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Effect of Cerulenin on Growth and Lipid Metabolism of Mycoplasmas

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Cerulenin markedly inhibited the growth of *Acholeplasma laidlawii*. *A. axanthum* and *A. granularum* were less susceptible, whereas the sterol-requiring *Mycoplasma* species examined showed very little susceptibility. The inhibition was not reversed by the addition of long-chain fatty acids to the medium. At a concentration of 20 μg/ml, cerulenin inhibited the incorporation of [^{14}C]acetate into *A. laidlawii* membrane lipids, but it had no effect on either protein or nucleic acid biosynthesis. Cerulenin inhibited both the de novo synthesis of long-chain fatty acids and the elongation of medium-chain fatty acids. As a result, carotenoid biosynthesis was stimulated, and increased amounts of oleic and elaidic acids were incorporated into membrane polar lipids. Our studies support the concept that cerulenin can serve as a useful tool for obtaining better control of fatty acid composition of *A. laidlawii* membranes.

Since mycoplasmas are capable of incorporating exogenous fatty acids, and thus enabling us to manipulate changes in their lipid composition (14, 15), the study of these organisms has provided a considerable amount of information on the molecular organization of membrane lipids (16). The most widely studied mycoplasma for this purpose is *Acholeplasma laidlawii*. This organism is dependent on an external source for its unsaturated fatty acids (9, 17), but it can readily synthesize saturated fatty acids from acetate (19). Thus, although exogenous fatty acids are incorporated into membrane lipids, as much as 50 to 70%, a biosynthetic background of lauric, myristic, and palmitic acids always exists (9, 11, 17). Furthermore, because *A. laidlawii* has the ability to elongate medium-chain fatty acids (C_{12} to C_{14}) to corresponding C_{16} and C_{18} isomers (11), an enrichment of membranes with medium-chain unsaturated fatty acids cannot be obtained.

Our studies were undertaken, therefore, in an attempt to obtain better control of fatty acid composition of *A. laidlawii* by inhibiting fatty acid biosynthesis and elongation with cerulenin. Cerulenin, isolated from the culture filtrate on *Cephalosporium caerulens* (7), has been shown to inhibit the growth of a variety of yeasts, fungi, and bacteria (8). Since this inhibition can be overcome by the addition of certain lipid molecules to the medium, it was proposed that cerulenin specifically interferes with lipid biosynthesis (3, 10). Recently, the specific, nonreversible inhibition of β-ketoacyl-acyl carrier protein synthetase, a key enzyme in fatty acid biosynthesis, was also ascribed to cerulenin (2, 23). In this report, the effects of cerulenin on the growth of representative *Acholeplasma* and *Mycoplasma* species, as well as on de novo biosynthesis and elongation of fatty acids by *A. laidlawii*, are described.

MATERIALS AND METHODS

Organisms and growth conditions. The mycoplasmas tested were *A. laidlawii* PG8, *A. axanthum* S743, *A. granularum* BTS39, *Mycoplasma capricolum* California kid, *M. anatis* 1340, *M. arginini* G230, *M. gallisepticum* PG31, and *M. pneumoniae* FH. The organisms were grown in 100 to 500 ml of a modified Edward medium (1) containing either 2% PPLO-serum fraction (Difco) or 0.5% fatty-acid-poor bovine serum albumin (BSA). The latter medium was supplemented with 5 to 15 μg of oleic acid per liter (*cis*-Δ^9-C_{18}) or elaidic (*trans*-Δ^9-C_{18}) acid with or without 15 μg of myristoleic acid per liter (*cis*-Δ^9-C_{14}). Cerulenin, kindly provided by S. Omura (Kitasato Institute, Tokyo, Japan), was added to the medium as an ethanolic solution to a final concentration of up to 100 μg/ml.

