Fatty Acid Synthesis in *Escherichia coli* Is Indirectly Inhibited by Phenethyl Alcohol†

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ABSTRACT: Experiments were performed to determine how phenethyl alcohol inhibits phospholipid synthesis in *E. coli*. At a nonbacteriostatic concentration, the drug reduces the rate of de novo fatty acid and phospholipid synthesis by 60 to 70%. The inhibition of fatty acid synthesis was found to be a secondary consequence of the inhibition of phospholipid synthesis. Phenethyl alcohol reduces the rate of incorporation of exogenous fatty acids into the phospholipids of a fatty acid auxotroph by 60%. These results indicate that this drug controls phospholipid synthesis beyond the level of fatty acid synthesis. Phenethyl alcohol inhibits the synthesis of phospholipids containing saturated fatty acids to a greater extent than it does the synthesis of phospholipids containing unsaturated fatty acids. It controls the synthesis of phospholipids containing saturated fatty acids at both the level of fatty acid synthesis and the level of incorporation of the saturated fatty acids into phospholipids. The synthesis of phospholipids containing unsaturated fatty acids is inhibited at the level of incorporation of the fatty acids into phospholipids.

Several laboratories have examined the effect of phenethyl alcohol on membrane phospholipid synthesis in *Escherichia coli* (Barbu et al., 1970; Nunn and Tropp, 1972; Tunaitis and Cronan, 1973; Nunn, 1975). These studies were of interest because other workers had shown that this drug causes alterations in the structure of the cell membrane (Lester, 1965; Silver and Wendt, 1967; Yura and Wada, 1968). Silver and Wendt (1967) suggested that the primary action of phenethyl alcohol is at the level of the cell membrane with resultant breakdown of the cellular permeability barriers. In studies to determine the effect of this drug on the lipid component of the cell membrane, we found that phospholipid synthesis is more sensitive to inhibition by low concentrations of phenethyl alcohol than is DNA, RNA, and protein synthesis (Nunn and Tropp, 1972). In addition, we have shown that the inhibition of phospholipid synthesis by this drug is not a secondary effect resulting from the perturbation of these cellular processes (Nunn and Tropp, 1972).

Recently we have shown that nonbacteriostatic concentrations of phenethyl alcohol drastically alter the phospholipid composition of the cell membrane (Nunn, 1975). The changes in phospholipid composition are due to the inhibitory effect of phenethyl alcohol on the de novo rates of synthesis of phosphatidylethanolamine and phosphatidylglycerol (Nunn, 1975). This drug also alters the fatty acid composition of membrane phospholipids by differentially inhibiting the rates of synthesis of saturated and unsaturated fatty acids (Nunn, 1975).

To date our studies (Nunn and Tropp, 1972; Nunn, 1975) indicate that the de novo rate of fatty acid synthesis decreases concomitantly with phospholipid synthesis when cells are treated with phenethyl alcohol. Several interpretations from these studies are possible: (i) phenethyl alcohol controls phospholipid synthesis by inhibiting the synthesis of fatty acids; (ii) phenethyl alcohol controls lipid synthesis at the levels of both fatty acid synthesis and phospholipid synthesis; and/or (iii) phenethyl alcohol inhibits de novo fatty acid synthesis as a secondary consequence of inhibiting phospholipid synthesis. The latter interpretation is quite feasible because fatty acid synthesis is tightly coupled to phospholipid synthesis in *E. coli* and virtually all of the fatty acids synthesized are esterified to phospholipids (Cronan, 1974). In our earlier studies, we showed that fatty acid supplementation of phenethyl alcohol treated cells does not reverse the inhibitory effect of this drug on phospholipid synthesis (Nunn, 1975). This result indicates that phenethyl alcohol does not control phospholipid synthesis by solely inhibiting the synthesis of fatty acids. However, this result does not indicate whether this drug inhibits fatty acid synthesis directly or indirectly.

