**In Vitro Alterations of the Product Distribution of the Fatty Acid Synthetase from Mycobacterium phlei**

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**Summary**

The spectrum of fatty acids produced by the fatty acid synthetase complex of *Mycobacterium phlei* under several conditions has been examined. The observed pattern is always bimodal, consisting of palmitate and tetracosanoate as the two principal products and of lesser amounts of myristate, stearate, arachidate, and behenate. However, the relative proportions of the shorter chain acids (C14 to C16) to longer chain acids (C18 to C20) can be varied over a wide range. Such alterations can occur either with or without changes in the over-all rate of synthesis. Raising the acetyl-CoA to malonyl-CoA ratio from 0.4 (8 μM acetyl-CoA) to 150 (3 mM acetyl-CoA) increases the percentage of shorter chain acids from 12% to 87%. The addition of mycobacterial 3-O-methylmannosone containing polysaccharide (MMP) or 6-O-methylglucose containing polysaccharide (MGLP) at standard assay conditions (300 μM acetyl-CoA) causes a similar shift from a low (25%) to a high (85%) proportion of shorter chain acids, concurrent with a 5- to 10-fold increase in the rate of over-all synthesis. Bovine serum albumin (BSA) has the same effect on the fatty acid pattern as MMP or MGLP; but does not stimulate over-all synthesis. The effect of free CoA depends on the concentration of both the coenzyme and of acetyl-CoA. At 50 μM acetyl-CoA and CoA concentrations up to 100 μM, the formation of short chain acids is favored, whereas at higher CoA concentrations there is a shift toward the longer (C20 to C24) acids. *M. phlei* palmitoyl thioesterase when added to the standard assay system (300 μM acetyl-CoA) roughly doubles the proportion of short chain acids but does not affect the over-all rate of synthesis. To account for the widely varying fatty acid patterns in response to experimental conditions, it is proposed that fatty acyl chains (C14 and C16) either enzyme bound or accumulating as free CoA derivatives regulate the over-all rate of synthesis, perhaps by feed-back inhibition. Reagents that lower the levels of free or enzyme bound C14-CoA (or C16-CoA) will therefore affect the synthetic rate, the product distribution or both. They may do so by competition (high concentrations of acetyl-CoA), by complexing acyl-CoA (MMP, MGLP and BSA), or by thioester hydrolysis (palmitoyl thioesterase). Explanations are offered for the ability of the polysaccharides and the failure of BSA to stimulate over-all synthesis.

The fatty acid synthetase complex from *Mycobacterium phlei* produces CoA derivatives of saturated straight chain fatty acids 14 to 26 carbon atoms long (1). The prominent feature of the *M. phlei* system is that it affords not only fatty acids of an unusual length, but also that their distribution pattern is bimodal. Palmitate and tetracosanoate predominate and C14, C16, C18, C20, and C22 are found in lesser amounts. It has been established that this bimodal pattern is due to the activity of a single homogeneous multienzyme complex and not due to the combined activities of two separate synthetase systems (2). Several other noteworthy properties of the *M. phlei* synthetase complex have been reported. Unusually high concentrations of acetyl-CoA and the presence of one of the mycobacterial polysaccharides MMP or MGLP are needed for optimal activity (3). The synthetase also catalyzes the elongation of palmitoyl-CoA or stearoyl-CoA to C16 and C18 acids (1) but this process requires, in addition to polysaccharide, the presence of free CoA or of a thioesterase which produces free CoA from the primer substrate (4). We now show that the spectrum of enzymatically produced fatty acids can be drastically altered by varying the conditions for assaying the synthetase. The pattern can be shifted either toward very low or very high relative proportions of shorter chain (C14 to C16) and longer chain (C20 to C24) acids. The direction of the shift depends on the concentration of acetyl-CoA, the ratio of acetyl-CoA to malonyl-CoA and on the presence or absence of polysaccharides (MMP, MGLP) or BSA or of CoA. Under all conditions the fatty acid pattern remains bimodal.

**Materials and Methods**

Materials—Acetyl-CoA was synthesized by the method of Simon and Shemin (5). [2-14C]Malonyl-CoA was purchased from New England Nuclear. Coenzyme A, palmitoyl-CoA, and dithiothreitol (DTT) were obtained from P-L Biochemicals, Inc.