For labeling the cells, media were supplemented with 10 μCi of 1-^{14}C-labeled acetic acid per liter (59.3 mCi/mmol), 1 μCi of 1-^{14}C-labeled oleic acid per liter (58 mCi/mmol), 50 μCi of L-[2,3-^{3}H]phenylalanine per liter (20 Ci/mmol), 100 μCi of [5-^{3}H]-uracil per liter (25 Ci/mmol), 1 μCi of [4-^{14}C]cholesterol per liter (56 mCi/mmol), or 100 to 300 μCi of [*methyl*-^{3}H]thymidine per liter (20 Ci/mmol). The radioactive compounds were obtained from the Radiochemical Centre, Amersham, England. After 16 to 40 h of incubation at 37 C, growth was esti-

mated by measuring the absorbancy of the culture at 640 nm. The organisms were harvested by centrifugation at 12,000 × *g* for 15 min and washed once with 0.2 M NaCl.

Assessment of susceptibility. The growth inhibition of mycoplasmas by cerulenin was determined by a modification of the microtiter metabolic inhibition test (12, 21), in which cerulenin was used in place of the specific antiserum. In brief, nine twofold dilutions of cerulenin (100 μg/ml) were made in U-shaped wells of microtiter plates in a total volume of 0.025 ml. One-tenth milliliter of fresh medium and 0.050 ml of the culture were then added to each well. Two wells (10 and 11) served as the positive mycoplasma controls (no cerulenin) and one well (12) was used as the medium control. The microtiter plates were incubated at room temperature for the fast-growing *Acholeplasma* species or at 37 C for the *Mycoplasma* species. Daily readings were made, and the final reading was taken when the medium in control wells containing no cerulenin showed a color change from orange to yellow or from orange to red for the fermentative or arginine-utilizing species, respectively. The end point was established as the lowest concentration of cerulenin that inhibited growth.

Preparation of cell membranes. Cell membranes were obtained by osmotic lysis of the organisms (13). The membranes were sedimented by centrifugation at 36,000 × *g* for 30 min, separated from the cytoplasmic fluid, washed twice, and suspended in deionized water. The membranes were kept at −20 C until used.

Analytical procedures. Protein content of cell and membrane preparations was determined by the method of Lowry et al. (6) with BSA as the stand ard. Carotenoid content was estimated by measuring the absorbancy at 442 nm of an ethanolic extract obtained by extracting a cell pellet with absolute ethanol at 75 C for 15 min. Results were expressed as absorbancy at 442 nm × 1,000 per mg of cell protein. Radioactivity in the cell or membrane preparation was determined with a Packard Tri-Carb liquid scintillation spectrometer with Aquasol (New England Nuclear Corp., Boston, Mass.) scintillation fluid.

Lipid analyses. Lipids were obtained from cells or membrane preparations (2 to 10 mg of protein) by two successive extractions with chloroform-methanol (2:1, vol/vol) at room temperature for 2 h. The extracts were combined and filtered through glass wool. The solvent was then evaporated under nitrogen, and the dried lipid was dissolved in 0.5 ml of chloroform. The separation of the neutral lipid from the polar lipid was achieved with an activated silicic acid column as previously described (20). Methyl esters of fatty acids were prepared by heating the lipid sample for 15 min at 80 C in bromine trifluoride-methanol solution (Supelco Inc., Bellefonte, Pa.). The resultant methyl esters were extracted with hexane and examined by capillary gas-liquid chromatography (11) in a Perkin-Elmer model 990 apparatus with a polar column (150 feet by 0.01 inch [45.7 m by 0.0254 cm]) coated with Carbowax k-20 plus V-93 (99:1). Fatty acids were then identified by determining their retention times relative to that of the standard methyl ester mixtures (Applied Science Laboratories, College Park, Pa.). Packed-column gas chromatography was performed as described previously (20). The newly synthesized radioactive fatty acids were collected from the column directly into scintillation vials containing 10 ml of scintillation fluid and counted.

Paramagnetic resonance spectroscopy. Membranes were spin-labeled with *N*-oxyl-4′,4′-dimethyloxazolidine derivatives of 5-ketostearic acid (5 nitroxystearate, Syva, Palo Alto, Calif.) by exchange from bovine serum albumin as previously described (18). Electron paramagnetic spectra of spin-labeled membranes were obtained with a Varian E-3 spectrometer. The molecular motion is reported as $2T_{\parallel}$, the hyperfine splitting that has been shown previously to be related to the freedom of motion of the nitroxide radical (5). A greater freedom of motion is associated with smaller values of $2T_{\parallel}$.

