Summary.—The development of α-amylase activity by isolated aleurone layers of barley endosperm is completely dependent upon added gibberellic acid and is a result of the *de novo* synthesis of the α-amylase molecule. The synthesis of α-amylase and of other heat-stable proteins is prevented by actinomycin D. It is therefore postulated that gibberellic acid controls the synthesis of α-amylase and of other heat-stable proteins in aleurone cells by causing the production of specific messenger RNA's.

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*THE MECHANISM OF FATTY ACID SYNTHESIS**

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Previous reports from this laboratory[1–4] and others[5–10] have indicated that long chain fatty acids (mainly palmitic acid) were synthesized from acetyl CoA, malonyl CoA, and TPNH according to the following over-all reaction:

$$CH_3CHOSCoA + 7HOOCCH_2COSCoA + 14TPNH + 14H^+ \rightarrow CH_3(CH_2)_{14}COOH + 7CO_2 + 8COASH + 14TPN^+ + 6H_2O. \quad (1)$$

Using a highly purified preparation from pigeon liver, Bressler and Wakil[2] showed that palmitic acid and not its CoA derivative was the main reaction product. Acetyl CoA contributed carbons 15 and 16 of palmitic acid, whereas malonyl CoA provided the C_2 units that comprise carbons 1–14. Short-chain acyl CoA derivatives (butyryl CoA, hexanoyl CoA, and octanoyl CoA) were incorporated into palmitic acid only 5 per cent as well as the equivalent amount of acetyl CoA. Moreover, they did not accumulate during synthesis from acetyl CoA. Bressler

and Wakil[2, 4] concluded that *free* acyl CoA derivatives were unlikely intermediates in the synthesis of long-chain fatty acids. Crotonyl CoA and β-hydroxybutyryl CoA could neither be reduced by TPNH in the presence of highly purified preparations from pigeon liver nor could they substitute for acetyl CoA in the conversion of malonyl CoA to palmitic acid. Acetoacetyl CoA, however, was reduced to D(−)-β-hydroxybutyryl CoA by preparations of the fatty acid-synthesizing enzyme system but only at relatively high concentrations and at a rate significantly lower than the rate of fatty acid synthesis. This activity has been attributed to a TPNH-acetoacetyl CoA reductase which does not appear to be part of the fatty acid-synthesizing enzyme complex.[3]

A requirement for a protein-linked sulfhydryl group in fatty acid synthesis was demonstrated with preparations from pigeon liver,[4, 11] yeast,[5] rat brain,[6] and rat adipose tissue.[7] Since substituted acyl CoA derivatives did not substitute for acetyl CoA as primers in palmitate synthesis, Lynen and Tada[5] concluded that acyl-S-enzyme(s) were intermediates in fatty acid synthesis. Indeed, they were able to isolate an acetoacetyl-S-enzyme when the yeast preparation was incubated with C^{14}-acetyl CoA and malonyl CoA in the absence of TPNH.

Recently, Vagelos *et al.*,[10] and Bloch and his group[9] prepared soluble extracts from *E. coli* that catalyzed synthesis of palmitic and *cis*-vaccenic acids from malonyl CoA and acetyl CoA. Manipulation of the soluble extracts yielded two fractions, a heat-labile protein fraction that precipitated between 55 and 70 per cent saturation of ammonium sulfate and a heat-stable protein fraction. Both fractions were required for the synthesis of long-chain fatty acids. Bloch designated the heat-labile fraction as the "fatty acid synthetase" and more recently reported the presence in this fraction of two dehydrase activities. One dehydrates β-hydroxydecanoyl CoA to α-β-decanoyl CoA, and the other dehydrates the same substrate to β-γ-decanoyl CoA. The presence of these two dehydrases in the *E. coli* preparations is compatible with Bloch's hypothesis for the anaerobic synthesis of *cis*-vaccenic acid.[12]

