Lipid Biosynthesis in the Sebaceous Glands: Synthesis of Multibranched Fatty Acids from Methylmalonyl-Coenzyme A in Cell-Free Preparations from the Uropygial Gland of Goose†

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ABSTRACT: Cell-free extracts from the uropygial gland of goose catalyzed the incorporation of malonyl-CoA and methylmalonyl-CoA into *n*- and multi-branched fatty acids, respectively, with NADPH as the preferred reductant. Methylmalonyl-CoA was shown to be incorporated almost exclusively into the acyl portion of wax esters by the cell-free extract while malonyl-CoA was incorporated into polar lipids and both the acyl and alcohol portions of the wax. The optimal pH for the synthesis of both *n*- and multi-branched acids was 6.0. Apparent K_m and V_{max} for malonyl-CoA were 2×10^{-4} *M* and 250 nmol per min per mg, respectively, while the K_m and V_{max} for methylmalonyl-CoA were 7.7×10^{-4} *M* and 0.8 nmol per min per mg, respectively, with 105,000*g* supernatant; but partial purification resulted in a tenfold decrease in K_m values. The partially purified synthetase preparation catalyzed the formation of *n*-C_{16} acid (80%) and *n*-C_{18} acid (20%) from acetyl-CoA and malonyl-CoA. With the same synthetase preparation and the appropriate primer methylmalonyl-CoA was converted into 2,4,6,8-tetramethyldecanoic acid and 2,4,6,8-tetramethylundecanoic acid which were identified by radio gas–liquid chromatography and combined gas chromatography–mass spectrometry. Experiments with an equimolecular mixture of acetyl-CoA and propionyl-CoA showed that the synthetase preferred acetyl-CoA as a primer. Since malonyl-CoA is known to be rapidly decarboxylated in the gland, acetyl-CoA and methylmalonyl-CoA are expected to be the major primer and elongating agent, respectively, available in the gland and therefore 2,4,6,8-tetramethyldecanoic acid should be the major product. Combined gas–liquid chromatography and mass spectrometry demonstrated that this acid was in fact the major acid of the gland.

Branched fatty acids occur in most biological systems, animals, including humans (Velick and English, 1945; Murray, 1962; Odham, 1967b; Nicolaides et al., 1970), bacteria (Kaneda, 1963a; Asselineau and Bennet, 1964), and higher plants (Mold et al., 1966). It has been quite clearly shown that *iso* and *anteiso* monomethyl branched acids originate from the appropriately branched starter chains related to the branched amino acids (Horning et al., 1960; Kaneda, 1963b; Kolattukudy, 1968). Introduction of a single methyl branch into a preformed acyl chain utilizing *S*-adenosylmethionine as the methyl donor is also known to occur in some organisms (Akamatsu and Law, 1970). However, the mechanism of synthesis of multi-branched fatty acids and fatty acids which contain methyl groups at C-2 and/or other even numbered carbon atoms has not been established. In vivo labeling studies suggested that such methyl branches arise from propionate (Noble et al., 1963). Under physiological conditions, which should lead to accumulation of methylmalonyl-CoA, such branched acids might be synthesized (Cardinale et al., 1970; Frenkel et al., 1973; Duncan et al., 1974). This observation is consistent with the notion that substitution of methylmalonyl-CoA for malonyl-CoA, in the reactions catalyzed by fatty acid synthetase, would give rise to the appropriately branched acids. However, no direct experimental evidence for this hypothesis has been presented heretofore.

Multi-branched fatty acids are synthesized in large quantities in the uropygial glands of water birds such as geese, ducks, and swans (Murray, 1962; Odham, 1963, 1965, 1967a). In the goose the bulk of the naturally occurring oil is wax ester in which multi-branched fatty acids are esterified to *n*-fatty alcohols. Therefore, the uropygial gland of goose provides a suitable model system for studying enzymatic synthesis of multi-branched fatty acids and the role of methylmalonyl-CoA in this process. In this paper we describe the isolation, partial purification, and some properties of a fatty acid synthetase from the 105,000*g* supernatant prepared from the uropygial gland of goose. This synthetase was shown to catalyze the incorporation of methylmalonyl-CoA and malonyl-CoA into multi-branched fatty acids and *n*-fatty acids, respectively. The multi-branched fatty acids produced by the partially purified synthetase were identical with those generated by the gland in vivo.

Experimental Section

Materials. Domestic white geese were purchased from the Richards Goose Hatchery, Outlook, Wash., and were raised and maintained on a low energy breeder ration. ATP, NADPH, NADH, glucose 6-phosphate, glucose-6-phosphate dehydrogenase (Type XI), acetyl-CoA, propionyl-CoA, malonyl-CoA, dithioerythritol, and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, Mo. Sepharose 4B was purchased from Pharmacia Fine Chemicals, Upsala, Sweden. Bio-Gel P-2 was purchased from Bio-Rad Laboratories, Richmond, Calif.

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Methylmalonyl-CoA was purchased from P-L Biochemicals, Milwaukee, Wis. [2-^{14}C]Malonic acid (5.6 Ci/mol), [*methyl*-^{3}H]methylmalonic acid (109.9 Ci/mol), sodium [1-^{14}C]acetate (59 Ci/mol), sodium [1-^{14}C]propionate (48 Ci/mol), [1-^{14}C]propionyl-CoA (25.7 Ci/mol), [^{3}H]toluene, [^{14}C]toluene, Omnifluor, and Aquasol were purchased from New England Nuclear, Boston, Mass. Boron trifluoride in butanol was prepared by bubbling BF_3 gas into 1-butanol until the weight of the mixture had increased by 14%. The reagent was stored at 4°.

Synthesis of Malonyl-CoA and Methylmalonyl-CoA. Monothiophenolic esters of [2-^{14}C]malonic acid and [*methyl*-^{3}H]methylmalonic acid were prepared by the method of Trams and Brady (1960). Purification of the monothiol esters by thin-layer chromatography and subsequent transacylation reactions to give the coenzyme A derivatives of the dicarboxylic acids were done as described by Khan and Kolattukudy (1973). [2-^{14}C]Malonyl-CoA and [*methyl*-^{3}H]methylmalonyl-CoA were purified by DEAE-cellulose column chromatography (Kusaka and Goldman, 1967). These preparations were desalted by gel filtration with Bio-Gel P-2. [1-^{14}C]Methylmalonyl-CoA was prepared enzymatically from HCO_3^- and [1-^{14}C]propionyl-CoA in the presence of partially purified goose uropygial gland carboxylase (Buckner and Kolattukudy, 1975). The enzymatically synthesized [1-^{14}C]methylmalonyl-CoA was purified by DEAE-cellulose column chromatography utilizing a linear LiCl gradient (Gregolin et al., 1968) followed by a Bio-Gel P-2 desalting step.

