Preliminary Communication

Evidence for Separate Pathways for the Biosynthesis of Saturated and Unsaturated Fatty Acids by an Avocado Enzyme System*

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There is growing evidence that in plant tissue the major pathway of oleic acid synthesis is not by dehydrogenation of stearic acid. Simmons and Quackenbush (1) have reported that in soybeans incubated *in vitro* with labeled sucrose, radioactivity appeared first in oleic acid, and later in saturated, linoleic, and linolenic acids, in that order. The fact that oleic acid retained the highest activity throughout the experiment led the authors to propose that it might be a precursor of the others. Also, Ballance and Crombie (2), working with mycelia of the fungus *Trichoderma veride*, found that acetate proceeds readily into palmitic and oleic acids whereas stearic acid is formed only in low concentrations. Moreover, specific activity measurements definitely eliminated stearate as a precursor of oleate. Similar results have been obtained by James[1] working with castor bean leaves. During time course studies with acetate-1-C^{14}, the specific activities of oleic and linoleic acids were high whereas that of stearic acid remained relatively low and palmitate remained unchanged.

Mudd and Stumpf (3) have shown recently that a particulate fraction, rich in mitochondria, obtained from avocado mesocarp readily synthesized saturated and unsaturated fatty acids from acetate. Barron, Squires, and Stumpf (4) have prepared a water-soluble system from these particles which synthesized only saturated fatty acids from acetyl coenzyme A and malonyl coenzyme A. Recently, it has been possible to obtain a soluble system from these particles which, depending on the initial substrate employed, synthesizes only saturated fatty acids or both unsaturated and saturated fatty acids.

The particles were isolated as previously described (4). They were then subjected to a gradient-density centrifugation, similar to that described by Kmetec and Newcomb (5), to remove contaminating microsomes. The pellet thus obtained was suspended in a small volume of a solution consisting of equal parts of 0.5 M sucrose and 0.2 M phosphate buffer, pH 7.15, and transferred to a dialysis bag. The dialysis bag was then placed in the cup of the transducer (maintained at 4°) of a Raytheon 10 kc. sonic oscillator, and the cup was flushed with H_2. The sample was treated at maximal output for 4 minutes in intervals of 30 seconds to minimize heating. The material was then centrifuged at 28,000 × g for 20 minutes at 4°, and the supernatant fluid was termed the "sonically solubilized enzyme." The enzyme preparation is very labile, rapidly losing activity when stored at 0° or − 15°.

The assay system was the same as previously employed (4) except that twice the amount of adenosine triphosphate was used. The extraction procedure for newly synthesized lipids, the counting techniques, and the procedure for quantitation have been described elsewhere (4).

When either acetate-1-C^{14} or acetyl-1-C^{14}-CoA was used as the substrate, both saturated and unsaturated fatty acids were synthesized. It was surprising to find, however, that when malonyl-1,3-C^{14}-CoA was used, only saturated fatty acids were synthesized (Table I). On reinvestigation of unsaturated fatty acid biosynthesis by intact particles, the same results were obtained, *i.e.* acetate but not malonate was incorporated into unsaturated fatty acids. Occasionally, there was a trace of radioactive unsaturated acids from labeled malonyl-CoA; this is probably related to the presence of malonyl-CoA decarboxylase, which is known to occur in these particles (4).

The fatty acids were identified by paper chromatography on siliconized paper with 80% acetic acid (6) or acetic acid-H_2O-formic acid-H_2O_2(7:1:1:1) (7). The predominant radioactive unsaturated fatty acid formed by the "sonically solubilized enzyme" or by the intact particles was oleic acid. However, when acetate-1-C^{14} was used as the substrate, an unidentified component, which moved close to the solvent front, was detected on the chromatograms developed with 80% acetic acid. Some characteristics of this unidentified component are as follows. It is not eluted from a silicic acid column with 10% ether in hexane, which removed normal fatty acids (8), but is slowly eluted with ether and rapidly eluted with 5% methanol in ether. This is typical of polar acids such as "hydroxy-acids." The unknown compound was isolated by silicic acid chromatography, and the methyl ester was subjected to gas-liquid chromatography on a diethylene glycol-succinate polyester column. Most of the radioactivity was eluted in the region where esters of normal saturated acids of 22 to 23 carbon atoms would be found. Known 12-hydroxystearic acid methyl ester behaved like the ester of a normal saturated acid of 23.7 carbon atoms. The unknown was not attacked by permanganate-periodate, thereby excluding an unsaturated or a vicinal dihydroxy fatty acid.

Moreover, it was found that the "sonically solubilized system" could convert oleic-1-C^{14} to a compound that behaves similarly to the acetate-derived unknown compound when chromatographed on paper or silicic acid. Adenosine triphosphate, CoA, and oxygen were required for this conversion. Since oleic acid can be synthesized from acetate, at least a portion of the unknown substance produced from acetate could be the same as the unknown compound derived from oleic acid. The complete identification of these unknown compounds is under investigation and the results will be reported in the future.

That the unsaturated fatty acid produced from acetate-1-C^{14}

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[1] A. T. James, personal communication.