Although starch gel electrophoresis of the purified fatty acid synthesis preparations from pigeon liver revealed the presence of at least five different protein moieties, our attempts to fractionate such preparations into components which catalyze partial reactions were unsuccessful. Accordingly, we have turned to the *E. coli* systems of Vagelos and Bloch for further fractionation studies. Using sonicated extracts of *E. coli*, we readily confirmed these authors' observations[9, 10] of heat-stable and heat-labile fractions required for fatty acid synthesis. Extension of these studies is the subject of this report. As will be reported below, the heat-stable protein has been found to serve as a coenzyme rather than as an enzyme; the sulfhydryl group of this protein acts as an acyl acceptor and donor, and all the reactions in which the fatty acid chain is elongated and reduced appear to occur while the chain is in acyl linkage to this protein. The enzymes proper have been fractionated into at least 3 components which catalyze the elongation reactions, and the reduction and intermediate dehydration steps. In view of the apparent role of the heat-labile protein, it has been tentatively designated as the "acyl carrier protein" which is abbreviated herein as "ACP."[13]

Purification of the Heat-Labile Protein.—The heat-labile protein fraction was prepared according to the procedure of Lennarz *et al.*[9] After dialysis against 0.01 *M* potassium phosphate (pH 7.4) for 4–10 hr it was fractionated on a DEAE-cellulose column with 0.01 *M* potassium phosphate

(pH 7.4) and increasing concentration of NaCl to yield three fractions here designated E_{II}, E_{III}, and E_{IV}. Fraction E_{IV} was eluted with 0.01 *M* phosphate (pH 7.4), E_{III} was eluted with 0.01 *M* phosphate (pH 7.4) and 0.1 *M* NaCl, and E_{II} was eluted with 0.01 *M* phosphate (pH 7.4) and 0.25 *M* NaCl.

In the presence of C^{14}-acetyl CoA, malonyl CoA, and TPNH and the heat-stable protein, no palmitic or *cis*-vaccenic acid was synthesized as depicted in Table 1. When E_{II} was added to this

TABLE 1

THE SYNTHESIS OF *cis*-VACCENIC, PALMITIC ACID, AND β-HYDROXY ACIDS BY FRACTIONS E_{II}, E_{III}, AND E_{IV}

Fractions	*Cis*-vaccenic (cpm)	Palmitic (cpm)	Unknown compound (cpm)	Ratio vaccenic:palmitic
E_{II}	100	300	1200	
$E_{II} + E_{III}$	600	4300	800	1:7
$E_{II} + E_{IV}$	3000	500	500	6:1
$E_{II} + E_{III} + E_{IV}$	9000	2000	300	4.5:1

Each reaction mixture contained 30 μmoles of potassium phosphate (pH 7.4), 2 μmoles of thioethanol, 1.0 μmole of TPNH, 97 mμmoles of acetyl CoA, 83 mμmoles of malonyl-2-C^{14} CoA (40,000 cpm), 0.63 mg of ACP, and, where indicated, the following enzyme fractions: 0.20 mg of fraction E_{II}, 0.16 mg of fraction E_{III}, and 0.14 mg of fraction E_{IV}. The final volume was 0.4 ml, and the reaction mixture was incubated at 38° for 1 hr. The fatty acids were isolated with pentane (from acid solution after alkaline hydrolysis), methylated, and chromatographed on $AgNO_3$-silica gel H (ether:hexane, 1:9).

reaction mixture, polar compounds were formed as shown in Table 1. Addition of a combination of fractions E_{II} and E_{III} yielded mainly palmitic acid, whereas addition of a combination of E_{II} and E_{IV} yielded mainly *cis*-vaccenic acid (cf. Table 1). When all three protein fractions were added, a mixture of *cis*-vaccenic and palmitic acids was formed. The ratio of *cis*-vaccenic to palmitic acids could readily be controlled by the relative amounts of E_{III} and E_{IV} added.

In these studies the synthesis of palmitic acid was followed by measuring the amount of radioactive substrate incorporated into palmitic acid in the presence of TPNH, heat-stable protein, E_{II}, and E_{III}. Measurement of the decrease in optical density of TPNH at 340 mμ afforded an alternate means of following the synthetic process.

