METABOLIC ALTERATIONS OF FATTY ACIDS

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INTRODUCTION

This article is a logical extension of last year's review by Volpe & Vagelos (1). These authors considered saturated fatty acid biosynthesis and its regulation, whereas this review covers the metabolic alteration of fatty acids and especially the introduction of double bonds and hydroxy groups into the fatty acyl chain. Hydrogenation and hydration of double bonds in the fatty acyl chain are also covered to the extent that these reactions are distinct from those involved in either fatty acid synthesis or β oxidation. The latter subject has been included in a number of recent reviews including those by Green & Allmann (2), Stumpf (3), and Bishop & Stumpf (4). The conversion of polyunsaturated fatty acids to prostaglandins has also been reviewed recently (5) and is not covered here. Those metabolic reactions of fatty acids that center at the carboxyl carbon are covered in part by a companion review by van den Bosch in this volume (6) and by a number of other recent reviews in this series (7–10).

UNSATURATED FATTY ACID BIOSYNTHESIS

Research on unsaturated fatty acid biosynthesis has accelerated rapidly in the past decade. This effort was stimulated by the initial studies of Bloch and his group (11) on monounsaturated fatty acid biosynthesis and by Mead and co-workers (12) on the metabolism of polyunsaturated fatty acids.

To date, many of the major metabolic pathways for the formation of unsaturated fatty acids have been mapped, a number of cell-free desaturation systems have been studied, and some initial success has been achieved in attempts to understand both the biochemical mechanisms of the desaturation reaction and those factors involved in the regulation of unsaturated fatty acid biosynthesis. In this review I hope to integrate the recent advances in these areas into the overall picture that has emerged within the past decade.

Metabolic Pathways

A large variety of naturally occurring unsaturated fatty acids can be distinguished by differences in the position, type and number of double bonds and by variations in chain length and carbon branching. Despite this diversity, however, there appear to be only two distinct biochemical mechanisms for the introduction of *cis* double bonds (13). In many bacteria, a *cis*-3 double bond is introduced into a medium chain length fatty acid (usually C_{10}) by dehydration of a fatty acid synthesis intermediate, the β-hydroxyacyl thioester derivative of acyl carrier protein (ACP), by a specific enzyme, β-hydroxydecanoyl thioester dehydrase (14–18). Chain elongation of the *cis*-3 derivative gives rise to long-chain unsaturated fatty acids (11). This so-called "anaerobic" pathway is identical (except for the formation of the β,γ-unsaturated intermediate) to the pathway for saturated fatty acid biosynthesis in *E. coli* (1) and is not considered in this review. A second mechanism for *cis* double bond formation involves the direct, oxygen-dependent desaturation of long-chain fatty acids (19, 20). Although certain bacteria and presumably all nonparasitic eucaryotic organisms utilize the O_2-dependent or aerobic mechanism to produce unsaturated fatty acids, differences in the nature and availability of the activated substrates, the cofactor requirements other than O_2, and specificities of the enzyme systems themselves probably account for the differences in metabolic pathways found among various groups of organisms. Recent work on the comparative aspects of these pathways is discussed below.

BACTERIA The anaerobic pathway for the formation of monounsaturated fatty acids (to date found only in bacteria) leads generally to palmitoleic (9-hexadecenoic) and vaccenic (11-octadecenoic) acids as the major products, while the aerobic pathway found in certain bacteria yields primarily palmitoleic and oleic (9-octadecenoic) acids (11, 21). There are exceptions to these generalizations, however, particularly in the aerobic pathway. The bacilli, for example, produce a variety of isomeric hexadecenoic acids by O_2-dependent desaturation. *B. megaterium* KM desaturates palmitate and stearate to form exclusively the *cis*-5 derivatives (22). Fulco (23, 24) showed that *B. megaterium, B. subtilis,* and certain strains of *B. pumilis, B.*

licheniformis, and *B. alvei* also desaturate palmitate exclusively in the 5 position, while other bacilli tested, including various strains of *B. brevis, B. stearothermophilus, B. macerans,* and *B. licheniformis* produce various mixtures of the 8-, 9-, and 10-hexadecenoic isomers. Dart & Kaneda (25) showed that *B. cereus* desaturates palmitic and stearic acids to the Δ^{10} isomers while Kaneda (26) found that three psychrophilic species of Bacillus (*B. insolitus, B. psychrophilus,* and *B. globisporus*) produce exclusively the Δ^5 isomers. Among the mycobacteria, the Δ^{10} and Δ^9 isomers of hexadecenoic and octadecenoic acids are often found together (27, 28). Apparently the positional isomers are not interconvertible but are produced independently by O_2-mediated desaturation of the saturated precursors (27). Curiously, although whole cells of *M. phlei* desaturate palmitate predominantly in the 10 position, a cell-free desaturating system obtained from the same organism produces only the Δ^9 isomer (29).

Asselineau and her co-workers (30) have isolated a series of monounsaturated fatty acids from *M. phlei* containing 20–27 carbon atoms. These included normal chain Δ^5-monoenoic acids of 22, 24, or 26 C atoms, branched-chain Δ^5 derivatives of 25 and 27 C atoms, and a series which included 4-eicosenoic, 6-docosenoic, and 8-hexacosenoic acids. They suggest that the Δ^5 acids may play a role in the synthesis of the mycolic acids of the same organism.

Leptospira canicola has been found to desaturate palmitate to a mixture of the *cis*-Δ^9- and the *cis*-Δ^{11}-hexadecenoic acids whereas stearate is desaturated at the 9 position only (31). This seems to be the first demonstration in bacteria of the formation of a *cis*-11-monoenoic acid by O_2-dependent desaturation of the corresponding saturated acid. Recently, the desaturation of (1-^{14}C)-labeled palmitate and stearate was reported to occur in *Alcaligenes faecalis* (32), an organism known to produce 11-octadecenoic acid and cyclopropane fatty acids (33), both typical products of the anaerobic pathway. However, an oxygen requirement for desaturation was not demonstrated and since there was some randomization of the (1-^{14}C) label, unambiguous interpretation of these results is not yet possible.

It was originally thought that polyunsaturated fatty acids could not be synthesized by bacteria (21), but with our present knowledge of the O_2-dependent pathway in these organisms, there seems to be no reason why this should be the case. Nevertheless, the occurrence of polyunsaturated fatty acids in bacteria is extremely rare. Apparently, the only unequivocal evidence for the synthesis of diunsaturated fatty acids in bacteria was reported by Fulco (34), who showed that 5,10-hexadecadienoic was produced by the direct desaturation of added (1-^{14}C) palmitate in a strain of *B. licheniformis.* He later showed (35) that the biosynthesis of this unique dienoic acid resulted from the cooperative action of two distinct desaturation systems. One system, present in the bacterium at both 35° and 20°, desaturated palmitate to 10-hexadecenoic acid, while a second system, active only at the lower temperature, resulted in the conversion of palmitic acid to 5-hexadecenoate. Both the Δ^5 and Δ^{10} monoenoic acids were shown to be precursors of the 5,10-dienoic acid. Recently Sklan, Budowski & Volcani (36) reported the synthesis of linoleate from stearate and oleate in a supernatant fraction from calf rumen liquor. Sklan & Budowski (37) found that rat colonic contents incubated aerobically carried out the same trans-

formation. In both cases, the biosynthesis of linoleic acid was attributed to bacteria but proof of this assumption must await further experimentation. This reviewer considers synthesis by protozoa a more likely possibility. Asselineau and others (38) have isolated from *M. phlei* several highly unsaturated fatty acids (the so-called phleic acids) of obscure metabolic origin, whose main member has the structure n-$C_{16}H_{33}[CH{=}CHCH_2CH_2]_5COOH$.

