Inhibition of Unsaturated Fatty Acid Synthesis in *Escherichia coli* by the Antibiotic Cerulenin†

Thomas M. Buttke*,‡ and Lonnie O'Neal Ingram

ABSTRACT: Low concentrations of cerulenin inhibit the growth of *Escherichia coli* by selectively blocking unsaturated fatty acid synthesis. This inhibition was relieved by unsaturated fatty acid supplements alone but not by saturated fatty acid supplements. The utilization of exogenous unsaturated fatty acids to sustain growth in the presence of cerulenin was confirmed by the analysis of bulk lipid composition. The effects of cerulenin on fatty acid synthesis were examined in vivo by pulse labeling with [^{14}C]acetate and in vitro using [^{14}C]malonyl-coenzyme A. In both cases, unsaturated fatty acid synthesis was inhibited by low concentrations of cerulenin with a stimulation of saturated fatty acid synthesis. Using mutant strains deficient in fatty acid synthesis, the effects of cerulenin on β-ketoacyl-[acyl-carrier-protein] synthetases I and II were examined. Our results indicate that β-ketoacyl-[acyl-carrier-protein] synthetase I is more sensitive to inhibition by cerulenin than β-ketoacyl-[acyl-carrier-protein] synthetase II.

Cerulenin is a potent antibiotic capable of inhibiting the growth of a variety of yeasts, fungi, and bacteria (for a review, see Ōmura, 1976). In yeast and bacteria, growth inhibition results specifically from an inhibition of lipid synthesis, and the effect of cerulenin can be overcome by providing exogenous fatty acids (Goldberg et al., 1973; Awaya et al., 1975). Studies with *E. coli* have shown that cerulenin binds irreversibly to the β-ketoacyl-[acyl-carrier-protein] synthetase (Vance et al., 1972; D'Agnolo et al., 1973), and a similar interaction has been proposed to occur in several other organisms (Vance et al., 1972). The ability of cerulenin to inhibit β-ketoacyl thioester synthetases in general suggests that synthetases from a wide variety of organisms possess structural similarities in their active sites.

The β-ketoacyl-ACP[1] synthetases have been proposed as the rate-limiting step in fatty acid synthesis by *E. coli* (Vance, 1976). Since the initial studies regarding cerulenin and fatty acid synthesis in *E. coli*, the presence of two β-ketoacyl-ACP synthetases in this organism have been demonstrated (D'Agnolo et al., 1975). Mutants deficient in the originally described synthetase (β-ketoacyl-ACP synthetase I) are unable to synthesize unsaturated fatty acids (D'Agnolo et al., 1975). In contrast, mutants in which the newly described synthetase (β-ketoacyl-ACP synthetase II) is defective do not elongate palmitoeoyl-ACP to *cis*-vaccenoyl-ACP, and produce low amounts of saturated fatty acids in vitro (Gelmann and Cronan, 1972). This suggested that in vivo the two β-ketoacyl-ACP synthetases may catalyze the elongation of different acyl chains.

During our studies on fatty acid synthesis in *E. coli*, we obtained evidence which suggested that cerulenin inhibits the synthesis of saturated and unsaturated fatty acids disproportionately. These studies were extended to further evaluate the effect of cerulenin on fatty acid synthesis in *E. coli*. Our results demonstrate that in *E. coli* cerulenin preferentially inhibits unsaturated fatty acid synthesis; low concentrations of the antibiotic do not inhibit saturated fatty acid synthesis either in vivo or in vitro. Using mutant strains, we have found that β-ketoacyl-ACP synthetase I is extremely sensitive to cerulenin, while the β-ketoacyl-ACP synthetase II is relatively resistant to this antibiotic.

Experimental Procedure

Bacterial Strains. The *E. coli* strains used in this study are all K12 derivatives. Strain CSH2 (*thi*$^-$, *trp*$^-$, *lac*$^-$) was obtained from a strain kit from Cold Spring Harbor Laboratory (Cold Spring Harbor, N.Y.). Strain TB4 is a *fadE*$^-$ derivative of strain CSH2 and has been described (Buttke and Ingram, 1978). Strain K1060 (*fabB*$^-$, *fadE*$^-$) was obtained from the *E. coli* Genetic Stock Center, and strain WN1 (*cvc*$^-$) was obtained from J. E. Cronan, Jr. (Yale University, New Haven, Conn.).