The heat-stable protein from sonicated extracts of *E. coli* cells was purified extensively by first heating the extract for 10 min at 100° followed by dialysis for 4–48 hr and chromatography on DEAE-cellulose. The active component was eluted with 0.35 *M* NaCl, 0.01 *M* potassium phosphate pH 7.4, and 5×10^{-3} *M* thioethanol, and filtered through Sephadex G-75. The material so obtained was a protein with a low molecular weight of less than 10,000. It was homogeneous in the ultracentrifuge and on electrophoresis on the cellulose acetate paper strips. Amino acid analyses revealed the presence of a minimum of one residue of each histidine, methionine, arginine, and tyrosine, or a total of 82 residues (i.e., a minimum molecular weight of 9,000–9,400). The protein is very acidic and contains 20 residues of glutamic, 10 residues of aspartic. It would seem from our present data that there is one sulfhydryl group in ACP that accepts the acyl group from their CoA derivatives. The nature of the sulfhydryl group in the protein is under investigation.

Acyl Protein as Intermediates in Fatty Acid Synthesis.—When the acyl carrier protein (ACP) was incubated with C^{14}-acetyl CoA and the E_{II} fraction, and the reaction mixture was filtered through Sephadex G-75, C^{14}-acetyl-labeled ACP was isolated as shown in Figure 1. The free CoA was released and could be separated, together with the unreacted acetyl CoA in fractions 50–110 (cf. Fig. 1). The amount of radioactivity in the protein was absolutely dependent upon the presence of the enzyme E_{II}. Fractionation of E_{II} on hydroxyl apatite yielded a protein fraction that catalyzed the transacylation of the acetyl groups from acetyl CoA to ACP but did not replace E_{II} in catalyzing fatty acid synthesis. This enzyme has been called acetyl CoA-acyl carrier protein transacylase.

The C^{14}-acetyl protein fraction isolated from the Sephadex column was concentrated by lyophilization, the residue was dissolved in minimal amounts of water,

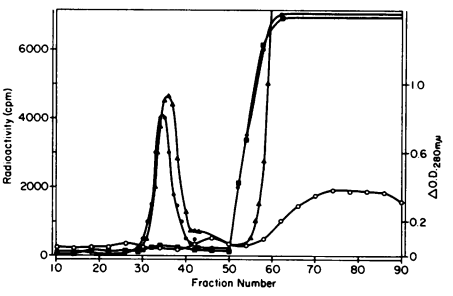

FIG. 1.—Isolation of C^{14}-acetyl ACP and C^{14}-malonyl ACP by gel filtration on Sephadex G-75. C^{14}-acetyl ACP (solid circles) was prepared by incubating 30 μmoles potassium phosphate, pH 7.4, 2 μmoles thioethanol, 50 mμmoles 1-C^{14}-AcCoA (47 μc/μmole), 30 μg E_{III}, 200 μg of ACP, and water to a final volume of 0.4 ml. This mixture was incubated for 30 min at 38° and was then filtered through Sephadex G-75 column (4 × 40 cm) using 0.05 *M*, potassium phosphate, pH 7.4 (10-ml fractions were collected). The open circles represent optical density measurement of the fractions at 280 mμ. C^{14}-malonyl ACP (solid triangles) was prepared in exactly the same way as that of C^{14} acetyl ACP, except that 90 mμmoles of malonyl 2C-^{14} CoA (5 μc/μmole) was used instead of C^{14}-acetyl CoA. The solid squares represent the results of an experiment with C^{14}-acetyl CoA but without E_{II}.

and the solution was dialyzed for several hours. During dialysis there occurred little or no loss of radioactivity. Alkaline hydrolysis of C^{14}-acetyl ACP yielded C^{14}-acetate as shown by paper chromatography in butanol:ammonia system.[2] Treatment of the C^{14}-acetyl ACP with hydroxylamine at pH 7.0 yielded acethydroxamic acid.