FUNGI, PROTOZOA, ALGAE, AND PLANTS Because of the extensive work in this area in the past few years, comprehensive coverage of recent developments is beyond the scope of this review. The reader is referred to the excellent review by Stearns (39) and to the book by Hitchcock & Nichols (20) for a more complete coverage of the pathways of unsaturated fatty acid biosynthesis in these organisms, particularly in algae and plants. The short review by Erwin & Bloch (21), although somewhat dated, is also helpful.

Recent work in blue-green algae and in higher plants has blurred the once clearcut distinction between the pathways leading to polyunsaturated fatty acids in these organisms and in the metazoa. It was originally proposed (13, 21) that, starting with oleic acid as a common precursor, algae and higher plants inserted additional double bonds between the pre-existing double bond and the terminal methyl group to produce successively linoleic acid (40) and α-linolenic (9,12,15-octadecatrienoic) acid, whereas animals could desaturate only between the original double bond and the carboxyl group to yield 6,9-octadecadienoic acid or, by chain elongation and further desaturation, 5,8,11-eicosatrienoic acid (41). The animal could also carry out the same reactions on dietary linoleic acid to obtain γ-linolenic (6,9,12-octadecatrienoic) acid and arachidonic acid (42, 43). Linoleate and α-linolenate were thus considered to be typical plant fatty acids while γ-linolenate and arachidonate were characteristic of animal pathways. If one excludes the protozoa, no one has yet demonstrated clearly de novo biosynthesis of linoleate and α-linolenate in animals. However, numerous representatives among the algae and metaphyta are now known to synthesize the typical animal polyunsaturated fatty acids. Among the algae, the unsaturated fatty acid composition and pathways have been most intensively studied in the blue-green algae. Compositional data has been used to assess certain geochemical relationships (44), as an aid in determining phylogenetic positions (45), and especially in the study of the possible evolutionary significance of polyunsaturated fatty acids within this group (46–49). Thus Kenyon, Rippka & Stanier (48) examined 32 axenic strains of filamentous blue-green algae and Kenyon (49) studied 34 strains of unicellular blue-green algae; they found that four metabolic groups could be recognized according to the major fatty acid of highest degree of unsaturation found in each strain. These groups included: 1. those in which there is little or no desaturation of oleate; 2. those in which linoleate is desaturated toward the methyl end of the molecule to give α-linolenate; 3. those in which linoleate is desaturated towards the carboxyl end to give γ-linolenate; and 4. those in which octadecatetraenoate is synthesized. Thus, within this group of prokaryotic organisms are found pathways which were considered typical of animals (i.e. pathway 3) and of higher plants (pathway 2). In addition, the blue-green algae of group 1 resemble

bacteria in their inability to desaturate oleate. These authors conclude that the blue-green algae are a living record of the transition stages between the bacteria and the higher protists with respect to fatty acid biosynthesis.

Holz and his co-workers have analyzed the polyunsaturated fatty acids of marine and freshwater cryptomonads (50) and marine dinoflagellates (51) and have found a diversity similar to the blue-green algae but with certain differences (including the synthesis of C_{20} and C_{22} polyunsaturated fatty acids) that suggest further evolution in the pathways for polyunsaturated fatty acid biosynthesis. In a sense, certain protozoa represent the evolutionary apex of biosynthetic pathways leading to polyunsaturated fatty acids. A number of phytoflagellates contain within the same organism the polyunsaturated fatty acids characteristic of animals (i.e. γ-linolenic, arachidonic) and of plants (linoleic, α-linolenic). The same is true for some amoebae and ciliates (21). Indeed, Gellerman & Schlenk (52) concluded from a study of unsaturated fatty acid metabolism in *Ochromonas danica* that chain elongation and desaturation of the proximal part of already unsaturated fatty acid chains appear to be subject to the same structural requirements as in the rat and the same effects apply to desaturation in the distal part of the chain.

One can surmise that separate plant and animal pathways arose during further evolution by the loss of either the ability to desaturate between the pre-existing double bond(s) and the terminal methyl group (animals) or between the pre-existing double bond(s) and the carboxyl group (most higher plants). A number of plants, particularly those low on the evolutionary scale, have retained the ability to desaturate on both the carboxyl and methyl sides of pre-existing double bonds. Schlenk & Gellerman (53) were the first to clearly demonstrate the presence of arachidonic acid in mosses and ferns, and these workers, in collaboration with Anderson, have recently broadened and extended these findings (54, 55). Among the fungi, the phycomycetes synthesize γ-linolenic acid while the ascomycetes and basidiomycetes produce α-linolenic acid (56).

ANIMALS The pathways for polyunsaturated fatty acid biosynthesis in animals have been elaborated in great detail in recent years. Fortunately for this reviewer, Mead (12) has summarized this work up to 1969. More recently Brenner and his co-workers (57–60), Sprecher et al (61–65), Bridges & Coniglio (66–68), and a number of other workers (69–72) have continued to study the biosynthetic pathways and interconversions of the polyunsaturated fatty acids in animals, particularly in the rat. Ayala et al (60), utilizing subcellular fractions from rat liver and testes, compared the metabolic fate of linoleic, arachidonic, and 7,10,13,16-docosatetraenoic acids. Conversion of linoleic to arachidonic by microsomes in both tissues proceeded through γ-linolenic and 8,11,14-eicosatrienoic acids as originally demonstrated by Mead et al in whole animals (12). Testicular mitochondria carry out the retroconversion of 7,10,13,16-docosatetraenoate to arachidonate, but there was no evidence in vitro of the conversion of 4,7,10,13,16,19-docosahexaenoic acid to 7,10,13,16,19-docosapentaenoate, a process demonstrated in whole animals by Schlenk, Sand & Gellerman (69).

Ayala et al propose that the synthesis of acids of the linoleic family proceeds in

two stages: a rapid one in which arachidonic acid is made and a second, slower stage in which the C_{22} and C_{24} acids are synthesized. They also suggest that there is a cycle between microsomes and mitochondria that acts to conserve essential polyunsaturated C_{20} and C_{22} acids by means of synthesis and partial degradation, respectively. Ullman & Sprecher (65) have concluded from both in vitro and in vivo studies that the Mead pathway for arachidonic acid synthesis from linoleate (i.e. through γ-linolenate and 8,11,14-eicosatrienoate) is the only significant pathway in the rat for synthesis of this essential fatty acid. The C_{24}-polyunsaturated fatty acids of rat testes, 9,12,15,18-C_{24}-tetraenoic and 6,9,12,15,18-C_{24}-pentaenoic acids, were shown by Bridges & Coniglio (66) to be derived from linoleic acid via C_{22}-tetraene and C_{22}-pentaene, respectively.

In insects, the pathways for unsaturated fatty acid biosynthesis are less well known than in vertebrates. The de novo biosynthesis of polyunsaturated fatty acids remains an open question, although there is little doubt that some insects produce monoenoic acids by the same pathway as higher animals. Municio and his co-workers (73) have studied in vitro the elongation and desaturation of C_{10}, C_{12}, C_{14}, and C_{16} saturated fatty acids during development in insects and have shown distinct differences between homogenates prepared from larvae or pharate adults of *Ceratitis capitata*. Larval homogenates desaturate and elongate the (^{14}C)-substrates according to their chain length, with elongation decreasing and desaturation increasing with increasing chain length of substrate. These reactions, however, are insignificant in homogenates prepared from adults. Thompson & Barlow (74) injected (1-^{14}C)-acetate in adult male *Galleria mellonella* and demonstrated significant incorporation of labeled acetate into 9-eicosenoic acid, as well as in myristic, palmitic, palmitoleic, stearic, and oleic acids. Degradative analysis indicated that synthesis of monounsaturated acids proceeded through direct desaturation of the saturated analogs. These workers also found that several insect parasites (*Itoplectis conquisitor* and *Exeristes comstockii*) could, to a limited extent, metabolize and desaturate fatty acids independently of the host (75, 76).