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fatty acid-free BSA from Sigma, and silver carbonate from Mallinckrodt Chemical Works. DPNH, TPNH, and FMN were products of Calbiochem. Carrier fatty acid methyl esters (C14, C16, C18, C20, C22, and C24) were purchased from Supelco, Inc.

Methods—The _M. phlei_ fatty acid synthetase complex, the mycobacterial polysaccharides MMP and MGLP-I and palmitoyl thioesterase were isolated and purified as previously described (3, 5). Methylated MGP was prepared as described by Vance et al. (2).

Determination of Fatty Acid Spectra—Reaction mixtures contained, in a total volume of 0.5 ml: 0.1 m potassium-phosphate buffer, pH 7.0; 5 μM DTT; 1 μM FMN; 30 μM TPNH; 30 μM DPNH; and 20 μM [2-14C]malonyl-CoA (20.6 mCi per mmole). Δ7-ly-CoA, polysaccharide, CoA, BSA, and thioesterase were added as indicated in the figures. Enzyme (4 μg) was added to start the reactions which were terminated after 15 min at 37° by the addition of 0.15 ml of 50% KOH. Carrier fatty acid methyl esters (50 μg of a mixture of C12 to C24) were then added to each tube and the mixtures heated on a boiling water bath for 20 min. After acidification with 6 N HCl, fatty acids were extracted with two 5 ml portions of petroleum ether. The solvent was transferred to screwcap culture tubes and evaporated under a stream of nitrogen. For converting the acids to methyl esters 1 ml of 0.75 N HCl in methanol, prepared by bubbling HCl gas into methanol was added and the tubes were capped (Teflon-lined caps) and heated at 75° overnight in a heating block. Solid silver carbonate was added to neutralize the acid and the methanol solution transferred to vials and evaporated under nitrogen. The residue was taken up in a small amount of hexane and assayed for total radioactivity prior to gas chromatographic analysis.

Methyl esters were analyzed by gas-liquid chromatography on a 6 ft. column of 12% stabilized diethylene glycol succinate with an F & M model 400 instrument equipped with a hydrogen flame detector and variable stream splitter. The column temperature was 160° for C14 to C18 esters and 180° for C20 to C24 esters. Effluent was either passed directly into a proportional counter (Nuclear Chicago) with methane as the carrier, or burned to CO2 and water and passed into a proportional counter (Packard model 894) with propane as carrier. The values for each acid are expressed as percentages of the total radioactivity in the sample.

Results

Under standard conditions the _M. phlei_ synthetase is assayed in the presence of relatively high acetyl-CoA concentrations because the _K_m for acetyl-CoA is very large in this system (2) unless one of the mycobacterial polysaccharides is added. At 300 μM acetyl-CoA and 20 μM malonyl-CoA and in the absence of either MPP or MGLP, the enzyme produces the fatty acid spectrum shown in Fig. 1. Under these conditions longer chain acids (C22 to C24) account for about 70% of the total and the shorter acids (C18 to C20) for the remainder. The most critical determinant of chain length as a function of substrate concentration appears to be the ratio of acetyl-CoA to malonyl-CoA rather than the absolute concentration of acetyl-CoA. As the ratio of these two substrates is increased from 0.40 (acetyl-CoA, 20 μM; malonyl-CoA, 50 μM) to 150 (acetyl-CoA, 3 mm; malonyl-CoA, 20 μM) the percentage of shorter chain acids rises from a low of 12% to a high of 87% (Figs. 2, 3, and 4). It should be noted that at the highest acetyl-CoA to malonyl-CoA ratio chosen the concentration of acetyl-CoA is 3 mm and that under these conditions the over-all rate of synthesis ([14C]malonyl-CoA incorporation) is the same as that obtained with 300 μM acetyl-CoA and optimal concentrations of polysaccharides.

Polysaccharide Effects—At an acetyl-CoA concentration of 300 μM the addition of graded amounts of mycobacterial polysaccharides progressively raises the proportion of shorter chain acids (Fig. 5). The latter comprise 85% of the total with optimal amounts of MMP and 80% with optimal MGLP-I. Palmitate, which increases from 15% to 60% under these conditions, accounts for most of the polysaccharide-induced increase in this group of shorter chain acids (Fig. 6). In the absence of polysaccharide the proportion of the C14 to C18 acids is only 25% (Fig. 1). As pointed out above, optimal polysaccharide affects the chain length pattern and the over-all synthetic rate in much the same way as do very high acetyl-CoA to malonyl-CoA ratios suggesting a common or similar mode of action.