In an effort to understand how phenethyl alcohol inhibits lipid synthesis, we decided to test if this drug controls phospholipid synthesis by directly inhibiting fatty acid synthesis and/or fatty acid incorporation into phospholipids. In this paper, we present evidence that suggests that phenethyl alcohol inhibition of phospholipid synthesis results in the secondary inhibition of overall fatty acid synthesis. However, when we tested the effect of this drug on the synthesis of saturated and unsaturated fatty acids, we found that it directly inhibits saturated fatty acid synthesis. These results, coupled with experiments showing that this drug inhibits the incorporation of fatty acids (both saturated and unsaturated) into phospholipids, suggest that phenethyl alcohol controls phospholipid synthesis at the level of saturated fatty acid synthesis and at the level of phosphatidic acid synthesis.

Experimental Procedure

Bacterial Strains. Bacterial strains used in this study are derivatives of *E. coli* K-12. Their derivations and complete genotypes have been described previously (Cronan et al., 1975; Nunn and Cronan, 1974b, 1976). Only the relevant genotypes are given here. Strain LW1 possesses defects in β oxidation (*fad*E$^-$)and in the utilization of endogenous *sn*-glycerol 3-phosphate (*pls*B). The defect in the *pls*B gene results in a *sn*-glycerol 3-phosphate acyltransferase with a K_m value for *sn*-glycerol 3-phosphate that is tenfold higher than normal

† From the Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717. *Received September 15, 1976.* This work was supported by a grant from the United States Public Health Service (GM 22466-1A), a grant from the California Division of the American Cancer Society (813), and a grant from the American Heart Association, Orange County Chapter.

(Bell, 1974; Cronan and Bell, 1974b). Since the endogenous supply in this strain is insufficient for normal phospholipid synthesis, it must be supplied with an exogeneous source of *sn*-glycerol 3-phosphate to allow growth (Bell, 1974). Strain LW3 is a *fad*E$^-$ *sn*-glycerol 3-phosphate auxotroph which owes its requirement to a lesion in the *gps*A$^-$ gene (Bell, 1974; Cronan et al., 1975). The *gps*A locus is the structural gene for the biosynthetic *sn*-glycerol 3-phosphate dehydrogenase of *E. coli* (Cronan and Bell, 1974a). Strains WN24 (*rel*$^+$) and WN25 (*rel*A) are derivatives of LW1 (Nunn and Cronan, 1976). Strain LA2-89 is a fatty acid auxotroph which requires both saturated and unsaturated fatty acids for growth at 37 °C (Semple and Silbert, 1975). Its phenotype is due to a thermolabile defect in malonyl-coenzyme A acyl carrier protein transacylase.

Bacteria were routinely grown in New Brunswick gyratory water bath shakers at 37 °C. The cultures were usually grown in standard medium consisting of medium E (Vogel and Bonner, 1956) supplemented with 0.4% sodium succinate and 0.05% casein hydrolysate (vitamin free). When appropriate, *sn*-glycerol 3-phosphate was added to the standard medium at a final concentration of 0.02%. Glycerol 3-phosphate starvation was accomplished by filtration and/or centrifugation as described previously (Nunn and Cronan, 1976). Cell growth was determined at 540 nm in a Klett-Summerson colorimeter (1 Klett unit is equivalent to 5×10^6 cells per mL). All experiments were initiated when the density of an exponentially growing culture reached 2.5 to 3.0×10^8 cells per mL.

Analysis of Radioactive Lipids. The incorporation of labeled acetate into lipid was assayed as previously described (Nunn and Cronan, 1974b). All analyses were done by thin-layer chromatography. Neutral lipids were separated from phospholipids as described by Cronan et al. (1975). In some cases, free fatty acids were separated from phospholipids by thin-layer chromatography with diethyl ether–acetic acid (100:1). The identification of various lipids was established by the simultaneous chromatography of known standards. The fatty acid composition was determined as previously described by Cronan et al. (1975).

Rate of Fatty Acid Synthesis. Samples (1 mL) of growing cultures were labeled for 5 min with 10 μCi of [1-^{14}C]acetate (57 mCi per mmol). After 5 min of incubation at 37 °C, 6 mL of chloroform–methanol (1:2, v/v) was added to the tube and the lipids were extracted as previously described (Nunn and Cronan, 1974a). The extracted phospholipids were converted to fatty acid methyl esters as previously described (Nunn and Cronan, 1974a). The radioactive fatty acid methyl esters were then analyzed by chromatography on 20% $AgNO_3$-impregnated silica gel thin-layer plates (Gelmann and Cronan, 1972). The plates were developed twice in toluene at −17 °C and autoradiographed, and the appropriate areas of silica gel were scraped into vials containing toluene scintillation solution (Gelmann and Cronan, 1972) and counted.