ALTERNATE PATHWAYS IN PLANTS AND ANIMALS There is always the temptation, after elucidating a biosynthetic pathway in one or more members of a particular class or family of organisms, to assume that all members of the group have the same pathway. Although this may often be true, two recent examples are enough to show the danger inherent in such generalizations. More than a decade ago, Fulco & Mead (77) showed that the rat could not convert *cis*-12-octadecenoic acid to linoleic acid, even though such a transformation would be expected if this unsaturated acid served as a substrate for the Δ^9-desaturase responsible for the conversion of stearate to oleate in the same animal. Although from these results it would seem likely that *cis*-12-octadecenoate would be an ineffective precursor of linoleate in other animal systems as well, such is not always the case. Gurr et al (78) have recently shown that goat, pig, hen, and *Chlorella vulgaris* convert *cis*-12-octadecenoate into linoleate in good yield; rabbit and mouse do so in very poor yield, whereas rat, hamster, and *Candida utilis* do not desaturate this substrate.

In the plant kingdom, the biosynthetic route for the formation of α-linolenate has

always been assumed to proceed as follows: oleate → linoleate → α-linolenate (i.e. the typical plant pathway). Although the generality of the oleate to linoleate conversion has been well demonstrated in a large number of systems ranging from algae, fungi, and protozoa to higher plants, the direct conversion of linoleate to α-linolenate has been clearly demonstrated only in algae and fungi (39). Recently, Jacobson, Kannangara & Stumpf (79, 80) have provided compelling evidence that the major pathway for the formation of α-linolenate in spinach is not by further desaturation of linoleate but rather by chain elongation of 7,10,13-hexadecatrienoic acid. Using a disrupted chloroplast system, these workers showed that under conditions where (^{14}C) acetate was readily converted to α-linolenate, (^{14}C) oleate was a totally ineffective precursor. Furthermore, a specific elongation system which converted 5,8,11-tetradecatrienoate to the C_{16} triene and this, in turn, to α-linolenate was demonstrated. This elongation system did not act on saturated or monounsaturated substrates. Thus the typical plant pathway leading from oleate to α-linolenate may, in higher plants, be the exception rather than the rule.

Enzymology and Substrate Specificity

The first cell-free system which carried out fatty acid desaturation was described by Bloomfield & Bloch (81) in 1960 and since that time more than a dozen distinct cell-free systems have been described. Most of these are listed in Table 1. Systems utilizing intact chloroplast preparations, those in which direct desaturation of a substrate was not clearly demonstrated or those which are similar in essentials to a system listed in Table 1, have been omitted. Several of these are described in recent books and reviews (4, 11, 12, 19, 20, 39).

It is obvious from Table 1 that the enzymology of O_2-mediated desaturation remains in a primitive state. Only two active systems have been solubilized; the soybean cotyledon preparation (90), which has not been fractionated, and the photoauxotrophic *Euglena gracilis* system (86), which has been separated into three protein components. The other systems are either particulate preparations or homogenates which have not been further characterized. Despite this, a great deal of information has been obtained that allows several general conclusions. First of all, both O_2 and a reduced pyridine nucleotide are required for activity in every cell-free system that carries out the direct removal of two hydrogens to form a *cis* double bond. Furthermore, it would seem that more or less complex electron transport chains are involved in the desaturation process in most of these systems. A number of notable differences among the various systems are also evident, including differences in the components of the electron transport chains and in the types of acyl derivatives that will serve as substrates. One may distinguish three types of acyl derivatives desaturated in one system or another. Animal microsomal systems seem to desaturate only acyl-CoA esters; when the free fatty acids are added, both CoA and ATP must generally be included for maximal desaturation. The particulate cell-free bacterial system studied (82) also utilizes the CoA esters, as do the particulate stearate desaturases of the yeast *Saccharomyces cerevisiae* (81), the fungus *Neurospora crassa* (84), and the etiolated heterotrophic alga *E. gracilis* (86, 87). When *E. gracilis* is grown photoauxotrophically, however, a soluble desaturating

Table 1 Cell-free fatty acid desaturation systems[a]

Source and type of cell-free system	Major substrate and product	Components of system and cofactors	Reference
Mycobacterium phlei (bacterium) particulate	stearyl-CoA → oleate[b]	100,000 × *g* particles, O_2, NADPH, Fe^{+2}, FAD (or FMN)	(29, 82)
Saccharomyces cerevisiae (yeast) particulate	palmityl-CoA → palmitoleyl-CoA	100,000 × *g* particles, O_2, NADPH (or NADH)	(81)
Torulopsis utilis (yeast) microsomal	oleyl-phosphatidyl choline → linoleyl-phosphatidyl choline	microsomes, 100,000 × *g* supernatant, O_2 NADPH	(83)
Neurospora crassa (fungus) microsomal	stearyl-CoA → (oleyl-CoA) → oleyl-phospholipid → linoleyl-phospholipid	microsomes, O_2, NADH	(84)
Euglena gracilis, photoauxotrophic (alga) soluble	stearyl-ACP → oleate[b]	desaturase protein, flavoprotein, ferredoxin, O_2, NADPH	(85, 86)
Euglena gracilis, dark grown heterotrophic (algal) particulate	stearyl-CoA → oleate[b]	particles, supernatant O_2, NADPH	(86, 87)
Carthamus tinctorius seeds (plant) membrane fragments	oleyl-CoA → linoleyl-CoA	particles, O_2, NADH	(88)

Potato tubers (plant) microsomal	oleyl-CoA → linoleyl-phospholipid	microsomes, O_2, NADPH (or NADH)	(89)
Soybean cotyledon (plant) soluble	stearate → oleate[b] oleate → linoleate[b]	100,000 × *g* supernatant, CoA, ATP, Mg^{+2}, O_2, NADPH	(90)
Bovine mammary tissue microsomal	stearyl-CoA → oleate palmityl-CoA → palmitoleate	microsomes, O_2, NADH	(91)
Rat brain homogenate	stearyl CoA → oleate[b]	homogenate, O_2, NADH	(92)
Rat liver microsomal	stearyl-CoA → oleyl-CoA	microsomes, O_2, NADH (or NADPH), (or ascorbate[c])	(93–97)
Hen liver microsomal	stearyl CoA → oleate[b]	acetone-extracted microsomes, lipid, O_2, NADH (or NADPH)	(98)
Rat liver microsomal	linoleyl-CoA → *γ*-linolenyl-CoA	microsomes, supernatant, O_2, NADPH (or NADH)	(99, 100)
Rat liver microsomal	Systems leading to other polyunsaturated fatty acids	microsomes, O_2, NADPH (or NADH)	(55, 60, 63–65, 101, 102)

[a] Limited to those systems that carry out the direct removal of 2H from a fatty acyl chain to form a *cis* double bond.
[b] Product hydrolyzed before analysis.
[c] In high concentrations can partially replace NADPH but desaturation activity is very low.

system can be isolated which desaturates stearyl-ACP much more efficiently than stearyl-CoA (stearyl-ACP is not a substrate for the particulate desaturase obtained from the etiolated heterotrophic organism). Surprisingly, the purified reconstituted soluble system desaturates the ACP and CoA derivatives of stearate equally well. In spinach chloroplasts (87) the desaturation system is specific for stearyl-ACP. In view of these results and less direct evidence by others (19) it seems likely that as more higher plant systems are investigated, additional monodesaturases will be found which preferentially utilize the saturated acyl-ACP thioesters as substrates.