Isolation of Subcellular Fractions. Geese were killed by exsanguination and the uropygial glands were excised from the birds. The residual muscle tissue and fat were carefully removed from the glands. The two lobes of the gland (4–8 g) were cut into thin slices with a razor blade and homogenized in a Ten-Broeck tissue grinder with approximately 10 ml of 100 m*M* phosphate buffer (pH 7.6) containing 250 m*M* sucrose, 0.5 m*M* dithioerythritol, and 1.0 m*M* magnesium chloride. Cell debris and fat were removed from the tissue homogenate by initial centrifugation at 3000*g* for 15 min. The resultant supernatant was centrifuged at 15,000*g* for 30 min to remove the mitochondrial fraction. The 15,000*g* supernatant was centrifuged at 105,000*g* for 90 min to sediment a microsomal fraction. The 105,000*g* soluble supernatant was filtered through two layers of cheesecloth for removal of the floating lipid materials and it was used as the enzyme source in most experiments. Mitochondrial and microsomal pellets were washed by suspension of the pellets in the grinding medium and centrifugation at the appropriate rotor speeds. For isolation of 105,000*g* supernatant from the liver, fresh liver was sliced into small pieces. A fresh weight amount of liver, equal to that of the gland of the same bird, was homogenized and centrifuged as described above for the glandular tissue. All subcellular fractionation procedures were done at 0–4°.

Enzyme Assay. The standard reaction mixture for incorporation of methylmalonyl-CoA into fatty acids with the 105,000*g* supernatant consisted of 0.26 μmol of NADPH, 3.9 μmol of glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, 0.05 μmol of acetyl-CoA or propionyl-CoA, 0.54 μmol of [*methyl*-^{3}H]methylmalonyl-CoA (0.66 Ci/mol), and enzyme in a total volume of 0.5 ml of 100 m*M* phosphate buffer (pH 7.0) containing 0.5 m*M* dithioerythritol. Reaction mixtures for malonyl-CoA incorporation contained the same components except for methylmalonyl-CoA which was replaced by 0.1 μmol of [2-^{14}C]malonyl-CoA (4.6 Ci/mol). Reaction mixtures for malonyl-CoA and methylmalonyl-CoA were usually incubated for 10 and 60 min, respectively, at 30°. The reaction was terminated by the addition of 10 ml of 10% KOH in 95% ethanol. The alkaline mixtures were refluxed for 2 hr and acidified with 6 *N* HCl and the saponified lipids were recovered by extraction with chloroform. The chloroform extract was washed with water and evaporated with a rotary evaporator. Portions of the chloroform solutions were subjected to thin-layer chromatography for recovery of labeled fatty acids. To aid in the location of fatty acids, authentic fatty acid samples were cochromatogramed with the reaction products. Chromatography was performed on silica gel G with hexane–ethyl ether–formic acid (40:10:1) as the developing solvent and the fatty acids were visualized under uv light after spraying the chromatogram with a 0.1% alcoholic solution of 2′,7′-dichlorofluorescein. The silica gel from the fatty acid region was transferred into counting vials containing scintillation solution and ^{3}H or ^{14}C was assayed in a scintillation spectrometer.

The activity of the partially purified fatty acid synthetase preparations was usually measured spectrophotometrically. Initial rates of NADPH oxidation were obtained by measuring the absorbance decrease at 340 nm using a Beckmann DU spectrophotometer with a Model 222 Gilford photometer. The assay was carried out in a total volume of 0.2 ml, contained in 0.5-ml cuvets with a 1.0-cm light path. The reaction mixtures contained 20 μmol of phosphate buffer (pH 7.0), 0.1 μmol of dithioerythritol, 0.04 μmol of NADPH, 0.01 μmol of acetyl-CoA, either 0.02 μmol of malonyl-CoA or 0.1 μmol of methylmalonyl-CoA, and partially purified fatty acid synthetase. The reactions with either malonyl-CoA or methylmalonyl-CoA were started by the addition of enzyme.

In order to express the synthetase activity in terms of nanomoles of malonyl-CoA or methylmalonyl-CoA incorporated into fatty acids per minute, it was assumed that 2 mol of NADPH was oxidized for every mole of malonyl-CoA or methylmalonyl-CoA incorporated. This assumption was experimentally verified by comparing the rates of NADPH oxidation with the rates of incorporation of [2-^{14}C]malonyl-CoA and [methyl-^{3}H]methylmalonyl-CoA into fatty acids by a radiochemical assay. First, initial rates of NADPH oxidation were measured spectrophotometrically with the reaction mixtures containing either [2-^{14}C]malonyl-CoA or [methyl-^{3}H]methylmalonyl-CoA. Then the reactions were stopped by mixing the contents of the cuvets with an excess of 2:1 mixture of chloroform and methanol. The labeled fatty acids were recovered, purified, and assayed for radioactivity as described above for the radiochemical assays. The results showed that the nanomoles of malonyl-CoA or methylmalonyl-CoA incorporated into fatty acids were equal to one-half of the number of nanomoles of NADPH oxidized. Specific activity for fatty acid synthetase was expressed as nanomoles of malonyl-CoA or methylmalonyl-CoA incorporated into fatty acids per minute per milligram of protein.

Protein was determined by the method of Lowry et al. (1951) after precipitation of the protein with 10% trichloroacetic acid. Bovine serum albumin served as standard.

Determination of Radioactivity. Radioactivity on thin-layer chromatograms was monitored with a Berthold thin-layer scanner. Aliquots of solutions containing labeled materials were transferred into counting vials, and the organic solvent or water was evaporated. The residue was dissolved

Table I: Incorporation of [*methyl*-^{3}H] Methylmalonyl-CoA and [2-^{14}C] Malonyl-CoA into Lipid Classes by the 15,000*g* Supernatant from Goose Uropygial Glands.[a]

Fraction	Incorporation (nmol per hr per mg)	
	[*methyl*-^{3}H] Methylmalonyl-CoA	[2-^{14}C] Malonyl-CoA
Polar lipid[b]	(1.08)[c]	1.55
Fatty alcohol	0.06	0.09
Fatty acid	0.95	0.24
Triglyceride	0.29	0.64
Wax ester	1.66	1.29
Acyl	1.65	1.02
Alcohol	0.01	0.27

[a] Reaction mixtures containing 0.09 μmol of [*methyl*-^{3}H] methylmalonyl-CoA (25 Ci/mol) or 0.07 μmol of [2-^{14}C] malonyl-CoA (4.6 Ci/mol), 0.52 μmol of NADPH, 0.66 μmol of NADH, 7.8 μmol of glucose 6-phosphate, 2 units of glucose-6-phosphate dehydrogenase, 0.5 μmol of dithioerythritol, and 4.6 mg of protein in a total volume of 1.0 ml of 100 m*M* phosphate buffer (pH 7.0) were incubated at 30° for 120 min. [b] The polar lipid fraction contained lipids which remained at the origin on thin-layer chromatographic plates developed with hexane–diethyl ether–formic acid (40:10:1). [c] Further analysis of the polar labeled material obtained from the incorporation of methylmalonyl-CoA showed that the bulk of radioactivity of this fraction was contained in an artefact and only a very small part of this label could be recovered following transesterification with 14% BF_3 in 1-butanol. On the other hand, following similar treatment of the labeled polar lipids derived from [2-^{14}C] malonyl-CoA, the bulk of the radioactivity was recovered in fatty acid methyl esters.

in 15 ml of 30% ethanol in toluene containing 4 g/l. of Omnifluor, and assayed for radioactivity in a Packard Model 3003 TriCarb scintillation spectrometer. Internal standards of [^{3}H]toluene and [^{14}C]toluene were used to determine counting efficiencies which were 17 and 74%, respectively, for ^{3}H and ^{14}C. Tritium in thin-layer chromatographic fractions was assayed in Aquasol with a counting efficiency of 29%.