Assessment of leakiness of cells. Cells grown with 200 μCi of [*methyl*-^{3}H]thymidine were harvested by centrifugation, washed once, and suspended in a modified Edward medium (1). Varying concentrations of cerulenin were then added to the media, and samples were incubated at 37 C. At various time intervals, 1-ml samples were withdrawn and centrifuged at 12,000 × *g* for 30 min. The supernatant fluid was separated from the pellet and tested for radioactivity.

RESULTS

Growth inhibition of mycoplasmas by cerulenin. The results presented in Table 1 show that cerulenin inhibited the growth of all mycoplasmas tested but that the *Acholeplasma* species were inhibited at much lower concentrations than were the *Mycoplasma* species.

TABLE 1. *Effect of cerulenin on growth of Mycoplasma and Acholeplasma species*[a]

Organism	Cerulenin concn (μg/ml)[b]
Acholeplasma laidlawii	3.1
A. axanthum	12.5
A. granularum	12.5
Mycoplasma capricolum	50.0
M. anatis	50.0
M. arginini	50.0
M. gallisepticum	50.0
M. pneumoniae	50.0

[a] Organisms were grown in Edward medium (1) containing horse serum and various concentrations of cerulenin.

[b] The lowest concentration of cerulenin required to inhibit the growth of the organisms as determined by a modification of the metabolic inhibition test (12, 21).

The activity of cerulenin varied considerably among the *Acholeplasma* species, with *A. laidlawii* being the most sensitive. As shown in Table 2, the lowest inhibitory concentration of cerulenin for *A. laidlawii* was affected by the serum content in the growth medium. The inhibition effects obtained in media containing horse serum or pleurpneumonia-like orga- horse serum or PPLO-serum fraction were greater than those obtained in media supplemented with BSA or fatty-acid-poor BSA. The inhibitory effect of cerulenin was not reversed by the addition of fatty acids, such as lauric, myristic, palmitic, elaidic, or oleic acids, to the growth medium. The fatty acids were added separately (5 to 20 μg/ml) or as a mixture containing 5 μg of each per ml.

Inhibition of the incorporation of radioactive precursors. Figure 1 shows the effect of cerulenin on the incorporation of radioactive precursors into *A. laidlawii* cells. Whereas the incorporation of [^{3}H]uracil, [^{3}H]thymidine, and [^{3}H]phenylalanine were either not, or only slightly, affected by as much as 30 μg of cerulenin per ml, [^{14}C]acetate incorporation was markedly inhibited with a concomitant increase in the incorporation of ^{14}C-labeled oleic acid. At concentrations up to 50 μg/ml, cerulenin had no effect on incorporation of any of the radioactive precursors by *M. capricolum*. However, at concentrations higher than 50 μg/ml, where growth inhibition could be demonstrated, the incorporation of radioactive uracil, phenylalanine, and thymidine was inhibited by 30 to 35%. These findings suggest that high concentrations of cerulenin may have a nonspecific inhibitory effect on the *Mycoplasma* species. The possibility that such an effect is due to damage to the permeability barrier of the mycoplasma cells was examined by determining the effect of high cerulenin concentrations on the leakiness of *M. capricolum* and *A. laidlawii* cells. Table 3 shows that when [^{3}H]thymidine-labeled cells were incubated in media containing 50 to 100 μg of cerulenin per ml, a 2.5-fold increase (from 6 to 16%) in the release of thymidine-labeled components was found with *M. capricolum*, but a much smaller release was obtained with *A. laidlawii*. Because only about 12% of the radioactivity of *M. capricolum* cells is extractable with cold trichloroacetic acid, it seems apparent that the high concentrations of cerulenin induce the release of not only small molecules, but also of some high-molecular-weight deoxyribonucleic acid.