Determination of the Rate of Fatty Acid Incorporation into Phospholipids. Cultures of strain LA2-89 were grown at 37 °C in standard medium supplemented with glycerol (4 mg per mL), potassium oleate (50 μg per mL), potassium palmitate (50 μg per mL), potassium glutamate (5 mM), yeast extract (1 μg per mL), thiamine (0.05 μg per mL), and Brij 58 (4 mg per mL). Upon reaching a cell density of 2.5×10^8 cells per mL, the cultures were divided into two equal portions. Phenethyl alcohol at a final concentration of 8 mM was added to one portion. The other portion was left untreated. At various time intervals after the addition of phenethyl alcohol, a 1-mL aliquot was removed from each culture and placed in a tube containing 25 μCi of [9,10-^{3}H]oleic acid (139 μCi per μmol) and 0.8 μCi of [1-^{14}C]palmitate (4.4 μCi per μmol). After 5 min of incubation at 37 °C, 6 mL of chloroform–methanol (1:2 v/v) was added to each tube and the lipids were extracted as previously described (Nunn and Cronan, 1974a). The extracted lipids were then resolved into phospholipid and fatty acid fractions by thin-layer chromatography on silica gel plates developed in diethyl ether–acetic acid (100:1). The phospholipid fraction was counted in a scintillation counter adjusted for dual label counting. The reciprocal experiment using [1-^{14}C]oleate and [9,10-^{3}H]palmitate gave similar results.

Fatty Acid Synthetase Assay. Fatty acid synthetase was assayed according to the procedure of Gelmann and Cronan (1972).

Samples of cerulenin were kindly provided by Dr. John E. Cronan, Jr.

Results

Effect of Phenethyl Alcohol on Free Fatty Acid and Fatty Acyl Phospholipid Synthesis. In order to determine if phenethyl alcohol directly inhibits fatty acid synthesis per se, we have used an in vivo system in which fatty acid synthesis has been uncoupled from phospholipid synthesis by the specific inhibition of phospholipid synthesis (Cronan et al., 1975). Glycerol 3-phosphate starvation of *E. coli sn*-glycerol 3-phosphate auxotrophs results in a profound inhibition of phospholipid synthesis (Bell, 1974). If the strain is also defective in β oxidation (to prevent fatty acid degradation), the synthesis of fatty acids in a free (nonesterified) form begins following a short time lag. The rate of free fatty acid synthesis then proceeds at a steadily increasing rate until fatty acids are synthesized at the rate observed in *sn*-glycerol 3-phosphate supplemented cultures. Free fatty acid synthesis continues at this high rate for an extended period of time (Cronan et al., 1975). The free fatty acids have been shown to be the direct product of de novo synthesis (Cronan et al., 1975).

Our first experiment was performed with strain LW1. This strain possesses defects in β oxidation (*fad*E$^-$) and in the utilization of endogenous *sn*-glycerol 3-phosphate that is tenfold higher than normal (Bell, 1974; Cronan and Bell, 1974b). Since the endogenous supply in these strains is insufficient for normal phospholipid synthesis, they must be supplied with an exogenous source of *sn*-glycerol 3-phosphate to allow growth (Bell, 1974).

The results in Table I show that 8 mM phenethyl alcohol inhibits by 60 to 70% the rate of [^{14}C]acetate incorporation into the phospholipids of strain LW1 growing in *sn*-glycerol 3-phosphate supplemented medium. Since virtually all of the fatty acids synthesized in LW1 during *sn*-glycerol 3-phosphate supplementation are esterified to phospholipids (Cronan et al., 1975), these results indicate that phenethyl alcohol inhibits the synthesis of fatty acyl phospholipids.