Incubation of the C^{14}-acetyl ACP with malonyl CoA, TPNH, E_{II}, and E_{III} fractions resulted in the incorporation of the radioactivity into long-chain fatty acids as shown in Table 2. The conversion of acetyl ACP to palmitic acid was

TABLE 2

CONVERSION OF C^{14}-ACETYL ACP (C^{14}-Ac ACP) AND C^{14}-MALONYL ACP (C^{14}-Ma ACP) TO LONG-CHAIN FATTY ACIDS

Expt. no.	Substrates added	C^{14}-fatty acids
I	C^{14}-Ac ACP + MaCoA + TPNH	600
	AcCoA + C^{14}-MaACP + TPNH	700
	C^{14}-Ac ACP + MaCoA	0
II	AcCoA + C^{14}-MaACP + TPNH	1,500
	C^{14}-Ac ACP + C^{14}-Ma ACP + TPNH	1,700
	C^{14}-ACP + C^{14}-MaACP	175
III	C^{14}-Ac ACP + MaCoA + TPNH	9,000
	C^{14}-AcCoA + MaACP + TPNH	11,000
	C^{14}-AcCoA + ACP + TPNH	600

Each reaction mixture contained 30 μmoles phosphate pH 7.0, 2 μmoles thioethanol, 80 μg E_{II}, 30 μg E_{III}, and, where indicated, the following substrates were added: 0.5 μmole TPNH, 90 mμmoles MaCoA, 30 mμmoles acetyl CoA, 2.0 mμmoles C^{14}-acetyl CoA (100,000 cpm), 20 mμmoles C^{14}-Ac ACP, (3000 cpm prepared enzymatically and used in expt. I only), 100 mμmoles C^{14}-Ma ACP (2500 cpm prepared enzymatically and used in expt. I only), 0.1 mg C^{14}-acetyl-ACP (80,000 cpm prepared chemically), 0.1 mg C^{14}-malonyl ACP (160,000 cpm prepared chemically), 0.3 mg malonyl ACP (prepared chemically), and 0.6 mg ACP. The final volume was 0.4 ml, and the reaction mixtures were incubated for 1 hr at 38°.

dependent upon the enzyme fractions E_{II} and E_{III}, and TPNH. The addition of nonacylated ACP (i.e., heat-stable protein) did not increase the incorporation of C^{14}-acetyl ACP into palmitate as shown in Table 2.

In the presence of E_{II}, malonyl CoA also donates its acyl group to the purified ACP. Incubation of C^{14}-malonyl CoA, ACP, and E_{II} resulted in the formation of C^{14}-malonyl ACP which was isolated by gel filtration on Sephadex G-75 as shown in Figure 1. Alkaline hydrolysis of the malonyl protein yielded malonic acid as shown by paper chromatography in the amyl alcohol:formic acid solvent system.[1] Incubation of C^{14}-malonyl ACP with acetyl ACP, TPNH, E_{II}, and E_{III} yielded C^{14}-labeled fatty acids as shown in Table 2. An absolute requirement was evident for

both TPNH and enzyme fraction E_{II} whereas acetyl ACP was only partially required, possibly due to decarboxylation of malonyl ACP to acetyl ACP.

Acetyl, and malonyl ACP were prepared chemically by reaction of ACP with acetic anhydride and malonyl thiophenol, respectively.[14] In the absence of coenzyme A, the chemically synthesized derivatives could readily be converted to palmitic acid and *cis*-vaccenic acids in the presence of TPNH and the enzyme fractions E_{II}, E_{III}, and E_{IV}, as shown in Table 2. This observation strongly suggests that the acetyl and malonyl derivatives of the ACP are the immediate substrates for fatty acid synthesis. This represents the first instance in which a rather large polypeptide appears to function as a coenzyme rather than as an enzyme.