TABLE 2. *Influence of serum components on growth inhibition of Acholeplasma laidlawii by cerulenin*[a]

Medium supplement (%)	Cerulenin (μg/ml)[b]
Horse serum (2)	3.1
Serum fraction (2)	3.1
BSA (1)	12.5
Fatty-acid-poor BSA (1)	12.5

[a] Cells were grown in Edward medium (1) supplemented with either horse serum, PPLO-serum fraction or BSA and various concentrations of cerulenin.

[b] The lowest concentration of cerulenin required to inhibit the growth of the organism as determined by a modification of the metabolic inhibition test (12, 21).

Effect of cerulenin on lipid metabolism. The results in Table 4 show that cerulenin markedly inhibits the incorporation of [^{14}C]acetate into the molar lipid fraction of *A. laidlawii*. However, the incorporation of label into the neutral lipid fraction, which represents a very small but consistent portion of the total acetate incorporated, was stimulated. Methanolysis of the polar lipid fractions showed the label to be confined to the fatty acid methyl esters, suggesting that the inhibitory effect of cerulenin is due to the inhibition of the synthesis of the long-chain fatty acids. The radioactivity

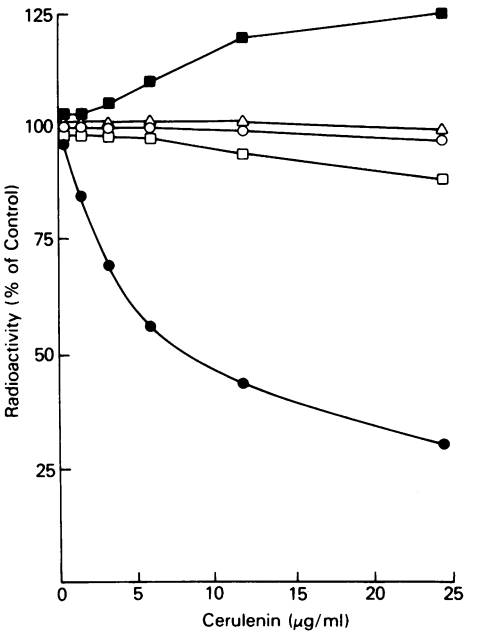

FIG. 1. *Effect of cerulenin on the incorporation of radioactive precursors into A. laidlawii cells. Symbols: (○) [5-^{3}H]uracil, (△) L-[2,3-^{3}H]phenylalanine, (□) [methyl-^{3}H]thymidine; (●) 1-^{14}C-labeled acetic acid; (■) 1-^{14}C-labeled oleic acid.*

TABLE 3. *Effect of cerulenin on the release of [methyl-^{3}H]thymidine-labeled material from mycoplasma cells*[a]

Cerulenin concn (μg/ml) in medium	Radioactivity released (% of total)[b]	
	A. laidlawii	*M. capricolumn*
None	4	6
10	5	5
50	5	12
100	7	16

[a] Organisms grown in Edward medium (1) containing [*methyl*-^{3}H]thymidine were harvested and resuspended to a concentration of about 1 mg of cell protein/ml in fresh media containing various concentrations of cerulenin. The cell suspensions were incubated at 37 C for various periods of time and centrifuged. Radioactivity was determined in the supernatant fluid. The data presented in the table are those obtained after 60 min of incubation at 37 C.

[b] Calculated from the values of total radioactivity in washed cells.

TABLE 4. *Effect of cerulenin on cell yield, carotenoids, and acetate incorporation by Acholeplasma laidlawii*[a]

Cerulenin concn (μg/ml)	Cell yield (mg of cell protein)	Carotenoids[b]	[1-^{14}C]acetate incorporated (counts/min)	
			Polar lipids	Neutral lipids
0	10.2	15.4	58.250	1,220
10	6.6	21.6	24.640	1,600
20	4.2	28.4	12.000	1,750

[a] Cells were grown in 100 ml of a modified Edward medium (1) containing 2% PPLO-serum fraction and 8 μCi of 1-^{14}C-labeled sodium acetate.