Growth Conditions. All bacterial strains were grown at 37 °C in a gyrotory shaker. The minimal medium used was M63 mineral salts (Miller, 1972) supplemented with glycerol (0.4%), thiamin (0.001%), and yeast extract (0.005%). When necessary, the medium was further supplemented with tryptophan (0.02%), Brij 58 (0.04%), and oleic acid (0.01%). Cell growth was monitored by measuring optical density at 550 nm using either a Spectronic 70 spectrophotometer or a Beckman Model 25 spectrophotometer.

For in vitro fatty acid synthesis studies, cells were grown in medium 63 mineral salts supplemented with glucose (0.2%), thiamin (0.001%), tryptophan (0.02%), and casamino acids (vitamin free; 0.1%). Cells were harvested when they had reached the late log phase (0.70 absorbance unit at 550 nm). When strain K1060 was grown for in vitro fatty acid synthesis studies, succinate (0.2%) was substituted for glucose.

Preparation of Fatty Acid Synthetase Enzymes. Late log phase cells were harvested by centrifugation and washed once with 10 mM phosphate buffer, disrupted by passage through a French pressure cell (12 500 psi) and centrifuged at 5000 rpm for 10 min to remove unbroken cells. The supernatant

† From the Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida 32611. *Received June 12, 1978.* Florida Agricultural Experiment Station Publication 1248. This investigation was supported by Grant 1 RO1 GM 24059-01 from the National Institutes of Health.

‡ Present address: James Bryant Conant Laboratories, Harvard University, Cambridge, Mass. 02138.

[1] Abbreviations used: 12:0, lauric acid; 14:0, myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; Δ17, *cis*-9,10-methylenehexadecanoic acid; 17:1, *cis*-9-hepadecenoic acid; 18:1, *cis*-vaccenic acid; ACP, acyl carrier protein; Mops, 4-morpholinepropanesulfonic acid.

0006-2960/78/0417-5282$01.00/0

contained the fatty acid synthesis enzymes (Lennarz et al., 1962). The crude enzyme preparation was centrifuged at 100 000*g* for 2 h to pellet the membranous material. The supernatant which contained the fatty acid synthesis enzymes after this step was further purified by ammonium sulfate fractionation as described by Lennarz et al. (1962). Ammonium sulfate was added to the supernatant to 55% saturation, and the precipitated protein was removed by centrifuging at 10 000 rpm for 10 min. The supernatant was brought to 75% saturation with additional ammonium sulfate, and the precipitated fatty acid synthetase enzymes were harvested by centrifugation. The pellet was resuspended in 10 mM phosphate buffer (pH 7.0) containing 1 mM dithiothreitol and frozen at −20 °C in 0.2-mL aliquots.

Analysis of Fatty Acids by Gas Chromatography. Cells were harvested by centrifugation and washed twice with media containing 0.04% Brij 58 and twice with media without detergent. The washed cells were extracted into chloroform–methanol (2:1) as described by Kanfer and Kennedy (1963). Extracted lipids were transesterified using 2% H_2SO_4 in methanol, as described by Silbert et al. (1973). The methyl esters were extracted into pentane and concentrated under N_2 prior to analysis. The conditions for gas chromatography have been described (Ingram, 1976).

In Vitro Fatty Acid Synthesis Assay. The conditions for assaying fatty acid synthesis in vitro were essentially as described by Silbert (1976), except that the phosphate buffer was replaced by Mops buffer (pH 7.1) and $NADH_2$ (100 nmol) replaced the NAD, ethanol, and alcohol dehydrogenase. Reactions were stopped and labeled fatty acids extracted by the procedure of Gelmann and Cronan (1972). After transesterification, an aliquot of the combined pentane extracts was removed to measure total lipid synthesis. The labeled methyl esters were separated into saturated, unsaturated, and polar fatty acids by argentation chromatography.

