

# CONTROL MECHANISMS IN THE SYNTHESIS OF SATURATED FATTY ACIDS<sup>1</sup>

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<sup>1</sup>The following abbreviations are used: ACP, acyl-carrier protein; BSA, bovine serum albumin; FAS, fatty acid synthetase; MGLP, 6-O-methylglucose-containing polysaccharide; MMP, 3-O-methylmannose-containing polysaccharide; PMSF, phenylmethane sulfonyl-fluoride; ppGpp, guanosine-5'-diphosphate-3'-diphosphate; pppGpp, guanosine-5'-triphosphate-3'-diphosphate.

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## PERSPECTIVES AND SUMMARY

Control mechanisms for the synthesis of long-chain fatty acids were last dealt with comprehensively by Volpe & Vagelos in the *Annual Review of Biochemistry* four years ago (1). Various aspects of the same topic have since been reviewed by the same authors (2) and by Numa & Yamashita (3). Acetyl-CoA carboxylase, the enzyme that catalyzes the committed step in fatty acid biosynthesis, and the fatty acid synthetases themselves are now available in pure form from a variety of sources, permitting a detailed analysis of ligand-induced (allosteric or short-term) control. The *in vitro* activity of acetyl-CoA carboxylases can be varied widely by positive as well as negative effectors, and the pivotal role of this enzyme in regulating fatty acid synthesis in animal tissues and in yeast is generally accepted. Positive allosteric effectors (citrate, isocitrate) for acetyl-CoA carboxylase are known with certainty only for the enzyme from some animal tissues, while negative control—e.g. inhibition by palmitoyl-CoA—appears to be more general, extending to acetyl-CoA carboxylases of some unicellular organisms (e.g. yeast). As for the regulation of fatty acid synthesis in bacteria, the discovery that *Escherichia coli* acetyl-CoA carboxylase is inhibited by ppGpp and pppGpp represents an important new development. The levels of these nucleotides are elevated in stringent *E. coli* strains, and hence bacterial fatty acid synthesis may be tightly coupled to cellular growth. This raises the following question. Fatty acid synthesis provides long-chain acyl groups for two principal, functionally distinct purposes: (a) for storage fat and (b) for the various more polar membrane-associated lipids. Membrane lipids are essential for the function and growth of all cells, whereas lipid storage is more variable and restricted to specialized tissues. Separate mechanisms may therefore control the synthesis of fatty acids destined for storage and for membrane lipids, respectively, and if so this might explain why only certain animal acetyl-CoA carboxylases (from liver adipose tissue and lactating mammary gland) respond to citrate, a response expressing the regulated flow of excess carbohydrate to fat. By contrast, bacterial cells do

not ordinarily deal with excess intracellular nutrient because the flux of carbon is controlled by saturable transport systems and geared to the rate of growth of the cell as a whole. Bacterial fatty acid synthesis may therefore be subject to signals that more generally affect growth-associated processes such as RNA synthesis.

Diverse rather than universal control mechanisms are also likely for fatty acid-synthesizing enzymes that are aggregated (type I) and for those that are not (type II). Thus, the absence of free intermediates—the special feature of multienzyme complexes—argues against the possibility that any one of the sequential steps catalyzed by type I synthetases is rate-limiting, even though there may be selective sensitivity to external reagents. On the other hand, it is conceivable that one of the component enzymes of the nonaggregated (type II) fatty acid synthetases employed by bacteria and plants controls the overall synthetic rate.

The interesting parallel now emerging is that in cells possessing multienzyme-complex fatty acid synthetases, the acetyl-CoA carboxylases are also tightly aggregated, polyfunctional systems. On the other hand, the bacterial carboxylase (*E. coli*) separates into three functional proteins as readily as the various components of the *E. coli* fatty acid synthetase.

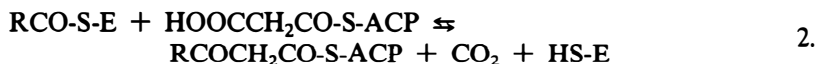
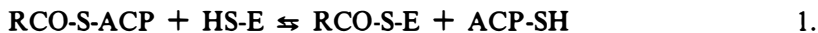
Fatty acid synthetase (FAS) multienzyme complexes were until recently believed to consist of six or seven separate catalytic entities held together by exceptionally strong noncovalent bonds. Persistent failures to dissociate these complexes into individual mono-functional components are now explained by the important discovery that the type I synthetases of yeast and animal tissues are composed of multifunctional polypeptide chains. A new definition of the term multienzyme complex as it applies to FAS systems is therefore needed.

Two sometimes temporally overlapping types of control mechanisms regulate the overall rates of metabolic pathways: short-term control of enzyme activity by small molecules and long-term or adaptive control exerted on enzyme quantity. Sensitivity to protein synthesis inhibitors and immunological techniques distinguish one from the other. In lipid biosynthesis adaptive changes occur in response to external stimuli (nutritional, hormonal) or in phase with development. For microorganisms that do not ordinarily accumulate storage fat, adaptive mechanisms are probably less significant or they may involve different signals than in multicellular organisms. Since such responses can be readily obtained in tissue culture or hepatocytes, the use of isolated animal cells holds great promise for the elucidation of adaptive mechanisms. At any rate since the adaptive response involves protein synthesis and degradation, it seems unlikely that the molecular events in the induction and repression of fatty acid synthesis will be known in detail before protein synthesis in general is better understood.

In this chapter we also review some topics that are more broadly related to the control of long-chain fatty acid synthesis. They include the microsome-associated synthetases, generally held to be elongation systems but probably also capable of de novo synthesis in some tissues; and the control of various lipogenic enzymes that provide substrates and coenzymes for fatty acid synthesis.

## CHEMICAL MECHANISM OF FATTY ACID SYNTHESIS

A few significant details have been added to the knowledge of the chemical mechanism of fatty acid synthesis, which otherwise is well understood. Condensing enzyme or  $\beta$ -ketoacyl-ACP synthetase, which is responsible for chain elongation, catalyzes the overall reaction



Reaction 2., the endergonic condensation, has long been suspected to be driven by synchronous decarboxylation (4). Compelling isotopic evidence for the concerted nature of reaction 2., has now been presented (5). Dideuteriomalonyl-CoA showed no primary kinetic isotopic effect when it was the substrate for fatty acid synthesis catalyzed by yeast FAS. In the interpretation of this result the assumption is made that the condensation reaction is the rate-limiting step in overall synthesis. This is true, however, only if the rates of partial reactions with model substrates are indicative of the rates occurring normally in the FAS complex (6). More convincingly, in the condensation reaction catalyzed by purified  $\beta$ -ketoacyl-ACP synthetase from *E. coli*, dideuteriomalonyl-ACP reacted at the same rate as the unlabeled analogue. A mechanism involving a malonyl carbanion intermediate and acylation of the carbanion is therefore excluded, and the formation of the new carbon-carbon bond and the cleavage of the carboxyl group from malonate are therefore concerted.

### *Primer Studies*

FAS systems that catalyze de novo fatty acid synthesis have been assumed to use acetyl-CoA preferentially for starting chains. There is now evidence that for mammalian mammary gland and liver FAS, butyryl-CoA is the more active primer. Cytosol from the mammary gland of lactating goats contains as separate entities three enzymes necessary for the synthesis of crotonyl-CoA from acetyl-CoA (reversal of  $\beta$ -oxidation). The mammary gland FAS proper catalyzes the reduction of crotonyl-CoA to butyryl-CoA

(7, 8). The same synthetase has also a markedly lower  $K_m$  and higher  $V_{max}$  for butyryl-CoA than for acetyl-CoA (7, 9, 10). Butyryl-CoA is superior to acetyl-CoA as a primer for the FAS systems of rat and rabbit liver as well (11). Whether one or more of the enzymes that produce butyryl-CoA is significant for regulating fatty acid synthesis has not been investigated.

## ACETYL-CoA CARBOXYLASE (IN VITRO)

### *Escherichia coli*

The reactions catalyzed by acetyl-CoA carboxylase and many of the molecular and regulatory features of this enzyme have been comprehensively reviewed (1, 12). The acetyl-CoA carboxylase activity from *E. coli* consists of three entities: biotin carboxyl carrier protein (molecular weight 22,500), biotin carboxylase (molecular weight 98,000, with two subunits of identical size), and carboxyltransferase (molecular weight 130,000, containing nonidentical polypeptides of 30,000 and 35,000 daltons) (1, 12–14). Biotin carboxylase and carboxyltransferase do not contain biotin but will catalyze model reactions with derivatives of *d*-biotin (12, 13). This has been exploited for studying the catalytic properties of these two enzymes (15). A two-step mechanism for carboxylation of acetyl-CoA has been substantiated. In addition, it has now been proven that the enzymatic carboxylation of biotin occurs at the 1'-ureido-N position and does not involve the ureido carbonyl-O (16).

Until recently regulatory mechanisms for fatty acid synthesis in *E. coli* were unknown. It has now been demonstrated that ppGpp (guanosine-5'-diphosphate-3'-diphosphate) controls phospholipid synthesis (17) and probably also fatty acid synthesis in stringent *E. coli* (18). Under conditions of amino acid starvation, the intracellular concentration of ppGpp [and (p) ppGpp] increases to 4 mM (18, 19). At the same time there is a dramatic cessation of the synthesis of RNA, carbohydrate, and nucleotides. Other effects seen in amino acid starvation (19) include restriction of unsaturated fatty acid and phospholipid synthesis (17, 20). The inhibition of fatty acid synthesis during amino acid starvation of *ret<sup>+</sup>E. coli* may be due primarily to reduced acetyl-CoA carboxylase activity. The ppGpp specifically inhibits the carboxyltransferase component but has no effect on biotin carboxylase activity (18).