The role of acyl-ACPs as desaturation substrates in nonphotosynthetic systems, on the other hand, would seem at best limited. Unlike green plants and bacteria, animals do not utilize ACP or an ACP-like protein in acyl transfer reactions or in fatty acid synthesis (1, 103) and all the animal desaturases so far studied require the CoA esters as substrates. Although yeasts do contain an ACP-like protein, it is tightly bound to the fatty acid synthetase complex and does not participate in the enzymatic desaturation of fatty acids (86, 104). The conversion of oleate to linoleate does not seem to involve the ACP derivative but here the picture is quite complex. Vijay & Stumpf (88, 105) have shown that oleyl-CoA is converted to linoleyl-CoA by a particulate desaturase from *Carthamus tinctorius* (safflower) seeds. Ben Abdelkader et al (89), using (1-^{14}C) oleyl-CoA as a substrate for a desaturase associated with a microsomal fraction from aged slices of potato tuber, were able to obtain 40% conversion to linoleate. However, all of the labeled linoleate and the remaining (1-^{14}C) oleate were incorporated into phospholipids, particularly phosphatidylcholine. Similar results were obtained by Baker & Lynen (84) using microsomes from *N. crassa.* They presented indirect evidence that oleyl phospholipid was an intermediate in the conversion of oleyl-CoA to linoleyl phospholipid. Gurr et al (106), using whole cells and isolated chloroplasts of *Chlorella vulgaris,* found a tight coupling between incorporation of (1-^{14}C) oleate into the 2 position of phosphatidylcholine and the appearance of label in linoleate. Direct proof that oleyl phospholipid can serve as a desaturation substrate has recently been obtained by Talamo, Chang & Bloch (83). They showed that a particulate enzyme system from the yeast *Torulopsis utilis* catalyzes the conversion of oleyl-CoA or oleyl phospholipid to linoleate. Incubation of particles with (^{3}H)-oleyl-CoA in the absence of a supernatant fraction yields oleyl phospholipid. This material is then desaturated to linoleate either in situ after addition of supernatant or after isolation by the complete enzyme system. Oleyl phosphatidylcholine was the most active of the various phospholipid fractions tested for desaturation. Furthermore, synthetic 1,2-di-(^{14}C)-oleyl phosphatidylcholine is converted to linoleate with high efficiency.

To this point we have considered substrate specificity in the desaturation reaction in terms of the group attached to the carboxy carbon of the fatty acid. Obviously, however, the nature of the fatty acyl chain is also of great importance. A. T. James and his co-workers (107, 108) have studied the effects of double bond position on the desaturation of monoenoic acids and the efficiency of conversion of homologous (^{14}C) labeled fatty acids to the corresponding Δ^9-monoenoic acids by goat mammary gland microsomes, hen liver microsomes, and whole cells of the yeast *Torulopsis bombicola* and of the alga *Chlorella vulgaris.* They also studied Δ^9-desaturation by

these systems of a series of isomeric methylstearic acids. Their findings can be summarized as follows: In each of the four systems tested with homologous saturated fatty acid substrates (C_{12}–C_{19}), C_{18} was the most active, followed by C_{17}. Activity with C_{19} was always low (except in hen) but activity with C_{16} varied from system to system. Thus in *T. bombicola* and with hen microsomes, C_{16} was desaturated almost as well as C_{17} and C_{18}, while in goat mammary microsomes and in *C. vulgaris* it was much less active. A second activity peak at shorter chain lengths was clearly observed in two of the systems: at C_{14} for hen microsomes and at C_{15} in chlorella. Indeed, in the former system, C_{14} was desaturated almost as well as C_{18}. The isomeric methyl stearates were tested in the hen microsomal Δ^9-desaturating system and in all cases activity was lower than with stearate itself. The two most active isomers were 2-methyl- and 18-methyl-stearate (i.e. *n*-nonadecanoate) but the 17-methyl and 4-methyl derivatives were also desaturated reasonably well. The 3 and 16 substituted isomers showed only very low activity, whereas there was no significant desaturation when the methyl group was located at or between carbon atoms 5 to 15 of the stearyl chain. On the basis of this finding, Brett et al (107) proposed that substrate (between C-5 and C-15) is so closely bound to the enzyme surface that the methyl groups cannot easily be accommodated. In a hen liver desaturating system [Johnson et al (109)] and in rat liver microsomes [Paulsrud et al (110)], maximal Δ^9 desaturation was observed with stearic acid; palmitic and margaric acids were less active.

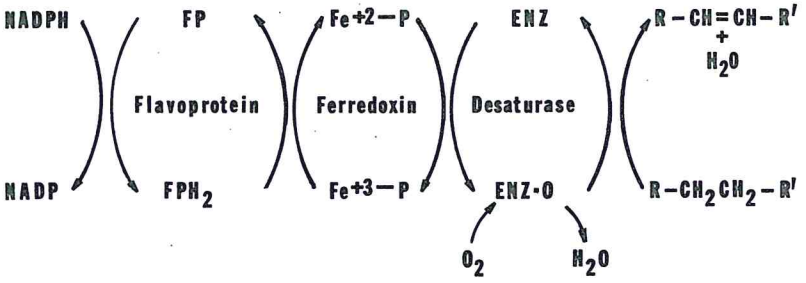
Similar specificity patterns were observed in several bacterial systems (22, 29), although in *Micrococcus lysodeikticus* palmitate was significantly favored over stearate for Δ^9 desaturation. Quint & Fulco (111) used a variety of methods to determine in vivo the substrate specificities for six desaturases from bacilli that insert a *cis* double bond at the 5 and 10 positions of the acyl chain, respectively. Five of the six Δ^5 desaturases and the Δ^{10} desaturase showed maximal activity with palmitate. Activity for the *n*-saturated substrates decreased in the order $C_{16} > C_{17} > C_{18}$. Myristic acid (C_{14}) was not desaturated, while C_{15}, tested with two Δ^5 desaturases, had about half the activity of C_{16} in both cases. The branched chain acids, iso-C_{16}, and iso- and anteiso-C_{17} were also desaturated but iso- and anteiso-C_{15} acids were not touched. Various monounsaturated C_{16} and C_{18} acids were also desaturated at the 5 position. The positional specificity of desaturation was always the same relative to the carboxyl carbon of the substrate, regardless of substrate chain length, branching, or the presence of a double bond in the 9 or 10 position. The authors concluded that the substrate is attached to the desaturase at the carboxyl carbon of the fatty acid and that the carboxyl binding site of the enzyme must be at a fixed distance from the active (hydrogen removal) site to account for the absolute positional specificity of double bond insertion. On the other hand, the efficiency of desaturation (substrate specificity) must depend on a second binding site which anchors the substrate to the enzyme near the terminal methyl group of the fatty acid. This site is visualized as a hydrophobic pocket on the enzyme surface which may vary slightly in shape from enzyme to enzyme but appears to be located, for the desaturases studied, about 15 carbon atoms away from the carboxyl binding site (assuming that the fatty acid substrate is fully extended along the enzyme surface).

Mechanisms of Desaturation

The chemical mechanism(s) of O_2-dependent desaturation of fatty acids have not yet been elucidated, primarily because no stable desaturation system has yet been fractionated into its components, rigorously purified, and then studied with this end in view. Ideally, such a study would elucidate the stereochemistry of hydrogen removal to form the *cis* double bond, the nature of the intermediates (if any) and the primary acceptors of the hydrogens removed, the role of oxygen in this process, and the nature and function of the individual components of the electron transport chain involved in the overall reaction. The stereochemistry of hydrogen removal, first studied by Schroepfer & Bloch (112) and later by Morris and his co-workers (113), has been considered in detail in reviews by Bloch (11) and Morris (114). Hence, this work is not considered here except to note that in all Δ^9-desaturating systems studied, the 9-D and 10-D hydrogens of the fatty acyl chain are stereospecifically removed to form the *cis* double bond. In *Corynebacterium diphtheriae,* isotope effects observed in the formation of oleate suggest that hydrogen removal at carbon 9 precedes hydrogen removal at carbon 10 of stearate (112). However, from similar experiments carried out with chlorella cells and goat mammary gland microsomes, Morris (114) concludes that desaturation reactions in these systems probably involve concerted removal of a pair of hydrogen atoms from the 9 and 10 positions.