Partial Purification of Synthetase. A Sepharose 4B column (2.2 × 90 cm) was equilibrated with 0.1 *M* phosphate buffer (pH 7.6) containing 20 m*M* citrate and 0.5 m*M* dithioerythritol. The 105,000*g* supernatant (3.5 ml) was placed atop the column and proteins were eluted with the same buffer with a flow rate of 0.25 ml/min. The absorbance at 280 nm of the column effluent was measured with an Isco Model UA-2 monitor and 3–5-ml fractions were collected with an Isco Model 270 fraction collector. Fractions were assayed for fatty acid synthetase activity spectrophotometrically.

Chromatography. Thin-layer chromatography (TLC) was performed with 20 × 20 cm plates coated with silica gel G and activated overnight at 110°. Lipid fractions (wax ester, triglyceride, free fatty acid, and primary alcohol) were separated by TLC with hexane–ethyl ether–formic acid (40:10:1) as the developing solvent. Lipids which remained at the origin of such chromatograms were designated as the polar lipid fraction.

Radio gas–liquid chromatography was performed with a Perkin-Elmer Model 801 gas chromatograph equipped with a flame ionization detector and an effluent splitter, attached to a Barber-Coleman radioactivity monitor. Fatty acid butyl esters were analyzed on a coiled stainless steel column (0.3 × 300 cm) packed with 5% OV-1 on 80–100 mesh Gas-Chrome Q and a carrier-gas flow of 80 ml/min. The column temperature was programed from 160 to 240° with a 2°/min rate. Authentic samples were used as standards for thin-layer and gas–liquid chromatography.

Gas–Liquid Chromatography–Mass Spectrometry (GLC–MS). GLC–MS of fatty acid butyl esters was done on 5% SE-30 on 80–100 mesh Gas Chrome Q packed in a 0.3 × 150 cm coiled glass column held at 180° with a Varian Aerograph Model 328 gas chromatograph attached to a Perkin-Elmer-Hitachi RMU 6D mass spectrometer using a Bieman separator interphase. Mass spectra were recorded at the apex of gas chromatographic peaks with 70 eV as the ionizing voltage. Spectra were also recorded at the ascending and descending side of each peak in order to make sure that the peak did not represent incompletely resolved compounds.

Preparation of Derivatives. Butyl esters of the glandular acyl lipids were prepared by refluxing each fraction with 14% BF_3 in 1-butanol for 4 hr. The butyl esters were recovered from the reaction mixture by chloroform extraction and they were purified by TLC with benzene as the developing solvent.

Results and Discussion

Incorporation of [1-^{14}C]Acetate and [1-^{14}C]Propionate into Lipids. Since the uropygial gland of goose contains large quantities of multi-branched fatty acids the glandular tissue should contain a fatty acid synthetase capable of utilizing precursors of branched fatty acids. In fact a 15,000*g* supernatant prepared from an extract of uropygial gland when incubated in phosphate buffer (pH 7.0) with ATP, HCO_3^-, Mg^{2+}, NADPH, NADH, and 0.34 μmol of [1-^{14}C]acetate or 0.42 μmol of [1-^{14}C]propionate for 4 hr at 30°, 0.04 μmol of acetate, and 0.02 μmol of propionate were incorporated into the total lipids. Thin-layer chromatographic analysis of the labeled lipids showed that approximately 25 and 37% of the total radioactivity of the total lipids derived from [1-^{14}C]acetate and [1-^{14}C]propionate, respectively, were in the wax esters. Treatment of the labeled wax ester fractions with BF_3–butanol followed by analysis of the products by thin-layer chromatography showed that virtually all the radioactivity was contained in the acyl portion of the wax. Radio gas–liquid chromatography of these labeled butyl esters indicated that the major products derived from both acetate and propionate were branched. These preliminary results showed that the 15,000*g* supernatant from goose uropygial gland could catalyze the synthesis of branched acids.

Incorporation of Malonyl-CoA and Methylmalonyl-CoA into Lipids. Since acetate and propionate have to be activated and carboxylated before incorporation into fatty acids they are not the direct precursors of fatty acids. Therefore, incorporation of synthetic [2-^{14}C]malonyl-CoA and [*methyl*-^{3}H]methylmalonyl-CoA into lipids by the 15,000*g* supernatant was examined. Both of these substrates were readily incorporated into lipids (5–6 nmol per hr per mg).

The bulk of the multi-branched fatty acids in the gland are found in the acyl portion of the wax esters. If the incorporation of methylmalonyl-CoA into lipids catalyzed by the cell-free preparation reflects the reactions in vivo, the major product derived from methylmalonyl-CoA should be wax esters. In order to examine this possibility, the radioactive lipids derived from [*methyl*-^{3}H]methylmalonyl-CoA were analyzed by thin-layer chromatography (Table I). The wax ester fraction constituted the major labeled component derived from [*methyl*-^{3}H]methylmalonyl-CoA. Free fatty

acids also contained substantial amounts of radioactivity. Very little incorporation of methylmalonyl-CoA into the other lipid fractions was observed. In contrast to these results, polar lipids constituted the major labeled component derived from [2-^{14}C]malonyl-CoA. Wax ester and triglyceride fractions also contained substantial amounts of radioactivity.

In the wax ester, synthesized in vivo by the uropygial glands of goose, multi-branched acids are esterified to *n*-fatty alcohols. That this specificity also holds in the cell-free extract was shown by the following observation. Virtually all of the radioactivity contained in the wax ester fraction derived from [*methyl*-^{3}H]methylmalonyl-CoA was recovered in the acyl moiety while a substantial portion of the radioactivity in the wax ester fraction derived from [2-^{14}C]malonyl-CoA was found in the alcohol moiety. Furthermore, radio gas–liquid chromatographic analysis of the acyl moieties of the wax ester as butyl esters revealed that the acids produced from methylmalonyl-CoA were branched and those derived from malonyl-CoA were *n*-fatty acids. The alcohol moiety of the wax ester produced from malonyl-CoA was a mixture of *n*-C_{16} and *n*-C_{18} alcohols (analyzed by radio gas–liquid chromatography of their acetates but data not shown). Thus it appears that the 15,000*g* supernatant can incorporate either malonyl-CoA or methylmalonyl-CoA into fatty acids and these fatty acids are available for esterification with alcohol. Only the *n*-fatty acids are reduced to alcohol. Since the in vivo specificities are reflected in this cell-free preparation, this extract provides a suitable system for studies on the mechanism of selection of fatty acids for the synthesis of various lipid classes.

Subcellular Localization of Multi-Branched Fatty Acid Synthesis. In order to determine the location of the methylmalonyl-CoA incorporating activity various subcellular fractions from goose uropygial glands were incubated with methylmalonyl-CoA and the results are summarized in Table II. Only the 105,000*g* supernatant incorporated methylmalonyl-CoA into fatty acids. The subcellular fractions were also analyzed for their ability to incorporate malonyl-CoA into lipid. The 105,000*g* supernatant contained the majority of the malonyl-CoA incorporating activity. Under the conditions of the assays for incorporation of methylmalonyl-CoA and malonyl-CoA by the 105,000*g* supernatant, specific activity values were considerably higher for the incorporation of malonyl-CoA.

