, i-C_{20}, n-C_{20}, and a-C_{21}. An admixture from The Hormel Institute contained normal fatty acids: n-C_8, n-C_{10}, n-C_{12}, n-C_{14}, n-C_{16}, n-$C_{16:1}$, n-C_{18}, n-$C_{18:1}$, and n-$C_{18:2}$. Identification of i-C_{13}, i-C_{15}, a-C_{15}, and i-C_{17} was made by comparing the linear relationship of the log retention time to the number of carbon atoms within a homologous series of long-chain fatty acids (9).

Analysis of radioactive fatty acids. Labeled methyl esters of the fatty acids were analyzed with a gas chromatography-liquid scintillation spectrometer system (5, 21). An Aerograph A-90 gas chromatograph equipped with a thermal conductivity detector was modified to provide a union of the effluent currents from both the sample and reference cells. The combined effluents were delivered to the exit port through an aluminum tube maintained at 250 to 260 C. The outer end of the tube was covered with Teflon and fitted with a glass tee. A toluene-base liquid scintillation mixture containing 7 g of 2,5-diphenyloxazole per liter was pumped through the tee past the exit port, mixing with the effluent stream. Liquid and gas flowed together to a fraction collector operated on a time basis by a 0- to 60-s interval timer. Fractions were collected directly in 20-ml scintillation counting vials. For the separation of the methyl esters a stainless-steel column (1/8 inch outer diameter by 20 feet) packed with 7% ethylene glycol adipate on 80/100 mesh G.H.P. was used. The column was maintained at 190 C with helium flowing at 100 ml/min. Under these conditions, the methyl ester of i-C_{16} has a retention time of 36 min. Fractions were collected at 30-s intervals, and the scintillation liquid was pumped at 20 ml/min. A total of 170 fractions was collected over the entire gas chromatographic analysis. The thermal conductivity signal was digitized using an Infotronics CS-30 digitizer that provided a punched paper tape representation of the curve as an interface to an IBM 1130 computer.

The filled vials were counted in a Beckman model LS-250 liquid scintillation spectrometer equipped with automatic external standardization and automatic quench compensation. Three voltage discriminator channels were provided, and data were presented on a teletypewriter printer and a punched paper tape. Computer programs developed by Thomas and Dutton (21) were utilized to process the data after appropriate modifications to provide for the increased number of fractions per sample. Each fraction collected was counted in duplicate, and the duplicate counts were compared. If a significant deviation was noted, an error message was printed to identify the fraction number. Counting efficiencies for each fraction were determined from an external standard ratio derived from quench correction curves. The quench correction curves were established by counting a series of quench standards, and coefficients of the curves were stored in the computer memory. Data from the scintillation spectrometer were reduced to a plot of disintegrations per minute in each fraction versus the relative retention time at the beginning of the fraction. Figure 1 depicts thermal conductivity and radioactivity data for individual fatty acids obtained using the computer methodology described. The exact position of the i-C_{15} methyl ester peak, determined by inspection of the recorder chart, was specified to the program in terms of the digital point and the fraction number. Each digital point and fraction was then assigned an x value corresponding to its position relative to the reference ester. A scale of relative retention values from 0 to 2.5 times that of i-C_{16} was divided into 1,250 increments. The computer, beginning at zero, calculated a y value at each increment to give a "percentage of full scale" figure that was recorded by the plotter as a point on the curve. The points were connected by straight lines, producing a graph of the data versus relative retention time. The plotting routine was run twice with the appropriate data for the thermal conductivity signal and the ^{14}C analysis. The plots were super-imposed on the same chart using different colored inks. Integration of the curves was obtained by summing the y values for each increment between specified values of the relative retention time. The area under each peak was then calculated and printed out as a percentage of the total. Relative specific activity of each ^{14}C-labeled