MGLP, a polysaccharide containing both glucose and 6-O-methylglucose and various acyl groups in sugar ester linkage, can be deacylated to a product designated as MGP (2). When treated with dimethylsulfate, MGP yields a methylated product which is only one-third as effective as MGLP in stimulating over all fatty acid synthesis (2). Methylated MGP also raises the relative proportion of the shorter chain acids but the effect is much smaller than with MGLP-I or MPP (Fig. 5).

Effects of BSA—BSA stimulates the rate of the reactions catalyzed by the fatty acid synthetase complexes of yeast and of Corynebacterium diphtheriae and enhances in these systems the accumulation of palmitate at the expense of stearate (6). Although BSA is without effect on over-all _M. phlei_ synthetase activity, it profoundly alters the spectrum of the fatty acids produced. At 300 μM acetyl-CoA, the proportion of shorter chain acids rises from 25% of the total in the absence of BSA to 70% in its presence (1.0 mg per ml) (Fig. 7). However, even at the highest levels of BSA tested (4 mg per ml) the longer chain acids (C24) continue to be synthesized.

Effects of CoA and Palmitoyl-CoA Thioesterase on Synthetase Rate and Fatty Acid Spectrum—Free CoA was previously found to be essential for supporting palmitoyl-CoA elongation as catalyzed by the _M. phlei_ synthetase (4). When the enzyme is assayed for de novo synthesis with 50 μM acetyl-CoA as the primer and in the absence of polysaccharide, 500 μM CoA will stimulate the synthetic rate by as much as 4-fold (Fig. 8). The concurrent
FIG. 2 (left). Effect of the molar ratio of acetyl-CoA to malonyl-CoA on the percentage of short chain fatty acids produced by the *M. phlei* synthetase. The lowest substrate ratio was 0.4. The data for the percentages of short chain acids refer to the sum of C₁₂, C₁₄, and C₁₆.

FIG. 3 (center). Fatty acid spectrum of the *M. phlei* synthetase with acetyl-CoA:malonyl-CoA = 0.40 (acetyl-CoA, 20 μM; malonyl-CoA, 50 μM in the standard reaction mixture described under "Materials and Methods").

FIG. 4 (right). Fatty acid spectrum of the *M. phlei* synthetase with acetyl-CoA:malonyl-CoA = 150 (acetyl-CoA, 3 mM; malonyl-CoA, 20 μM in the standard reaction mixture described under "Materials and Methods").

 changes in the fatty acid spectrum are complex. At 250 μM, CoA raises the ordinarily very low percentage of myristate to 13% but has little influence on the level of palmitate. When the concentration of CoA is raised to 1 mM, an increase in the proportion of C₁₄ to 81% of the total is the major result. At higher primer concentrations (300 μM acetyl-CoA) CoA does not change the fatty acid spectrum substantially nor does it stimulate the over-all synthetic rate.

Addition of 6 μg of partially purified palmitoyl-CoA thioesterase (4) to reaction mixtures containing 300 μM acetyl-CoA (total volume 0.5 ml) elevates the proportion of shorter chain acids from 25% to 50% of the total without any change in the over-all synthetic rate. The effects of graded amounts of thioesterase at varying acetyl-CoA concentrations have not been examined.
The problem, chain termination in fatty acid synthesis catalyzed by the mycobacterial enzyme by Sumper et al. (8), has been analyzed in detail for the enzymes which terminate growth of the fatty acid chain.

One of the variables tested by multienzyme complexes, has been analyzed in detail for the synthesis of fatty acids as previously described (1). The ratio of long chain end products was determined by the relative velocities of the condensing enzyme which lengthens the chain and the terminal transacylase which discharges the fatty acid from the complex.