If the methylmalonyl-CoA incorporation into fatty acids observed in goose gland extract reflects the in vivo situation, where the bulk of the fatty acids are branched, then other tissues such as liver which do not contain multi-branched fatty acids should not be expected to incorporate methylmalonyl-CoA into fatty acids. The results in Table II show that methylmalonyl-CoA was incorporated into fatty acids only by the uropygial gland extract and not by the liver extract, while malonyl-CoA was incorporated into fatty acids by both preparations. These results suggest that the incorporation of methylmalonyl-CoA into fatty acids is specific for only those organs which are capable of synthesizing multibranched fatty acids. It is possible that the glandular tissue contains either two enzyme complexes, one for methylmalonyl-CoA incorporation and another for malonyl-CoA, or a single enzyme complex that can incorporate either of these substrates.

Identification of the Fatty Acids Synthesized by the 105,000g Supernatant. In order to determine the nature of the fatty acids synthesized from [2-^{14}C]malonyl-CoA and [*methyl*-^{3}H]methylmalonyl-CoA the total lipids derived from these were transesterified with BF_3–butanol and the resulting fatty acid butyl esters were analyzed by radio gas–liquid chromatography. The labeled fatty acids derived from [2-^{14}C]malonyl-CoA contained three labeled components which had retention times identical with those of the butyl esters of tetradecanoic (C_{14}), hexadecanoic (C_{16}), and octadecanoic (C_{18}) acids (Figure 1).

Radio gas–liquid chromatogram of the labeled fatty acids derived from [*methyl*-^{3}H]methylmalonyl-CoA showed that the radioactivity was resolved into three distinct peaks all of which had retention times shorter than that of the butyl ester of tetradecanoic acid (Figure 1). One component had a retention time between those of undecanoic (C_{11}) and dodecanoic (C_{12}) acids. The retention of a second component was close to that of dodecanoic acid while the third radioactive component was located between tridecanoic (C_{13}) and tetradecanoic acids. Since the retention times of these compounds were not identical with those of authentic *n*-fatty acid butyl esters, these acids are either branched or unsaturated. The latter possibility was ruled out by the fact that these components were unaffected by treatment with OsO_4. The radio gas–liquid chromatograms showed that the three labeled components were identical with the butyl esters of three of the major endogenous fatty acids A, B, and C.

In order to determine the structures of fatty acids A, B, and C, they were subjected to analysis by combined gas chromatography–mass spectrometry. The mass spectra of A and B showed molecular ions at *m/e* 284 and 296, respec-

Table II: Subcellular Localization of Fatty Acid Synthetase Activity in the Uropygial Gland and Liver of Goose.[a]

Subcellular Fractions	Incorporation into Fatty Acid (nmol per min per mg)	
	Methylmalonyl-CoA	Malonyl-CoA
Experiment 1		
Homogenate	0.36	12.43
Cell debris and fat pad	0.05	2.10
Mitochondria	0.04	2.34
Microsomes	0.04	3.04
105,000*g* supernatant	0.58	17.58
Experiment 2		
105,000*g* supernatant		
Goose liver	<0.01	6.07
Goose gland	0.33	7.78

[a] In experiment 1, reaction mixtures contained 0.26 μmol of NADPH, 0.33 μmol of NADH, 3.9 μmol of glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, 0.05 μmol of acetyl-CoA, 0.05 μmol of propionyl-CoA, 0.54 μmol of [*methyl*-^{3}H] methylmalonyl-CoA (0.66 Ci/mol) or 0.05 μmol of [2-^{14}C] malonyl-CoA (3.0 Ci/mol), 0.25 μmol of dithioerythritol, and protein from each fraction in a total volume of 0.5 ml of 50 m*M* phosphate buffer (pH 7.0). Reaction mixtures with malonyl-CoA and methylmalonyl-CoA were incubated at 30° for 12 and 60 min, respectively. The protein used for each subcellular fraction with methylmalonyl-CoA as substrate represented 0.3% of the glandular homogenate and 0.03% of the homogenate was used with malonyl-CoA. After the incubation period fatty acids were recovered from the lipids as described in the Experimental Section. In experiment 2, assay conditions with [*methyl*-^{3}H] methylmalonyl-CoA (0.07 μmol, 3.8 Ci/mol) and [2-^{14}C] malonyl-CoA (2.1 Ci/mol), as substrates, were the same as those described above except for a 15-min incubation period and addition of 0.3 mg of 105,000*g* supernatant protein from either the liver or the uropygial gland.

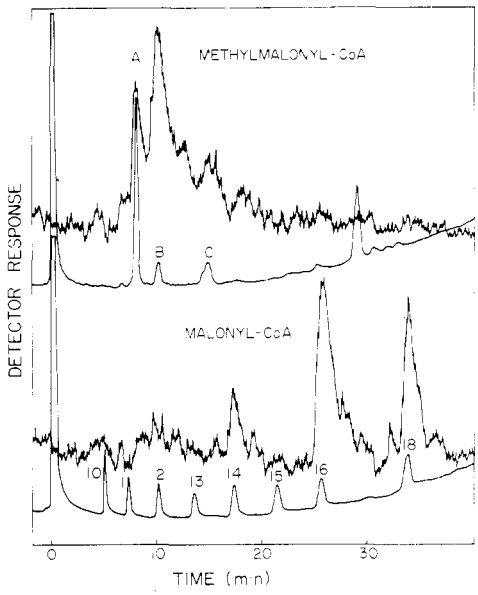

FIGURE 1: Radio gas-liquid chromatogram of fatty acids (as butyl ester) derived from [*methyl*-^{3}H]methylmalonyl-CoA and [2-^{14}C]malonyl-CoA in the 105,000*g* supernatant from goose uropygial glands. Reaction mixtures containing 0.16 μmol of [*methyl*-^{3}H]methylmalonyl- CoA (0.66 Ci/mol) or 0.16 μmol of [2-^{14}C]malonyl-CoA (1.85 Ci/mol), 0.26 μmol of NADPH, 0.3 μmol of NADH, 0.05 μmol of acetyl-CoA, 0.25 μmol of dithioerythritol, and enzyme in 0.5 ml of 100 m*M* phosphate (pH 7.0) were incubated at 30° for 120 min. Assay with methylmalonyl-CoA and malonyl-CoA contained 3.6 and 0.36 mg of protein, respectively. For both chromatograms the top tracing is radioactivity and the bottom tracing is flame ionization detector response. In the upper chromatogram (methylmalonyl-CoA) the letters above mass peaks denote major fatty acids from the uropygial gland of goose. The numbers above the mass peaks in the lower chromatogram (malonyl-CoA) represent chain lengths of authentic *n*-fatty acid butyl esters. The procedures for the recovery of fatty acids and radio gas-liquid chromatography conditions are described in the Experimental Section.