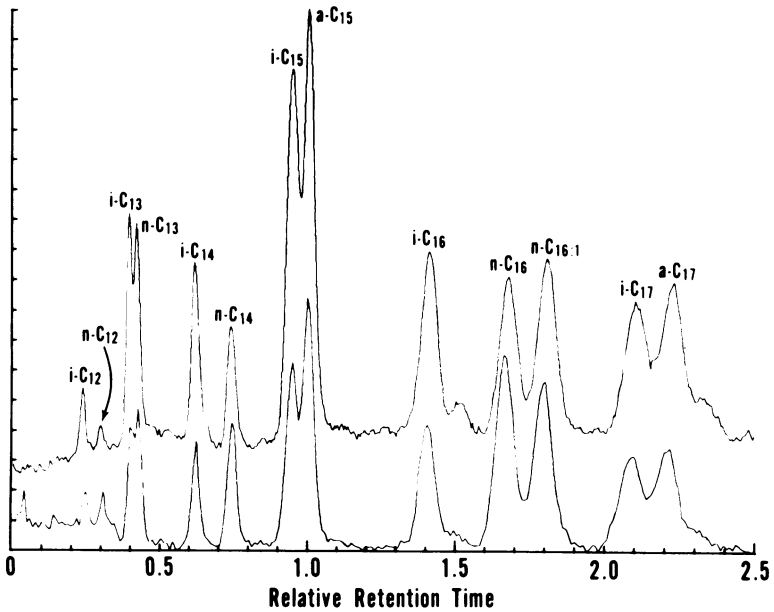

FIG. 1. *Thermal conductivity (upper curve) and radioactivity (lower curve) of fatty acid methyl esters obtained from B. thuringiensis cells.*

ester was calculated by dividing the radioactivity percent by the area percent (6).

RESULTS

Relative abundance of individual fatty acids in spores and vegetative cells. *B. thuringiensis* is typical of the gram-positive bacteria in that its membranes contain a high proportion of branched-chain fatty acids. These branched-chain fatty acids are designated as either iso- or anteiso-isomers depending on whether their methyl branch is at the ultimate or penultimate carbon atom. Table 1 compares the percentage of relative abundance of individual fatty acids in spores (T_0) and vegetative cells (post-T_{120}) of *B. thuringiensis*. Twelve fatty acids were present in both cases with the iso-C_{13}, i-C_{15}, a-C_{15}, and a-C_{17} isomers predominating. However, the levels of branched-chain C_{13} and C_{15} homologues differed in the spore and vegetative cell membranes. In spores, the i-C_{13} and i-C_{15} fatty acids were far more prevalent than the corresponding a-C_{13} and a-C_{15} isomers, whereas the values were approximately equal in the vegetative cells. Overall, the iso fatty acids decreased during the transition from spore to vegetative cell, whereas the anteiso isomers increased; normal isomers remained constant. Scandella and Kornberg (19) observed similar differences between spores and vegetative cells of *B. megaterium*.

TABLE 1. *Percentage of relative abundance of fatty acids in total lipids of spores and vegetative cells of B. thuringiensis*

Fatty acid	Spores	Vegetative cells
i-C_{12}	1.3	1.3
n-C_{12}	0.4	0.4
i-C_{13}	11.8	7.5
a-C_{13}	2.2	6.0
i-C_{14}	9.8	5.9
n-C_{14}	4.4	3.5
i-C_{15}	27.4	18.9
a-C_{15}	10.1	19.4
i-C_{16}	7.8	7.7
n-C_{16}	5.7	7.4
i-C_{17}	6.1	7.1
a-C_{17}	11.7	14.7
Σ	iso-64.2	iso-48.4
	n-10.5	n-11.3
	ante-24.1	ante-40.1

Differential synthesis of individual fatty acids pulse labeled with acetate. Many biochemical changes occur in a short time (2 h) during spore germination and outgrowth. The membrane fatty acid composition must revert from those ratios characteristic of spore membrane to those characteristic of vegetative cells. In a typical time sequence, all spores complete germination by T_{15}. They subsequently swell, elongate, undergo septum formation at T_{110}, and enter cell division at T_{130}. Spores of *B. thuringiensis* have already begun to incorporate

TABLE 2. *Differential synthesis of individual fatty acids pulse labeled with radioactive acetate during germination and outgrowth of B. thuringiensis*

Fatty acid	[U-^{14}C]acetate incorporation (counts/min)			
	0-30 min[a]	30-60 min	60-90 min	90-120 min
i-12		6,100		
n-12		1,200		
i-13	35,900	7,700		2,400
a-13		20,800		3,500
i-14	2,400	19,800	77,100	24,800
n-14		28,300	162,400	46,100
i-15		24,800	106,600	93,500
a-15		55,100	229,600	160,900
i-16	8,300	26,300	196,800	159,700
n-16		64,500	495,300	260,300
i-17		18,400	267,300	243,700
a-17		19,000	105,000	158,500
Σ iso (%)	99.9	35.4	39.5	45.4
n (%)	0	32.2	40.1	26.6
anteiso (%)	0	32.5	20.4	28.0

[a] Pulse time.

exogenous acetate into fatty acids by T_{20}; acetate incorporation is preceded only by that of uridine into ribonucleic acid (14).