Since the M. phlei product spectrum is bimodal and since such mode consists of one major and of several minor acids, we shall consider any shifts in the distribution pattern in terms of the two groups, shorter (C₅ to C₁₃) and longer (L C₁₄ to C₂₄) acids. Changes in the acetyl-CoA to malonyl-CoA ratio affect the M. phlei synthetase in the expected fashion. The higher the ratio the shorter the length of the fatty acids produced. At one extreme when the substrate ratio [acetyl-CoA to malonyl-CoA] is 0.4, the L component amounts to 85 to 90%, of the total, and at the other, with a 150 molar excess of acetyl-CoA, the S:L ratio is the reverse, the S group accounting for 80% of the total. Thus, the direction of the change in response to varying substrate ratios is the same for the M. phlei synthetase as for the yeast enzyme (8) except that in the mycobacterial system one observes relative increases or decreases of groups of fatty acids differing in length by 6 to 8 carbon atoms, whereas in yeast the relative proportions of neighboring fatty acids (e.g., C₁₈ and C₁₉) are affected.

There is good evidence that the malonyl transacylase and palmitoyl transacylase components of the yeast synthetase are identical (10) and this seems to be true also for the M. phlei system. Therefore, malonyl-CoA at very high concentration may competitively inhibit palmitoyl transacylation and favor chain extension beyond C₁₆. This will result in an increase of the longer chain acids at the expense of palmitate.

BSA causes the synthetases of yeast and Corynebacterium diphtheriae to produce more palmitate and less stearate (6). In the presence of BSA the fatty acid spectrum of the M. phlei enzyme also shifts in favor of shorter acids. The effect is much more pronounced in M. phlei than in the two other microbial systems. Moreover, the principal change is in the concentration of C₁₄ relative to C₂₄ rather than of C₁₈ relative to C₁₆. Early chain termination in the presence of BSA can be attributed to sequestration of fatty acyl-CoA by protein on the assumption that the strength of the interaction increases with the length of fatty acyl-CoA and that it reaches a maximum at C₁₆. Removal of product in the complexed form will shift the equilibrium of the reversible transacylase reaction toward palmitoyl-CoA production and thereby retard the further extension of C₁₆ chains.

The two mycobacterial polysaccharides MGLP and MMP have recently been shown to duplicate the effects of BSA on fatty acid synthesis catalyzed by the enzyme from yeast and Corynebacterium (6). They also cause an increase in the production of myristate and palmitate relative to stearate in these systems. On the basis of these observations it was proposed (6) and then demonstrated that MMP and MGLP, like BSA, form adducts with acyl-CoA derivatives of the appropriate chain length (11).

The product pattern of the M. phlei fatty acid synthetase is very markedly altered by MMP or MGLP. In the absence of MMP or MGLP, C₁₄ predominates (80% of total) while in their presence, the shorter acids (C₁₃ to C₁₄) account for 80% of the total. The very similar alterations in the M. phlei acid spectrum caused by MMP or MGLP on the one hand and by BSA on the other can both be explained by palmitoyl-CoA binding which in turn will shift the transacylase equilibrium. However, as previously noted, BSA does not share the property of MMP or MGLP to stimulate the over-all rate of M. phlei synthetase reactions.

FIG. 8. Effect of free CoA on the fatty acid spectrum and on the over-all synthetic rate of the M. phlei synthetase. The standard reaction mixture described under "Materials and Methods" contained 50 μM acetyl-CoA and CoA as indicated. The over-all synthetic rate was measured by incorporation of [2-¹⁴C]malonyl-CoA into fatty acids as previously described (1). The values (Vₘₐₓ) for Cl₂ acids. For the acids intermediate between C₁₃ and C₁₈ the values (Vₘₐₓ) are very much lower.

DISCUSSION

The regulation of the M. phlei synthetase activity poses a number of problems that are not encountered with functionally analogous multienzyme complexes from other sources. The apparently unique properties of the M. phlei system include (a) the unusually high Kₘ for acetyl-CoA (1); (b) the requirement for DPNH as well as TPNH due to the different pyridine nucleotide specificities of the β-keto-acyl reductase and the α,β-enzyme reductase steps (7); (c) the sensitivity of the synthetase to solutions of low ionic strength (1); (d) the marked stimulation of the system by the mycobacterial polysaccharides MGLP and MMP (3), and (e) the bimodal nature of the fatty acid spectrum. Among these features of the M. phlei system two seem to us of central interest. They are the mechanism underlying the effects of polysaccharides on enzyme activity and the bimodal fatty acid pattern. Conceivably these two phenomena are related.