Initially, the ppGpp effects on phospholipid and fatty acid formation in *E. coli* appeared to be redundant since fatty acids are only utilized for phospholipid synthesis. This apparent paradox may have been resolved by the demonstration that *sn*-glycerol-3-phosphate acyltransferase was inhibited by ppGpp when palmitoyl-CoA was a substrate but not when palmitoyl-ACP was the acyl donor (21). It is therefore speculated that when de novo fatty acid synthesis supplies fatty acyl moieties for phospholipids,

reduction of phospholipid synthesis would result from ppGpp inhibition of acetyl-CoA carboxylase. When fatty acids are supplied by phospholipid turnover or by the growth medium, phospholipid synthesis from acyl-CoAs would be decreased by ppGpp inhibition of the acyltransferase (21). Although more evidence for this hypothesis is required, it is clear that inhibition of the acetyl-CoA carboxylase is not the sole mechanism for regulating phospholipid synthesis in *E. coli* (22).

### *Eukaryotes*

Progress in determining the structure of acetyl-CoA carboxylase from liver has been slower than for the *E. coli* enzyme. It is well-established that the active enzyme exists as a polymer with a molecular weight of  $4-8 \times 10^6$  and that this polymer can be dissociated into inactive protomers of  $4-5 \times 10^5$  daltons (1, 12). However, agreement on the subunit structure of the protomeric form of acetyl-CoA carboxylase has not been reached. The initial studies on the rat liver enzyme provided evidence for two subunits of 215,000 daltons susceptible to further dissociation by sodium dodecyl sulfate into two species, one of 118,000 and the other 125,000 daltons (23). According to more recent studies the rat liver enzyme consists of two subunits of molecular weight 230,000 and smaller species (124,000 and 118,000 daltons) arising from this larger subunit due to cleavage by endogenous liver proteases during isolation (24). Both groups of researchers have found one molecule of biotin per large subunit (215,000 or 230,000 daltons) (23, 24). Hence, in the rat liver enzyme the carboxylase and transferase activities as well as the biotin carboxyl carrier protein may be associated with a single multifunctional polypeptide.

The acetyl-CoA carboxylase from chicken liver seems to have a different subunit structure even though the active form is also a high-molecular-weight polymer. The protomeric form of the enzyme has a molecular weight of 470,000 to 500,000 and has four subunits—two with molecular weights of 117,000 and one each with molecular weights of 129,000 and 139,000 (25). Biotin was found in only one of the two 117,000-dalton subunits (25). The physical differences between the rat and chicken liver enzyme may relate to the fact that citrate activates chicken liver acetyl-CoA carboxylase instantly, whereas the rat liver enzyme requires an incubation period of 15–20 min for full activation (1).

In addition to the *in vitro* regulation by citrate and palmitoyl-CoA (1, 12) discussed below, prosthetic group metabolism and inactivation by phosphorylation may also play a role in the regulation of liver acetyl-CoA carboxylase.

It was previously suggested that an apo-acetyl-CoA carboxylase accumulates in liver and adipose tissue of biotin-deficient rats (1). This apoenzyme

has now been isolated by affinity chromatography (26). The apoenzyme from rat adipose tissue does not appear to form polymeric structures, but will polymerize after in vitro conversion to holoenzyme (27). Hence, the biotinyl prosthetic group appears to be required for the polymerization of the carboxylase to the active form.

Control of acetyl-CoA carboxylase by phosphorylation and dephosphorylation of the enzyme has been reported (28–30). A rat liver protein fraction inactivated the enzyme with concomitant incorporation of  $^{32}\text{P}$  from [ $\gamma$ - $^{32}\text{P}$ ]ATP. This phosphorylation can be reversed by a magnesium-activated phosphatase activity (29). Inhibition of acetyl-CoA carboxylase activity in rat liver slices on incubation with cyclic AMP has also been reported (31). However, neither the evidence for phosphorylation nor the cAMP effects have been verified [unpublished experiments cited in (12)].

A novel mechanism for controlling the supply of alternate substrates has been described for the uropygial gland of the goose (32, 33). Cell-free extracts from this gland catalyze the carboxylation of propionyl-CoA but not of acetyl-CoA, yet a partially purified preparation carboxylates both substrates. These results were explained by the presence of a highly active and specific malonyl-CoA decarboxylase in the gland. Methylmalonyl-CoA is a very poor substrate for the decarboxylase. Hence, fatty acid synthesis would be expected to proceed from acetyl-CoA and methylmalonyl-CoA when the decarboxylase is present. This is consistent with the finding that 2,4,6,8-tetramethyldecanoic acid is normally the major fatty acid found in the uropygial gland of the goose (33). It appears therefore that the activity of an ancillary enzyme, malonyl-CoA decarboxylase, determines whether multibranched or straight-chain fatty acids are produced in this specialized organ.

Some unusual physical properties of the acetyl-CoA carboxylase from *Euglena gracilis* have been described (34, 35). From this organism acetyl-CoA carboxylase can be isolated as an aggregate that also contains phosphoenolpyruvate carboxylase and malate dehydrogenase (34). The complex has a molecular weight of 360,000 and dissociates on DEAE-cellulose chromatography into active constituent enzymes with molecular weights of 183,000 (phosphoenolpyruvate carboxylase), 67,000 (malate dehydrogenase), and 127,000 (acetyl-CoA carboxylase) (35). The kinetic parameters for the acetyl-CoA carboxylase were similar whether the enzyme was part of the complex or the dissociated species. However, for phosphoenolpyruvate carboxylase the  $K_m$  for  $\text{HCO}_3^-$  was much lower in the multienzyme complex (0.7–1.3 mM) than for the separated enzyme (5.4–7.3 mM) (35). It is not yet clear whether the association of these enzymes in a complex also has advantages for the carboxylation of acetyl-CoA (34).

## FATTY ACID SYNTHETASES (TYPE II)

The previous comprehensive review in this series (1) summarized the properties of the seven individual fatty acid synthetase enzymes from *E. coli*. Several noteworthy contributions have since been made to this subject.

The 4'-phosphopantetheine moiety of *E. coli* ACP is more rapidly metabolized than the ACP protein (1). In exponentially growing cells the protein portion of ACP does not appear to be degraded at all (36). Also there is no apparent relationship between prosthetic group exchange and the cell cycle (37). As for the possible regulatory significance of prosthetic group turnover, it is noteworthy that under normal growth conditions only holo-ACP was present in *E. coli*. In a pantetheine-requiring mutant apo-ACP could be detected but only after the CoA pool was exhausted (38). Thus, extreme conditions are required to generate apo-ACP in *E. coli*, and the significance of prosthetic group turnover remains to be established.

A major incentive for continued studies of *E. coli* fatty acid synthesis is the relative ease of selecting mutants with useful properties. Two temperature-sensitive mutants with defective malonyl-CoA-ACP transacylase activity and hence with impaired fatty acid synthesis have been identified (39). The isolation of another *E. coli* mutant, designated *fabB*, has disclosed some unexpected complexities in  $\beta$ -ketoacyl-ACP synthesis. The *fabB* mutant is defective in the synthesis of unsaturated fatty acids but normal in the synthesis of saturated fatty acids (40, 41). The suspicion that the defect is in some way associated with  $\beta$ -ketoacyl-ACP synthesis led to a reexamination of wild-type  $\beta$ -ketoacyl-ACP synthetase. It was found that the preparation previously thought to be homogenous is in fact composed of two forms, synthetase I and II (41). Synthetase I (mol wt 66,000) has the properties of the enzyme previously described (1, 42), whereas synthetase II is slightly larger (76,000), has a lower pH optimum (5.5 instead of 6.1), and is less sensitive to heat denaturation (41). Synthetases I and II have similar affinities for saturated and unsaturated acyl-ACP thioesters except that for palmitoleyl-ACP, enzyme II has a  $K_m$  about one tenth as high as, and three times the  $V_{max}$  of, enzyme I. This suggests that synthetase II functions specifically in the elongation of palmitoleyl-ACP to *cis*-vaccenyl-ACP. An "elongating system" had previously been postulated to explain the inability of an *E. coli* mutant to convert palmitoleate to *cis*-vaccenyl-ACP (43). With this background information from wild-type cells it could be shown that the *fabB* mutant lacks  $\beta$ -ketoacyl-ACP synthetase I. The addition of wild-type synthetase I, the enzyme that synthesizes only saturated acids, to the *fabB* synthetase—now identified as enzyme II—permitted the latter to synthesize unsaturated fatty acids (41). The manner in



which the two condensing enzymes I and II interact or cooperate in unsaturated fatty acid synthesis in *E. coli* is not yet understood.

A logical control point for unsaturated fatty acid synthesis is  $\beta$ -hydroxydecanoyl thioester dehydrase. Indeed, under certain conditions the unsaturated fatty acid content of *E. coli* phospholipids is directly proportional to the amount of dehydrase (44, 45). While a double dosage of the *fabA* gene (which codes for the dehydrase) had little effect on the rate of unsaturated fatty acid synthesis in cells grown at 37°C, this rate increased almost twofold when the temperature was lowered to 15°C (45).

*E. coli* mutants that are glycerol auxotrophs and also defective in  $\beta$ -oxidation respond to changes in growth temperature by producing saturated and unsaturated fatty acids in a different ratio (46). Hence the type of fatty acids produced in *E. coli* appears to be thermally controlled. Likely sites for thermal control are either  $\beta$ -hydroxydecanoyl thioester dehydrase or  $\beta$ -ketoacyl-ACP synthetases I or II. The earlier observations on temperature-sensitive transacylation in phosphatidic acid synthesis (47) have been confirmed by another approach (46). Thus, there appear to be at least two control points for thermally regulating the fatty acid composition of *E. coli* membranes.

Many bacteria synthesize cyclopropane fatty acids by alkylation of mono-unsaturated fatty acids (48). In *Lactobacillus plantarum* the controlling enzymes appear to be cyclopropane synthetase and S-adenosyl-homocysteine hydrolase (48). The latter enzyme degrades S-adenosyl-homocysteine and may thereby relieve feedback inhibition of the cyclopropane synthetase. More recently, two *E. coli* mutants deficient in cyclopropane fatty acids but without impaired physiological function have been isolated (49).

Type II fatty acid synthetase is also found in eukaryotic algae and plants, e.g. in *E. gracilis* when the phytoflagellate is grown in the light but not in the dark (1). Another ACP-dependent synthetase has been identified in cells grown in both light and dark (50). Myristoyl-CoA and palmitoyl-CoA are the preferred primers for this system, and the major product of palmitoyl-CoA elongation is stearoyl-ACP. Since the type I synthetase, which is also present in *Euglena*, produces C<sub>16</sub> as the main product, this elongation system may be required for the production of longer-chain fatty acids (50).