Table 2 indicates the relative amounts of each individual fatty acid synthesized during four successive 0.5-h pulses after germination. Only three fatty acids, i-C_{13}, i-C_{14}, and i-C_{16}, were synthesized during the first 0.5-h pulse. Thus, there is an obvious dichotomy between the limited number of fatty acids (all iso fatty acids) synthesized prior to T_{30} and the greater variety synthesized during the later time periods. In addition, there is an apparent trend toward the synthesis of short-chain fatty acids before T_{60} and of long-chain fatty acids after T_{60}.

At no time did the proportions of fatty acids synthesized during outgrowth resemble the iso-anteiso-normal ratios found in Table 1 for either intact spores or vegetative cells. The fatty acids synthesized during T_{0-30} were exclusively iso isomers, and during the latter three pulse times, when all three classes of fatty acids were being synthesized, the normal fatty acids were synthesized to a far greater extent than would be predicted from their final relative abundance in either spores or vegetative cells. As expected, the synthesis of iso fatty acids exceeded that of the anteisos throughout outgrowth.

Differential synthesis of individual fatty acids continuously labeled with acetate. Specific fatty acid synthesis was also monitored during outgrowth by continuous labeling with acetate. Radioactive acetate was added at T_0. Those individual fatty acids labeled at T_{30}, T_{60}, T_{90}, and T_{120} are listed in Table 3. Once again the extent of acetate incorporation into fatty acids was vastly greater in the later stages of outgrowth as the spores approached first cell division. Significantly, the relative specific activities of the individual fatty acids were roughly the same at each time period regardless of whether the acetate was added continuously or was pulsed. That is, although the amount of acetate incorporation at T_{90}, for instance, differed in the pulse and continuous experiments, the approximate contribution of an individual fatty acid to the total acetate incorporation did not. The same patterns were observed within the groups of fatty acids formed. In both cases n-C_{16} exceeded n-C_{14}, which in turn exceeded n-C_{12}; more normal fatty acids were synthesized than expected, and there was a sharp rise in a-C_{17} at T_{120}.

Two major differences were present, however. The monounsaturated fatty acid n-$C_{16:1}$ was synthesized only in the continuous label experiment, appearing at T_{120}, whereas in none of the pulse-label experiments was acetate converted to n-$C_{16:1}$. Furthermore, the total amount of acetate incorporated into fatty acids was far greater in the pulse than in the continuous experiments; e.g., cells harvested at T_{90} had 10 times more acetate present in their fatty acids if the acetate was added at T_{60} (Table 2) than if it was added at T_0 (Table 3). This phenomenon is seen more dramatically in Fig. 2, where the extent of acetate incorporation into branched-chain and normal fatty acids is compared for pulse and continuous labeling. For both types of

TABLE 3. *Differential synthesis of individual fatty acids continuously labeled with radioactive acetate during germination and outgrowth of B. thuringiensis*

Fatty acid	[U-^{14}C]acetate incorporation (counts/min)			
	0-30 min[a]	0-60 min	0-90 min	0-120 min
i-12		1,500	2,800	7,300
n-12		1,100	1,000	2,700
i-13	35,900	6,100	6,500	7,100
a-13		7,600	11,000	16,500
i-14	2,400	7,500	13,100	47,200
n-14		10,900	21,700	72,600
i-15		8,100	14,300	79,800
a-15		18,900	26,600	148,700
i-16	8,300	7,100	16,000	104,300
n-16		14,800	32,200	118,800
n-16:1				39,900
i-17		1,200	12,800	143,300
a-17		2,700	2,100	93,400
Σ iso (%)	99.9	36.0	40.9	44.1
n (%)	0	30.0	34.3	26.5
anteiso (%)	0	33.4	24.8	29.3

[a] Labeling period.