In Vivo Fatty Acid Synthesis Assay. The incorporation of [^{14}C]acetate into fatty acids was assayed as described previously (Buttke and Ingram, 1978). The radioactive lipids were tranesterified as described above, and the methyl esters were analyzed by argentation chromatography.

Argentation Chromatography. Thin-layer chromatography plates coated with silica gel G were impregnated with 10% aqueous silver nitrate and activated as described by Cubero and Mangold (1965). The labeled methyl esters were applied to the argentation plate in pentane and developed twice in toluene at −17 °C (Morris et al., 1967). Radioactive regions were identified by autoradiography using Kodak X-OMAT R film, scraped into vials, and counted.

Reversed-Phase Chromatography. The chain lengths of the saturated fatty acids synthesized in vivo were determined by reversed-phase chromatography (Bergelson et al., 1964). Regions on the argentation plates corresponding to saturated fatty acids were scraped and the methyl esters were eluted with pentane. The methyl esters were applied in pentane to silica gel G plates impregnated with dodecane. Impregnation was achieved by ascending in petroleum ether containing 10% dodecane. The solvent system used for development consisted of acetone–acetonitrile (1:1) saturated with dodecane (Bergelson et al., 1964). Radioactive regions were located by autoradiography.

Chemicals. Heptadecenoic acid was purchased from Nu Chek Prep, Inc. (Elysian, Minn.). [1-^{14}C]Acetate was the product of the Amersham Corp. (Arlington Heights, Ill.). [2-^{14}C]Malonyl-CoA was purchased from New England Nuclear. Unlabeled malonyl-CoA, acetyl-CoA, NADH, NADP, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, and dodecane were the products of the Sigma Chemical Co. Acyl carrier protein (ACP) was purified by the procedure of Majerus et al. (1969) from 1 kg of *E. coli* K12 (Grain Processing Co., Muscatine, Iowa). Cerulenin was purchased from Makor Chemicals Ltd. (Jerusalem, Israel).

TABLE I: Effect of Cerulenin on Fatty Acid Synthesis in Vivo.[a]

cerulenin (mg/L)	fatty acids synth (cpm)		
	sat.	unsat.	total
0	4 400	9 400	13 800
5	22 000	5 200	27 200
10	23 000	3 600	26 600
20	21 500	1 800	23 300
25	7 900	500	8 400
35	2 100	100	2 200
45	1 600	100	1 700
50	1 700	200	1 900
100	400	200	600

[a] Strain CSH2 was grown in 37 °C in the absence of cerulenin to an optical density of 0.3 absorbance unit at 550 nm. The culture was split and aliquots were added to flasks containing various concentrations of cerulenin. Cultures were incubated at 37 °C for 60 min, at which time 1-mL samples were removed from each flask and added to tubes containing 10 μCi of [1-^{14}C]acetate (61 mCi/mmol). Samples were pulsed for 10 min. Fatty acids were separated using argentation chromatography.

Results

Effects of Cerulenin on Fatty Acid Synthesis in Vivo. The effects of cerulenin on fatty acid synthesis in cells pulse labeled with [^{14}C]acetate are demonstrated in Table I. In agreement with previous reports (Vance et al., 1972; Goldberg et al., 1973), we observed a dose-dependent inhibition of fatty acid synthesis by cerulenin. This inhibition of bulk fatty acid synthesis resulted from a marked preferential inhibition of unsaturated fatty acid synthesis. In the presence of low concentrations of cerulenin (5–20 mg/L), the inhibition of unsaturated fatty acid synthesis was accompanied by an increased synthesis of saturated fatty acids. Separation of [^{14}C]acetate-labeled saturated fatty acids by reversed-phased chromatography demonstrated that the increased amount of saturated fatty acids was due to an increase in myristic acid with no change in the amount of palmitic acid synthesized (data not shown). Even at the highest concentrations of cerulenin, unsaturated fatty acid synthesis was preferentially inhibited.