When C^{14}-acetyl CoA plus malonyl CoA were incubated with acyl carrier protein and the E_{II} enzyme fraction, and the reaction mixture was filtered through Sephadex G-75, then C^{14}-acetoacetyl derivative of the acyl carrier protein was isolated. The formation of the acetoacetyl ACP was demonstrated by measuring the increase in absorbancy at 305 mμ at pH 9.0 and in the presence of Mg^{++},[15] as shown in Table 3. Further, treatment with KHB_4 yielded the β-hydroxylbutyryl derivative, which, on alkaline hydrolysis followed by acidification and extraction with diethyl ether, yielded β-hydroxybutyric acid which was identified by gas-liquid chromatography. The formation of acetoacetyl-ACP was absolutely dependent upon the presence of malonyl CoA, acetyl CoA, ACP, and enzyme fraction E_{II} (cf. Table 3). Acetoacetyl-ACP was readily converted to palmitic and *cis*-vaccenic acids in the presence of TPNH and the enzyme fractions E_{II}, E_{III}, and E_{IV} as shown in Table 4. This formation of the acetoacetyl protein and its conversion to the long-chain fatty acids is analogous to the formation of "enzyme-bound" acetoacetate by Goldman *et al.*[10] who demonstrated the formation of an acetoacetyl derivative of "enzyme II," the heat-stable protein, and its conversion to long-chain fatty acids in the presence of TPNH, malonyl CoA, and the heat-labile fraction.

TABLE 3

REQUIREMENTS FOR THE FORMATION OF ACETOACETYL POLYPEPTIDE

Components omitted	Acetoacetyl-ACP (Δ O.D.$_{305}$ mμ)
None	0.210
Thioethanol	0.275
E_{II}	0.020
AcCoA	0.170
MaCoA	0.020
ACP	0.040

The complete system contained 30 μmoles potassium phosphate pH 7.4, 2 μmoles thioethanol, 20 mμmoles acetyl CoA, 100 mμmoles malonyl CoA, 0.1 mg ACP, and water to a final volume of 0.4 ml. The reaction was started by the addition of 60 μg of E_{II} and was incubated at 25°. At the end of 20 min the absorption at 305 mμ was measured, before and after addition of 2 μmoles of $MgCl_2$ and KOH to pH 9.0.

TABLE 4

CONVERSION OF ACETOACETYL ACP TO FATTY ACIDS

Component omitted	C^{14} fatty acids (cpm)
None	720
MaCoA	240
TPNH	80
E_{II}	100

The complete system contained 30 μmoles of potassium phosphate pH 7.4, 2 μmoles of thioethanol, 1 μmole of TPNH, 90 mμmoles of malonyl CoA, 80 μg of E_{II}, 50 μg of E_{III}, and 10 mμmoles of acetoacetyl ACP (about 7000 cpm). The reaction was incubated for 20 min at 38°.

β-Hydroxyacyl-Polypeptides as Intermediates in the Synthesis of Palmitic and Cis-Vaccenic Acids.—As indicated in Table 1, incubation of acetyl CoA, malonyl CoA, TPNH, the heat-stable protein, and the enzyme fraction E_{II} yielded after hydrolysis polar fatty acids and very little or no palmitic or vaccenic acids.