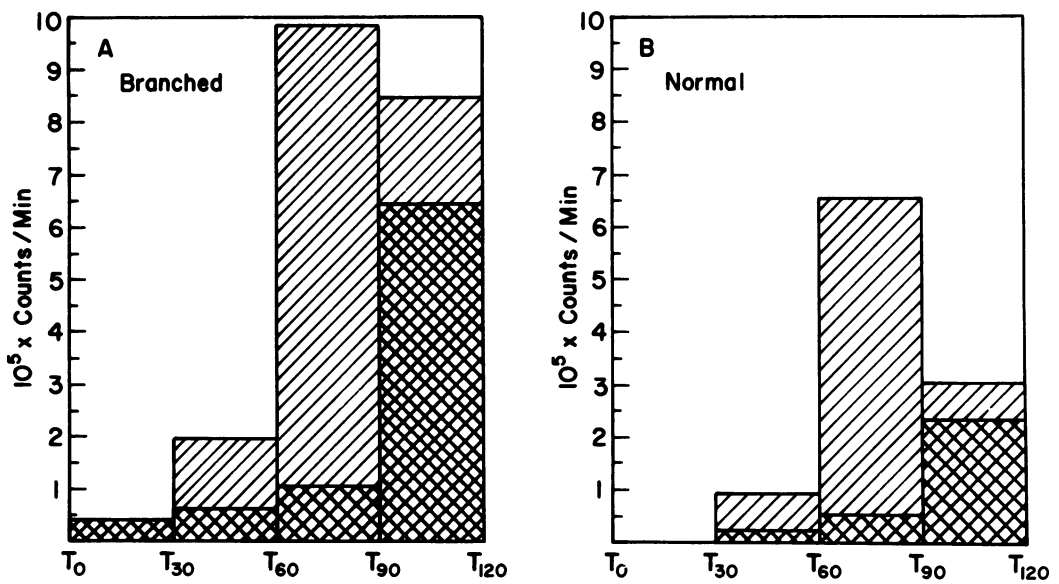

FIG. 2. *Acetate incorporation into (A) branched- and (B) normal-chain fatty acids: pulse versus continuous labeling. Heat-activated spores were germinated in GYS medium and incorporation of [U-^{14}C]acetate was monitored as described in Materials and Methods. Symbols: slant hatching, pulse labels; cross hatching, continuous label.*

fatty acids, a dramatic rise was seen for continuous labeling between T_{90} and T_{120}, whereas peak synthesis by pulse labeling occurred between T_{60} and T_{90}. These differences cannot be due to prior utilization of the acetate added at T_0 for purposes other than fatty acid synthesis, because high levels of radioactivity did appear in the continuously labeled fatty acid fractions when the cells were harvested at T_{120}. We showed earlier by radiorespirometry that the acetate added at T_0 was not metabolized to CO_2 via the tricarboxylic acid cycle (14). Intermediate conversion to an acetate storage product or fatty acid precursor is indicated. The levels of

acetate incorporation into purified fatty acids observed during this continuous labeling experiment were in good agreement with the kinetics of acetate and glycerol incorporation into trichloroacetic acid-precipitable material we observed previously (14).

DISCUSSION

The outgrowing cell of *B. thuringiensis* has a marked capacity to synthesize new fatty acids and this new synthesis is differential. The relative specific activity of each fatty acid varies with time as the cell progresses through outgrowth. Differential synthesis implies that regulatory mechanisms control membrane fatty acid composition, and such changes in composition could determine developmental changes in membrane function. One obvious regulatory mechanism would involve the availability of the branched-chain precursor molecules α-keto isovalerate, α-keto isocaproate, and α-keto methylvalerate (11). Kaneda (11) demonstrated that in *Bacillus subtilis* the relative abundance of the branched-chain fatty acids is determined by the availability of their respective precursors.

As an example of how membrane function depends on composition, Read and McElhaney (16) have shown that in *Acholeplasma laidlawii* the rate at which D-glucose is transported into the cell is markedly dependent on the membrane fatty acid composition. Both active and passive permeability rates are directly dependent on the fluidity of the membrane lipids as measured by the temperature at which the reversible thermo-tropic gel-to-liquid crystalline phase transition occurs. The incorporation of branched-chain or unsaturated fatty acids, or fatty acids of shorter chain length, increased permeability. Overath et al. (15) have made similar observations for the dependence of transport on membrane fatty acid composition in *Escherichia coli*.