The occurrence of small proteins that function as carriers of covalently linked substrates or intermediates seems to be more widespread. In addition to ACP, which serves as a vehicle for biosynthetic fatty acyl intermediates, and biotin carboxyl-carrier protein, which functions as a CO<sub>2</sub> carrier in the carboxylation of acetyl-CoA, an ACP-like (4'-phosphopantetheine-containing) protein has now been separated from the citrate ligase of *Klebsiella*

*aerogenes* (51). This ACP carries citrate from the transferase component to the ligase component of the multifunctional enzyme (51). The *Klebsiella* ACP differs markedly in structure from *E. coli* ACP (52).

## SUBUNIT STRUCTURE OF FATTY ACID SYNTHETASES (TYPE I)

The molecular weights of FAS multienzyme complexes from various sources range from  $0.4 \times 10^6$  to  $2.5 \times 10^6$  (see Table 1). All mammalian synthetases are small and of approximately the same size, whether isolated from liver (53–56), lactating mammary gland (57–63), or brain (64). The same is true for the enzymes of pigeon liver (65, 66), chicken liver (67), goose uropygial gland (68, 69), and insects (*Ceratitidis capitata*) (70). The FAS from rabbit mammary gland is one notable exception ( $0.91 \times 10^6$ ) (59).

**Table 1** Molecular weights of fatty acid synthetases (multienzyme complexes) and their subunits (mol wt  $\times 10^{-3}$ )<sup>a</sup>

Source	Native FAS	Subunit
<b>Mammalian Liver</b>		
Human	410 (53)	—
Rat	540 (54)	250 (55)
Rabbit	453 (56)	225 (56)
<b>Mammalian Mammary Gland</b>		
Rat	530 (57)	250 ? (58)
Rabbit	910 (59)	—
Guinea pig	400 (60)	—
Cow	450–530 (61–63)	—
<b>Mammalian Brain</b>		
Mouse	~500 (64)	—
<b>Avian Liver</b>		
Pigeon	450 (65)	220 (66)
Chicken	508 (67)	250 (67)
Goose, uropygial gland	547 (69)	270 (68, 69)
<b>Insect</b>		
<i>Ceratitidis capitata</i>	560 (70)	250 (70)
<b>Microbial</b>		
Yeast	2200 (71)	180, 185 (72)
<i>Neurospora crassa</i>	2300 (73)	200 (73)
<i>Corynebacterium diphtheriae</i>	2500 (74)	—
<i>Mycobacterium smegmatis</i>	1400 (75)	200 (75)
<i>Euglena gracilis</i>	1700 (76, 77)	—

<sup>a</sup>References are given in parentheses.

The largest synthetases are found in yeast (71, 72) ( $2.3 \times 10^6$ ), *Neurospora crassa* (73), and *Corynebacterium diphtheriae* ( $2.5 \times 10^6$ ) (74), while those from *Mycobacterium smegmatis* (75) and *E. gracilis* (76, 77) are intermediate in size. Functional consequences attributable to these molecular weight differences are not apparent. Regardless of source and size, all FAS complexes appear to contain either single or multiple copies of multifunctional polypeptide chains of similar molecular weight (185,000–250,000). Whether these subunits are identical and hence contain all six or seven catalytic centers in a single polypeptide chain or whether there are two functionally distinct subunits of similar or identical size is at present a matter of controversy. It is certainly possible that the FAS complexes from various sources are not of uniform design, the subunit structures corresponding to  $\alpha_n$  in some cases and to  $\alpha_n\beta_n$  in others.

Lynen visualized yeast synthetase as an aggregate of seven distinct, monofunctional components (polypeptide chains), each present in more than one copy (78). The chains were thought to be held together firmly, but by noncovalent forces. However, attempts to dissociate the complex from yeast (or any other source) to monomeric, monofunctional components never succeeded; these failures could be attributed to the exceptional tightness of the complex. The resistance of FAS systems to dissociation clearly distinguishes these "aggregates" from the pyruvate dehydrogenase multienzyme complexes, which separate into three monofunctional components with relative ease and without the aid of detergents. Active pyruvate dehydrogenase can be reassembled from the three constituent enzymes (79).

A new model for yeast FAS and possibly all FAS multienzyme complexes has recently emerged. It is based on a comprehensive genetic analysis of yeast fatty acid auxotrophs by Schweizer et al (72, 80–83), which shows that the FAS enzymes are coded by no more than two genetically unlinked gene loci designated *fas 1* and *fas 2*. Each of these polycistronic gene clusters codes for a set of sequential enzymes or functions, the sum of which accounts for all of the chemical events catalyzed by the complex. Thus yeast FAS consists of multiple copies of only two polypeptides, and each of these chains is multifunctional. Genetic complementation studies together with biochemical characterization of various complementation groups led to the following functional assignments for the two polycistronic gene clusters.

*fas 1*

(Subunit B)

Acetyl transacylase  
 Malonyl (palmitoyl) transacylase  
 Dehydratase  
 Enoyl reductase

*fas 2*

(Subunit A)

4'-phosphopantetheine-binding  
 region  
 $\beta$ -ketoacyl synthetase  
 $\beta$ -ketoacyl reductase

Deficiencies in malonyl and palmitoyl transacylase are induced by a single lesion and therefore must be encoded by the same structural gene (83). This result confirms the identity of the peptide maps obtained earlier from synthetic [ $^{14}\text{C}$ ]malonyl- and [ $^{14}\text{C}$ ]palmitoyl enzyme (84, 85).

SDS-polyacrylamide gel electrophoresis of native as well as of various mutant synthetases (done with the necessary precautions to prevent proteolysis) yields only two bands, A and B, with molecular weights of 185,000 and 175,000, respectively. Synthetase labeled by growing yeast in the presence of [ $^{14}\text{C}$ ]pantetheine affords a single radioactive electrophoretic component coincident with peak A (Subunit A). Hence band A corresponds to *fas 2*, which also contains  $\beta$ -ketoacyl synthetase. This assignment has been confirmed by the demonstration that both the "central" pantetheine SH group and the "peripheral" SH group (of  $\beta$ -ketoacyl synthetase) are associated with the same polypeptide chain, *fas 2* or subunit A (86). Mutants that fail to incorporate [ $^{14}\text{C}$ ]pantetheine yield synthetase that nevertheless affords the same electrophoretic A, B pattern. This mutant synthetase also lacks  $\beta$ -ketoacyl synthetase activity, whereas all other partial activities are unimpaired. Thus, the catalytic transformations of the model substrates (N-acetyl cysteamine derivatives) for the two reductase steps and for dehydratase appear not to require covalent attachment to the 4'-phosphopantetheine moiety. The carrier function of this "prosthetic group" is thereby emphasized. Since acetyl-CoA and malonyl-CoA are the normal substrates for the two transacylations (to non-thiol sites),  $\beta$ -ketoacyl synthetase is the only reaction for which substrate linkage to 4'-phosphopantetheine is mandatory. This could explain why condensation is several orders of magnitude slower than the rate of overall synthesis and by far the slowest of the partial reactions observed with artificial substrates (78). Clearly the relative velocities of partial reactions measured with model substrates give no information on the rate-limiting step (6).

Interestingly, of the reactions catalyzed by the individual *E. coli* enzymes—all of which normally use ACP derivatives as substrates—only  $\beta$ -ketoacyl synthetase has an absolute ACP specificity (87). Some features of the more primitive bacterial systems have therefore been retained in the yeast multienzyme complex in spite of the profound differences in enzyme organization.

The synthetase isolated from the 4'-phosphopantetheine yeast mutant was electrophoretically indistinguishable from the wild-type enzyme. This finding is consistent with the assumption that in the FAS complex "acyl carrier protein" is not encoded separately by a structural gene. It may represent a region of one of the multifunctional polypeptide chains (*fas 2*), presumably coincident with the  $\beta$ -ketoacyl synthetase segment. How-

ever, the issue whether or not "ACP" is covalently linked to the *fas 2* product has not been unequivocally settled (88).

If, as seems certain, only one of the two sets of yeast FAS polypeptide chains (*fas 2*) contains the 4'-phosphopantetheine-binding region, one would expect a total of six residues of 4'-phosphopantetheine per mole of intact synthetase, ( $6 \times 185,000 + 6 \times 178,000 = 2.2 \times 10^6$ ). Five such residues have been found (89), a finding that is in fair agreement with an  $A_6B_6$  stoichiometry for the native synthetase.

Because of their small size, animal tissue FAS cannot contain more than one copy each of two separate multifunctional polypeptide chains or alternatively two copies of a single chain. In fact SDS gel electrophoresis of the synthetases from rat, pigeon, or chicken liver shows only a single band (if proteolysis is avoided) with an  $R_F$  value corresponding to a molecular weight of 240,000 (55). However, a successful separation of FAS subunits of pigeon liver synthetase labeled by [ $^{14}C$ ]phosphopantetheine has been reported (90). This was achieved by affinity chromatography on  $\epsilon$ -amino caproyl pantetheine-Sepharose. Separately, the two subunits (I and II) were active for some of the partial reactions, but they catalyzed overall synthesis only on reassociation (91). Comparison of the partial activities associated with the two liver FAS subunits with the activities allocated to yeast *fas 1* and *fas 2* shows some, but not complete, correspondence. Yeast *fas 1* and liver subunit II both carry acetyl transacylase, malonyl transacylase, and dehydratase activities, while yeast *fas 2* and liver subunit I contain 4'-phosphopantetheine,  $\beta$ -ketoacyl reductase, and presumably  $\beta$ -ketoacyl synthetase. However, enoyl reductase is associated with "noncorresponding" subunits (yeast *fas 1* and liver subunit I). Also, in pigeon liver FAS dehydratase activity is present in both subunits I and II, as is long-chain deacylase. It is worth noting that in yeast FAS, palmitoyl transacylase and malonyl transacylase share the same catalytic center (84, 85) and are coded by the same gene (83). Animal FAS systems thus contain one more catalytic or functional center (eight centers, including the ACP region) than does yeast FAS. It may therefore be significant that the subunit molecular weight of all animal FAS systems is consistently somewhat larger ( $>200,000$ ) than it is in yeast (see Table 1).