We then examined the effect of phenethyl alcohol on lipid synthesis under conditions which uncouple fatty acid synthesis from phospholipid synthesis. These conditions (*sn*-glycerol 3-phosphate starvation) result in the synthesis of fatty acids as free (nonesterified) acids rather than as fatty acyl phospholipids and thus the effect of this drug on fatty acid synthesis per se can be determined. Under such conditions, the rate of [^{14}C]acetate incorporation into the fatty acids of phenethyl alcohol treated cells was reduced by less than 5% (Table I). Comparable results were obtained with another *sn*-glycerol 3-phosphate auxotroph, strain LW3 (Cronan et al., 1975), which owes its phenotype to a defective anabolic *sn*-glycerol 3-phosphate dehydrogenase (data not shown).

TABLE I: Effect of Phenethyl Alcohol on Esterified and Nonesterified (Free) Fatty Acid Synthesis in *E. coli* Strain LW1.[a]

Condition	Rate of Fatty Acid Synthesis (cpm per 10^7 cells)					
	Esterified			Nonesterified (Free)		
	Total	Saturated	Unsaturated	Total	Saturated	Unsaturated
Untreated	1620	840	780	1180	760	420
Phenethyl alcohol	540	190	340	1140	420	720

[a] See Experimental Procedures section for details. Data are presented for 30-min time periods.

TABLE II: Effect of Various Treatments on Esterified and Nonesterified (Free) Fatty Acid Synthesis.

Condition	Rate of Fatty Acid Synthesis (cpm per 10^7 cells)[d]			
	Esterified	%	Non-esterified (Free)	%
Untreated[a]	1606	100	1200	100
Phenethyl Alcohol[a]	516	32	1141	95
Cerulenin[b]	81	5	48	4
Amino acid starvation[c]				
rel^+	688	43	533	44
relA	1525	95	1104	92

[a] The growth conditions and experimental protocol were identical with those described in Figures 1a and 1b. [b] The growth conditions and experimental protocol were identical with that described in Figures 1a and 1b, except that cerulenin at a final concentration of 100 μg per mL was used instead of phenethyl alcohol. [c] Strains WN24 (rel^+) and WN25 (relA) were grown at 37 °C in standard medium supplemented with arginine (20 μg per mL) and 0.02% *sn*-glycerol 3-phosphate. The experimental procedure for measuring the rates of synthesis of fatty acyl phospholipids and free (unesterified) fatty acids during amino acid deprivation of *sn*-glycerol 3-phosphate starved and/or unstarved cultures of WN24 and WN25 was identical with that described by Nunn and Cronan (1976). [d] Data presented are for the 30-min time points.

TABLE III: Composition of Fatty Acid Fractions.[a]

Fatty Acid	+Glycerol 3-Phosphate (%)		−Glycerol 3-Phosphate (%)	
	−PEA	+PEA	−PEA	+PEA
Palmitoleic	13.6	10.9	2.3	1.8
cis-Vaccenic	37.1	51.7	27.8	45.8
cis-Eicosenoic	0.4	0.8	9.0	15.4
Lauric	0.5	0.5	0.3	0.4
Myristic	0.4	0.5	2.1	1.4
Palmitic	43.6	32.2	25.7	16.4
Stearic	4.0	3.0	24.6	14.8
Arachidic	0.4	0.4	5.9	2.3
Behenic	0.0	0.0	2.3	1.6
SFA	48.9	36.6	60.9	37.0
UFA	51.1	63.4	39.1	63.0
Ratio UFA/SFA	1.04	1.73	0.64	1.70

[a] See Experimental Procedure section for details. Data are presented for 30-min time points.

In another experiment, we tested the effect of phenethyl alcohol on fatty acid synthesis, in vitro. We found that the drug inhibits the incorporation of [2-^{14}C]malonyl CoA into fatty acids by less than 5% (data now shown). Since phenethyl alcohol reduces the in vivo synthesis of fatty acyl phospholipids by 60 to 70% (Table I), these in vitro results, in addition to the above in vivo results (Table I), suggest that the drug inhibits fatty acid synthesis as a consequence of inhibiting phospholipid synthesis.