The data in Tables 2 and 3 indicate that fatty acid synthesis occurs in two distinct patterns. A sharp division exists at T_{30}. Prior to T_{30}, all the acetate is incorporated specifically into the three iso fatty acids i-C_{13}, i-C_{14}, and i-C_{16}. No normal or anteiso synthesis occurs. It is attractive to speculate that this dichotomy results from early fatty acid synthesis in the absence of de novo protein synthesis and is accomplished by fatty acid biosynthetic enzymes preexisting in the dormant spore. Kinetic data (14) indicate that acetate incorporation into fatty acids commences at T_{20}, whereas protein synthesis, as determined by incorporation of exogenous phenylalanine, does not start until after T_{30}. Dawes and Halvorson (4) also concluded that enzymes for lipid synthesis were present in spores of the closely related bacterium *Bacillus cereus*, because early lipid synthesis was insensitive to chloramphenicol. They further speculated that this early lipid synthesis was necessary to maintain membrane integrity during the spore transition to the hydrated swollen state. In addition, early fatty acid synthesis is probably required for the membrane alterations observed by Fitz-James (7). Protoplasts obtained from dormant *Bacillus megaterium* spores require divalent calcium ions to maintain membrane integrity. This instability is quickly repaired after germination.

Subsequent to T_{30}, *B. thuringiensis* synthesizes the full range (Table 2) of branched- and normal-chain fatty acids displayed in Fig. 1 and Table 1. Also, the overall rate of synthesis is greater. There was 35 times more acetate incorporated into fatty acids during T_{60-90} than during T_{0-30}. Presumably, this increased lipid synthesis is the consequence of new protein synthesis initiated about T_{30}. Dawes and Halvorson (4) found that a similar burst in lipid synthesis in *B. cereus* was dependent on transcription and new protein synthesis.

Concomitant to this large increase in the rate of fatty acid synthesis, there is a marked shift from the synthesis of short-chain fatty acids during the early stages of outgrowth to that of long-chain fatty acids in the later stages. The increase in a-C_{17} during T_{90-120} is especially dramatic (Tables 2 and 3). Another significant conclusion from Tables 2 and 3 is that normal fatty acids are synthesized in excess throughout outgrowth. Straight-chain fatty acids are present in spores and vegetative cells of *B. thuringiensis* only to the extent of 10 to 11%, and yet 40% of the fatty acids synthesized during the T_{60-90} pulse (Table 2) are normal fatty acids, predominantly n-C_{16}. It would be desirable to know whether these changes in membrane fatty acid composition are related to a change in membrane function as the cell approaches septum formation, cell division, and the onset of replicative deoxyribonucleic acid synthesis. Souza et al. (20) observed a similar large shift in the percentage of normal fatty acids in the membrane of a thermophilic *Bacillus*. The percentage of normal fatty acids increase from 20 to 57% as the growth temperature was raised from 42 to 65 C. They speculated that such a shift to fatty acids with higher melting points would give the membrane greater thermal stability. Correspondingly, the shifts in fatty acid composition we have observed, i.e., the shift from shorter to longer fatty

acids and the eventual preponderance of the n-C_{16} and a-C_{17} fatty acids, is consistent with a requirement at some critical stage during outgrowth, possibly elongation, for a stronger membrane with a greater percentage of high-melting-point fatty acids.

There are both qualitative and quantitative diferences between the pulse-labeled fatty acids (Table 2) and the continuously labeled fatty acids (Table 3). The monounsaturated fatty acid n-$C_{16:1}$ is labeled only between T_{90-120} in the continuous experiment. It is not synthesized from acetate during the T_{90-120} pulse period (Table 2). Apparently the desaturase enzyme is not present prior to T_{90}, and when present it preferentially uses preexisting fatty acids or fatty acid precursors rather than ones newly synthesized from acetate. In conjunction with the data in Fig. 2, this phenomenon provides additional evidence for multiple pools of fatty acid precursors and the possible existence of an acetate storage product that is later converted into fatty acids. Such a process would be especially intriguing because of the supposed role of monounsaturated fatty acids such as n-$C_{16:1}$ in the replication of deoxyribonucleic acid and in cell division (8).