[b] Absorbance at 442 nm × 1,000 per milligram of cell protein.

incorporated into the neutral-lipid fraction was confined to the non-saponifiable lipids, probably the carotenoids. The threefold increase in radioactivity of the neutral-lipid fraction calculated per milligram of cell protein obtained with cerulenin was also reflected in an increase in carotenoid content. The spectral analysis of the carotenoids extracted from cells grown with or without cerulenin showed a small change from a 426/442-nm peak ratio of 0.9 for extracts of control cells to 1.1 observed for extracts from cells grown with 20 μg of cerulenin per mg.

The inhibition of acetate incorporation into long-chain fatty acids was reflected in the fatty acid composition of membrane polar lipids, as shown in the quantitative data of capillary gas chromatograms tabulated in Table 5. Aside from the high content of oleic or elaidic acids, which were incorporated from the media, myristic (14:0) and palmitic (16:0) acids were the major fatty acids of membrane polar lipids, representing the products of de novo fatty acid biosynthesis by *A. laidlawii*. The content of these saturated fatty acids was dramatically decreased in cells grown with cerulenin. The decrease was accompanied by an increase in the relative amounts of the unsaturated fatty acid incorporated by the cells, i.e., 42 versus 60% for cells grown with oleic acid and from 62 versus 80% for cells grown with elaidic acid. The fatty acid content of membrane phospholipids, however, remained unchanged. The incorporation of [^{14}C]acetate into long-chain fatty acids by cells grown with or without cerulenin is shown in Table 6. Most of the label was incorporated into lauric, myristic, and palmitic acids. The relative intensity of the label among these fatty acids closely resembled the profile of saturated fatty acids obtained from gas-liquid chromatograms, indicating that under our experimental conditions most of the saturated fatty acids detectable in the cells are products of a de novo synthesis. As it is apparent from the data, in the presence of a cerulenin, the

TABLE 5. *Effect of cerulenin on fatty acid composition of Acholeplasma laidlawii cells*[a]

Fatty acid	Fatty acid composition (% of total) of cells grown with:			
	Oleic acid		Elaidic acid	
	Without cerulenin	With cerulenin (20 μg/ml)	Without cerulenin	With cerulenin (20 μg/ml)
12:0[b]	2.0	17.9	1.2	8.2
14:0	14.8	12.7	14.4	6.1
16:0	38.1	5.8	12.6	3.6
18:0	3.1	3.1	8.0	3.0
18:1	42.0	60.0	62.4	80.7
Saturated/unsaturated[c]	1.38	0.63	0.57	0.25

[a] Cells were grown in a modified Edward medium (1) containing 0.5% fatty-acid-poor BSA as the sole serum component and either 15 μg of elaidic acid (*trans*-Δ^9-C_{18}) per ml or 15 μg of oleic acid (*cis*-Δ^9-C_{18}) per ml. Analyses were performed by capillary-column gas chromatography.

[b] The first number indicates chain length and the second indicates the number of double bonds.

[c] Ratio of saturated to unsaturated fatty acids.

TABLE 6. *Effect of cerulenin on the incorporation of [1-^{14}C]acetate into long-chain fatty acids by Acholeplasma laidlawii cells*[a]

Fatty acid	Radioactivity (counts/min)	
	Cells grown without cerulenin	Cells grown with cerulenin (20 μg/ml)
12:0[b]	80	210
14:0	540	105
16:0	1,310	70
18:0	45	10

[a] Cells were grown in a modified Edward Medium (1) containing 0.5% fatty-acid-poor BSA, 15 μg of oleic per ml, and 10 μCi of 1-^{14}C-labeled acetic acid per liter. Fatty acid methyl esters were analyzed by a packed-column gas chromatography (20).