Table II shows the effects of the antibiotic on [^{14}C]acetate incorporation at various times after the addition of cerulenin. Within 10 min of adding the antibiotic, there is a significant inhibition of unsaturated fatty acid synthesis coupled with a stimulation of saturated fatty acid synthesis. After 15 min in the presence of cerulenin, the inhibition of unsaturated fatty acid synthesis was complete, while saturated fatty acid synthesis was only inhibited 40–50%. Although total fatty acid synthesis was 96% arrested by 100 mg/L cerulenin after 60 min, again the preferential inhibition of unsaturated fatty acid synthesis was maintained. These results indicate that, in vivo, cerulenin preferentially inhibits unsaturated fatty acid synthesis in *E. coli*.

Effects of Cerulenin on Fatty Acid Synthesis in Vitro. Cerulenin has been shown to inhibit fatty acid synthesis in vitro (Vance et al., 1972; D'Agnolo et al., 1973). In agreement with previously described results, we observed a 60% inhibition of fatty acid synthesis in vitro by 10 mg/L cerulenin (Table III). However, an analysis of the acyl products formed indicates that this inhibition results primarily from a block in unsaturated fatty acid synthesis (Table III). At the highest concentration

TABLE II: Time Course of Inhibition of Fatty Acid Synthesis in Vivo by Cerulenin.[a]

time (min)[b]	cerulenin (mg/L) 0 sat.	0 unsat.	50 sat.	50 unsat.	100 sat.	100 unsat.
0	5 700	16 000	37 000	11 500	14 400	6 800
15	9 000	21 000	5 600	200	4 500	200
30	13 200	31 000	3 000	100	1 900	100
60	13 600	34 000	1 700	100	400	100

[a] Strain CSH2 was grown at 37 °C to an optical density of 0.30 absorbance unit at 550 nm. The culture was split and aliquots were added to flasks containing different concentrations of cerulenin. At various times after cerulenin addition, samples were removed from the cultures and added to tubes with 10 μCi of [1-^{14}C]acetate and incubated at 37 °C for 10 min. Fatty acids were separated by argentation chromatography. [b] Time after cerulenin addition.

TABLE III: Effect of Cerulenin on Fatty Acid Synthesis in Vitro.[a]

cerulenin (mg/L)	fatty acids synth (cpm) sat.	unsat.	polar	total
0	22 900	53 000	16 700	92 600
1	39 500	28 600	15 500	83 600
2	38 000	18 000	11 600	67 600
10	22 900	3 300	5 500	31 700

[a] Fatty acid synthesis was measured by [2-^{14}C]malonyl-CoA incorporation as described under Experimental Procedure. Fatty acid synthesis enzymes were isolated from strain CSH2 and added to tubes containing various concentrations of cerulenin. Assays were performed at 37 °C for 20 min. Fatty acids were separated by argentation chromatography.

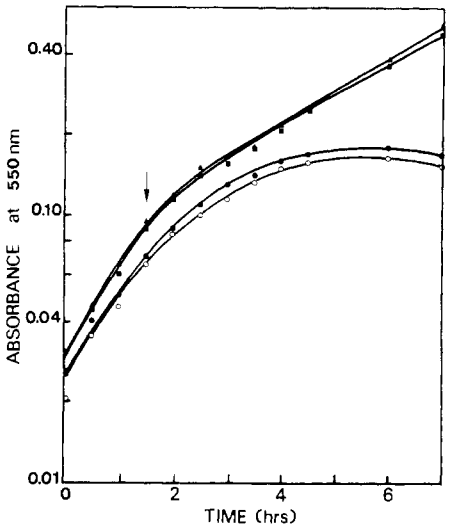

FIGURE 1: Growth of *E. coli* in the presence of cerulenin and exogenous fatty acid supplements. Strain CSH2 was grown in minimal media in the presence of various fatty acid supplements. At the time indicated (arrow), cerulenin was added to a final concentration of 25 mg/L. Growth was monitored by measuring the absorbance at 550 nm. Symbols: (○) no fatty acid supplements, (●) palmitic acid, (■) heptadecenoic acid, (▲) oleic acid.

of cerulenin (10 mg/L), there was a 90% inhibition of unsaturated fatty acid synthesis with no effect on saturated fatty acid synthesis. The inhibition of unsaturated fatty acid synthesis was accompanied by a decrease in polar fatty acids. Since these polar fatty acids are presumed to be the hydroxy intermediates of fatty acid synthesis (Silbert, 1975), the absence of large amounts of these intermediates suggests that they were used for saturated fatty acid synthesis (See Discussion). These results in vitro further demonstrate that cerulenin preferentially inhibits unsaturated fatty acid synthesis in *E. coli*.