To date, no intermediate in the desaturation reaction has been detected. A wealth of data gathered chiefly from work with the systems listed in Table 1 would seem to rule out hydroxystearate as an intermediate or a precursor to oleate; whether some enzyme-bound oxygenated intermediate will eventually be implicated remains to be seen. Recent work has shed little additional light on this problem, and the reader is referred to several excellent books and reviews (4, 11, 19, 20, 114) for its further discussion.

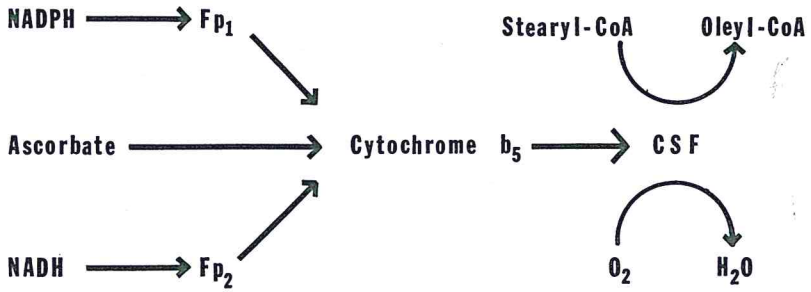
Recent research has partially elucidated the nature and function of the individual components of the electron transport chains involved in O_2-mediated desaturation of fatty acids. Nagai & Bloch (85, 86) showed that desaturation of stearyl-ACP by a soluble system from *E. gracilis* required at least three distinct protein components, a flavoprotein which catalyzed the oxidation of NADPH, ferredoxin, and the desaturase, in addition to O_2 and NADPH. Inhibition was observed with KCN (50% at 10^{-5} *M*) but desaturation was not affected by high CO concentrations (an inhibitor of cytochrome P-450). NADPH could not be replaced by high ascorbate concentrations or by formamidine sulfinic acid, an electron donor for certain ferredoxin-requiring enzyme reactions. However, low concentrations of dithionite partially replaced NADPH in the ferredoxin-dependent stearyl ACP desaturation by spinach chloroplasts. Based on these results, Bloch (11) proposed the electron transport chain for oxygen activation in the desaturation reaction in these systems shown in Scheme 1. In the particulate preparation from *M. phlei* (82) the necessary cofactors included FAD and Fe^{+2} in addition to O_2 and NADPH. Thus at first glance one might propose a similar electron transport scheme for this system as well, assuming that the FAD was a component of a NADPH oxidase while the Fe^{+2} served as the active portion of a nonheme iron protein. However, detailed comparison



Scheme 1

of the two systems leads to certain conflicts. For example, in *M. phlei,* NADPH, FAD (or FMN), and Fe^{+2} are all absolute requirements. Fe^{+3} does not replace Fe^{+2}, even in the presence of all other components. If Scheme 1 holds, it is not clear why NADPH and FAD are needed in the presence of Fe^{+2}, since they presumably function only to reduce iron to the ferrous state. Similarly, if this is their function, then Fe^{+3} should serve in place of Fe^{+2} when NADPH and FAD are present. Also, in the *M. phlei* system, neither KCN nor EDTA were good inhibitors.

Oshino and his co-workers (93–97) have presented evidence that the microsomal desaturation system from rat tissue, responsible for the conversion of stearate to oleate, consists of at least four protein components which include NADH-cytochrome b_5 reductase (F_{P_2}), NADPH-cytochrome *c* reductase (F_{P_1}), cytochrome b_5, and a terminal component called cyanide-sensitive factor (CSF) which seems to function as an oxygen-activating enzyme (93–97). Other workers, including Holloway & Wakil (115), Gaylor et al (116), and Brenner (58), have also made significant contributions to our developing concepts of the electron transport system involved in desaturation and in general their findings supplement and confirm those of Oshino's group. Oshino & Sato (96) propose the following scheme for electron transport among the various components of the rat liver microsomal desaturating system.



Scheme 2

This scheme is essentially the same as that proposed by Bloch (Scheme 1) for the *E. gracilis* system except that cytochrome b_5 replaces ferredoxin, and two flavoproteins, specific either for NADPH or NADH, mediate electron transport between the reduced pyridine nucleotides and the nonheme iron protein. Indeed, in the soluble system of Nagai & Bloch (86), the crude (but not the purified) preparation could utilize NADH as well as NADPH. Brenner's postulated scheme for electron transport involved in the desaturation of linoleate to γ-linolenate (58) again is in essential agreement with Schemes 1 and 2. Thus, the overall process of electron transport may well be the same, in the broad sense, for all O_2-dependent desaturations (although there are still unresolved problems with the bacterial system). However, the specifics of O_2 involvement remain completely unknown and the solution to this problem must await the purification and rigorous study of the desaturase itself.

Regulation and Control

A multitude of interlocking controls and regulatory mechanisms are known to operate in various organisms which act to maintain or adjust the level of specific unsaturated fatty acids in lipids of structural or functional importance in biological systems, particularly membranes (117–119). Of special interest to this reviewer are those regulatory mechanisms which adjust the composition of the unsaturated fatty acid component of membranes to compensate for changes in the environmental temperature. The commonly observed inverse relationship between temperature and unsaturated fatty acid composition almost certainly represents an adaptation on the part of the organism to maintain the integrity and function of cell membranes. Rose (120) has reviewed the work in this area to 1968. A number of more recent studies illustrate this adaptation process in bacteria (23, 24, 34, 35, 121–130), in yeast and fungi (131–135), in algae (136), in higher plants (137, 138), and in vertebrates (139–142). Until quite recently, however, the mechanism of these effects remained obscure. A number of possibilities exist; these include changes in 1. relative rates of β oxidation of saturated and unsaturated fatty acids, 2. relative rates of incorporation of saturated or unsaturated fatty acids into membrane lipids (including rates of transfer of lipids, particularly phospholipids, from one pool to another), and 3. relative rates of biosynthesis of saturated and unsaturated fatty acids (including both desaturation and biohydrogenation). Examples of the last two of these processes are now known. Sinensky (122) has shown that the proportion of oleate to stearate incorporated from the medium into *E. coli* phospholipids increased with decreasing temperature. In *E. coli* extracts, the relative rates of transacylation of palmityl CoA and oleyl CoA were shown to vary as a function of temperature. Temperature in turn was shown to affect the relative activity of a transacylase (as opposed to an effect on enzyme synthesis). The net result of this mechanism, then, is to increase the ratio of unsaturated to saturated fatty acids in the cell membrane as the environmental temperature is lowered.

A direct effect of temperature on unsaturated fatty acid biosynthesis seems to be the more common means of changing membrane composition. Thus, Harris & James (138, 143) have shown in several plant tissues that the temperature effect can be

explained in terms of the increased solubility of O_2 (an obligate cosubstrate) as the temperature decreases. At constant temperature, desaturation increased with increasing oxygen tension, while at constant oxygen tension the rate of desaturation actually decreased somewhat with decreasing temperature. The results reported by Rinne (137) in an enzyme system obtained from developing soybean cotyledons and by Brown & Rose (132) in yeast also suggest that the increase in unsaturated fatty acid biosynthesis at lower temperatures in these systems can be ascribed to increased solubility of O_2.