Further studies of the polar compounds indicated that they were mixtures of various β-hydroxyl acids, that they pre-existed as acyl derivatives of the heat-stable protein, and that they were intermediates in fatty acid synthesis. Both C^{14}-malonyl CoA and C^{14}-acetyl CoA were incorporated into these compounds, and TPNH is required for their synthesis. The polar compounds were extracted with pentane from the reaction mixture after alkaline hydrolysis followed by acidification with HCl. The pentane extracts were separated, and the solvent was removed under N_2. The residues were methylated with diazo-methane and were chromatographed on thin layer using $AgNO_3$-silica gel H with ether hexane (1:9) as solvents. The methyl esters of the polar compounds remained at the origin, whereas *cis*-vaccenic and palmitic esters chromatographed with an R_f of 0.32 and 0.64, respectively. The silica gel was scraped from the plate, and the "polar" esters were extracted with diethyl ether. The ether was removed under nitrogen, and the esters were rechromatographed on silica gel G with ether-hexane (4-6) as solvent. Usually two spots were obtained. One spot (containing 30–40 per cent of the total radioactivity) remained at the origin; 60–70 per cent of the radioactivity chromatographed with an R_f of 0.42 similar to that of methyl β-hydroxydecanoate. The radioactive materials from the latter spot were eluted from the silica gel and further identified as a mixture of the β-hydroxy derivatives of C_{10}, C_{12}, and C_{14} acids as follows: (1) gas-liquid chromatography of the methyl esters coupled with simultaneous determination of the radioactivity in the effluent gas revealed the presence of the β-hydroxy acids; (2) the acetate derivatives of the mixture of β-hydroxy acids were prepared and on thin layer chromatography[17] gave one spot corresponding to authentic methyl ester of the β-hydroxydecanoyl acetate (derivatives of C_8, C_{10}, and C_{12} cannot be separated from each other in this system); (3) treatment of the β-hydroxymethyl esters with PBr_3 and potassium *ter*-butoxide followed by catalytic hydrogenation[18] yielded the parent methyl esters of the saturated acids which were identified in gas-liquid chromatogram; and (4) the dehydrated methyl esters that were obtained after treatment with PBr_3 and K-*ter*-butoxide, on ozonolysis and subsequent reduction with triphenyl phosphine, yielded C^{14}-labeled aldehydes of chain lengths of 2 or 3 carbon atoms less than the original β-hydroxy acids, indicating the presence of α-β and β-γ unsaturated acids which arose by the dehydration of the β-hydroxy acids.

At the end of the enzymic incubations, the β-hydroxy acids were not free but bound to ACP as acyl-derivatives. This was shown by the inability of pentane or diethyl ether to extract these acids from the reaction mixture at pH 4.5 unless it had first been subjected to alkaline hydrolysis. When the reaction mixture, consisting of ACP, enzyme fraction E_{II}, C^{14}-acetyl CoA, malonyl CoA, and TPNH was incubated at 37° for 1 hr and then filtered through a Sephadex G-75 column, a mixture of the C^{14}-β-hydroxy acyl derivatives of the ACP was obtained. The amount of C^{14} incorporated into the β-hydroxyacyl-ACP was absolutely dependent upon the presence of all five components. Omission of TPNH from the reaction mixture yielded C^{14}-acetyl protein and C^{14}-acetoacetyl protein, whereas omission of malonyl CoA yielded only C^{14}-acetyl ACP. Alkaline hydrolysis of the acyl-ACP which was derived from the complete reaction mixture yielded a mixture of β-hydroxy acids similar to those isolated from the reaction mixture. When the β-hydroxyacyl-ACP was incubated with fraction E_{III} and TPNH, and the products

were hydrolyzed and extracted with pentane, saturated acids of varying chain length were obtained in proportions similar to that of the mixture of β-hydroxyacyl derivatives. Addition of malonyl CoA to this reaction did not result in elongation of the saturated fatty acids unless fraction E_{II} was also added (cf. Table 5). Under

TABLE 5

CONVERSION OF β-HYDROXYACYL-ACP TO PALMITIC AND *Cis*-VACCENIC ACIDS

Additions		Fatty Acids (cpm)		
Enzymes	Cofactors	β-Hydroxy acids	Unsaturated	Saturated
None	None	550	100	100
E_{III}	None	170	115	100
E_{III}	TPNH	50	100	500*
E_{III}	TPNH + MaCoA	115	115	600*
E_{III} and E_{II}	TPNH + MaCoA	100	250	500†

* C_{10}, C_{12}, and C_{14} acids.
† C_{16} acid.

these conditions palmitic acid was formed. This observation suggests that fraction E_{III} contains the dehydration and reduction enzyme but not the elongation enzyme which appears to be present in E_{II}. When E_{IV} was added instead of E_{III}, both *cis*-vaccenate and palmitate were formed. Here again E_{IV} appears to contain the dehydration and reduction enzymes for the synthesis of *cis*-vaccenic acid. The partial separation of enzymatic activities in these three fractions is thus indicated.