As already mentioned, fatty acid synthesis catalyzed by animal tissue FAS complexes terminates with the release of free fatty acids, rather than by transacylation to CoA as in yeast and in some other microbial multienzyme complexes. The acylenzyme hydrolase (deacylase) of pigeon liver FAS is rapidly and selectively inactivated by the serine esterase inhibitor phenylmethane sulfonyl-fluoride (PMSF) (92). Native FAS enzyme catalyzes the hydrolysis of the model substrate palmitoyl-CoA, and this is the only

partial activity sensitive to PMSF. Two moles of [ $^{14}\text{C}$ ]PMSF are covalently bound per mole of enzyme, suggesting that each of the two FAS subunits (mol wt  $\sim 250,000$ ) contains a deacylase site. These observations agree with the finding that the separated two subunits of pigeon liver FAS (90) both catalyze palmitoyl-CoA deacylation (91).

A synthetase that catalyzes palmitate formation from malonyl-CoA and multiply branched acids from methylmalonyl-CoA has recently been isolated from the uropygial gland of the goose (69). Remarkably, a one-step gel filtration on Sepharose-4B yielded homogenous enzyme (mol wt 547,000). The synthetase contained one 4'-phosphopantetheine residue and also one "active" serine or terminal deacylase site per subunit (mol wt 269,000), i.e. two per mole of enzyme. As previously mentioned, pigeon liver FAS also contains two PMSF-sensitive deacylase sites per mole of native enzyme, i.e. presumably one such site per subunit (92).

Treatment of lactating rat mammary gland FAS with trypsin or chymotrypsin inhibits overall synthetase activity and releases a palmitoyl-CoA thioesterase activity (mol wt  $\sim 32,000$ ) from the complex (93) without impairing any other partial activity. Trypsinized FAS produces longer-chain acids than the native enzyme.

Significant structural differences between animal and microbial FAS complexes clearly exist. In the former, malonyl transacylase and palmitoyl transacylase are distinct entities (92), whereas in the latter, e.g. yeast (84, 85), the two activities are associated with the same catalytic site (serine-OH).

Termination of the synthetic process by transacylation of acyl enzyme to water rather than to CoA has one important consequence. Free fatty acids are readily released from enzyme protein, whereas acyl-CoAs are released only sluggishly, particularly when the carbon chains are very long. Product diffusion can therefore become rate-limiting as shown by experiments with yeast FAS. This enzyme system had been routinely assayed in the presence of bovine serum albumin (BSA) (4). It is now clear why BSA affects this system beneficially. When BSA is omitted, enzymatic activity declines abruptly after a period of less than 1 min to about 5% of the rate seen in the presence of BSA (94). As shown by sucrose density-gradient centrifugation, product formed in the absence of BSA (125–150 molecules of acyl-CoA per mole of yeast FAS) remains associated with the synthetase; when BSA is present it binds the palmitoyl-CoA formed quantitatively, removing the inhibitory ligand from the enzyme. The importance of product diffusion and its facilitation by sequestering lipid-accepting agents for regulation of FAS activity in *M. smegmatis* is discussed below.

Whether or not all FAS multienzyme complexes conform to the same general design remains the most important structural issue to be resolved.

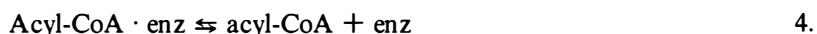
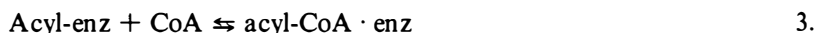
For yeast FAS, the nonidentity of the two multifunctional polypeptides, only one containing the 4'-phosphopantetheine binding or ACP regions, has been convincingly demonstrated (83). On the other hand, definitive proof for the subunit structure of animal FAS systems is still lacking. Although the separation of dissociated pigeon liver FAS into two distinct subunits seems to have been achieved (90, 91), the sharing of some partial activities by both polypeptide chains (e.g. deacylase) is not easily explained. Central for distinguishing between the two alternative subunit structures, i.e.  $\alpha_n$  and  $\alpha_n\beta_n$ , is the stoichiometry of the prosthetic group, which is difficult to determine with precision. Earlier analytical data indicated a content of only one 4'-phosphopantetheine residue per mole in all animal FAS systems (2), a finding consistent with nonidentity of the two subunits. However, according to more recent reports, this number is closer to two in some instances [FAS from uropygial gland (69), rat liver, and chicken liver (95)], which suggests two identical subunits, each containing the entire set of catalytic activities for fatty acid synthesis. Alternatively if the two polypeptide chains should prove to be nonidentical yet each contains one 4'-phosphopantetheine residue, one of these "prosthetic" groups may be redundant or function in a manner not yet known. Clarification of this important issue must await the unambiguous separation—or proof of identity—of the FAS subunits in each instance.

Multienzyme complexes are well designed for catalytic efficiency. This is achieved by avoidance of the accumulation of free intermediates that are not otherwise needed in intermediary metabolism. Moreover, multifunctional polypeptide chains constitute an elegant device for permanent subunit assembly and for establishing one-to-one stoichiometry of individual catalytic activities.

## FATTY ACID SYNTHETASE OF MYCOBACTERIUM SMEGMATIS

Purified *M. smegmatis* FAS (mol wt  $1.4 \times 10^6$ ) shows the following properties atypical for such systems: (a) dual pyridine nucleotide requirement, NADPH for  $\beta$ -ketoacyl reduction and NADH for  $\alpha,\beta$  enoyl-acyl reduction (96, 97); (b) an unusually high  $K_m$  (400  $\mu\text{M}$ ) for acetyl-CoA in the steady state (98); (c) a bimodal product pattern with peaks at  $\text{C}_{16}$ - and  $\text{C}_{24}$ -CoA (98); and (d) marked rate acceleration and lowering of the  $K_m$  for acetyl-CoA by mycobacterial polysaccharides (99). These polysaccharides are of two types known as MMP (containing 3-O-methylmannose) (99, 100) and MGLP (containing 6-O-methylglucose) (101). The molecular weight of MMP is 2100 (100) and that of MGLP is 4100 (101). It has now been established that the production of unusually long ( $\text{C}_{24}$  and  $\text{C}_{26}$ ) CoA deriva-

tives is responsible for the complex kinetics of the system and for the rate acceleration by MMP and MGLP. In aqueous solution these two polysaccharides form stoichiometric adducts with long-chain ( $C_{16}$ - $C_{24}$ ) acyl CoA (102, 103). This suggests that MMP and MGLP interact with the FAS system, removing product that would otherwise arrest synthesis by blocking active enzyme sites. Recent kinetic experiments support this hypothesis (104). The rate profile for de novo synthesis in the absence of polysaccharide is biphasic, showing a burst of activity that lasts for 5–10 sec and produces 5–6 moles of  $C_{24}$ -CoA per mole of enzyme. During this initial phase the  $K_m$  for acetyl-CoA is 6  $\mu$ M. Thereafter (in the steady state), synthesis slows to one sixth of the initial rate with a concomitant rise of the  $K_m$  for acetyl-CoA to 400  $\mu$ M. The  $C_{24}$  chains formed during the initial 5–10 sec period remain tightly bound to protein, preventing or greatly slowing enzyme turnover. If MMP is present in the system at  $t_0$  or is added after transition to the steady state, synthesis continues linearly and at the initial rate for very much longer periods. These results indicate that the rate-limiting step is not elongation but occurs during termination, i.e. acyl transfer and/or release of  $C_{24}$ -CoA. MMP promotes synthesis by facilitating one or the other of these terminal events. Specifically, release of  $C_{24}$ -CoA from the enzyme (enz) has been identified as the step that is rate-limiting and under control by polysaccharide. Termination in the presence or absence of MMP is therefore described by the following equations:



Reaction 3. is the catalyzed step in long-chain transacylation, 4. the ordinarily slow and therefore rate-limiting release of product, and 4a. diffusion facilitated by ternary complex formation between enzyme-bound acyl-CoA and polysaccharide. In support of the above formulation it has been found that the very slow exchange reaction  $C_{24}\text{-CoA} + *CoA \rightleftharpoons C_{24}\text{-CoA}$ , a measure of reaction 3. plus 4., is accelerated up to 100-fold by MMP (105). Secondly, reaction 3. (acyl transfer from synthetic [ $^{14}$ C] $C_{24}$ -enzyme to CoA) is likewise promoted by polysaccharide. Product (acyl-CoA) release is slow and becomes rate-limiting only with acyl-chains longer than  $C_{16}$ . Release of palmitoyl-CoA from the enzyme is nearly instantaneous and only slightly accelerated by MMP. This explains the abrupt rise in  $K_m$  for acetyl-CoA after the initial 5–10 sec burst since high ratios of acetyl-CoA to malonyl-CoA favor the production of shorter ( $C_{16}$ ) chains (106). Although BSA



(102) and lecithin liposomes (94) also complex acyl-CoA, they do not duplicate the rate acceleration in *M. smegmatis* fatty acid synthesis shown by MMP or MGLP (98, 107). It appears that in order to be effective the sequestering agent must be able to form a ternary complex with enzyme-bound acyl-CoA and this requires access to the transacylase site. Their large size and perhaps other structural features may prevent BSA and liposomes from reaching this site.

For the stoichiometric acyl-CoA · MMP complexes, a helical polysaccharide conformation has been proposed (108). Favorable conformations for hydrophobic interaction between the two ligands are obtained by rotating hexose residues around the glycosidic bond so that all ten of the methyl groups of the 3-O-methylmannose segment point into the interior of a channel about 5 Å in diameter. The length of the resulting channel, about 29 Å, is sufficient to include 15 paraffinic carbon atoms. A similar helical structure has been postulated for the inclusion complexes formed between MGLP and acyl-CoA (109). Certain cyclic glycosides (cyclohexa- and cycloheptaamyloses or  $\alpha$ - and  $\beta$ -cyclodextrins and their alkyl derivatives) duplicate all the effects on *M. smegmatis* fatty acid synthesis shown by MMP and MGLP (108, 110). The cyclodextrins form well-defined inclusion complexes with guest molecules of appropriate size (111) including fatty acyl-CoA (108). These properties support the inclusion complex structure proposed for the MMP- or MGLP-acyl-CoA adducts.