This explanation, however, apparently does not apply to all systems. Fulco (24, 129, 130, 144) has intensively investigated the temperature-mediated control of desaturation in *Bacillus megaterium* and has demonstrated three control mechanisms which regulate the level of Δ^5-desaturating enzyme, and hence the rate of unsaturated fatty acid biosynthesis, in response to temperature changes in the growth or incubation medium. One control process is that of desaturase induction. A culture growing at 35° does not synthesize unsaturated fatty acids. When the culture is transferred to 20°, however, the synthesis of desaturase begins and continues at a high rate for at least one hour. This hyperinduction, so-called because levels of desaturating enzyme far exceed those found at normal 20° growth, is blocked by chloramphenicol, and the evidence suggests that both the desaturase-synthesizing system and the desaturase itself are absent in cultures growing at 35° and must be induced at 20° by a process requiring protein synthesis. Wide variations in O_2 levels do not affect hyperinduction (145). A second control process responsive to temperature is the irreversible inactivation of desaturating enzyme, which follows first order kinetics at all temperatures. Near 20°, a decrease of less than 2° in temperature of the incubation medium results in a twofold increase in the half-life of the desaturating enzyme. Temperature-mediated irreversible inactivation of the desaturating system is probably the most important mechanism for regulating the steady-state level of desaturase during the exponential phase of cell growth at temperatures near 20°. A third process, the zero-order decay of the desaturase synthesizing system, is observed when hyperinduced cultures are transferred from 20° to 35°. Present evidence (145) suggests that the rapid turn off of desaturase synthesis at 35° as well as the eventual cessation of hyperinduction at 20° involve the synthesis of a repressor which is in some way coupled to new DNA synthesis. Kepler & Tove (146) have recently studied a complementary system in bacilli, namely the induction of biohydrogenation of oleic acid in *B. cereus* by an increase in culture temperature. Oleate is reduced to stearate at 37° but not at 20°, and the appearance of the biohydrogenation system after an increase in temperature is blocked by either chloramphenicol or rifampin.

Several factors other than temperature operate in various systems to affect unsaturated fatty acid biosynthesis. In plants, these include light (147–151), aging or development (89, 152), and the levels of various ions (79, 80, 153). In higher animals, the effects of diet (and particularly the competitive effects of various fatty acids in the diet) have been intensively studied (58, 63, 64, 154–157), as have the roles of age, cofactor levels, and levels of acyl acceptors such as glycero-3-phosphate (59, 91, 158–160). Actis-Dato, Catala & Brenner (161) report the presence of a circadian rhythm of fatty acid desaturation in liver microsomes from mice exposed to light-dark cycles.

One rhythm was observed for stearate desaturation and a second for the desaturation of linoleate or γ-linolenate.

HYDROGENATION AND HYDRATION OF UNSATURATED FATTY ACIDS

The hydrogenation of an isolated double bond in the hydrocarbon chain of a fatty acid (as opposed to the reduction of *trans*-2 double bonds as in fatty acid biosynthesis) appears to be a relatively rare event in nature. Reiser (162) was probably the first to clearly demonstrate that sheep rumen contents could hydrogenate polyunsaturated fatty acids, and he attributed this action to rumen bacteria. Wright (163, 164) later showed that both rumen bacteria and rumen protozoa could hydrogenate unsaturated lipids. However, it was not until the work of Tove and colleagues (146, 165–170) that a specific organism (*Butyrivibrio fibrisolvens*) was identified and some details of the hydrogenation process elucidated. They showed that in the rumen two systems are involved in the complete hydrogenation of linoleic acid: one specific for the conversion of linoleic acid to a monoenoic acid and the other for the hydrogenation of the monoene to stearic acid (165). *B. fibrisolvens* carries out only the former reaction yielding *trans*-11-octadecenoic acid via the intermediate *cis*-9, *trans*-11-octadecadienoic acid (166). They later (167) isolated and partially purified an enzyme from the cell envelope of *B. fibrisolvens* that carried out the first step, the conversion of linoleic acid to *cis*-9,*trans*-11-octadecadienoic acid. The same enzyme could convert linolenic acid to the *cis*-9,*trans*-11,*cis*-15 isomer. Stereospecific addition of hydrogen to carbon atom 13 of linoleic acid in the D configuration was demonstrated and it was deduced that the mechanism of isomerization involves either the protonation of an enzyme-bound carbanion or a concerted reaction (169). The presence of the *cis*-9,*cis*-12-diene system and a free C-1 carboxyl group was an absolute requirement for isomerization (168). No enzyme has yet been isolated that hydrogenates the isomerized diene to *trans*-11-octadecenoic acid, but Rosenfeld & Tove (170) showed that in whole cells incubated in D_2O, deuterium was incorporated at the *cis* double bond reduced by the organism. This reduction, which takes place stereospecifically, was found to occur by *cis* addition to the D side of *cis*-9,*trans*-11-octadecadienoic acid. The distribution of deuterium at the reduced carbon atoms shows an isotope effect that can be explained if the reduction occurs by addition of a proton and hydride ion mediated by an unknown carrier. Both a rumen spirochete, *Treponema* (*Borrellia*) sp. (171) and rat gut (172) have also been shown to hydrogenate linoleic acid (171).

Hydration of an isolated fatty acid double bond, like hydrogenation, seems to be a relatively rare phenomenon. In 1962, Wallen, Benedict & Jackson (173) reported the isolation of a pseudomonad which converted oleate to 10-hydroxy stearate. Schroepfer and his co-workers (174–179) subsequently studied this conversion both in vivo and in cell-free preparations. In a deuterium-oxide enriched medium, the organism forms 10-D-hydroxystearic acid from oleic acid with the incorporation of one atom of stably bound deuterium at carbon atom 9 in the L configuration (174, 175). The isolation of a soluble preparation which catalyzed the formation of 10-D-hydroxystearate from oleate (176) facilitated the elucidation of the reaction

mechanism. The enzyme (oleate hydratase) catalyzed the addition of water across the *cis* double bond of oleic acid and also catalyzed the formation of *trans*-10-octadecenoic acid from either oleic acid or 10-D-hydroxystearic acid. Although 9-D-hydroxystearic acid was not a substrate for the enzyme, palmitoleic acid was converted to 10-hydroxypalmitic acid and linoleic acid to 10-D-hydroxy-12-*cis*-octadecenoic acid (177). In addition, the same enzyme carried out the stereospecific hydration of 9,10-*cis*-epoxystearic acid to yield one isomer of *threo*-9,10-dihydroxystearic acid, and of 9,10-*trans*-epoxystearic acid to yield one isomer of *erythro*-9,10-dihydroxystearic acid (178). Kisic, Miura & Schroepfer (179) prepared nine positional isomers of DL-hydroxystearic acid (ranging from 5-hydroxy- to 15-hydroxystearic acids) and found that only the D isomer of 10-hydroxystearic acid serves as a substrate for the reverse (dehydration) reaction. Mortimer & Niehaus (180) have recently shown that *trans*-10-octadecenoic acid is formed by the pseudomonad enzyme by direct isomerization of the *cis*-9 double bond of oleate and that the alternate pathway, hydration of oleate to 10-hydroxystearate followed by dehydration to *trans*-10-octadecenoic acid, does not occur. Thus, the formation of the *trans*-10-acid from 9-D-hydroxystearic acid (176) must proceed via oleic acid as an intermediate. Other work in this area includes that by Wallen et al (181), who have reported the formation of three new 10-D-hydroxy fatty acids by anaerobic microbiological hydration of linoleic, linolenic, and ricinoleic acids and by Thomas (182), who identified several enteric bacteria that convert oleic acid to hydroxystearic acid in vitro.

HYDROXYLATION OF FATTY ACIDS

Hydroxy fatty acids occur widely in nature as intermediates in synthesis or oxidation of fatty acids or as endproducts which are incorporated into various lipids. Here we are concerned only with direct hydroxylation reactions. The distribution of naturally occurring aliphatic hydroxy acids has been reviewed by Downing (183) and Pohl & Wagner (184), while recent reviews that consider hydroxylation mechanisms include those by Ulrich (185), Morris (114), and the book edited by Boyd & Smellie (186).