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TABLE I

Comparison of acetate-1-C^{14} and malonyl-1,3-C^{14} in oleic acid synthesis

The reaction mixtures consisted of: acetate-1-C^{14} (1.5 × 10^6 d.p.m.), 200 mμmoles; or malonyl-1,3-C^{14}-CoA (151,600 d.p.m.), 205 mμmoles; ATP, 10 μmoles; CoA, 0.13 μmole; TPN, 0.13 μmole; glucose-6-P, 0.6 μmole; HCO_3^-, 30 μmoles; Mn^{++}, 2.8 μmoles; potassium phosphate buffer, pH 7.15, 50 μmoles; particle suspension, 0.5 ml; or sonically dispersed protein, 3 mg. Free CoA was not added when malonyl-CoA was used. The reaction volume in each case was 1.25 ml. The reactions were allowed to occur in 10-ml Erlenmeyer flasks at 37° for 1 hour in a shaking water bath.

Enzyme preparation	Substrate	Total incorporation	Distribution of radioactivity				
			Arachidic	Stearic	Palmitic	Oleic	"Hydroxy acid"*
		d.p.m.	%	%	%	%	%
Particles	Acetate-1-C^{14}	163,829	1	24	44	24	6
Particles	Malonyl-1,3-C^{14}-CoA	32,076		69	31	<1	0
Sonically solubilized enzyme	Acetate-1-C^{14}	392,000	4	59	14	9	13
Sonically dispersed enzyme	Malonyl-1,3-C^{14}-CoA	88,960	2	79	18	0	0

* Although the identification of this polar acid(s) is uncertain, it has tentatively been listed as "hydroxy-acid".

TABLE II

Effect of avidin on incorporation of acetate-1-C^{14} into fatty acids

The reaction mixtures were the same as in Table I, except that 2.5 mg of sonically solubilized protein was used. The avidin was obtained from Nutritional Biochemicals Corporation and contained 2.5 units per mg.

Avidin added	Total inhibition	Inhibition of oleic acid synthesis	Distribution of radioactivity*				
			Arachidic	Stearic	Palmitic	Oleic	"Hydroxy-acids"
μg	%	%	%	%	%	%	%
0	0	0	8	41	12	13	26
15	83	71	0	9	24	35	32
75	91.5	81	0	0	12	41	47

* The relative percentage of radioactivity of each fatty acid is reported for the product of each reaction mixture.

by the "sonically solubilized system" is oleic acid was confirmed by subjecting a sample of the biosynthesized fatty acids to permanganate-periodate oxidation (9). Seventeen per cent of the radioactivity of the sample to be oxidized, from which the "hydroxy-acids" had been removed by silicic acid chromatography, was in oleic acid as determined by paper chromatography. A portion of the oxidation products was chromatographed on Whatman No. 1 filter paper with propanol-NH_3 (80:20) as the solvent, and 6% of the radioactivity was found in an area that corresponded to azelaic acid. Another fraction of the product was chromatographed on siliconized paper with 70% acetic acid as the solvent. (Under these conditions, short chain monocarboxylic and dicarboxylic acids move together.) The short monocarboxylic acid-azelaic acid area of the chromatogram contained 14.5% of the radioactivity. Thus, the monocarboxylic acid, presumably pelargonic acid, contained 8.5% of the radioactivity.

The synthesis of oleic acid, as well as saturated fatty acids, from acetate by the "sonically solubilized enzyme" seems to involve biotin. As shown in Table II, the amounts of labeled unsaturated fatty acids relative to saturated fatty acids increase when increasing amounts of avidin are added to the reaction mixtures. It may also be noted that when larger amounts of avidin are used, there is synthesis of oleic acid without any apparent synthesis of stearic acid. It might have been anticipated that if oleic acid is derived from stearic acid and the synthesis of stearic acid is inhibited the relative amount of oleic acid synthesized would also decrease. The concomitant rise in the relative amount of "hydroxy acids" with that of oleic acid is a further indication that these substances may be metabolically related. The total amount of acetate incorporated into unsaturated fatty acids is decreased by avidin.

It has not been possible to show the conversion of stearate-1-C^{14}, palmitate-1-C^{14}, 10-hydroxystearate-1-C^{14}, stearoyl-1-C^{14}-CoA, and palmitoyl-1-C^{14}-CoA to unsaturated fatty acids. These results, plus the fact that stearic acid is synthesized when malonyl-CoA is the substrate without a resulting synthesis of oleic acid, suggest that stearic acid is not a precursor of the oleic acid synthesized by the solubilized enzyme system. Furthermore, it has not been possible to obtain chain elongation of octanoate-1-C^{14}, palmitate-1-C^{14}, or palmitoyl-1-C^{14}-CoA in the presence of unlabeled acetate or acetyl-CoA by the soluble system or by intact particles.

The inability to obtain unsaturated fatty acid synthesis from malonyl-CoA, the difference in the effects of avidin on the relative amounts of saturated and unsaturated acids synthesized, and the ineffectiveness of stearoyl-CoA as a precursor of oleic acid indicate that, in higher plants, the synthesis of oleic acid *de novo* can occur by a different pathway from that of saturated fatty acids.

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