Requirement for a Heat-Stable Polypeptide in the Fatty Acid-Synthesizing System from Pigeon Liver.—The fatty acid-synthesizing system from animal tissues appears to form tighter complex than does the *E. coli* system, since the complex from pigeon liver was isolated as one unit over 2000-fold purification. However, "aging" of such preparations (stored at −18° for 2–6 weeks) resulted in loss of enzymatic activity which could be partially restored by addition of either the *E. coli* acyl-carrier protein or a heat-stable extract of pigeon liver preparations. Furthermore, both enzymatically and synthetically prepared C^{14} acetyl ACP and C^{14}-malonyl ACP were incorporated into palmitic acid when incubated with TPNH and the pigeon liver preparations as shown in Table 5. These observations suggest that the pigeon liver system behaves similarly to that from *E. coli* and that the requirement for a polypeptide coenzyme is a general characteristic of fatty acid-synthesizing systems.

TABLE 6

CONVERSION OF C^{14}-ACETYL ACP AND C^{14}-MALONYL ACP TO PALMITATE BY THE PIGEON LIVER ENZYME SYSTEM

Substrates	C^{14}-palmitic acid
C^{14}-AcCoA + MaCoA	7,500
C^{14}-AcCoA + MaACP	10,000
AcACP + C^{14}-MaACP	5,600

Each reaction mixture contained 30 μmoles phosphate pH 7.4, 0.5 μmoles TPNH, 2 μmoles thioethanol, 20 μg pigeon liver enzyme system R_{2a}, and, where indicated, the following substrates: 2 mμmoles C^{14}-acetyl CoA (100,000 cpm), 90 mμmoles malonyl CoA, 0.1 mg C^{14}-acetyl ACP (80,000 cpm), 0.2 mg C^{14}-malonyl ACP (320,000 cpm), and 0.3 mg malonyl ACP. (All the ACP derivatives were chemically synthesized.) The final volume was 0.4, and the reaction mixture was incubated for 30 min at 38°.

Summary.—(1) A heat-stable protein (referred to as acyl carrier protein-ACP) participates in fatty acid synthesis. (2) This protein accepts acetyl and malonyl groups from their CoA derivatives, forming covalently linked acetyl and malonyl derivatives. (3) Acetyl ACP and malonyl ACP react to form acetoacetyl ACP. (4) In the presence of TPNH, malonyl ACP and acetoacetyl ACP react to form long-chain fatty acids. (5) In the presence of enzyme fraction E_{II}, acetyl CoA, malonyl CoA, TPNH, and ACP yielded a mixture of β-hydroxyacyl ACP of chain

lengths C_8, C_{10}, C_{12}, and C_{14}. These β-hydroxyacyl derivatives were converted to their saturated homologues or palmitic acid on incubation with TPNH, appropriate enzyme fractions, and malonyl CoA. (6) The final product in fatty acid synthesis is free palmitate, but in the presence of stoichiometric amounts of ACP, palmityl ACP can be isolated.

These observations suggest the following scheme for fatty acid synthesis:

(1) $CH_3COSCoA + HSACP \longrightarrow CH_3COSACP + CoASH$

(2) $HOOCCH_2COSCoA + HSACP \longrightarrow HOOCCH_2COSACP + CoASH$

(3) $CH_3COSACP + HOOCCH_2COSACP \rightarrow CH_3COCH_2COSACP + CO_2 + HSACP$

(4) $CH_3COCH_2COSACP + TPNH + H^+ \longrightarrow CH_3CHOHCH_2COSACP + TPN^+$

(5) $CH_3CHOHCH_2COSACP \longrightarrow CH_3CH{=}CHCOSACP + H_2O$

(6) $CH_3CH{=}CHCOSACP + TPNH + H^+ \longrightarrow CH_3CH_2CH_2COSACP + TPN^+$

Repetition of steps 3–5 six more times yields palmityl SACP which is then hydrolyzed to palmitic acid and the acyl carrier protein.