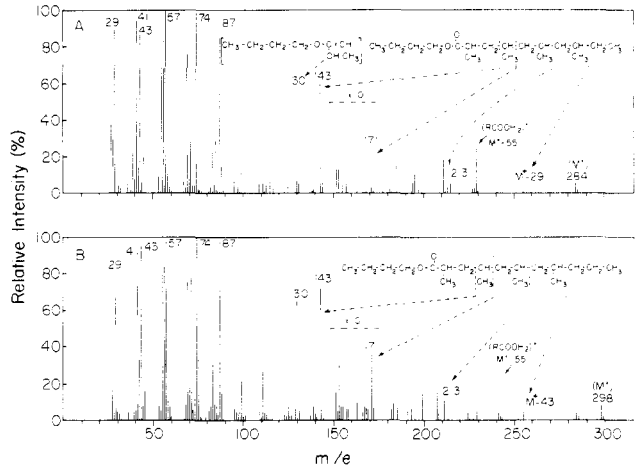

FIGURE 2: Mass spectra of the butyl esters of fatty acids A and B shown in Figure 1. The spectra were recorded at the apex of peaks A and B with an ionizing voltage of 70 eV as described in the Experimental Section.

tively (Figure 2). Since butyl esters have characteristic fragmentation resulting in protonated carboxylic acid ions, $RCOOH_2^+$ (Budzikiewicz et al., 1967), molecular weights of the acids A and B were also obtained from the major ions at *m/e* 229 and 243 ($M^+ - 55$), respectively. The two mass spectra show major ions at *m/e* 130 and 143. The fragment at *m/e* 130 represents β cleavage of the acyl chain with a γ-hydrogen transfer (McLafferty rearrangement) and it strongly suggests that a methyl branch is present on carbon-2 of the acyl chain. A methyl branch at carbon-2 is also indicated by the strong ion at *m/e* 74, a result of a one hydrogen transfer from the alcohol chain of the McLafferty ion with loss of the alkyl chain (Budzikiewicz et al., 1967). The intense ions at *m/e* 143 and 171 in both spectra indicate the presence of a methyl branch at C-4 position of both A and B. A methyl branch at C-6 of both acids is indicated by significant fragment ions at *m/e* 185 and 213. Significant ions corresponding to $M^+ - 29$ and $M^+ - 43$ for A and B, respectively, suggest a methyl branch at C-8 of both acids. From these mass spectral data, components A and B are identified to be butyl esters of 2,4,6,8-tetramethyldecanoic acid and 2,4,6,8-tetramethylundecanoic acid, respectively. These two acids were previously identified to be major components of the acyl moieties of the wax esters isolated from the free flowing preen gland secretion of domestic geese (Odham, 1963). These wax esters were reported to be approximately 90% of the gland secretion and were identified as the octadecyl esters of 2,4,6,8-tetramethyldecanoic (C_{14}) and 2,4,6,8-tetramethylundecanoic (C_{15}) acids in relative proportions of 94 and 6%, respectively (Odham, 1963).

The gas-liquid chromatogram of the butyl esters from total glandular lipids (Figure 1), in addition to revealing the presence of the multi-branched C_{14} and C_{15} acids (peaks A and B), shows the presence of peak C and other fatty acid butyl esters of longer retention times. Since methylmalonyl-CoA was not incorporated into the acids with retention time longer than that of C_{14} such long acids were not further examined. The mass spectrum of butyl ester C indicated a molecular weight of 312, a value obtained from both the molecular ion (M^+) and the protonated carboxylic acid ion ($M^+ - 55$). The fragmentation pattern for this acid was also consistent with the presence of multiple methyl branches in this molecule but it was difficult to ascertain whether 4 or 5 methyl branches were present. On the basis of the relative retention time and the mass spectrum, component C is tentatively identified as 2,4,6,8,10-pentamethyldodecanoic acid. Thus the radio gas-liquid chromatographic data (Figure 1) and the mass spectral data (Figure 2) show that the naturally occurring fatty acids, which coincided with the labeled acids derived from [*methyl*-^{3}H]methylmalonyl-CoA, are 2,4,6,8-tetramethyldecanoic acid, 2,4,6,8-tetramethylundecanoic acid, and probably 2,4,6,8,10-pentamethyldodecanoic acid.

Cofactor Requirements for the Incorporation of Methylmalonyl-CoA into Fatty Acids. Incorporation of methylmalonyl-CoA into fatty acids by the 105,000*g* supernatant required reduced pyridine nucleotide and showed a preference for NADPH (Table III). NADH either alone or together with NADPH gave lower rates of incorporation. Addition of a NADPH regenerating system resulted in a slight increase in activity as did addition of ATP or propionyl-CoA, while acetyl-CoA had no effect. Flavine mononucleotide resulted in inhibition of incorporation of methylmalonyl-CoA. The lack of appreciable increases in activity with the addition of acetyl-CoA or propionyl-CoA and the NADPH regenerating system could be due to the presence of significant amounts of these materials in the 105,000*g* supernatant. The same NADPH dependence was shown for malonyl-CoA incorporation into fatty acid by the 105,000*g* supernatant (data not presented). This requirement of NADPH for incorporation of both methylmalonyl-CoA and malonyl-CoA is similar to the NADPH dependence shown

Table III: Cofactor Requirement for the Incorporation of Methylmalonyl-CoA into Fatty Acids by the 105,000*g* Supernatant from Goose Uropygial Glands.[a]

Cofactor Additions	Incorporation into Fatty Acids (cpm × 10^{-4})
None	0.5
NADH	3.7
NADPH	5.3
NADH + NADPH	4.5
NADPH + regenerating system	5.9
NADH + NADPH + ATP	6.2
NADH + NADPH + propionyl-CoA	5.1
NADH + NADPH + acetyl-CoA	4.6
NADH + NADPH + FMN	2.7

[a] Reaction mixtures contained 0.83 μmol of ATP, 0.13 μmol of NADH, 0.13 μmol of NADPH, 3.9 μmol of glucose 6-phosphate, 1.0 unit of glucose-6-phosphate dehydrogenase, 0.06 μmol of FMN, 0.02 μmol of acetyl-CoA, 0.03 μmol of propionyl-CoA, 0.08 μmol of [*methyl*-^{3}H] methylmalonyl-CoA (3.8 Ci/mol), and 1.0 mg of 105,000*g* supernatant protein in 0.5 ml of 50 m*M* phosphate buffer (pH 7.0) containing 250 m*M* sucrose, 1 m*M* $MgCl_2$, and 0.5 m*M* dithioerythritol. Reaction mixtures were incubated for 60 min at 30°. Products were isolated as described in the Experimental Section.

for the fatty acid synthetases from pigeon liver (Hsu et al., 1965), rat liver (Burton et al., 1968), and mammary glands (Smith and Abraham, 1970). The uropygial gland synthetase is different from other synthetases which show a stimulation with added NADH (White et al., 1971; Khan and Kolattukudy, 1973).

pH Dependence. The effect of pH on methylmalonyl-CoA incorporation into fatty acids by the 105,000*g* supernatant is shown in Figure 3. The synthetase was active in a broad pH range between 5.5 and 8.0 with optimal activity at about pH 6.0. Data are also presented for the pH dependence of malonyl-CoA incorporation into fatty acids and a pH optimum of 6.0 is indicated. Both activities decreased sharply below pH 5.5 and the incorporatin of malonyl-CoA decreased above pH 7.0. The pH dependence for both activities is generally similar to that observed with other animal fatty acid synthetases (Hsu et al., 1965; Burton et al., 1968; Smith and Abraham, 1970). However, a discernible difference was observed between the pH dependence of malonyl-CoA incorporation and that of methylmalonyl-CoA incorporation.