The indirect inhibition of overall fatty acid (saturated plus unsaturated) synthesis by phenethyl alcohol appears to be different from other known modes of inhibiting fatty acid synthesis (Nunn and Cronan, 1976; Goldberg et al., 1973). For instance, cerulenin, a specific inhibitor of β-ketoacyl carrier protein synthetase (D'Angnolo et al., 1973), and thus of fatty acid synthesis (Goldberg et al., 1973), inhibits both free fatty acid and fatty acyl phospholipid synthesis by 95% (Table II). Since cerulenin's effect on fatty acyl phospholipd synthesis can be reversed by the addition of exogenous fatty acids (Goldberg et al., 1973), this drug, unlike phenethyl alcohol, directly inhibits fatty acid synthesis and as a secondary consequence inhibits phospholipid synthesis (Goldberg et al., 1973). Another means of inhibiting lipid synthesis, amino acid starvation of stringent bacteria (rel^+), results in the inhibition of both free fatty acid and fatty acyl phospholipid synthesis (Table II; Nunn and Cronan, 1976). Thus rel^+ control, unlike phenethyl alcohol, of phospholipid synthesis operates at two sites (Nunn and Cronan, 1976).

Effect of Phenethyl Alcohol on the Synthesis of Saturated and Unsaturated Fatty Acids. In our present and earlier work (Nunn, 1975), we observed that phenethyl alcohol reduced the saturated fatty acyl content of phospholipids to a greater extent than it reduced the unsaturated fatty acyl content of phospholipids (Table I). Although the results in Table I show that there is no significant direct effect of phenethyl alcohol on overall fatty acid synthesis, these studies do not indicate how this drug affects the synthesis of saturated and unsaturated fatty acid species. Therefore, we determined if the effect of phenethyl alcohol on the fatty acyl content of phospholipids (Table I) results from this drug's direct inhibition of saturated and/or unsaturated fatty acid synthesis. We found that the drug inhibits the rate of synthesis of phospholipids containing saturated fatty acids by 75 to 80% in *sn*-glycerol 3-phosphate supplemented cultures (Table I). The results in Table I show that it inhibits the rate of saturated fatty acid synthesis in *sn*-glycerol 3-phosphate starved cultures by approximately 45%. The latter results indicate that phenethyl alcohol directly inhibits saturated fatty acid synthesis. Although the rate of synthesis of phospholipids containing unsaturated fatty acids in *sn*-glycerol 3-phosphate supplemented cells was reduced 50 to 60% by phenethyl alcohol, the rate of free (unesterified) unsaturated fatty acid synthesis in *sn*-glycerol 3-phosphate starved cells increased in the presence of this drug (Table I). The results in Table III show the overall effect of this drug on the fatty acid composition of *sn*-glycerol 3-phosphate sup-

TABLE IV: Effect of PEA on the Incorporation of Fatty Acids into Phospholipids in *E. coli* Strain LA2-89.[a]

Conditions	Rate of Fatty Acid Incorp. into Phospholipids (pmol of fatty acid per min per 10^7 cells)		
	Total	Saturated	Unsaturated
Untreated	51	26	25
Phenethyl alcohol	23	11	12

[a] See Experimental Procedure section for details. Data are presented for 30-min time points.

plemented and starved cultures. Phenethyl alcohol treatment altered the unsaturated to saturated ratio of free (unesterified) fatty acids in *sn*-glycerol 3-phosphate starved cultures to the same extent as the unsaturated to saturated ratio of fatty acids in the phospholipids of *sn*-glycerol 3-phosphate supplemented cultures (Table III).

Effect of Phenethyl Alcohol on the Rate of Incorporation of Saturated and Unsaturated Fatty Acids. The results presented above indicate that the phenethyl alcohol inhibits the synthesis of phospholipids containing saturated fatty acids to a greater extent than it inhibits the synthesis of saturated fatty acids (Table I). In addition, the inhibition of the synthesis of phospholipids containing unsaturated fatty acids in phenethyl alcohol treated cells cannot be explained as being due to the direct inhibition of unsaturated fatty acid synthesis (Table I). These findings suggest that: (i) phenethyl alcohol inhibits the synthesis of phospholipids containing saturated fatty acids at two sites, at the level of fatty acid synthesis, per se, and at the level of saturated fatty acid incorporation into phospholipids; and that (ii) it inhibits the synthesis of phospholipids containing unsaturated fatty acids by inhibiting the incorporation of unsaturated fatty acids into phospholipids. To test these possibilities, we examined the effect of this drug on the incorporation of exogenously supplied fatty acids into phospholipids. For this experiment we used a strain deficient in the synthesis of both saturated and unsaturated fatty acids. The strain, LA2-89, synthesizes fatty acids at about one-fourth the normal rate (at 37 °C) due to the defect in malonyl transacylase (Semple and Silbert, 1975). Hence, this strain requires supplementation with both an unsaturated and saturated fatty acid to allow phospholipid synthesis and growth to proceed at 37 °C.