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LITERATURE CITED

1. Bishop, D. G., L. Rutberg, and B. Samuelsson. 1967. The chemical composition of the cytoplasmic membrane of *Bacillus subtilis*. Eur. J. Biochem. **2**:448-453.
2. Bulla, L. A., G. A. Bennett, and O. L. Shortwell. 1970. Physiology of sporeforming bacteria associated with insects. II. Lipids of vegetative cells. J. Bacteriol. **104**:1246-1253.
3. Daniels, M. J. 1969. Lipid synthesis in relation to the cell cycle of *Bacillus megaterium* KM and *Escherichia coli*. Biochem. J. **115**:697-701.
4. Dawes, I. W., and H. O. Halvorson. 1972. Membrane synthesis during outgrowth of bacterial spores, p. 449-455. *In* H. O. Halvorson, R. Hanson, and L. L. Campbell (ed.), Spores V. American Society for Microbiology, Washington, D.C.
5. Dutton, H. J. 1963. Monitoring gas chromatography for H^3- and C^{14}-labeled compounds by liquid scintillation counting, p. 147-152. *In* S. Rothschild (ed.), Advances in tracer methodology, Plenum Press, Inc., New York.
6. Dutton, H. J., and T. L. Mounts. 1966. Desaturation of fatty acids in seeds of higher plants. J. Lipid Res. **7**:221-225.
7. Fitz-James, P. C. 1971. Formation of protoplasts from resting spores. J. Bacteriol. **105**:1119-1136.
8. Fralick, J. A., and K. G. Lark. 1973. Evidence for the involvement of unsaturated fatty acids in initiating chromosome replication in *Escherichia coli*. J. Mol. Biol. **80**:459-475.
9. Hawke, J. C., R. P. Hansen, and F. B. Shorland. 1959. Gas liquid chromatography: retention volumes of the methyl esters of fatty acids with special reference to n-odd-numbered, iso and (+)-anteiso acids. J. Chromatogr. **2**:547-551.
10. Jacob, F., S. Brenner, and F. Cuzin. 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28**:329-348.
11. Kaneda, T. 1966. Biosynthesis of branched-chain fatty acids. IV. Factors affecting relative abundance of fatty acids produced by *Bacillus sublilis*. Can. J. Microbiol. **12**:501-514.
12. Kaneda, T. 1968. Fatty acids in the genus *Bacillus*. II. Similarity in the fatty acid compositions of *Bacillus thuringiensis*, *Bacillus anthracis*, and *Bacillus cereus*. J. Bacteriol. **95**:2210-2216.
13. Keynan, A. 1973. The transformation of bacterial endospores into vegetative cells. Symp. Soc. Gen. Microbiol. **23**:85-123.
14. Nickerson, K. W., J. DePinto, and L. A. Bulla. 1975. Lipid metabolism during bacterial growth, sporulation, and germination: kinetics of fatty acid and macromolecular synthesis during spore germination and outgrowth of *Bacillus thuringiensis*. J. Bacteriol. **121**:227-233.
15. Overath, P., H. U. Schairer, and W. Stoffel. 1970. Correlation of *in vivo* and *in vitro* phase transitions of membrane lipids in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **67**:606-612.
16. Read, B. D., and R. N. McElhaney. 1975. Glucose transport in *Acholeplasma laidlawii* B: dependence on the fluidity and physical state of membrane lipids. J. Bacteriol. **123**:47-55.
17. Ryter, A. 1968. Association of the nucleus and the membrane of bacteria: a morphological study. Bacteriol. Rev. **32**:39-54.
18. Ryter, A., Y. Hirota, and F. Jacob. 1968. DNA-membrane complex and nuclear segregarion in bacteria. Cold Spring Harbor Symp. Quant. Biol. **33**:669-676.
19. Scandella, C. J., and A. Kornberg. 1969. Biochemical studies in bacterial sporulation and germination. XV. Fatty acids in growth, sporulation, and germination of *Bacillus megaterium*. J. Bacteriol. **98**:82-86.
20. Souza, K. A., L. L. Kostiw, and B. J. Tyson. 1974. Alterations in normal fatty acid composition in a temperature-sensitive mutant of a thermophilic bacillus. Arch. Microbiol. **97**:89-102.
21. Thomas, P. J., and H. J. Dutton. 1969. Improved resolution in high-sensitivity dual-label gas radiochromatography. Anal. Chem. **41**:657-660.
22. Yousten, A. A., and M. H. Rogoff. 1969. Metabolism of *Bacillus thuringiensis* in relation to spore and crystal formation. J. Bacteriol. **100**:1229-1236.