α Hydroxylation and α Oxidation

The hydroxylation of fatty acids at the α-carbon may be considered an intermediate step in α oxidation by which fatty acids are degraded one carbon at a time. In higher animals, several distinct α-oxidation systems have been demonstrated. Steinberg and co-workers (187–196) have intensively investigated the metabolic defect responsible for Refsum's disease in humans, a clinical entity characterized by the accumulation of a dietary constituent, phytanic acid (3,7,11,15-tetramethylhexadecanoic acid), in the tissues. They found that the disease state was caused by the genetic lack of an enzyme, phytanic acid α-oxidase, which carries out a one-carbon degradation of phytanic acid to yield the next lower homolog, pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) which, unlike its precursor, can be totally degraded by the β-oxidation pathway (191). Most of this work has been covered in a review by Stumpf (3) and is not considered here. Tsai et al (196) describe the stereospecificity of the mitochondrial α-oxidation system for phytanic acid which proceeds through

the intermediate formation of α-hydroxyphytanic acid. Although the enzymatic introduction of the α-hydroxyl group is highly stereospecific (absolute configuration unknown), the oxidative decarboxylation of α-hydroxyphytanic acid is not. Both isomers are readily oxidized although one form is decarboxylated about 50% faster than the other. A change in the configuration of the methyl group at the 3 position of phytanic acid also has little effect either on hydroxylation or decarboxylation.

A second α-oxidation system found in higher animals is that associated with brain microsomes. This system, elucidated primarily by Mead and colleagues (197–205), is responsible for the oxidative decarboxylation of the very long-chain α-hydroxy acids of brain and is distinct in several ways from the system which decarboxylates α-hydroxyphytanic acid. The latter system is located in liver mitochondria, requires NADPH and O_2, is stimulated by Fe^{+3} but inhibited by Fe^{+2}, and does not require ascorbic acid (192). The brain system, on the other hand, is microsomal and requires O_2 but no pyridine nucleotide. Mead & Hare (204, 205) have recently shown that Fe^{+2} but not Fe^{+3} is a requirement for decarboxylation, as is ascorbic acid. Decarboxylation of (1-^{14}C)-2-hydroxytetracosanoic acid was greatly reduced in brain preparations from scorbutic guinea pigs. Ascorbic acid supplementation, both in vivo and in vitro, restored enzymatic activity in the preparations from scorbutic animals, but did not affect the preparations from supplemented animals in vitro.

The α-hydroxylation step responsible for the conversion of tetracosanoic to α-hydroxytetracosanoic acid in brain has recently been elucidated by Hoshi & Kishimoto (206). They showed that cell-free homogenates from rat brain carried out α hydroxylation in the presence of O_2, Mg^{+2}, pyridine nucleotides, and a heat-stable water-soluble cofactor. Both NADPH and NADH were equally effective, the oxidized forms less so. The product (α-OH-C_{24}) was detected only as a component of ceramide or cerebroside and not as the free acid or CoA ester. The presence of CO did not inhibit activity but all heavy metal ions (except Mg^{+2}) were strongly inhibitory. The apparent K_m value for tetracosanoic acid was 4.2×10^{-6} M and the enzyme seemed quite specific for the C_{24} chain length. Although the configuration of the α-hydroxy group in the product was not determined, Hammarström (207) has shown that all of the α-hydroxy acids of beef brain cerebrosides have the D configuration.

In other work in animal systems, Levis (208) separated and partially purified two α-hydroxy fatty acid oxidases from the $100{,}000 \times g$ supernatant fraction from a rat kidney homogenate. These enzymes formed α-keto acids and were specific for either the D or L isomers of α-hydroxystearic acid. NAD was a requirement for full activity. The α-keto acids formed by these enzymes were readily decarboxylated by a microsomal fraction from the same homogenate. Ushijima & Nakano (209) have purified 600-fold an FMN-containing enzyme present in the light mitochondrial fraction of rat liver cells which is specific for the oxidation of short-chain aliphatic L-α-hydroxy acids.

A number of α-oxidation systems have been described in plants and micro-organisms within the past few years. Most recently the sphingolipids of certain protozoa have been shown to contain large amounts of α-hydroxy fatty acids (210), and the presence of an α-hydroxy fatty acid oxidation system has been demonstrated

in the protozoan, *Crithidia fasciculata* (211). In yeast, Vesonder et al (212) have shown that *Hansenula sydowiocum* excretes free α-hydroxy fatty acids (primarily α-OH-C_{26}) into the medium when grown on a glucose solution. The α hydroxylation and decarboxylation of long-chain fatty acids in a number of yeasts had been demonstrated previously by Fulco (213).

α-Hydroxy fatty acids are common in bacteria (214–219) and cell-free extracts of *Arthrobacter simplex* have been obtained by Yano, Furukawa & Kusunose (220), which catalyze the conversion of palmitic acid to α-hydroxypalmitic acid and also the conversion of the latter to pentadecanoic acid.

Several fatty acid α-oxidation systems have been demonstrated in plants and much of this work has been reviewed by Stumpf (3) and by Morris (114). Hitchcock & Morris (221) and Hitchcock & Rose (222) have studied the stereochemistry of α oxidation of fatty acids in leaves. When (U-^{14}C)-palmitate was incubated with a particulate fraction from young pea leaves, (^{14}C) pentadecanal accumulated. L-(U-^{14}C, 2-^{3}H)-Palmitate similarly yields (^{14}C) pentadecanal which retains 90% of the tritium. D-(U-^{14}C, 2-^{3}H)-Palmitate gives pentadecanal with 87% loss of tritium (221). These results suggest that D-2-hydroxypalmitic acid is an intermediate in this reaction but that L-2-hydroxypalmitic and 2-ketopalmitic acid are not. Later work (222) confirmed that D-2-hydroxypalmitic acid accumulates in small amounts during the course of α oxidation of palmitate. Earlier work by Hitchcock, Morris & James (223), however, seemed to contradict these results by showing that the L-2-hydroxy isomer is preferentially α-oxidized in a pealeaf system. Hitchcock & Morris (221) suggest that the apparent contradiction can be explained if α oxidation in leaf systems occurs by two routes: (*a*) via D-2-hydroxypalmitate and pentadecanal, both of which can accumulate as intermediates, and (*b*) via L-2-hydroxypalmitate which is rapidly oxidized with no accumulation of intermediates. Pentadecanal would not be an intermediate in pathway (*b*) although 2-ketopalmitate, excluded as a possible intermediate in pathway (*a*), could be involved in pathway (*b*).

Markovetz & Stumpf (224) have carried out a sixtyfold purification of an α-oxidation system from germinated peanut cotyledons. Two activities (formation of 2-hydroxypalmitate and CO_2 formation from palmitate) remained with a single protein fraction. An H_2O_2-generating system was required but, unlike the pealeaf system mentioned above, O_2 was ineffective, D-2-Hydroxypalmitic acid accumulates in the reaction mixture as α oxidation proceeds, whereas L-2-hydroxypalmitate does not. Competition studies with unlabeled L-2-hydroxypalmitate would suggest that it is the intermediate substrate for further breakdown. Morris (114) suggests that α-oxidation systems in general may be stereoselective rather than completely stereospecific. Thus, the D-2-hydroxy acid may be the favored hydroxylation product but the L isomer may be the favored substrate for decarboxylation. At present, the bulk of experimental evidence would seem to favor this hypothesis.

ω and ω-Type Hydroxylations

These hydroxylations are characteristically of the mixed function oxidase type (i.e. involving both molecular oxygen and a reduced pyridine nucleotide) and are widespread in nature. Hitchcock & Nichols (225) have reviewed most of the work in this area up to 1971.