[b] The first number indicates chain length and the second indicates the number of double bonds.

cells synthesize shorter-chain saturated fatty acids, i.e., mainly lauric acid. In the absence of cerulenin, the label was found predominantly in the palmitic acid fraction. The decrease in the saturated/unsaturated fatty acid ratio and in the relative amount of shorter-chain saturated fatty acids in the phospholipids fraction of cells grown with cerulenin resulted in an increase in the freedom of motion of a spin-labeled fatty acid incorporated into the membranes (Fig. 2). Thus, the hyperfine splitting ($2T_{\parallel}$) was slightly but consistently higher in membranes from cells grown without cerulenin than in cells grown with cerulenin, indicating a slightly higher membrane fluidity in cells grown with cerulenin.

The effect of cerulenin on the elongation of a C14 unsaturated fatty acid is shown in Table 7. The data indicate that *A. laidlawii* is capable of elongating exogenous myristoleic acid (Δ^9-C_{14}) to form the corresponding C_{16} acid (Δ^{11}-C_{16}), with a small amount of the C_{18} acid (Δ^{13}-C_{18}) isomer. When cerulenin was added to the growth medium, the efficiency of elongation was markedly decreased. In cells grown with cerulenin, about 60% of the myristoleic acid and its elongated products were incorporated as myristoleic acid, whereas about 40% were further elongated. In cells grown without cerulenin only about 25% remained as myristoleic acid, whereas about 75% were further elongated.

DISCUSSION

Cerulenin is a unique, potent inhibitor of fatty acid synthetase (2, 23). Our findings that the growth of *A. laidlawii*, but not of *Myco-*

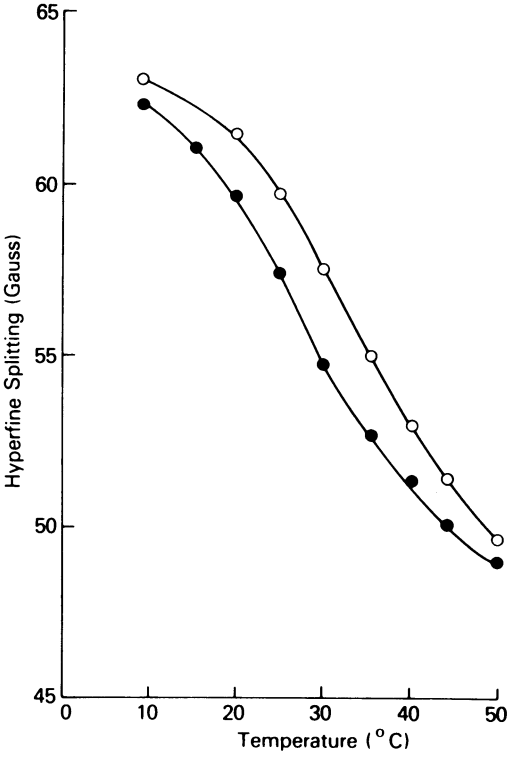

FIG. 2. *Temperature dependence of the hyperfine splitting ($2T_{\parallel}$) of 5-nitroxystearate in membranes of A. laidlawii cells grown with (●) or without (○) cerulenin (20 μg/ml).*

TABLE 7. *Effect of cerulenin on elongation of myristoleic acid in Acholeplasma laidlawii cells*[a]

Fatty acid	Fatty acid composition (% of total)	
	Cells grown without cerulenin	Cells grown with cerulenin (20 μg/ml)
12:0[b]	<1.0	3.0
14:0	1.8	5.5
14:1	12.1	28.1
16:0	14.1	5.2
16:1	38.4	18.1
18:0	4.0	2.1
18:1		
trans-Δ^{11}	15.0	24.4
cis-Δ^{13}	1.5	<1.0

[a] Cells were grown in a modified Edward medium (1) containing 0.5% fatty-acid-poor BSA as the only serum component, 5 μg of elaidic acid (*trans*-Δ^{11}-C_{18}) per ml, and 15 μg of myristoleic acid (*cis*-Δ^9-C_{14}) per ml. Analyses were performed by capillary-column gas chromatography.