Growth of E. coli in the Presence of Cerulenin and Exogenous Fatty Acids. Previous workers have shown that in the presence of high concentrations of cerulenin the growth of *E. coli* can be sustained by the addition of both exogenous saturated and unsaturated fatty acids (Goldberg et al., 1973). Based upon our pulse-labeling studies, unsaturated fatty acid supplements alone should relieve the growth inhibition caused by low concentrations of cerulenin. To examine this possibility, *E. coli* was grown in the presence of cerulenin (25 mg/L) and the cultures were supplemented with either saturated or unsaturated fatty acids. The results of this experiment are presented in Figure 1. In the absence of any exogenous fatty acids, the addition of cerulenin (25 mg/L) inhibited the growth of *E. coli* after 1 h and completely eliminated growth after 3 h. This inhibition of growth could not be overcome by the addition of exogenous palmitic acid (Figure 1). However, in the presence of either heptadecenoic acid (17:1) or oleic acid (18:1), *E. coli* could continue to grow in the presence of cerulenin. These results provide further evidence that low concentrations of cerulenin lead to a specific inhibition of unsaturated fatty acid synthesis.

E. coli does not normally synthesize heptadecenoic acid. Therefore, this fatty acid can be used to distinguish unsaturated fatty acids derived exogenously from those synthesized endogenously; 16:1, 17:1, and 18:1 can be easily resolved by gas chromatography.[2] The fatty acid compositions of cells grown with exogenous 17:1 in the presence and absence of cerulenin (10 mg/L) are shown in Table IV. In the absence of cerulenin, *E. coli* contained large amounts of unsaturated fatty acids derived from biosynthesis (16:1 and 18:1) as well as from exogenous supplements (17:1; Table IV). When low concentrations of cerulenin were added to the culture, the amount of unsaturated fatty acids derived from biosynthesis was signif-

[2] In the absence of fatty acid supplements, *E. coli* synthesizes low amounts of *cis*-9,10-methylenehexadecanoic acid which has a retention time by gas chromatography identical with that of heptadecenoic acid. However, these two fatty acids can be distinguished by separation of the methyl esters using argentation chromatography prior to analysis by gas chromatography. The *cis*-9,10-methylenehexadecanoic acid migrates with saturated fatty acids in argentation chromatography.

TABLE IV: Effect of Cerulenin on Bulk Lipid Composition.[a]

cerulenin (mg/L)	suppl	fatty acid composition 12:0	14:0	?	16:0	16:1	Δ17[b]	17:1	18:1
0	none		1.6	1.5	41.6	32.2	3.6		19.6
0	17:1	1.5			27.4	24.6	trace	20.5	25.9
10	17:1		12.9	1.6	30.6	1.6	trace	50.4	2.8

[a] Strain TB4 was grown in the presence and absence of 17:1 and cerulenin. Cells were harvested by centrifugation and washed to remove free fatty acids. Fatty acids were analyzed by gas chromatography and are expressed as the percentage of total fatty acids. [b] In duplicate samples, Δ17 and 17:1 were resolved by argentation chromatography of the methyl esters prior to quantitation by gas chromatography.

icantly reduced (Table IV). In contrast, there was a marked increase in the relative amount of exogenous unsaturated fatty acid (17:1) incorporated. Associated with the inhibition of unsaturated fatty acid synthesis, we observed an increase in the amount of myristic acid (14:0), analogous to the increase observed in [^{14}C]acetate pulse-labeling studies. A reduction in the concentration of cerulenin did not eliminate this increase in 14:0. Over a concentration range of 10–25 mg/L, the fatty acid composition of cells supplemented with 17:1 remained approximately the same.