Time-Course and Effect of Protein Concentration. Incorporation of methylmalonyl-CoA into fatty acids by the 105,000*g* supernatant was linear up to a protein concentration of 10 mg/ml and an incubation period of 90 min. Routinely, assays were run for 30 min with 2–4 mg/ml of protein. The effects of incubation time and protein concentration on the incorporation of malonyl-CoA into fatty acids by the 105,000*g* supernatant was also examined. Incorporation of this substrate was linear up to a protein concentration of 0.1 mg/ml and an incubation period of 15 min (data not presented). Assays were usually run for 10 min with 0.05 mg/ml of protein.

Preliminary experiments on the incorporation of methylmalonyl-CoA into lipids by cell-free extracts of the uropygial gland suggested that longer incubation periods favored synthesis of wax esters. Therefore, a time-course of methylmalonyl-CoA incorporation into various lipid classes was determined and the results are summarized in Figure 4. Methylmalonyl-CoA was rapidly incorporated into free fatty acids up to an incubation period of 30 min; during this

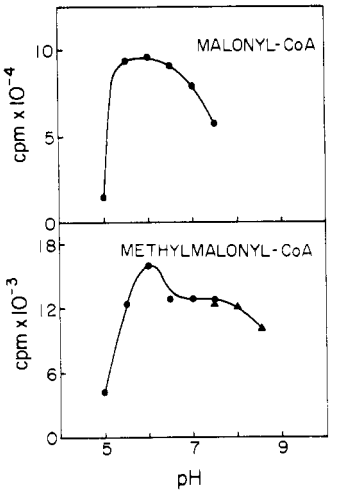

FIGURE 3: Effects of pH on the incorporation of malonyl-CoA and methylmalonyl-CoA into fatty acids by the 105,000*g* supernatant from goose uropygial glands. Reaction mixtures contained 50 μmol of citrate–phosphate (●) or phosphate (▲) buffer at the indicated pH, 0.25 μmol of dithioerythritol, 0.4 μmol of NADPH, 0.05 μmol of acetyl-CoA, 0.12 μmol of [2-^{14}C]malonyl-CoA (0.78 Ci/mol) or 0.04 μmol of [*methyl*-^{3}H]methylmalonyl-CoA (8.4 Ci/mol), and enzyme in a total volume of 0.5 ml. Reaction mixtures with malonyl-CoA contained 0.03 mg of protein and they were incubated at 30° for 10 min, while those with methylmalonyl-CoA contained 0.6 mg of protein and they were incubated for 60 min at 30°.

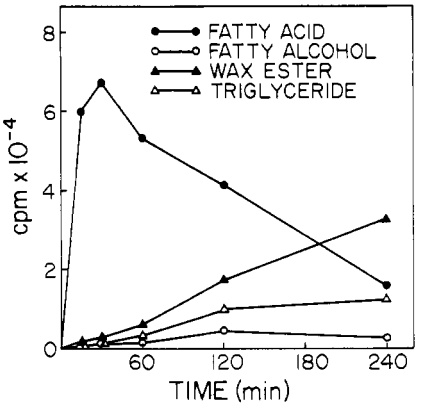

FIGURE 4: Time-course for methylmalonyl-CoA incorporation into lipids by the 105,000*g* supernatant from goose uropygial glands. Reaction mixtures containing 0.26 μmol of NADPH, 3.9 μmol of glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, 0.1 μmol of [*methyl*-^{3}H]methylmalonyl-CoA (6.3 Ci/mol), 0.1 μmol of acetyl-CoA, 0.25 μmol of dithioerythritol, and 2 mg of 105,000*g* supernatant protein in a total volume of 0.5 ml of 50 m*M* phosphate buffer (pH 7.0) were incubated at 30° for the times indicated above. Extraction and separation of the lipid classes are described in the Experimental Section.

time no other products contained significant amounts of label. Successively longer incubation periods resulted in a steady decrease in the proportion of label contained in the free fatty acids and a gradual increase in the proportion of ^{3}H found in the wax ester and triglyceride fractions. Methylmalonyl-CoA was not incorporated into the fatty alcohol fraction. These results suggest that the radioactivity found in the wax ester and triglyceride fractions results from incorporation of free fatty acids released from the fatty acid synthetase. Fatty acid synthetases from other animal sources also generate free fatty acids (Hsu et al., 1965; Burton et al., 1968; Smith and Abraham, 1970).

Effects of Substrate Concentration. The effects of in-

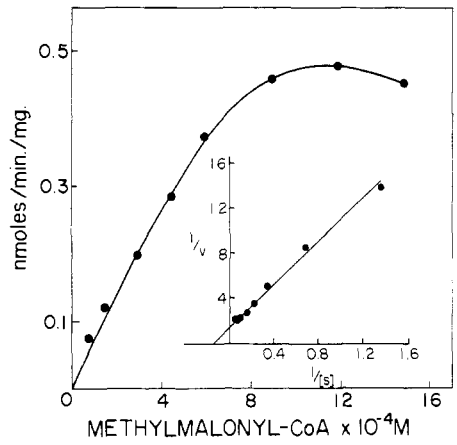

FIGURE 5: The effect of methylmalonyl-CoA concentration on the rate of synthesis of multi-branched fatty acids. Reaction mixtures containing 0.26 μmol of NADPH, 3.9 μmol of glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, the indicated amounts of [*methyl*-^{3}H]methylmalonyl-CoA (0.55 Ci/mol), 0.25 μmol of dithioerythritol, and 3 mg of 105,000*g* supernatant protein in a total volume of 0.5 ml of 100 m*M* phosphate buffer (pH 7.0) were incubated for 60 min at 30° and the lipids were recovered as described in the Experimental Section.

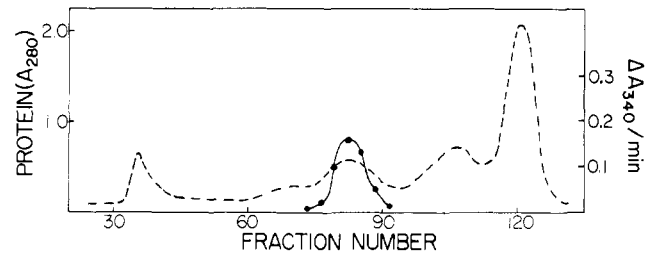

FIGURE 6: Elution profile of 105,000*g* supernatant filtered through a Sepharose 4B column (2.3 × 90 cm). Each fraction contained approximately 4 ml. Absorbance at 280 nm (- - -) and fatty acid synthetase activity (●) are plotted. Synthetase activity was assayed spectrophotometrically with NADPH, malonyl-CoA, or methylmalonyl-CoA and acetyl CoA as described in the Experimental Section. Reaction mixtures for incorporation of malonyl-CoA and methylmalonyl-CoA into fatty acids contained 5 and 40 μl of each fraction, respectively.

creasing concentrations of methylmalonyl-CoA on the synthesis of multi-branched fatty acids by the 105,000*g* supernatant are shown in Figure 5. A typical substrate saturation curve was obtained and the double reciprocal plots were linear. From these data an apparent K_m of 7.7×10^{-4} and a V_{max} of 0.8 nmol per min per mg were calculated. The 105,000*g* supernatant was also incubated with increasing concentrations of malonyl-CoA (data not presented) and from a double reciprocal plot an apparent K_m and V_{max} of 2×10^{-4} *M* and 250 nmol per min per mg, respectively, were calculated. Reaction mixtures used in these assays with increasing concentrations of methylmalonyl-CoA and malonyl-CoA did not contain added acetyl-CoA or propionyl-CoA since addition of these primers did not substantially affect the rate of the reaction.