Coon and co-workers (226, 227) have recently succeeded in solubilizing and resolving three functional components of the liver microsomal enzyme system which catalyzes the ω hydroxylation of fatty acids (as well as the hydroxylation of alkanes and various drugs). These include cytochrome P-450, NADPH-cytochrome P-450 reductase, and phosphatidylcholine (PC). The PC is necessary for electron transfer from NADPH to P-450. Hydroxylation in this soluble system is inhibited by superoxide dismutase, while a superoxide generating system can partially substitute for NADPH and the reductase. Based on these and previous observations (228), Coon et al (227) have proposed a mechanism of substrate hydroxylation catalyzed by cytochrome P-450. The initial step would involve the combination of the oxidized form of the cytochrome with the substrate, followed by reduction (by NADPH) of the cytochrome iron. PC is presumably required to facilitate reduction. Oxygen then combines with substrate-reduced P-450 to give a ternary complex. Estabrook et al (229) have provided experimental evidence for both the initial substrate-oxidized P-450 complex and the ternary complex with O_2. Intramolecular electron transfer to yield the superoxide radical (bound to oxidized P-450) followed by attack of superoxide on the substrate would then complete the reaction. The uptake of a second electron (donated either by the reductase or a second molecule of superoxide) would yield the hydroxylated product and water. Duppel, Lebeault & Coon (230) have studied a soluble system from the yeast, *Candida tropicalis,* which ω-hydroxylates lauric acid and contains cytochrome P-450 as a functional component as well as a phospholipid and NADPH-cytochrome P-450 reductase. Most recently Gallo et al (231) showed that alkane oxidation in this organism is catalyzed by the same P-450 ω-hydroxylating system described above.

A microsomal ω and $\omega-1$ hydroxylating system for fatty acids has been isolated from a torulopsis species of yeast (232) which may also be cytochrome P-450 dependent. Stereochemical study of this system (233) shows that the hydroxylations take place without double bond formation and with retention of configuration.

Björkhem (234, 235) and Hamberg & Björkhem (236) have studied the mechanism of ω and $\omega-1$ hydroxylation of fatty acids by a rat liver microsomal system. Hydroxylation in the ω or $\omega-1$ position of decanoic acid occurred with the loss of one hydrogen atom from the carbon hydroxylated. 10-Hydroxydecanoic acid accounted for 92% of the products formed, while L-9-hydroxydecanoic (6%) and the D-stereoisomer (2%) accounted for the remainder. Substitution of two deuteriums at carbon 9 of the substrate resulted in significant isotope effects in the formation of the 9-hydroxy derivatives, but there was no detectable isotope effect in the formation of the 10-hydroxy acid from the substrate fully deuterated at the 10 position. The two hydroxylations at carbon 9 proceeded stereospecifically with retention of the absolute configuration. These and previous results (237) suggested that $\omega-1$ hydroxylation of fatty acids in microsomes may involve enzymes that differ from those responsible for the cytochrome P-450 dependent ω hydroxylations. The most recent work (235) does not directly answer this point but does demonstrate that in the $\omega-1$ hydroxylation of laurate to 11-hydroxylauric acid, the rate-limiting step is the cleavage of the C–H bond and thus differs from the rate-limiting step in ω hydroxylation, which is probably the reduction of cytochrome P-450 (238). Ellin et al (239) have recently shown that still another system (rat kidney cortex

microsomes) carries out the ω and $\omega-1$ hydroxylation of laurate (at a ratio of about 2:1) and that both hydroxylations are a function of a cytochrome P-450-like hemoprotein called P-450_K by these authors.

Ichihara, Kusunose & Kusunose (240–246) have examined ω and ω-type hydroxylations in a large number of systems and have found evidence that all are of the mixed function oxidase type and that most seem to involve cytochrome P-450 or a similar hemoprotein. In several solubilized microsomal systems (porcine kidney, rat liver), the hydroxylating preparation was separated into two fractions, I and II. Fraction I contained a cytochrome P-450 type protein while fraction II contained NADPH-cytochrome *c* reductase. Fraction II, however, could be replaced by ferredoxin plus ferredoxin-NADP reductase. There was no evidence for a phospholipid requirement in the reconstituted (ferredoxin) systems, although ether extraction of fraction I reduced activity for laurate ω-hydroxylation. Activity was restored by the addition of various detergents (i.e. Triton X-100).

Fatty acid and alkane ω oxidation in the bacterium *Pseudomonas oleovorans* has been studied by Coon and his co-workers (247–250). Three protein components (NADH-rubredoxin reductase, rubredoxin, and the ω-hydroxylase) are required for activity and NADH is highly superior to NADPH as an electron donor. Spinach ferredoxin, adrenodoxin, and other nonheme iron proteins could not substitute for rubredoxin. Cytochrome P-450 is not a component of this system.

Most recently, Miura & Fulco (251) have described a unique soluble system from *Bacillus megaterium* which carries out the $\omega-2$ hydroxylation of fatty acids. When palmitic acid is used as a substrate, the major product is 14-hydroxy palmitate (55%), while the 15-hydroxy (27%) and the 13-hydroxy (18%) isomers are also produced. Activity is highest for the C_{15} chain length (pentadecanoic acid) and decreases in the order $C_{15} > C_{16} > C_{14} > C_{17} > C_{18} > C_{12}$. Two protein components of the system have been separated, one of which can be completely replaced by ferredoxin (from *Clostridium pasteurianum*). NADPH and O_2 are required for activity.

Although cell-free ω-type hydroxylating systems from higher plants have not been isolated, the work by Kolattukudy and his co-workers (252–256) demonstrates that such systems must exist there.

A number of fatty acid hydroxylating systems are known which are neither ω-type hydroxylases nor α-hydroxylating systems. One recent example is a soluble preparation from avocado mesocarp which carries out the β hydroxylation of medium chain length fatty acyl-CoAs in a reaction quite distinct from the usual β-oxidation pathway. In this new system, O_2 is required, whereas NAD and CO strongly inhibit (257, 258). Unfortunately, lack of space does not permit additional discussion of these systems, or of much interesting work peripheral to the subject matter of this review.

CONCLUDING REMARKS

The present state of our knowledge of fatty acid desaturation can be summarized as follows: The major pathways for the biosynthesis of the common unsaturated fatty acids in various organisms are now known and further work in this area will

serve chiefly to add details to the overall picture. Also, enough cell-free desaturating systems have now been studied to conclude that the direct removal of two hydrogens from a fatty acyl chain to produce a *cis* double bond four or more carbon atoms removed from the carboxyl group always requires both O_2 and a reduced pyridine nucleotide as cofactors; a two (or more) component electron transport chain (including both a flavoprotein and an iron-containing protein) is also involved. CoA and ACP thioesters and certain phospholipids (but not free fatty acids) have been found to serve as substrates in one or another of the desaturation systems.

On the other hand, no desaturation system has been extensively purified and we know nothing of the actual desaturation mechanism or of the role of O_2 in this process. With few exceptions, the desaturases have proven to be particle bound, relatively unstable, and resistant to purification by classical techniques. Until significant purification of a desaturase can be accomplished, there seems little hope of elucidating the actual mechanism(s) of *cis* double bond formation, and thus strong future efforts should be directed toward developing methods suitable for the fractionation and purification of fatty acid desaturases.

Like the desaturases, the ω-type fatty acid hydroxylating systems are characteristically mixed function oxidases and require O_2 and a reduced pyridine nucleotide for activity. They are also generally microsomal in eucaryotes and contain both a flavoprotein and an iron protein. Unlike the desaturases, however, the free fatty acids are utilized as substrates. Furthermore, several systems have been solubilized and partially purified, and Coon and his co-workers have proposed a hydroxylation mechanism based on their work with a cytochrome P-450 system solubilized from liver. They consider the active species to be a ternary complex of P-450, O_2, and substrate which, by intramolecular electron transfer, yields P-450-bound superoxide radical. Attack of the bound superoxide radical on the fatty acid substrate followed by the uptake of a second electron would then yield the ω-hydroxy fatty acid and water. Although the evidence for this mechanism is not conclusive, it is strongly suggestive and future work might well center on efforts to determine whether superoxide may be involved in other ω-type hydroxylating systems.

There are a number of α-hydroxylating systems that act on fatty acids but they appear to differ widely among themselves and, as a group, differ from the ω-type hydroxylases. None have been significantly purified and, as with the fatty acid desaturases, future progress in determining their mechanisms of action may well depend on the successful development of methods for the purification and characterization of these intractable systems.

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