Effect of Cerulenin on Mutants with Altered β-Ketoacyl-ACP Synthetases. Cerulenin has been shown to inhibit fatty acid synthesis in *E. coli* by binding irreversibly to the β-ketoacyl-ACP synthetase (Vance et al., 1972; D'Agnolo et al., 1973). However, more recent studies have demonstrated that *E. coli* contains two such synthetases (D'Agnolo et al., 1975) and in vivo these may be responsible for elongating different acyl intermediates.

Strain K1060 is an unsaturated fatty acid auxotroph deficient in β-ketoacyl-ACP synthetase I activity (D'Agnolo et al., 1975). Strain WN1 is a fatty acid mutant with a defective β-ketoacyl-ACP synthetase II (Gelmann and Cronan, 1972). In vivo, strain WN1 is defective in the elongation of 16:1 to 18:1; in vitro, this strain is deficient in both 18:1 synthesis and in the synthesis of saturated fatty acids. Both strains were examined for their sensitivity to low concentrations of cerulenin in vivo and in vitro. Table V demonstrates the effects of cerulenin on total fatty acid synthesis in these organisms. Strain K1060 was more resistant to cerulenin both in vivo and in vitro than the wild-type organism, strain CSH2. However, strain WN1 was as sensitive to cerulenin in vivo as strain CSH2 and even more sensitive to the antibiotic in vitro (Table V). These results implicate the β-ketoacyl-ACP synthetase I as the primary site of inhibition by cerulenin in vivo and in vitro, blocking unsaturated fatty acid synthesis.

TABLE V: Effect of Cerulenin on Fatty Acid Synthesis in Mutants Deficient in β-Ketoacyl-ACP Synthetase I or II.

strain	synthetase present	cerulenin (mg/L)	fatty acid synth (cpm) in vivo[a]	in vitro[b]
CSH2	I & II	0	13 800	43 700
		2		33 000
		25	8 400	
K1060	II	0	39 700	42 800
		2		45 700
		25	40 000	
WN1	I	0	70 500	58 700
		2		5 800
		25	40 600	

[a] The strains were grown at 37 °C in the absence of cerulenin to an optical density of 0.03 absorbance unit at 550 nm. The cultures were split and aliquots were added to flasks containing cerulenin, and incubation was continued. After 60 min, samples were removed and added to tubes containing 10 μCi of [^{14}C]acetate and pulsed for 10 min. Fatty acids were separated by argentation chromatography. [b] Fatty acid synthesis in vitro was measured by the incorporation of [^{14}C]malonyl-CoA as described under Experimental Procedure. Fatty acid synthesis enzymes were isolated from each of the strains and added to assay mixtures in the presence and absence of cerulenin. Assay mixtures were incubated at 37 °C for 20 min. Fatty acids were separated by argentation chromatography.

Discussion

Previous studies regarding the effects of cerulenin on fatty acid synthesis in *E. coli* have been concerned with the effects of the antibiotic on total fatty acid synthesis (Vance et al., 1972; D'Agnolo et al., 1973; Goldberg et al., 1973). In contrast, the experiments described in this paper were designed to examine the effects of cerulenin on saturated vs. unsaturated fatty acid synthesis. Based on the results of these experiments, we conclude that in *E. coli*, cerulenin preferentially inhibits unsaturated fatty acid synthesis. This effect was demonstrated in vivo using [^{14}C]acetate (Tables I and II) and in vitro using [^{14}C]malonyl-CoA (Table III). The growth inhibitory effects of cerulenin in vivo were overcome by providing exogenous unsaturated fatty acids alone (Table IV and Figure 1). The incorporation of externally supplied unsaturated fatty acids in place of normally synthesized fatty acids was confirmed by gas chromatography in cerulenin-inhibited cells (Table IV). The inability to sustain growth with palmitic acid indicates that the cerulenin was not being inactivated by an association with the exogenous fatty acids.