Partial Purification of Fatty Acid Synthetase. One of the initial steps of fatty acid synthesis is the condensation of malonyl-CoA with a primer such as acetyl-CoA. The experimental evidence presented thus far suggests that the fatty acid synthetase activity in the 105,000*g* supernatant from the uropygial gland is capable of forming multi-branched fatty acids from methylmalonyl-CoA. The involvement of acetyl-CoA and/or propionyl-CoA as primers could not be established since the 105,000*g* supernatant probably contained substantial amounts of these compounds. In order to overcome this problem the 105,000*g* supernatant was subjected to gel filtration chromatography with Sepharose 4B and an elution profile is shown in Figure 6. The fatty acid synthetase activity was located in fractions that coincided with the second major protein peak eluted from the column. The same elution profile of activity was obtained with either [2-^{14}C]malonyl-CoA or [*methyl*-^{3}H]methylmalonyl-CoA. This gel filtration step resulted in a three- to six-fold purification of the fatty acid synthetase activity. This preparation incorporated about 480 nmol of malonyl-CoA per min per mg of protein. This specific activity compares favorably with the specific activity values reported for the purified fatty synthetases from pigeon liver (Hsu et al., 1965), rat liver (Burton et al., 1968), and rat mammary gland (Smith and Abraham, 1970). Electrophoresis of the fatty acid preparation from the uropygial gland on 4% polyacylamide gels showed two major bands whch presumably represent subcomplexes. Only a few very minor bands were observed. Similarly, sucrose density gradient centrifugation studies also indicated that this preparation did not contain appreciable amounts of impurities. Furthermore, ultracentrifugation showed that this enzyme preparation was essentially pure. Since fatty acid synthesis is the major activity of the uropygial gland, it is not surprising that fatty acid synthetase constitutes an unusually large portion of the total proteins of this gland.

Products Generated by the Partially Purified Fatty Acid Synthetase. The results discussed in a previous section showed that the 105,000*g* supernatant from the uropygial gland catalyzed incorporation of methylmalonyl-CoA into 2,4,6,8-tetramethylundecanoic acid and 2,4,6,8-tetramethylundecanoic acid (Figures 1 and 2). Structural considerations suggest that the former is most probably formed from one molecule of acetyl-CoA and four of methylmalonyl-CoA while the latter could be derived from one molecule of propionyl-CoA and four molecules of methylmalonyl-CoA. However, since the crude preparations probably contained unknown quantities of acetyl-CoA and propionyl-CoA the effects of primers on the structure of the products of the fatty acid synthetase could not be defined. Since the fatty acid synthetase from Sepharose 4B should be free from endogenous primers this partially purified enzyme was used to determine the role of acetyl-CoA and propionyl-CoA as primers.

Radio gas chromatograms of the butyl esters of fatty acids, derived from [*methyl*-^{3}H]methylmalonyl-CoA in the presence of acetyl-CoA, propionyl-CoA, and an equimolecular mixture of the two, are shown in Figure 7. With acetyl-CoA as the primer two radioactive acids were formed. The major labeled fatty acid cochromatographed with 2,4,6,8-tetramethyldecanoic acid and the minor component with 2,4,6,8,10-pentamethyldodecanoic acid. The major labeled fatty acid synthesized in the presence of propionyl-CoA cochromatographed with 2,4,6,8-tetramethylundecanoic acid. One of the two minor components obtained from the reaction mixture containing propionyl-CoA is tentatively identified as 2,4,6,8,10-pentamethyltridecanoic acid while the other minor component could not be identified. Thus it appears that this synthetase catalyzes elongation of either acetyl-CoA or propionyl-CoA by four successive condensations with methylmalonyl-CoA. Formation of the small amounts of longer products show that this enzyme shows some tendency to add five C_3 units to the primer.

In the presence of an equimolecular mixture of acetyl-CoA and propionyl-CoA, [*methyl*-^{3}H]methylmalonyl-CoA gave rise to 2,4,6,8-tetramethyldecanoic acid and 2,4,6,8,10-pentamethyldodecanoic acid, the products expected from acetyl-CoA, as well as 2,4,6,8-tetramethylundecanoic acid, the product expected from propionyl-CoA. However, the quantitative distribution of label among these three acids (Figure 7) strongly suggests that this synthetase prefers acetyl-CoA over propionyl-CoA as a primer. Such a preference is also manifested by the fact that the major fatty acid of the gland is 2,4,6,8-tetramethyldecanoic acid.

The purified synthetase was also incubated with [2-^{14}C]malonyl-CoA with acetyl-CoA as primer to determine if the enzyme could synthesize *n*-fatty acids. The fatty acids resulting from such experiments were analyzed by radio gas–liquid chromatography and the chromatograms showed only two radioactive components, which were identified as hexadecanoic acid and octadecanoic acid. The former contained 80% of the total radioactivity. This chain length distribution of the straight chain products formed by the fatty acid synthetase of the goose uropygial gland is similar to that observed with the fatty acid synthetases from other biological systems (Volpe and Vagelos, 1973).

Properties of the Partially Purified Fatty Acid Synthetase. The rate of malonyl-CoA incorporation into fatty acids increased with increasing concentration of the substrate resulting in a typical substrate saturation pattern. An apparent K_m of 3×10^{-5} *M* and V_{max} of 500 nmol per min per mg were calculated from a linear double reciprocal plot. This K_m value is about one-tenth of the K_m calculated for the 105,000*g* supernatant. This difference is most probably due to the removal of a highly active malonyl-CoA decarboxylase (Buckner and Kolattukudy, 1975) from the fatty acid synthetase. The K_m value obtained for the purified enzyme is similar to that reported for the mammary gland synthetase from the rat (Smith and Abraham, 1970).

The effects of methylmalonyl-CoA concentration on the partially purified enzyme were also examined and the data resulted in a sigmoidal curve instead of a typical Michaelis–Menton type substrate saturation pattern. A maximum velocity of 1 nmol per min per mg was observed at a methylmalonyl-CoA concentration of 1×10^{-4} *M*. Since the 105,000*g* supernatant did give a typical substrate saturation pattern (Figure 5), these results suggest that the purification step removed an allosteric effector which might have a significant role in the gland, where the enzyme is likely to function with subsaturating levels of methylmalonyl-CoA.