The product pattern of *M. smegmatis* fatty acid synthesis is highly variable but persistently bimodal. Under appropriate conditions more than 80% of the fatty acids formed are C<sub>24</sub> and C<sub>26</sub>, or at the other extreme, more than 80% C<sub>16</sub> and C<sub>18</sub> (106). Shifts in favor of shorter acids occur at high concentrations of acetyl-CoA (>300  $\mu$ M), in the presence of BSA, MMP or MGLP, lecithin liposomes, or on addition of a mycobacterial long-chain acyl-CoA hydrolase (106, 107). In all instances, chain shortening can be attributed to shifts in the equilibrium of the transacylase-catalyzed reaction: palmitoyl-enzyme  $\rightleftharpoons$  palmitoyl-CoA. Shifts in the forward direction will cause early chain termination and suppress C<sub>16</sub> elongation. In essence the mechanism advanced for chain-length regulation in yeast fatty acid synthesis (112) appears to apply also to the *M. smegmatis* system.

A model that accounts for the bimodal product pattern assumes a single long-chain transacylase component in *M. smegmatis* FAS, the activity of which declines sharply with increasing length of the acyl chain. The transacylase competes for acyl chains with the more broadly specific condensing enzyme. A bimodal product pattern is generated because of the divergent, nonoverlapping chain-length specificities of these two partial activities of the FAS complex (105). Various aspects of *M. smegmatis* fatty acid synthesis are discussed elsewhere in greater detail (113).

## MEMBRANE-ASSOCIATED ELONGATION SYSTEMS

### *Microsomal Systems*

A third organizational arrangement of fatty acid synthetases (Type III) is associated with microsomal membranes of eukaryotic cells. To date none of the components of microsomal systems from whatever source have been separated or otherwise characterized. It is not even known whether they are individual entities or organized as multienzyme complexes.

Microsomal fatty acid synthetases appear to function primarily as elongation systems for saturated fatty acids produced by cytosolic Type I synthetase and for dietary (essential) unsaturated fatty acids (114). Thus chain elongation of linoleic acid by hepatic microsomes appears to be the major pathway in the formation of arachidonic and other polyunsaturated fatty acids (115).

Recent studies on the microsomal elongation of saturated fatty acids have focussed on the system from mammalian brain because of the relatively large quantities of  $C_{22}$  and  $C_{24}$  fatty acids found in the sphingolipids of myelin (116). Of special interest is the fact that elongation of stearoyl-CoA in brain is negligible prior to myelination but rises rapidly during myelinogenesis (117-119). The cause for this increase in elongation activity is either enzyme synthesis, activation of preexisting enzyme, or alteration in the supply of available substrate. The latter possibility has been examined by assay of the activities of acyl-CoA ligase and acyl-CoA hydrolase in rat brain microsomes (120). Microsomal hydrolase that acts on  $C_{16}$ - and  $C_{18}$ -CoA was markedly decreased during myelination, which would preserve these substrates for elongation. However, this explanation for the enhanced elongation activity during myelinogenesis is inconsistent with the finding that bovine serum albumin interfered with palmitoyl-CoA cleavage by microsomal thioesterase without markedly affecting the elongation activity of microsomes isolated from rat brains prior to the myelination period (120). The most likely explanation for enhanced elongation activity during development is the production of new enzyme.

Some insight into developmental aspects has been gained by comparative studies with brain microsomes from normal and quaking mice. The quaking mouse is a recessive, autosomal mutant deficient in myelination of the central nervous system (121). The activities of the soluble Type I synthetase and the microsomal system that catalyzes the elongation of  $C_{16}$ -CoA to  $C_{18}$  fatty acids were only slightly below normal in the quaking mouse (122, 123). The rate of stearoyl-CoA elongation was also nearly normal in mutant mice, but the product was almost entirely  $C_{20}$  fatty acid (123). Subsequently, it was demonstrated that the elongation of  $C_{20}$ -CoA by microsomes

from the quaking mouse was reduced (117, 124). This might explain the lowered content of C<sub>24</sub> acids in myelin from the quaking mouse (125) but may not be the prime reason for defective myelination in these mice. However, these studies suggest that in brain at least three separate microsomal synthetases are present: a system that elongates C<sub>16</sub>-CoA to C<sub>18</sub>, a second system that elongates C<sub>18</sub>-CoA to C<sub>20</sub>, and a third system that forms C<sub>22</sub> and C<sub>24</sub> acids from C<sub>20</sub>-CoA. It is possible that these systems share component enzymes for elongation and only differ with respect to one or two activities (e.g. transacylase or condensing enzyme).

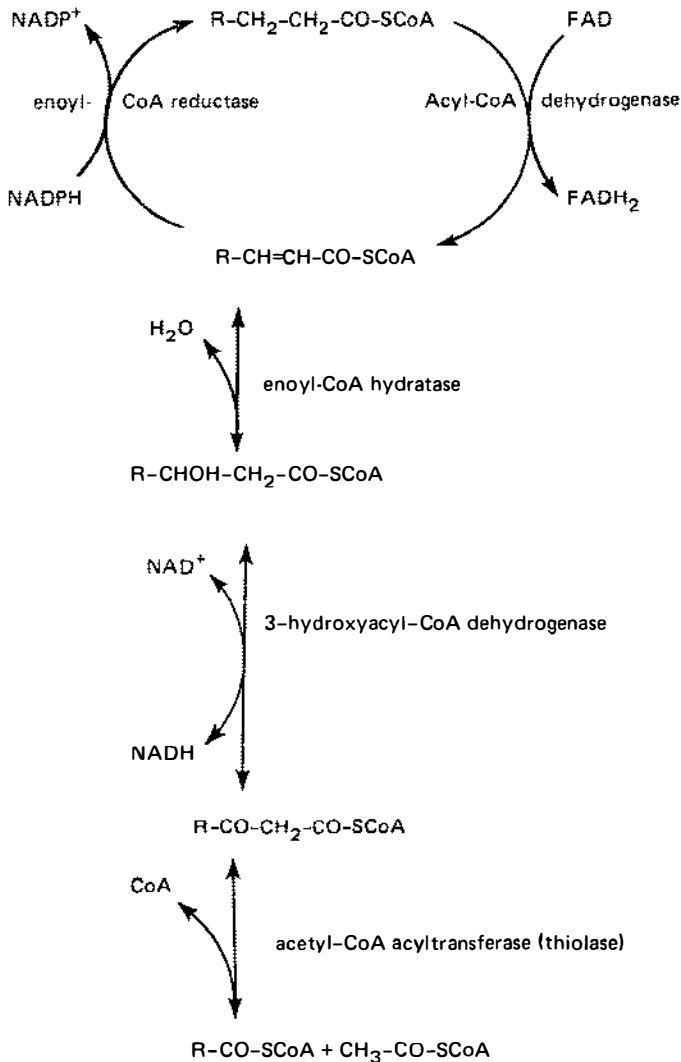
### *Mitochondrial Systems*

Prior to the discovery of what is now known as a Type I synthetase in liver cytoplasm, it was suggested that fatty acid synthesis might occur by reversal of mitochondrial  $\beta$ -oxidation (126). Subsequently, the function of the mitochondrial system was thought to be restricted to chain elongation of C<sub>16</sub>-CoA to C<sub>18</sub>-CoA and elongation of polyunsaturated fatty acids (126). It now appears that even these more limited functions are performed by microsomal systems and not by mitochondria.

However, the elongation of shorter-chain fatty acids can occur in the mitochondrial matrix by reversal of three  $\beta$ -oxidation activities: thiolase,  $\beta$ -hydroxyacyl-CoA dehydrogenase, and enoyl-CoA hydratase (Figure 1). The fourth  $\beta$ -oxidation enzyme (acyl-CoA dehydrogenase) appears to be replaced by an enoyl-CoA reductase activity (115). This mitochondrial enzyme (*trans*-2,3-decenoyl-CoA reductase) has been separated from a similar microsome-associated activity (115, 127). Microsomal enoyl-CoA reductases from beef adrenal and rat liver have been partially purified and characterized (127, 128). The optimal substrate is *trans*-2,3-hexenoyl-CoA, and either NADPH or NADH are utilized as hydrogen donors. In contrast, the mitochondrial enoyl-CoA reductase is specific for NADPH and is optimally active with *trans*-2,3-dodecenoyl-CoA (129). These differences in pyridine nucleotide specificity provided a means for evaluating the purity of the mitochondrial preparations. Highly purified mitochondrial enzymes from liver exhibited minimal elongation activity with C<sub>16</sub>-CoA, C<sub>18</sub>-CoA or CoA derivatives of polyunsaturated fatty acids but were fully active with decanoyl-CoA (130). It has therefore been postulated that the reversal of fatty acid oxidation does not function in the elongation of palmitate or stearate but conserves reducing power (NADPH and NADH) or funnels energy from NADPH into the electron-transport chain (115, 129, 130). This would only occur when the cell experiences low levels of oxygen or high levels of ATP (115, 129–131). It seems clear in any case that the ratios

of NADPH/NADP<sup>+</sup> and of NADH/NAD<sup>+</sup>, the rate of  $\beta$ -oxidation, and the reversal of this process in mitochondria are closely interrelated.

Reversal of  $\beta$ -oxidation appears to be the major if not the only mechanism for fatty acid synthesis in heart muscle and it may be governed only by the NADH/NAD<sup>+</sup> ratio since NADPH is not required for this reversal (129, 131).



*Figure 1*  $\beta$ -Oxidation and reversal of  $\beta$ -oxidation in liver mitochondria (130).