Experiments described in this paper utilizing mutants deficient in one or the other synthetases indicate that cerulenin preferentially inhibits the synthetase responsible for unsaturated fatty acid synthesis, β-ketoacyl-ACP synthetase I. Mutants deficient in this enzyme were more resistant to cerulenin than the wild-type organism. However, mutants which depend primarily on synthetase I for acyl chain elongation were at least as sensitive to the antibiotic as the wild type in vivo and even more sensitive in vitro (Table V). Since both β-ketoacyl-ACP synthetases are capable of elongating saturated as well as unsaturated fatty acid intermediates in vitro (D'Agnolo et al., 1975), it is not readily apparent how this selective inhibition can be occurring. However, results obtained with mutants deficient in either of these enzymes would seem to indicate that in vivo these enzymes may catalyze specific condensations (Gelmann and Cronan, 1972; D'Agnolo et al., 1975). Our results with cerulenin provide additional evidence for this proposal. The inhibition of both saturated and unsaturated fatty acid synthesis by high concentrations of cerulenin probably results from the inhibition of both synthetase enzymes.

In cerulenin-inhibited cells, we also observed an increased amount of myristic acid (14:0). Pulse-labeling studies indicated that the increase in 14:0 is due to an increased amount of saturated fatty acid synthesis and did not reflect an inhibition of

the elongation of 14:0 to 16:0. Apparently, the inhibition of unsaturated fatty acid synthesis by cerulenin permits an increased amount of acyl intermediates to be utilized for saturated fatty acid synthesis. This is not totally unexpected in view of the pathway for fatty acid biosynthesis in *E. coli*. In this organism, the intermediates leading to saturated and unsaturated fatty acids are identical up to and including β-hydroxydecanoyl-ACP (Volpe and Vagelos, 1976). The dehydration of this intermediate by the β-hydroxydecanoyl-ACP dehydrase yields both *cis*-β,γ-decenoyl-ACP and *trans*-α,β-decenoyl-ACP (Rando and Bloch, 1968). Only the former intermediate is utilized for unsaturated fatty acid synthesis, while the latter is used for saturated fatty acid synthesis. The β-hydroxydecanoyl-ACP dehydrase also exhibits isomerase activity (Rando and Bloch, 1968). Thus, an inhibition of *cis*-β,γ-decenoyl-ACP utilization may result in the isomerization of this intermediate to *trans*-α,β-decenoyl-ACP, which would provide increased amounts of substrate for saturated fatty acid synthesis. This is consistent with the lack of accumulation of polar intermediates.

The presence of two condensing enzymes with different susceptibilities to cerulenin in a single organism is not unprecedented. In *Mycobacterium*, safflower seed extracts, and barley chloroplasts (Vance et al., 1972; Jaworski et al., 1974), the presence of cerulenin-resistant elongating enzymes has been demonstrated. These elongating enzymes are responsible for specifically elongating palmitoyl thioesters to stearoyl thioesters via a reaction analogous to the condensing reactions catalyzed by the β-ketoacyl-ACP synthetases (Vance et al., 1972; Jaworski et al., 1974). Although *E. coli* does not possess such an elongating system per se, the β-ketoacyl-ACP synthetase II in this organism may represent a primitive elongating system. As organisms developed oxygen-dependent mechanisms for desaturating fatty acyl chains, these organisms also lost the ability to synthesize unsaturated fatty acids anaerobically (Bloch et al., 1961). Thus, in contrast to *E. coli*, which depends upon an anaerobic desaturating system (Kass and Bloch, 1967), more highly evolved organisms synthesize palmitic acid as the single product of fatty acid synthesis (Volpe and Vagelos, 1976). Such a reduction in the different species of fatty acids synthesized would be expected to eliminate the need for two distinct β-ketoacyl thioester synthetases, while creating a requirement for an enzyme capable of elongating 16:0 to 18:0 prior to aerobic desaturation. This may explain the results of Awaya et al. (1975), who reported that the growth of *Saccharomyces* could be sustained in the presence of cerulenin by providing either a short-chained saturated fatty acid or a long-chained unsaturated fatty acid. The shorter chained fatty acids could be elongated to 16:0 and 18:0 prior to desaturation; desaturase activity is not inhibited by cerulenin (Ōmura, 1976). Long-chained unsaturated fatty acids could be utilized directly. If our proposal regarding the evolutionary development of elongating enzymes from an enzyme similar to the β-ketoacyl-ACP synthetase II of *E. coli* is correct, then structural similarities should exist between the binding sites of the β-ketoacyl-ACP synthetase II of *E. coli* and the elongating enzymes present in other organisms.