[b] The first number indicates chain length and the second indicates the number of double bonds.

plasma species, was markedly inhibited by low concentrations of cerulenin are in accord with the observations that the *Acholeplasma* species are capable of synthesizing long-chain saturated fatty acids from acetate, whereas the *Mycoplasma* species are not (15). The growth inhibition of the *Mycoplasma* species with high cerulenin concentrations was probably due to a nonspecific effect resulting in a damage to the cell membrane. This concept was suggested from the marked increase in leakiness of *M. capricolum* cells when incubated with cerulenin (Table 3). By virtue of its hydrophobic nature, cerulenin, a 12-carbon atom unsaturated fatty acid amide, may interact with the lipid backbone of the membrane and damage it in a manner similar to that produced by high concentrations of long-chain unsaturated fatty acids. In fact, we have shown that high concentrations of oleic, linoleic, or linolenic acid can readily inhibit growth of all *Mycoplasma* species tested (M. W. Grabowski, unpublished data). The relative susceptibility of the three *Acholeplasma* species to cerulenin is also in accord with their ability to synthesize saturated fatty acid from acetate. Thus, *A. laidlawii,* the most susceptible *Acholeplasma* species, possessed the most active fatty acid synthetase system (19, 22), whereas *A. granularum* or *A. axanthum*, which are less susceptible, possessed only 10 to 20% of such biosynthetic activity (22). However, the question of whether the growth inhibition of *Acholeplasma* species is attributed only to the inhibition of fatty acid biosynthesis is not as yet resolved, because this effect could not be reversed by the addition of fatty acids to the growth medium.

In yeast cells, cerulenin inhibits the incorporation of [^{14}C]acetate into both fatty acids and non-saponifiable lipids (10). In *A. laidlawii* cells, however, the incorporation of acetate into long-chain fatty acid was markedly inhibited, whereas the incorporation into the non-saponifiable lipid fraction was unaffected or even stimulated. These findings point to a specific effect of cerulenin on the fatty acid synthetase system. Such an effect may be due to the inactivation by cerulenin of the β-ketoacyl-acyl carrier protein synthetase, a key enzyme in fatty acid biosynthesis, which was described for both the multienzyme complex and the non-associated fatty acid synthetases (2, 23). A specific block in *A. laidlawii* fatty acid synthetase system will result in an increased concentration of coenzyme A and of acetate available for the synthesis of non-saponifiable lipids.

In our studies with *A. laidlawii,* cerulenin interfered with both the de novo synthesis of saturated fatty acids from acetate and with the chain elongation of unsaturated fatty acid, beginning with myristoleic acid. Since the label derived from [^{14}C]acetate is accumulated in lauric acid rather than in myristic and palmitic acids (see Table 6), it appears that a chain-length specificity might play a role in the activity of cerulenin on the fatty acid synthetase system of *A. laidlawii.*

Our studies indicate that cerulenin can provide a useful tool for a better control of the fatty acid composition in *A. laidlawii* membranes. The partial inhibition of fatty acid synthesis by cerulenin caused a considerable increase in the homogeneity of membrane fatty acid (see Table 5). Nonetheless, even though a higher degree of unsaturation and a higher content of medium-chain fatty acids (C_{12} and C_{14}) appeared in cells grown with cerulenin, the fluidity of the hydrophobic region was only slightly increased. This might be due to the increase in carotenoid content of the cell membrane, which has been shown to restrict the motion of one of the hydrocarbon chains of membrane phospholipids (4). The increase in the carotenoid content of cells grown with cerulenin may be an attempt by the cells to compensate for the increase in membrane fluidity and, as a result, permit the cell to maintain membrane lipid fluidity within a narrow range.