## INHIBITION OF FATTY ACID SYNTHESIS BY END PRODUCT

Palmitoyl-CoA and other long-chain acyl-CoA derivatives inhibit a wide variety of enzymes, some related and others unrelated to lipid biosynthesis (132). Because palmitoyl-CoA is a potent surface-active molecule (133), the specificity and physiological significance of these effects have been questioned (132). However, some enzymes respond to long-chain acyl-CoA selectively, in a manner distinguishable from their responses to synthetic detergents. Correlations between intracellular levels of palmitoyl-CoA in various nutritional states and the direction of coincident changes in enzyme activities (see section on adaptive control mechanisms) suggest strongly that palmitoyl-CoA can play an effector role in regulating various phases of lipid biosynthesis and perhaps also of energy metabolism. Inhibition by long-chain acyl-CoA may be competitive, uncompetitive or noncompetitive, accompanied by conformational changes or by perturbation of the quaternary enzyme structure. Ordinarily, the structural features necessary for inhibition include a saturated or unsaturated acyl chain at least 14 carbon atoms long and a negatively charged thioester moiety. CoA derivatives are usually the most effective, but inhibitory activity is retained to varying degrees in certain CoA analogues. As is the case for other detergent molecules, palmitoyl-CoA inhibition is a function of the molar ratio of inhibitor to protein rather than of absolute inhibitor concentration. Often, but not always, palmitoyl-CoA affects enzyme activity only near or above its critical micelle concentration, which is low (2–4  $\mu\text{M}$ ) and varies with pH and ionic strength (133).

### *Inhibition of Acetyl-CoA Carboxylase by Palmitoyl-CoA*

Purified acetyl-CoA carboxylases and fatty acid synthetases from various sources (rat, pigeon, chicken liver, adipose tissue, yeast) are strongly inhibited by long-chain acyl CoA (1). In general, a given acetyl-CoA carboxylase will show greater sensitivity to palmitoyl-CoA than the fatty acid synthetase from the same source. In a system containing the two enzymes the carboxylase will therefore successfully compete for the inhibitor and the supply of malonyl-CoA will become rate-limiting for fatty acid synthesis. This has been demonstrated in a reconstituted yeast system designed to simulate fatty acid synthesis and product removal under physiological conditions (134). In earlier studies it had been shown that yeast FAS activity proceeds linearly only in the presence of either BSA or dimyristoyl lecithin liposomes (94). Both reagents prevent end-product inhibition by sequestering palmitoyl-CoA.

The system studied contained purified yeast FAS, acetyl-CoA, NADPH, and  $\text{KHCO}_3$ , and either acetyl-CoA carboxylase or malonyl-CoA. When malonyl-CoA was generated enzymatically by the carboxylase the rate of fatty acid synthesis declined rapidly but could be maintained somewhat longer in the presence of liposomes. Long-chain acyl-CoA formation was substantially faster when acetyl-CoA carboxylase was replaced by its product, malonyl-CoA, and lecithin liposomes were also present. Thus acetyl-CoA carboxylase activity is much more sensitive to palmitoyl-CoA than is the activity of FAS. In another series of experiments, increasing amounts of palmitoyl-CoA were added to a similar system. When malonyl-CoA was generated by carboxylase, 50% inhibition of fatty acid synthesis occurred at  $1 \mu\text{M}$   $\text{C}_{16}$ -CoA in the absence of liposomes and at  $10 \mu\text{M}$   $\text{C}_{16}$ -CoA in the presence of liposomes. On the other hand, when malonyl-CoA was supplied as such and liposomes were included, no inhibition of fatty acid synthesis was observed up to  $15 \mu\text{M}$  palmitoyl-CoA. Thus in a system containing FAS, acetyl-CoA carboxylase, and an artificial membrane, there is effective competition for the end product between acetyl-CoA carboxylase and membrane lipid. The concentration of free  $\text{C}_{16}$ -CoA remaining in the cytoplasm is too low to affect the activity of FAS. While these results were perhaps predictable from the relative  $K_i$  values of the two enzymes for  $\text{C}_{16}$ -CoA, these experiments point more directly at acetyl-CoA carboxylase as the control point in the allosteric regulation of yeast fatty acid synthesis. Since positive allosteric modifiers for yeast acetyl-CoA carboxylase are not known, the central regulatory role of palmitoyl-CoA is emphasized.

In the same study (134) the palmitoyl-CoA acceptor capacity of various randomly chosen proteins was found to be either nil or much less than that of acetyl-CoA carboxylase. BSA was 55% (on a weight basis) or 5% (on a molar basis) as active as acetyl-CoA carboxylase; yeast glucose-6-P dehydrogenase, which is known to bind palmitoyl-CoA tightly (135), was 27% as active (on a weight basis). From these results a dual role can be assigned to yeast acetyl-CoA carboxylase. Apart from furnishing malonyl-CoA, the enzyme also serves as an effective temporary acceptor for the long-chain acyl-CoA product. In turn carboxylase delivers the ligand to the membrane sites where phospholipid synthesis takes place. Cytoplasmic palmitoyl-CoA levels are thus kept at low, noninhibitory levels assuring continued operation of the carboxylase. The system can be regarded as self-regulatory as follows. Membrane lipids will competitively accept long-chain acyl-CoA by direct transfer from the acetyl-CoA carboxylase-acyl-CoA complex and deliver it to the sites for phospholipid synthesis. If this acceptor capacity or the capacity to synthesize phospholipid is exceeded, palmitoyl-CoA will remain bound to and inhibit acetyl-CoA carboxylase. As malonyl-CoA levels decline, FAS will stop producing palmitoyl-CoA until the acceptor capacity of the lipid membrane is restored.

Inhibition of acetyl-CoA carboxylase activity rather than of fatty acid synthetase by palmitoyl-CoA appears also to be the physiologically significant event in some animal tissues. In a 100,000  $\times$  g supernatant of chicken liver, 100  $\mu$ M palmitoyl-CoA inhibited the conversion of [ $^{14}$ C]citrate (source of acetyl-CoA) into long-chain fatty acids even in the presence of BSA (25 mg/ml) (136, 137). Of the various enzyme activities assayed in this cytoplasmic system (FAS, ATP-citrate lyase, malic enzyme, NADP-linked isocitrate dehydrogenase, and acetyl-CoA carboxylase), only carboxylase activity was diminished. The reversal of this inhibition by citrate, which opposes the palmitoyl-CoA effects on isolated acetyl-CoA carboxylase (138, 139), strengthens the conclusion that in the system under study, carboxylase is the only lipogenic enzyme subject to control by palmitoyl-CoA.

Although acetyl-CoA carboxylase appears to be the target for palmitoyl-CoA both in yeast and in liver (or other animal tissues such as adipose tissue and lactating mammary gland), one major difference between these FAS systems must be mentioned. In yeast the end products are fatty acyl-CoA derivatives, whereas the animal synthetases produce free fatty acids, which are not inhibitory. Since liver contains substantial concentrations of long-chain acyl-CoA [about 15–140  $\mu$ M, depending on the nutritional state of the animal (140)], generation of inhibitor requires intervention of microsomal long-chain acyl-CoA ligase (141), another potentially regulatory enzyme. Although the ligase is inhibited by palmitoyl-CoA with a  $K_i$  of 4  $\mu$ M (142), the "acceptor capacity" of this enzyme is unknown, and it is therefore not clear whether hepatic acetyl-CoA carboxylase plays a similar role in carrying and delivering cytoplasmic long-chain acyl-CoA to the membrane as proposed for the yeast system. Liver and other animal tissues contain various proteins ( $y$  and  $z$ ) with a high capacity for binding fatty acids or their CoA derivatives (143–145). Whether they are involved in the intercompartmental transfer of fatty acyl-CoA is not known.

### *Effects of Palmitoyl-CoA on FAS Systems*

Acetyl-CoA carboxylases do not always show higher affinity and sensitivity to palmitoyl-CoA than the FAS systems for which they supply malonyl-CoA. In *Mycobacterium smegmatis*, the relative sensitivities appear to be reversed. The mycobacterial FAS is highly sensitive to palmitoyl-CoA [ $K_i = 10 \mu$ M (97)], whereas the partially purified acetyl-CoA carboxylase is relatively resistant to this inhibitor [ $K_i > 100 \mu$ M (146)]. Palmitoyl-CoA not only inhibits but also can dissociate the mycobacterial FAS (mol wt  $1.3 \times 10^6$ ) into inactive subunits [mol wt approximately 200,000–250,000 (147)]. The inhibitor attaches firmly to the subunits (100–125 moles per mole of subunit protein) but not to the intact complex. Native synthetase is regenerated with 40% recovery of activity when the isolated palmitoyl-CoA subunit adduct is treated with one of the mycobacterial

polysaccharides MMP or MGLP, which serve as high-affinity palmitoyl-CoA acceptors (148). Dialysis and treatment with BSA or dimyristoyl lecithin do not regenerate activity. The reversibility of the palmitoyl-CoA-induced dissociation (147), the presence of MMP and MGLP in the mycobacterial cytoplasm (97) in sufficiently high concentrations to effect reversal, and the relative resistance of acetyl-CoA carboxylase to palmitoyl-CoA suggest that in this organism FAS itself may be under end-product control.

The mode of regulation for the ACP-dependent nonaggregated elongation system of *M. smegmatis* (98) is not known. Primer concentrations up to 200  $\mu\text{M}$  (palmitoyl-CoA or stearoyl-CoA) are tolerated by this system (149).

Although inhibitory concentrations of palmitoyl-CoA have been reported to depolymerize FAS from liver (150) and yeast (151), regeneration of active enzyme has either been unsuccessful or has not been attempted.

### *Regulation of Other Lipogenic Enzymes*

Palmitoyl-CoA not only antagonizes the positive effects of citrate on acetyl-CoA carboxylase, but also controls mitochondrial citrate production. Citrate synthases from various animal sources are strongly inhibited by long-chain acyl-CoA. A recent study deals with and largely resolves the question of whether or not such effects are specific or due to general detergency (152). The test molecule was the analogue oleoyl (1,N<sup>6</sup>-etheno) CoA (oleoyl- $\epsilon$ -CoA). The critical micelle concentration of oleoyl- $\epsilon$ -CoA is 3.2  $\mu\text{M}$ , and by this criterion the analogue is a slightly more effective detergent than oleoyl-CoA (critical micelle concentration 4.7  $\mu\text{M}$ ). Nevertheless, the analogue was at most one tenth as potent an inhibitor of citrate synthase as oleoyl-CoA itself. In contrast to oleoyl-CoA, it inhibited only at concentrations well above the critical micelle concentration. The relative effects of oleoyl-CoA and oleoyl- $\epsilon$ -CoA on citrate synthase clearly show that the critical micelle concentration and hence detergency need not be the determining factor in enzyme inhibition by long-chain acyl-CoA.