As shown in Table IV, phenethyl alcohol inhibits the rate of incorporation of total exogenous fatty acids (both saturated and unsaturated) into phospholipids by approximately 60%. The results in Table IV also show that this drug inhibits the rate of saturated and unsaturated fatty acid incorporation into phospholipids by 60 and 50%, respectively. The ratio of unsaturated to saturated fatty acids in the phospholipids of phenethyl alcohol treated cells was 1.04 as opposed to 0.92 in untreated cells. These experiments show that phenethyl alcohol inhibits the rate of synthesis of saturated and unsaturated fatty acyl phospholipids at the level of fatty acid incorporation into phospholipids.

Discussion

The studies presented in this paper were performed to determine how phenethyl alcohol controls phospholipid synthesis in *E. coli*. In our earlier studies (Nunn and Tropp, 1972; Nunn, 1975), we observed that a nonbacteriostatic concentration of phenethyl alcohol (8 mM) reduced concomitantly the de novo rate of fatty acid and phospholipid synthesis by two- to threefold. Thus, we decided to test if this drug controls phospholipid synthesis by directly inhibiting fatty acid synthesis. The experiments in this paper show that: (i) the de novo rate of fatty acid synthesis is reduced two- to threefold in phenethyl alcohol treated cells only when fatty acid synthesis is coupled to phospholipid synthesis (Table I). When fatty acid synthesis is uncoupled from phospholipid synthesis (Table I), the rate of fatty acid synthesis in phenethyl alcohol treated cells is only slightly inhibited (approximately 5%); (ii) the drug (8 mM) inhibits the in vitro activity of the fatty acid synthetase by less than 5%; and (iii) the drug reduces the synthesis of fatty acyl phospholipids by 60% in bacteria which depend on exogenous fatty acids for phospholipid synthesis (Table IV). These results indicate that this drug does not control phospholipid synthesis at the level of overall fatty acid synthesis. They also suggest that phenethyl alcohol inhibition of overall fatty acid synthesis is a secondary consequence of the drug's inhibitory effect on phospholipid synthesis.

The results showing that phenethyl alcohol directly inhibits saturated fatty acid synthesis (Table I) indicate that the synthesis of phospholipids containing saturated fatty acids is controlled by this drug at two sites, at the level of fatty acid synthesis and at the level of fatty acylation to *sn*-glycerol 3-phosphate. Since phenethyl alcohol does not affect the turnover of saturated and/or unsaturated fatty acids (Nunn, unpublished results), the experiments showing that this drug inhibits the synthesis of saturated fatty acids cannot be explained as being due to selective degradation. Studies in our laboratory are now being performed to determine if it inhibits saturated fatty acid synthesis at the level of the α-β-dehydrase.

As indicated in Table I, the inhibition of free saturated fatty acid synthesis was accompanied by a compensatory increase in the formation of free unsaturated fatty acids. These results can be explained by the fact that β-hydroxydecanoate is the last intermediate prior to the divergence of saturated and unsaturated pathways (Brock et al., 1967). Thus the inhibition of α-β-dehydrase would be expected to cause the observed diversion of β-hydroxydecanoate toward unsaturated long-chain product.