Other properties of the partially purified enzyme did not appreciably change as a result of the purification step. The effects of pH and added cofactors on the incorporation of methylmalonyl-CoA and malonyl-CoA were similar to those shown for the 105,000*g* supernatant. However, with the partially purified enzyme, the addition of acetyl-CoA or propionyl-CoA showed a greater stimulation of fatty acid synthesis than that observed with the 105,000*g* supernatant.

Stereospecificity of Methylmalonyl-CoA Incorporation. Propionyl-CoA carboxylase is known to give rise to the the *S* isomer of methylmalonyl-CoA (Overath et al., 1972). If such a stereospecificity applies to the carboxylase of the uropygial gland, only one of the stereoisomers of methylmalonyl-CoA would be available to the fatty acid synthetase of this tissue in vivo. However, the synthetic methylmalonyl-CoA used in our experiments contained most probably equal amounts of the two stereoisomers. The presence of the nonbiological isomer could have influenced the incorporation of the other isomer into the fatty acids. In order to test this possibility a competition experiment was performed in the following manner. The naturally formed isomer of methylmalonyl-CoA was synthesized from [1-^{14}C]propionyl-CoA with the carboxylase isolated from the gland as catalyst. The ^{14}C-labeled methylmalonyl-CoA was mixed with chemically synthesized [*methyl*-^{3}H]methylmalonyl-CoA. This doubly labeled substrate was incubated with partially purified fatty acid synthetase isolated from the uropygial gland. If the biosynthetically produced [1-^{14}C]methylmalonyl-CoA is preferred over the *R,S* mixture present in the chemically synthesized [*methyl*-^{3}H]methylmalonyl-CoA the ^{14}C/^{3}H ratio of the product acids should be higher than that of the substrate. However, experiments with three different mixtures of substrates with ^{14}C/^{3}H ratios of 3.0, 1.0, and 0.3 gave rise to fatty acids of isotopic ratios 2.8, 1.0, and 0.32, respectively. Furthermore, with the subsatur-

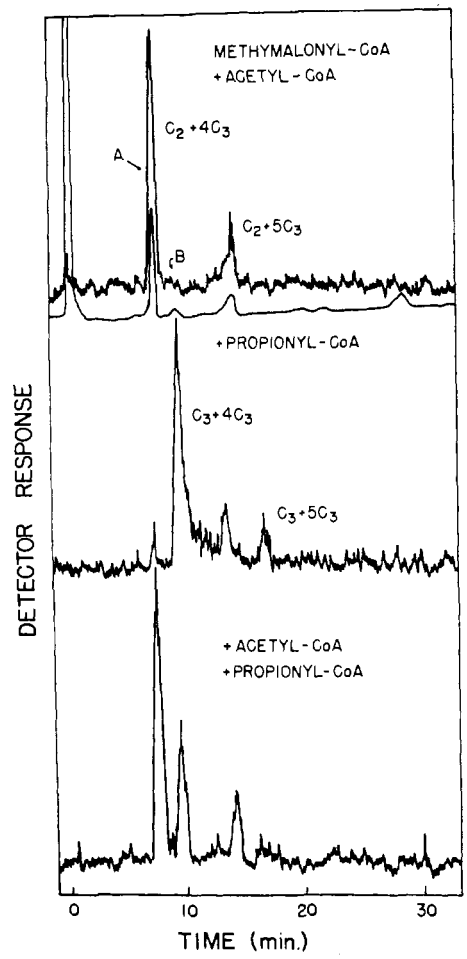

FIGURE 7: Gas–liquid chromatograms of fatty acids (as butyl esters) synthesized from acetyl-CoA or propionyl-CoA as primer by the partially purified fatty acid synthetase from goose uropygial glands. Reaction mixtures contained 0.26 μmol of NADPH, 3.9 μmol of glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, 0.54 μmol of [*methyl*-^{3}H]methylmalonyl-CoA (0.63 Ci/mol), 0.05 μmol of acetyl-CoA and/or propionyl-CoA, 0.25 μmol of dithioerythritol, and 2.8 mg of partially purified enzyme in a total of 0.5 ml of 100 m*M* phosphate buffer (pH 7.0). Procedures for the recovery of fatty acids and conditions for radio gas–liquid chromatography are as described in the Experimental Section. In the upper chromatogram (methylmalonyl-CoA + acetyl-CoA) the top tracing is radioactivity and the bottom tracing is flame ionization detector response. The letters A and B above the mass peaks denote the two major fatty acids from the goose uropygial gland. $C_2 + n(C_3)$ and $C_3 + n(C_3)$ denote fatty acids formed from acetyl-CoA + *n*(methylmalonyl-CoA) and propionyl-CoA + *n*(methylmalonyl-CoA), respectively.

ating levels of substrate used in these experiments, equal amounts of fatty acids were synthesized in all these reaction mixtures showing no inhibition due to the presence of the unnatural isomer. These results suggest that both stereoisomers of methylmalonyl-CoA are utilized for the synthesis of multi-branched acids by the fatty acid synthetase of the uropygial gland. However, the possibility that a racemase such as that reported earlier (Mazumder et al., 1962; Allen et al., 1963) was present in the synthetase preparation cannot be ruled out. The apparent stereochemical purity of the products synthesized in vivo (Odham, 1967b) could result from the stereospecificity of the carboxylase enzyme. In any case it appears that the presence of the nonbiological stereoisomer did not adversely affect the synthesis of multi-branched fatty acids.

Conclusions

If the composition of the fatty acids in the gland were determined by the properties of the fatty acid synthetase, straight chain fatty acids should predominate as the V_{max} for straight chain synthesis is far greater than that for branched chain synthesis. However, multi-branched fatty acids actually predominate in this gland. Therefore, the inherent specificity of the synthetase does not explain the composition of the fatty acids generated in the gland. As described in the accompanying paper, the availability of the precursors, namely malonyl-CoA and methylmalonyl-CoA, most probably determines the fatty acid composition (Buckner and Kolattukudy, 1975). A gland specific and substrate specific malonyl-CoA decarboxylase renders malonyl-CoA unavailable to the synthetase without significantly affecting the availability of methylmalonyl-CoA. Therefore, if the results of these in vitro studies are relevant to the in vivo activities of the gland, acetyl-CoA should be the major primer and methylmalonyl-CoA the major elongating agent available in the gland. Under these conditions the major fatty acid formed by the synthetase should be 2,4,6,8-tetramethyldecanoic acid according to the results of our specificity studies on the isolated enzyme described in this paper. Our results as well as previous reports (Murray, 1962; Odham, 1963) show that 2,4,6,8-tetramethyldecanoic acid is, in fact, the major acid of the goose uropygial gland.

The difference in the relative rates of incorporation of malonyl-CoA and methylmalonyl-CoA could be either a result of substrate specificity of a single fatty acid synthetase complex or due to the presence of two enzyme complexes, one specific for malonyl-CoA and the other for methylmalonyl-CoA. Although all attempts to resolve the two activities have failed thus far and all results thus far obtained indicate that both activities might be due to the same enzyme complex, the possibility of the presence of a second complex specific for methylmalonyl-CoA cannot be ruled out at the present time.

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