Note Added in Proof

After submission of this manuscript, Rottem et al. reported their results with cerulenin and fatty acid synthesis in *Proteus mirabilis*. In their analysis of the bulk fatty acid composition of cerulenin-grown cells, these authors observed an increase in myristic acid similar to the increase we observed in *E. coli*. It is possible that the mode of action of cerulenin in *P. mirabilis* is similar to its action in *E. coli*.

References

Awaya, J., Ohno, T., Ohno, H., and Ōmura, S. (1975), *Biochim. Biophys. Acta 409*, 267–273.

Bergelson, L. D., Dyatlovitskaya, E. V., and Voronkova, V. V. (1964), *J. Chromatogr. 15*, 191–199.

Bloch, K., Baronowsky, P., Goldfine, H., Lennarz, W. J., Light, R., Norris, A. T., and Scheuerbrandt, G. (1961), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 20*, 921–927.

Buttke, T. M., and Ingram, L. O. (1978), *Biochemistry 17*, 637–644.

Cubero, J. M., and Mangold, H. K. (1965), *Microchem. J. 9*, 227–236.

D'Agnolo, G., Rosengeld, I. S., Awaya, J., Ōmura, S., and Vagelos, P. R. (1973), *Biochim. Biophys. Acta 326*, 155–166.

D'Agnolo, G., Rosengeld, I. S., and Vagelos, P. R. (1975), *J. Biol. Chem. 250*, 5289–5294.

Gelmann, E. P., and Cronan, J. E., Jr. (1972), *J. Bacteriol. 112*, 381–387.

Goldberg, I., Walker, J. R., and Bloch, K. (1973), *Antimicrob. Agents Chemother. 3*, 549–554.

Ingram, L. O. (1976), *J. Bacteriol. 125*, 670–678.

Jaworski, J. G., Goldschmidt, E. E., and Stumpf, P. K. (1974), *Arch. Biochem. Biophys. 163*, 769–776.

Kanfer, J., and Kennedy, E. P. (1963), *J. Biol. Chem. 238*, 2919–2922.

Kass, L. R., and Bloch, K. (1967), *Proc. Natl. Acad. Sci. U.S.A. 58*, 1168–1173.

Lennarz, W. J., Light, R. J., and Bloch, K. (1962), *Proc. Natl. Acad. Sci. U.S.A. 48*, 840–845.

Majerus, P. W., Alberts, A. W., and Vagelos, P. R. (1969), *Methods Enzymol. 14*, 43–53.

Miller, J. H. (1972) *Experiments in Molecular Genetics*, pp 230–234, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Morris, L. J., Wharry, D. M., and Hammond, E. W. (1967), *J. Chromatogr. 31*, 69–76.

Ōmura, S. (1976), *Bacteriol. Rev. 40*, 681–697.

Rando, R. R., and Bloch, K. (1968), *J. Biol. Chem. 243*, 5627–5634.

Rottem, S., Markowitz, O., and Razin, S. (1978), *Eur. J. Biochem. 85*, 451–456.

Silbert, D. F. (1976) *Methods Membr. Biol. 6*, 151–182.

Silbert, D. F., Ulbright, T. M., and Honeggar, J. L. (1973), *Biochemistry 12*, 164–171.

Vance, D. E. (1976), *J. Theor. Biol. 59*, 409–413.

Vance, D., Goldberg, I., Mitsuhashi, O., Bloch, K., Ōmura, S., and Nomura, S. (1972), *Biochem. Biophys. Res. Commun. 48*, 649–656.

Volpe, J. J., and Vagelos, P. R. (1976), *Phys. Rev. 56*, 339–417.