The following experiments suggest that the rate-limiting step(s) in phospholipid synthesis inhibited by phenethyl alcohol is at the level of the *sn*-glycerol 3-phosphate acyltransferase or beyond the acyltransferase step: (1) the synthesis of phospholipids was reduced 60 to 70% in phenethyl alcohol treated *sn*-glycerol 3-phosphate supplemented cells (Table I). This result indicates that the drug must have a site of action beyond *sn*-glycerol 3-phosphate synthesis. (2) Phenethyl alcohol reduces the rate of exogenous fatty acid incorporation into the phospholipids of a fatty acid auxotroph by 70% (Table IV). This result indicates that it inhibits phospholipid synthesis beyond the level of fatty acid synthesis. This result also suggests that this drug controls phospholipid synthesis at the level of *sn*-glycerol 3-phosphate acylation. In support of the latter conclusion, we have found that phenethyl alcohol inhibits by two- to threefold the activity of the *sn*-glycerol 3-phosphate acyltransferase with palmitoyl-CoA and/or palmitoyl-ACP as acyl donors (Nunn et al., 1977). The latter finding, coupled with in vitro studies (Nunn et al., 1977), showing that this drug does not inhibit several other phospholipid biosynthetic enzymes (e.g., CDP-diglyceride synthetase, acyl-CoA:lysophosphatidic acid acyltransferase, phosphatidylglycerol phosphate synthetase, and the phosphatidylserine synthetase), suggests that the primary site of action of phenethyl alcohol

on phospholipid synthesis may be at the level of the acyltransferase.

In conclusion, the inhibition of overall fatty acid synthesis by phenethyl alcohol appears to be mediated by this drug's inhibitory effect on phospholipid synthesis. Since phospholipid synthesis is not completely inhibited in a phenethyl alcohol treated cell (Table I), it is conceivable that fatty acid synthesis may be feedback inhibited by the accumulation of some phospholipid intermediate or product. At present we believe that the drug controls phospholipid synthesis by inhibiting the *sn*-glycerol 3-phosphate acyltransferase. We are currently attempting to isolate and characterize mutants resistant to phenethyl alcohol in hope that they will provide further information on the primary site of action. Another tantalizing possibility would be that some of the phenethyl alcohol resistant mutants will have high levels of the enzyme(s) that this drug affects. Such mutants may owe their phenotype to altered regulatory genes.

Acknowledgments

We thank Drs. Calvin McLaughlin and Dennis Fujii for the helpful discussions and comments on this manuscript.

References

Barbu, E., Polonovski, J., Rampini, C., and Lux, M. (1970), *C. R. Acad. Sci. 270,* 2596–2599.

Bell, R. M. (1974), *J. Bacteriol. 117,* 1065–1076.

Brock, D. J. H., Kass, L. R., and Bloch, K. (1967), *J. Biol. Chem. 242,* 4432.

Cronan, J. E., Jr. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 3758–3762.

Cronan, J. E., Jr. (1975), *J. Biol. Chem. 250,* 7075–7077.

Cronan, J. E., Jr., and Bell, R. M. (1974a), *J. Bacteriol. 118,* 598–605.

Cronan, J. E., Jr., and Bell, R. M. (1974b), *J. Bacteriol. 120,* 227–233.

Cronan, J. E., Jr., Weisberg, L., and Allen, R. G. (1975), *J. Biol. Chem. 250,* 5835–5840.

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.

Lester, G. (1965), *J. Bacteriol. 90,* 29–37.

Nunn, W. D. (1975), *Biochim. Biophys. Acta 348,* 63–75.

Nunn, W. D., and Cronan, J. E., Jr. (1974a), *J. Biol. Chem. 249,* 724–731.

Nunn, W. D., and Cronan, J. E., Jr. (1974b), *J. Biol. Chem. 249,* 3994–3996.

Nunn, W. D., and Cronan, J. E., Jr. (1976), *J. Mol. Biol. 102,* 167–172.

Nunn, W. D., and Tropp, B. E. (1972), *J. Bacteriol. 109,* 162–168.

Nunn, W. D., et al. (1977), *J. Bacteriol.* (in press).

Overath, P., Paule, G., and Schairer, H. U. (1969), *Eur. J. Biochem. 7,* 559–574.

Semple, K. S., and Silbert, D. F. (1975), *J. Bacteriol. 121,* 1036–1046.

Silver, S., and Wendt, L. (1967), *J. Bacteriol. 93,* 560–566.

Tunaitus, E., and Cronan, J. E., Jr. (1973), *Arch. Biochem. Biophys. 155,* 420–427.

Vogel, H. J., and Bonner, D. M. (1956), *J. Biol. Chem. 218,* 97–106.