The translocation of mitochondrial citrate to the cytoplasm is mediated by the so-called mitochondrial tricarboxylate anion carrier. This process is also inhibited by palmitoyl-CoA in relatively low concentrations (153, 154). Thus the same molecule may exert dual control over citrate levels affecting both citrate synthesis and delivery to the cytoplasm.

Citrate not only modulates hepatic fatty acid synthesis as a positive effector for acetyl-CoA carboxylase but also appears to be the major immediate carbon source for this process. Citrate is cleaved to acetyl-CoA and oxaloacetate by ATP-citrate lyase (155). In crude extracts, ATP-citrate lyase activity is only moderately sensitive to palmitoyl-CoA (50% inhibi-



tion at 0.1 mM) (155). Thus citrate cleavage does not appear to be a primary control point for regulating the supply of a fatty acid precursor. This is borne out by the previously mentioned experiments on fatty acid synthesis in chicken liver cytosol (136, 137).

A number of pyridine nucleotide-linked dehydrogenases that provide reducing equivalents for fatty acid synthesis are inhibited or inactivated by long-chain acyl-CoA (132). Palmitoyl-CoA inhibition of yeast glucose-6-P dehydrogenase ( $K_i = 3 \mu\text{M}$ ) is competitive with glucose-6-phosphate and of the mixed type with respect to NADPH (132). When treated with relatively high concentrations of [ $^{14}\text{C}$ ]palmitoyl-CoA (1 mM, 1.2 mg protein per ml), the tetrameric yeast enzyme dissociates into a protomeric species (135) which shows the same sedimentation behavior as the dimers produced by treating the dehydrogenase with acid ammonium sulfate (156). The molecular weight of this species,  $1.1 \times 10^5$ , is one half that of the native dehydrogenase (157). Changes in quaternary structure occur also at low inhibitor concentrations. At  $3 \mu\text{M}$  [ $^{14}\text{C}$ ]palmitoyl-CoA and  $1.1 \mu\text{g}$  of enzyme per ml the dehydrogenase was 50% inhibited. No radioactivity was associated with density-gradient fractions containing the native, tetrameric dehydrogenase, whereas the dimer region contained about 10 moles [ $^{14}\text{C}$ ]palmitoyl-CoA per mole of protein. Since the enzyme dissociates even when partially inactivated, dehydrogenase inhibition may be a direct consequence of palmitoyl-CoA-induced changes in quaternary structure.

Palmitoyl-CoA remains bound to dimeric enzyme on dialysis but can be removed by the complexing agent 2,6-di-O-methyl- $\beta$ -cyclodextrin (135), whereupon the dimer reaggregates to a NADP-free, enzymatically inactive tetrameric species. Addition of NADP regenerates enzymatically active dehydrogenase.

Relatively high concentrations of sodium dodecyl sulfate dissociate the dehydrogenase directly to monomeric subunits. Subsequent removal of the bound detergent fails to regenerate activity. There is evidence that palmitoyl-CoA and sodium dodecyl sulfate bind to different protein sites. Thus, the responses of yeast glucose-6-P dehydrogenase to the "natural" detergent and to the synthetic detergent are clearly different. Palmitoyl-CoA is the more potent and the more selective inhibitor and the effects are reversible.

The glucose-6-P dehydrogenases from *Torulopsis utilis* (135), from *Leuconostoc mesenteroides* (158), and from mammary gland (159, 160) are also sensitive to palmitoyl-CoA, but each dehydrogenase appears to respond differently to the inhibitor.

Although glutamate dehydrogenase from bovine liver mitochondria is not ostensibly linked to lipogenesis, it is more sensitive to palmitoyl-CoA [ $K_i = 0.15\text{--}0.30 \mu\text{M}$  (132)] than any other enzyme tested. At high concen-

trations, palmitoyl-CoA depolymerizes the hexameric enzyme to dimeric protomers, mol wt  $1.2 \times 10^5$  (161), which is twice the size of the subunit produced by sodium dodecyl sulfate [mol wt 56,000 (162)]. The existence of dimeric glutamate dehydrogenase is unexpected because the native hexameric enzyme is thought to consist of two trimeric protomers (162). Conditions for regenerating the native hexamer from the dimeric species by removal of bound palmitoyl-CoA have not been found (161).

Among the various animal malate dehydrogenases the enzyme from pig heart is the most susceptible to palmitoyl-CoA [ $K_i = 1.8 \mu\text{M}$  (132)]. This enzyme retains the native dimeric structure in presence of palmitoyl-CoA but is completely inactivated and irreversibly converted to monomer by 70–100  $\mu\text{M}$  sodium dodecyl sulfate (161). [ $^{14}\text{C}$ ] Palmitoyl-CoA binds only weakly to mitochondrial enzyme since inhibition is reversed on dilution.

When mitochondrial malate dehydrogenase is assayed by measuring malate oxidation instead of oxaloacetate reduction, palmitoyl-CoA inhibits much less ( $K_i = 50 \mu\text{M}$  instead of  $1.8 \mu\text{M}$ ) either at pH 10, which is optimal, or at pH 7.4. This fact may be of significance in the regulation of mitochondrial  $\beta$ -oxidation of long-chain fatty acids (161). In the citric acid cycle, malate dehydrogenase operates in the direction malate  $\rightarrow$  oxaloacetate. Since palmitoyl-CoA inhibits this reaction only weakly it will not impair the regeneration of oxaloacetate, which is necessary for continued operation of the cycle. Malate dehydrogenase catalyzes a reversible reaction, and therefore the potential for malate accumulation exists. Because the mitochondrial membrane is freely permeable to malate but not to oxaloacetate, any accumulating malate might be lost to the extramitochondrial space. By inhibiting the backward reaction (oxaloacetate  $\rightarrow$  malate), palmitoyl-CoA will prevent malate depletion and hence conserve an essential citric cycle intermediate. This conservation may be important when fatty acid oxidation is a major energy source, i.e. when intramitochondrial palmitoyl-CoA levels rise sufficiently to inhibit malate dehydrogenase. But if only the backward reaction (oxaloacetate  $\rightarrow$  malate) is impaired, the continued operation of the citric acid cycle necessary for processing products of fatty acid oxidation will be assured.

From the examples given it is clear that long-chain acyl-CoA derivatives affect the structure and activity of a wide variety of enzymes and do so with a high degree of selectivity. Also, the resistance of numerous monomeric or polymeric enzymes to high concentrations of palmitoyl-CoA [lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase (132)] is inconsistent with the contention that the action of palmitoyl-CoA is that of an indiscriminate anionic detergent.

An assessment of the effective intracellular concentrations of palmitoyl-CoA is complicated by the presence of widely distributed noncatalytic

proteins with a high affinity for palmitoyl-CoA [ $\alpha$ - and  $\beta$ -protein (143–145)]. The measured acyl-CoA concentrations do not indicate, therefore, which fraction of the potential inhibitor is available for interaction with susceptible enzymes. Only in the instances of yeast (134) and liver acetyl-CoA carboxylase (136, 137) is it clear that these enzymes compete successfully for palmitoyl-CoA with whatever other proteins are present.

## ADAPTIVE CONTROL MECHANISMS

The ultimate control of fatty acid synthesis *in vivo* occurs at the level of gene expression, and this appears to be subject to developmental changes, diet, and hormones. Two mechanisms are temporally distinguishable: (a) short-term effects relating to changes in substrate and cofactor concentration or activation-inactivation of pre-formed enzymes, and (b) long-term or adaptive changes usually involving alteration of enzyme content. The effector molecules that regulate the adaptive response in fatty acid synthesis and the site of this response remain to be identified. On the other hand, it seems reasonably certain that changes in substrate, cofactor, and product concentration are responsible for short-term, including allosteric, control of enzyme activity.

Control of enzyme activity may also be mediated by an activation of dormant enzymes; evidence for two such mechanisms has been presented. In both instances the completed FAS polypeptide chains are modified by posttranscriptional events. Thus, a rapid turnover of the 4'-phosphopantetheine prosthetic group of the fatty acid synthetase (holo $\rightleftharpoons$ apoenzyme) from rat liver, brain, and adipose tissue has been observed (163). During starvation the rate of this prosthetic group exchange diminishes in liver but not in brain (163). Several other studies deal with prosthetic group turnover, but the regulatory significance of this phenomenon is not yet clear (164–166). The *in vitro* conversion of apo- to holoenzyme has been demonstrated with extracts from rat liver (165). Subsequently, the apo- and holo- forms of FAS from pigeon liver were separated by affinity chromatography, and the apo- form converted to the holoenzyme by incubation with ATP, CoA, and a pigeon liver enzyme system (166).

A second mechanism for interconverting active and inactive synthetase species involving phosphorylation has been reported (167). Two forms of the holo-fatty acid synthetase of pigeon liver (holo-*a*, which is fully active, and holo-*b*, which is less active) were separated by affinity chromatography. Holo-*a* can be converted to holo-*b* by phosphorylation with a protein kinase and ATP, and holo-*b* transformed to holo-*a* by a phosphatase (167). In apparent conflict with these results, attempts to detect a phosphorylated form of fatty acid synthetase in rat liver were unsuccessful (168).

*Dietary Effects on Fatty Acid Synthesis*

It is well-established that the rate of fatty acid synthesis is a function of the nutritional state of the animal. What is not yet understood are the signals and precise control mechanisms that modulate fatty acid synthesis in response to dietary changes. Fluctuation in the concentration of substrates and cofactors and their effects on rates of fatty acid synthesis have been evaluated by measuring levels of a large number of small molecules in rat liver under different dietary conditions (169). No relationship was found between the rate of fatty acid synthesis and the concentration of citrate, ATP, ADP, glucose, glucose-6-phosphate, or  $\alpha$ -glycerol phosphate (169). On the other hand, in short-term experiments with rats starved for 45 hr and then refed for 3 hr, fatty acid synthesis seemed to correlate positively with the concentration of malonyl-CoA and inversely with the levels of long-chain acyl-CoA (169). Hence, *in vivo* short-term control of fatty acid synthesis could be accounted for by long-chain acyl-CoA inhibition of acetyl-CoA carboxylase. Results obtained with other experimental systems are at variance with this hypothesis (170). Thus, when livers from rats maintained on different diets were perfused with glucose, there was no correlation between long-chain acyl-CoA levels and the rate of fatty acid synthesis. However, fatty acid synthesis and citrate concentrations decreased in parallel (170). On the other hand in rat liver slices, the levels of both citrate and palmitoyl-CoA were correlated with rates of fatty acid synthesis (171).