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[14] C^{14}-acetyl ACP and malonlyl ACP were prepared as follows: a solution of the polypeptide (10 mg/ml) was adjusted to pH 8.0 with $NaHCO_3$, and stoichiometric amounts of C^{14}-acetic anhydride in diethyl ether were added to it. The mixture was mixed thoroughly, allowed to stand at 0° for 10 min., and lyophilized to remove the excess C^{14}-acetate. The residue was then dissolved in water and assayed enzymatically for its conversion to fatty acids.

C^{14}-malonyl ACP was also synthesized by the interaction of C^{14}-malonyl thiophenol with the polypeptide at pH 8.0 in a manner similar to the malonyl transfer from thiophenol to coenzyme A. For each mole of polypeptide at pH 8.0 was added a slight excess of the C^{14}-malonyl thiophenol, and the reaction mixture was placed in ice bath and mixed vigorously by bubbling N_2 through. The pH was maintained at 8.0 at all times. At the end of 3 hr, the pH was carefully lowered to 6.0 with HCl, and the mixture was extracted four times with diethyl ether and then lyophilized. The residue was dissolved in water and characterized as malonyl ACP by its hydrolysis to malonic acid. Interaction with hydroxylamine yielded the hydroamine acid derivative.

[15] Lynen, F., *Federation Proc.*, **12**, 683 (1953).

[16] Goldman, P., A. W. Alberts, and P. R. Vagelos, *J. Biol. Chem.*, **238**, 3579 (1963).

[17] Kishimoto, Y., and N. S. Radin, *J. Lipid Res.*, **4**, 130 (1963).

[18] The authors wish to thank Dr. Veeravagu for making this procedure available to us before publication.

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*THE REPLICATIVE FORM OF MS2 RNA: AN X-RAY DIFFRACTION STUDY**

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During the multiplication of single-stranded RNA[1] viruses a specific replicative form of RNA, with the characteristics of a double helix, is synthesized.[2–10] Such a replicative form, first identified by Montagnier and Sanders[2] in animal cells infected with encephalomyocarditis virus, has since been reported for poliovirus[4] and RNA-containing bacteriophages such as MS2,[3, 5–8] R17,[9] and fr.[10] The replicative form consists of complementary RNA strands, one of which is of the parental type.[3, 5] It appears to be an obligatory intermediate in the reproduction of RNA viruses[3, 5] and accumulates in large quantities in the later stages of infection. In the case of phage MS2, it has been shown[3, 6] by *in vitro* experiments that the replicative form serves as template for the synthesis of MS2 RNA by the phage-induced RNA synthetase.[11]

In this paper we report on an X-ray diffraction study of the purified replicative form of MS2 RNA. The detailed patterns obtained, which are identical to those reported for the double-stranded RNA of reovirus,[12] conclusively establish the double-helical structure of the MS2 replicative form.

Materials and Methods.—Preparation of replicative form of MS2 RNA: A typical preparation was carried out as follows. *E. coli* Hfr 3000 infected with MS2 virus was homogenized with glass beads. The homogenate was treated with DNAase and fractionated with $MgCl_2$ exactly as described for the purification of RNA synthetase.[6] After addition of sodium dodecyl sulfate to a final concentration of 0.5%, this preparation, rich both in enzyme and replicative form,[6] was extracted three times with phenol, washed with ether, and the RNA was collected after precipitation with ethanol. The precipitate was dissolved in 0.15 *M* sodium chloride, 0.015 *M* sodium citrate to give an RNA concentration not exceeding 1 mg/ml and incubated with 50 μg/ml of pancreatic ribonuclease A for 30 min at 25°. After addition of sodium dodecyl sulfate, the digest was concentrated by precipitation with ethanol. It was dissolved in 0.15 *M* sodium chloride, 0.015 *M* sodium citrate, extracted four times with phenol, and the RNA then precipitated with