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LITERATURE CITED

1. Barile, M. F. 1973. Mycoplasmal contamination of cell cultures: mycoplasma-virus-cell culture interactions, p. 131-172. *In* J. Fogh (ed.), Contamination of cell cultures. Academic Press Inc., New York.
2. D'angnolo, G., I. S. Rosenfeld, J. Awaya, S. Ōmura, and P. R. Vagelos. 1973. Inhibition of fatty acid synthesis by the antibiotic cerulenin: specific inactivation of β-ketoacyl-acyl carrier protein synthetase. Biochim. Biophys. Acta 326:155-166.
3. Goldberg, I., J. R. Walker, and K. Bloch. 1973. Inhibition of lipid synthesis in *Escherichia coli* cells by the antibiotic cerulenin. Antimicrob. Agents Chemother. 3:549-554.
4. Huang, L., and A. Hang. 1974. Regulation of membrane lipid fluidity in *Acholeplasma laidlawii*: effect of carotenoid pigment content. Biochim. Biophys. Acta 352:361-370.
5. Hubbell, W. L., and H. M. McConnell. 1969. Orientation and motion of amphiphilic spin labels in membranes. Proc. Natl. Acad. Sci. U.S.A. 64:20-27.
6. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the

Folin phenol reagent. J. Biol. Chem. 193:265-275.
7. Matsumae, A., Y. Kamio, and T. Hata. 1963. Studies on cerulenin. I. Studies on a cerulenin producing strain. J. Antibiot. 16:236-238.
8. Matsumae, A., S. Nomura, and T. Hata. 1964. Studies on cerulenin. IV. Biological characteristics of cerulenin. J. Antibiot. 17:1-7.
9. McElhaney, R. N., and M. D. Tourtellotte. 1969. Mycoplasma membrane lipids: variations in fatty acid composition. Science 164:433-434.
10. Nomura, S., T. Horiuchi, S. Ômura, and T. Hata. 1972. The action mechanism of cerulenin. I. Effect of cerulenin on sterol and fatty acid biosynthesis in yeast. J. Biochem. (Tokyo) 71:783-786.
11. Panos, C., and S. Rottem. 1970. Incorporation and elongation of fatty acid isomers by *Mycoplasma laidlawii*. Biochemistry 9:407-412.
12. Purcell, R. H., D. Taylor-Robinson, D. C. Wong, and R. M. Chanock. 1966. A color test for the measurement of antibody to T-strain mycoplasmas. J. Bacteriol. 92:6-12.
13. Razin, S. 1963. Osmotic lysis of mycoplasma. J. Gen. Microbiol. 33:471-475.
14. Razin, S. 1969. Structure and function in mycoplasma. Annu. Rev. Microbiol. 23:317-356.
15. Razin, S. 1973. Physiology of mycoplasmas, p. 1-80. *In* A. H. Rose and D. W. Tempest (ed.), Advances in microbial physiology, vol. 10. Academic Press Inc., London.
16. Razin, S. 1975. The mycoplasma membrane, p. 257-313. *In* D. A. Cadenhead, J. F. Daniellis, and M. D. Rosenberg (ed.), Progress in surface and membrane science, vol. 9. Academic Press Inc., New York.
17. Razin, S., M. E. Tourtellotte, R. N. McElhaney, and J. D. Pollack. 1966. Influence of lipid components of *Mycoplasma laidlawii* membranes on osmotic fragility of cells. J. Bacteriol. 91:609-616.
18. Rottem, S., W. L. Hubbell, L. Hayflick, and H. M. McConnell. 1970. Motion of fatty acid spin labels in the plasma membrane of mycoplasma. Biochim. Biophys. Acta 219:104-113.
19. Rottem, S., and C. Panos. 1970. The synthesis of long-chain fatty acids by a cell-free system from *Mycoplasma laidlawii* A. Biochemistry 9:57-63.
20. Rottem, S., and S. Razin. 1973. Membrane lipids of *Mycoplasma hominis*. J. Bacteriol. 113:565-571.
21. Taylor-Robinson, D., R. H. Purcell, D. C. Wong, and R. M. Chanock. 1966. A color test for the measurement of antibody to certain *Mycoplasma* species based upon the inhibition of acid production. J. Hyg. 64:91.
22. Tully, J. G., and S. Razin. 1969. Characterization of a new sterol-nonrequiring *Mycoplasma*. J. Bacteriol. 98:970-978.
23. Vance, O., I. Goldberg, O. Mitsuhashi, K. Bloch, S. Ômura, and S. Nomura. 1972. Inhibition of fatty acid synthetases by the antibitoic cerulenin. Biochem. Biophys. Res. Commun. 48:649-656.