In order to shed more light on these regulatory aspects, we have now examined the factors that determine the M. phlei fatty acid chain length pattern. The spectrum is highly variable but persistently bimodal with one maximum at C₁₆ or C₁₈ and another at C₂₄. Obviously, the relative abundance of acids of different chain length is determined by the activities and specificities of the enzymes which terminate growth of the fatty acid chain. The problem, chain termination in fatty acid synthesis catalyzed by multienzyme complexes, has been analyzed in detail for the yeast enzyme by Sumper et al. (8). One of the variables tested was the ratio of acetyl-CoA to malonyl-CoA. When this ratio was abnormally low, stearate was the principal product, whereas at high substrate ratios, palmitate predominated and the shorter acids accounted for 80% of the total.

The ratio of long chain end products was determined by the relative activities of two enzymes, acetyltransacylase which is active with primers up to C₁₇, and the "long chain" (C₁₈ to C₂₄) transacylase in response to varying acetyl-CoA to malonyl-CoA ratios (8). There is, however, one unequivocal example of enzyme specificity displaying a bimodal pattern. The homogeneous β-hydroxyacyl-ACP-α,β-trans-acyl-ACP dehydrase isolated by Birge and Vagelos (9) shows two activity maxima, one for C₁₅ and the other for C₂₃ acids. For the acids intermediate between C₁₃ and C₁₈ the values (Vₘₐₓ) are very much lower.

2 Under certain conditions the pattern of the fatty acid synthetase from yeast also becomes bimodal with maxima at C₁₅ and C₁₈, respectively. This may reflect changes in the relative activities of two enzymes, acetyltransacylase which is active with primers up to C₁₇, and the "long chain" (C₁₈ to C₂₄) transacylase in response to varying acetyl-CoA to malonyl-CoA ratios (8). There is, however, one unequivocal example of enzyme specificity displaying a bimodal pattern. The homogeneous β-hydroxyacyl-ACP-α,β-trans-acyl-ACP dehydrase isolated by Birge and Vagelos (9) shows two activity maxima, one for C₁₅ and the other for C₂₃ acids. For the acids intermediate between C₁₃ and C₁₈ the values (Vₘₐₓ) are very much lower.

3 T. Esders, unpublished.
The derivatives of the cyclic glucosides mimic the effects of the mycobacterial polysaccharides, while sharing with BSA certain regulatory properties the basis of which is long chain acyl-CoA binding. MMP and MGLP exert additional and more specific effects on M. phlei fatty acid synthesis not explainable on that basis. Attempts to pinpoint these additional effects of MMP or MGLP have included studies of the various partial reactions catalyzed by the M. phlei synthetase (acyetyl- and malonyltransacylation, condensation, etc.) but none of these individual events are significantly stimulated or inhibited by MMP or MGLP.

A working hypothesis for the mode of action of the mycobacterial polysaccharides is outlined in Fig. 10. First, in order to explain the bimodal fatty acid pattern, we postulate the existence of a complex of two long chain transacylases catalyzing the reversible reaction

\[ \text{acyl-}CoA + E \rightleftharpoons \text{acyl-CoA} + E \]  

The first of the two long chain transacylases would operate optimally at the Cl6 level and the second at C8. It is proposed that the over-all rate and the product pattern (Cl6 versus C8) is controlled by the relative activities of the two long chain transacylases and also by the activity of the condensing enzyme.

The chain shortening effects of both BSA and MMP or MGLP are readily explained by adduct formation between the complexing molecule and palmitoyl-CoA once this product has dissociated from the enzyme. The relief of inhibition by external palmitoyl-CoA is similarly explained. Complex formation will shift the equilibrium of the reversible Cl6 transacylase (Reaction 1) in favor of free palmitoyl-CoA and, therefore, compete with the enzyme activities that lengthen the Cl6 chain. In order to rationalize the specific effects of the polysaccharides on over-all reaction rates, we propose that chain termination at the Cl6 level is the rate limiting step. A fatty acid chain that has grown to this length will be removed from the multienzyme complex by the following events (13-16):

\[ \text{C}_{16}-\text{ACP-E} \rightleftharpoons \text{C}_{16}-\text{Ser-E} \]  

We further assume that Cl6-ACP-E or Cl6-Ser-E are accessible to and available for interaction with MMP or MGLP. If the formation of such complexes facilitates removal of palmitoyl residues from the enzyme, perhaps by weakening hydrophobic interactions, the effect would be to accelerate the over-all rate of synthesis. These ad hoc assumptions are made to offer an explanation not only for the enhancement of over-all synthesis by MMP or MGLP but also for the failure of BSA to act similarly. BSA, a very much larger molecule than MMP or MGLP, may not have access to enzyme-bound palmitoyl chains. If this is so, it will form complexes only with palmitoyl-CoA that is free after release of this product from the enzyme. Since this event is subsequent to the rate-limiting (transacylase) step, BSA will not change the over-all rate of synthesis.