Experiments with cell cultures offer an alternative approach for evaluating changes in fatty acid synthesis due to nutritional manipulation. Fatty acid synthesis in human skin fibroblasts was reduced by 67% ten minutes after the cells were switched from a lipid-deficient medium to a medium supplemented with albumin-bound stearic acid; inhibition was only 26% with albumin-bound linoleic acid (172). However, this inhibition occurred without intracellular changes in the concentrations of long-chain acyl-CoA or citrate (172). Stearate was a much better inhibitor of *de novo* fatty acid synthesis than linoleate when added to Ehrlich cell cultures, and stearoyl-CoA (0.68  $\mu$ M for 50% inhibition) inhibited purified acetyl-CoA carboxylase from these cells more strongly than linoleoyl-CoA [37  $\mu$ M for 50% inhibition (173)]. Stearate was also better than linoleate as an inhibitor of fatty acid synthesis when added to rat hepatocytes (174) and more inhibitory than palmitate when added to chick hepatocytes (137). In rat feeding experiments, however, polyunsaturated fatty acids show a greater dampening effect than stearate (175, 176), in contrast to what is found in isolated cells. Conceivably, the response to stearate represents short-term regulation, whereas the linoleate effects are adaptive.

### *Hormones*

Not only the nutritional state but also hormones initiate short-term as well as adaptive mechanisms for controlling fatty acid synthesis (177). The five hormones that can be shown to influence fatty acid synthesis are insulin, glucagon, epinephrine, thyroid hormone, and prolactin.

The greatly impaired hepatic fatty acid synthesis in the diabetic state is corrected by administration of insulin (177–180). The defect caused by diabetes may ultimately result from a disturbance in hexose metabolism, i.e. reduced levels of inducer, increased levels of repressor, or both. Since in fructose-fed diabetic animals, fatty acid synthesis is induced normally (180), insulin per se does not appear to be the direct messenger for the adaptive response.

Glucagon antagonizes insulin effects not only in carbohydrate metabolism but in fatty acid synthesis as well. Administration of glucagon inhibits fatty acid synthesis immediately (short-term) as well as over longer periods (adaptive) (177, 179, 180). Both effects appear to be mediated by way of cAMP (179, 181). Thus feeding of dibutyryl cAMP duplicates the glucagon effects (179). Since glucagon drastically reduces glucose oxidation in liver slices (182) and since administered glucose raises hepatic cAMP levels (183), no direct action of the hormone at the level of protein synthesis need be postulated.

Glucagon also raises intracellular levels of long-chain acyl-CoA (137), possibly by way of stimulating a cAMP-sensitive triglyceride lipase. Glucagon may therefore set in motion two sets of events, ultimately inhibitory to fatty acid synthesis, reducing the level of inducer (carbohydrate metabolite) and raising the level of repressor (acyl-CoA) (177). Epinephrine, which has also been reported to impair fatty acid synthesis (178), may do so by the same mechanism as glucagon.

The inferential involvement of cAMP in the conversion of active pigeon liver fatty acid synthetase (holo-*a*) to less active holo-*b* by phosphorylation (167) is consistent with the notion that glucagon and epinephrine regulate fatty acid synthesis via the common second messenger cAMP. The stimulated event would then be protein-phosphorylation (posttranscriptional) rather than protein synthesis. However, further work is needed to establish FAS phosphorylation more firmly as a significant regulatory mechanism.

Prolactin appears to regulate fatty acid synthesis in the mammary gland but probably not elsewhere. After approximately two trimesters of pregnancy, the mammary gland of the rabbit begins to produce short-chain (octanoic and decanoic) fatty acids with a 12-fold increase in rate (184). Prolactin induces this change also in mammary gland explants from pseudopregnant rabbits (185). Corticosterone antagonizes the prolactin

effect (186). Effects of prolactin on both the synthesis and the degradation of FAS have been examined (187, 188). The hormone appears to stimulate the synthesis of the enzyme and of protein more generally in particulate-free supernatant (187). More importantly, under the influence of the hormone the degradation of FAS (but not of total supernatant protein) virtually stops until the concentration of enzyme reaches a maximum, and then degradation begins again (187, 188).

### *Mechanisms of Adaptive Control*

Long-term control involving changes in enzyme content as a result of protein synthesis and degradation has been previously reviewed (1, 2). More recently, the mechanism that causes the level of acetyl-CoA carboxylase in chick liver to increase after hatching has been examined (189). As determined by immunochemical techniques, an accelerated rate of synthesis of the carboxylase accounts for the increased amount of enzyme (189). Conversely, a decreased rate of enzyme synthesis was responsible for lower carboxylase activity in hepatocytes (JTC-25.P3 cells) grown on exogenous fatty acids (190).

If long-term regulation of fatty acid synthesis involves changes in enzyme concentration, then the controlled events may occur either at the stage of transcription or at translation. This regulatory aspect has been studied in rat liver with techniques developed by Taylor & Schimke (191). The binding of  $^{125}\text{I}$ -labeled antibody against rat FAS to polysomes was demonstrated and a correlation between the amount of antibody bound and the rate of synthesis of the FAS was established (192). Subsequently, an *in vitro* protein synthesis system was devised. This consisted of rat liver polysomes and a 30,000  $\times g$  supernatant from lysed Chang liver cells. In this system polysome-associated nascent peptides were completed and shown by SDS gel electrophoresis to have a molecular weight of 240,000 (193). Thus after fat-free refeeding of starved rats, the critical event in adaptive synthesis of FAS is the increase in the rate of *in vitro* translation of polysome-associated FAS peptides (193). It may also be noted that the first recognizable products of FAS synthesis are of the same molecular size as the multifunctional polypeptide chains obtained by dissociating native FAS. The further fate of the completed peptides has been studied recently (194). Pulse-labeling experiments suggest that the newly produced peptides enter into a pool of subunits in equilibrium with the intact FAS complex. FAS peptides have also been made in a cell-free system from pigeon liver (195).

The limited information on the mechanism of the adaptive response can be summarized as follows. Rat liver FAS is assembled in three stages. First, multifunctional polypeptide chains are synthesized from amino acids. In the second step these subunits (mol wt about 240,000) interact to form an immunologically reactive FAS duplex (apoenzyme), which lacks the

4'-phosphopantetheine prosthetic group and is therefore enzymatically inert. Finally, active FAS or holoenzyme is generated by enzymatic attachment of 4'-phosphopantetheine (163–166, 194). Collectively, this three-stage assembly process constitutes the events that occur adaptively in response to a variety of nutritional, hormonal, and developmental signals and that determine the quantity of active FAS. Some or perhaps all of these steps are reversible and lead to inactive enzyme forms, e.g. holoenzyme → apoenzyme. The tissue level of FAS—as of all proteins—is therefore a function of several synthetic and degradative rates.

The key problem in the adaptive response remains the identification of the inducer or repressor molecules and the sites at which they act (transcription, translation, or posttranslational). The most massive induction of fatty acid synthesis, or more specifically of enzyme synthesis, occurs in animals refed glucose or fructose after a preceding fast (1). The question is therefore being asked whether a normal carbohydrate metabolite serves as the signal in the adaptive response. Although the available information is scant, it points to glycolytic three-carbon intermediates or perhaps citrate as the inducer. Since citrate controls acetyl-CoA carboxylase allosterically and since the adaptive syntheses of the carboxylase and of FAS are often coordinated, it is an attractive hypothesis that the same effector molecule controls both the short-term and the long-term alterations of fatty acid synthesis (2, 136, 166, 177, 196).

Similarly it can be argued that the repressors causing the negative adaptive response of FAS synthesis are long-chain acyl derivatives, molecules that also inhibit isolated acetyl-CoA carboxylase and to a lesser extent FAS activity. All conditions inhibitory for fatty acid synthesis such as starvation, high-fat diets, and the diabetic state are accompanied by elevated levels of long-chain acyl-CoA. However, it should be kept in mind that fatty acids are subject to a variety of metabolic transformations in isolated cells as well as in the intact animal. The repressor could therefore be a fatty acid metabolite of perhaps still unknown structure. The recent clarification of the role of cholesterol in the repression of  $\beta$ -hydroxyl- $\beta$ -methylglutaryl-CoA reductase (197, 198) may provide an instructive analogy. First of all, the cell surface receptor for the repressor in fibroblasts is specific for lipoprotein-linked cholesterol rather than cholesterol itself (197). Secondly, hitherto unknown and accidentally discovered derivatives (25-hydroxy-cholesterol and 7-keto-cholesterol) exceed the repressor potency of cholesterol by several orders of magnitude (198).

### *Control of Fatty Acid Synthesis in Yeast*

Studies with yeast mutants have been instrumental for much of the recent progress made in the elucidation of the subunit structure of fatty acid synthetases (see earlier section on subunit structure of FAS). A large num-

ber of noncomplementing FAS mutants of *Saccharomyces cerevisiae* have been isolated in which FAS proteins or peptides could not be detected by the isolation procedures or immunological techniques applicable to wild-type enzyme (199). Whether these mutants are defective in the coordinate production of the two distinguishable FAS peptides (A and B) or defective in the association and formation of intact synthetase is not yet established (199). The finding that the content of acetyl-CoA carboxylase is reduced in yeast cells grown on a medium supplemented with fatty acids (200) has been followed by work with a mutant of *S. cerevisiae* defective in acyl-CoA synthetase. In order to repress the synthesis of acetyl-CoA carboxylase, activation of the exogenous fatty acid is required (201). These results are intriguing because they point to the possibility already raised that long-chain CoA is both an allosteric effector and a repressor.

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