The above scheme may also help to clarify the previous finding that the excessively high K_m for acetyl-CoA in the M. phlei synthetase system can be drastically lowered by polysaccharide (from 900 to 80 \mu M). We suggest that acetyl-CoA, at very high concentrations (or high acetyl-CoA to malonyl-CoA ratios) may compete with Cl6 chains for a common site. This competition would have the effect of accelerating Cl6 transacylation, increasing the proportion of shorter acids and stimulating the over-all rate of synthesis. The acetyl-CoA concentration will then become equivalent to fortifying the system with polysaccharide at low acetyl-CoA concentrations, both with respect to over-all rate and alteration of the fatty acid pattern. At 3 \mu M acetyl-CoA the synthetase does in fact exhibit the same over-all rate of synthesis.6 These ad hoc assumptions are made to offer an explanation not only for the enhancement of over-all synthesis by MMP or MGLP but also for the failure of BSA to act similarly.
fatty acid pattern and activity as with lower levels of acetyl-CoA (300 μM) and optimal amounts of polysaccharide.

Effects of Coenzyme A- and Palmitoyl-CoA Thioester—The response of the M. phlei synthetase to free CoA is complex and depends on the concentrations of acetyl-CoA as well as of CoA. At 300 μM acetyl-CoA the coenzyme neither stimulates the overall synthetic rate nor alters the fatty acid spectrum. When the acetyl-CoA concentration is lowered to 50 μM, optimal levels of CoA (500 μM) produce a 4-fold increase in the rate of de novo synthesis and at 1 mM CoA the relative proportion of the long chain acids rises to about 90%, the highest observed under any experimental conditions. This result is consistent with the earlier finding that CoA is limiting in chain elongation of palmitoyl-CoA to C14 acids, presumably because the transacylase operating at the C14 level has a high K_m for this coenzyme (4). CoA is the only reagent found so far which under certain specified conditions stimulates the over-all rate of de novo synthesis without affecting the chain length pattern.

A palmitoyl-CoA thioesterase, earlier referred to as elongating factor (EF, (4)), was shown to be necessary for supporting the chain extension of palmitoyl-CoA or stearoyl-CoA to C14 acids (4). Since free CoA had the same effect on elongation, it was concluded that the thioesterase functioned by hydrolyzing some of the substrate, thereby furnishing free CoA. The changes seen on addition of thioesterase to the de novo synthetase assay system can be explained on the same basis. Removal of released palmitoyl- or stearoyl-CoA by hydrolysis will shift the equilibrium favoring early chain termination. The specificity of the thioesterase (4) is consistent with these effects. Activity is highest with palmitoyl-CoA and stearoyl-CoA.

In summary, we describe here experimental conditions resulting in drastically altered product patterns of the M. phlei fatty acid synthetase complex. The synthetase activity can be modulated to furnish almost exclusively either the shorter (C14 to C16) or the longer (C18 to C20) acids and this can be achieved either with or without change in the over-all rate. The bimodal character of the fatty acid spectrum persists under all experimental conditions. A specific acyl-CoA transacylase active with acyl chains longer than C14 may be responsible for some of the singular properties of the M. phlei synthetase. To what extent the various factors are important for regulating the activity and specificity of the synthetase under physiological conditions is difficult to assess. It seems reasonable to assume, however, that the intracellular polysaccharides which are produced in relatively large amounts by the M. phlei cell (3, 17, 18) play a major role in controlling fatty acid synthesis in this organism.

In enzyme systems that produce or utilize palmitoyl-CoA or stearoyl-CoA, the tendency of these surface-active compounds to form micelles must be taken into account. Moreover, the values for the critical micelle concentration of a given acyl-CoA derivative vary greatly as a function of ionic strength, pH, and perhaps other factors (19). However, calculation shows that under the assay conditions for de novo fatty acid synthesis which have been examined in the present investigation, the concentration of acyl-CoA product remains far below the critical micelle concentration. It therefore appears that the variations in fatty acid spectrum described here are unrelated to this phenomenon.

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