ON THE BIOSYNTHESIS OF DIENOIC FATTY ACID BY ANIMAL TISSUES*

P. W. Holloway, Raul Peluffo and Salih J. Wakil

Department of Biochemistry
Duke University Medical Center
Durham, North Carolina

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Recent evidence (1,2) shows that C^{14}-acetyl CoA can be incorporated into a variety of saturated and unsaturated fatty acids by enzymes associated with subcellular particles of animal tissues. Several mechanisms appeared to be involved in this conversion. Saturated fatty acids (C_{16}, C_{18}) were synthesized via a pathway similar to the non-particulate de novo system with malonyl CoA as an intermediate. Acetyl CoA could elongate fatty acyl CoA derivatives by one or more carbon units and finally acetyl CoA could be incorporated into unsaturated fatty acids. Examining the latter process we found in conformity with the results of others (3-6) that the desaturating system of animal tissues is located in the microsomal fraction of the cells. Thus, in the presence of microsomes, reduced pyridine nucleotides and oxygen, palmityl CoA or stearyl CoA were desaturated to palmitoleyl CoA or oleyl CoA respectively, as shown in Table I. The cofactors required for this system appeared to be similar to the yeast system of Bloomfield and Bloch (7). The desaturation system appears to be non-specific with respect to the reduced pyridine nucleotides as reflected by the equal rates of desaturation of the acyl CoAs in the presence of either TPNH or DPNH (cf. Table I). The desaturating enzyme(s) appears to be a

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TABLE I

Cofactor Requirements for the Desaturation of Fatty Acids

Substrate	System	Corresponding $\Delta^{9,10}$ monounsaturated fatty acids
		mμmoles
Palmityl CoA	Complete	1.8
	no TPNH	0.1
	no TPNH + DPNH	1.8
	no Oxygen	0.2
Stearyl CoA	Complete	2.0
	no TPNH	0.1
	no TPNH + DPNH	2.0
	no Oxygen	0.3
	no Stearyl CoA + 20 mμmoles Stearic Acid	0.2
	Complete	2.0
	+ 0.1 μmoles p-hydroxy-mercury benzoate	0.5
	+ .10 μmole PHMB + 0.1 μmoles mercaptoethanol	1.8

The complete system contained 10 mμmoles 1-C^{14} acyl CoA (100,000 cpm), 50 mμmoles TPNH or DPNH, 30 μmoles potassium phosphate and water to a final volume of 0.5 ml. The reaction was started with the addition of 0.5 mg of rat liver microsomes and the flask was incubated for 10 minutes at 37° with constant shaking.

thiol-enzyme as evidenced by the ability of p-hydroxymercurybenzoate (PHMB) at concentrations of 10^{-4} M to completely inhibit the reaction and that the addition of a thiol compound such as mercaptoethanol to the inhibited system restored full activity. It is too early to state whether the thiol group of the enzyme is involved in the formation of an acyl-S-enzyme prior to the desaturation of the fatty acid or it is generally required for the maintenance of the proper conformation of the molecule.

Further desaturation of oleyl CoA to form $C_{18:2}$ by the microsomal fraction of rat liver cells is shown in Table II. The desaturation of oleyl CoA to the corresponding dienoic acid required the same cofactors as that for the desaturation of acyl CoA, namely oxygen and reduced pyridine nucleo-

tide. This observation indicated that the same general type of reactions are involved in the formation of mono- and polyunsaturated fatty acids.

TABLE II

Cofactor Requirements for the Desaturation of Oleyl CoA

System	$C_{18:2}$ Fatty Acids (mμmoles)
Complete	0.5
no TPNH	0.0
no TPNH + DPNH	0.5
no Oxygen	0.2

The complete system contained the same components as in Table I except 10 mμmoles oleyl CoA (100,000 cpm) was added.

Whether the same enzyme system is involved in both reactions remains to be determined. The desaturation of oleyl CoA by rat liver microsomes appears to occur equally well with either TPNH or DPNH at comparable concentrations (cf. Table II). Whether this response reflects the non-specific nature of the system as far as the reduced pyridine nucleotides are concerned, or, on the presence of transhydrogenase activity in the microsome preparations remains to be determined.

The products of desaturation of C^{14}-palmityl CoA, C^{14}-stearyl CoA or C^{14}-oleyl CoA were isolated from the reaction mixture by the usual extraction techniques and were separated as their methyl esters from the other fatty acid esters by thin layer chromatography using silica gel H impregnated with $AgNO_3$ (8). The monoenoic and dienoic esters of the fatty acids were extracted separately from the silica and were further resolved by gas-liquid chromatography. The effluent gas was passed through glass cartridges filled with glass wool to collect the C^{14}-labeled esters and the radioactivity content of the cartridges was measured in a scintillation counter. The results show that palmityl CoA and stearyl CoA were desa-

turated to $C_{18:1}$ and $C_{18:1}$ respectively. When C^{14}-oleyl CoA was the substrate and the product was isolated as described above and resolved by gas-liquid chromatography, a C^{14}-labeled peak was obtained in the general position of $C_{18:2}$ (linoleate in this case) although it had a distinct shoulder with which the radioactivity was associated (cf. Fig. 1).

Catalytic hydrogenation (9) of the isolated products of desaturation ($C_{18:1}$, $C_{18:1}$ and $C_{18:2}$) yielded the corresponding saturated acids which contained all of the radioactivity. Decarboxylation of the products via the Schmidt reaction released over 80 per cent of the radioactivity as CO_2, indicating the directness of the desaturation process with each substrate.

Finally, oxidation of the unsaturated products by periodate permanganate yielded C^{14}-labeled azelaic acid when 1-C^{14}-palmityl CoA or 1-C^{14} stearyl CoA were used indicated the formation of the Δ^9 unsaturated homologs, whereas the product of desaturation of the 1-C^{14} oleyl CoA yielded

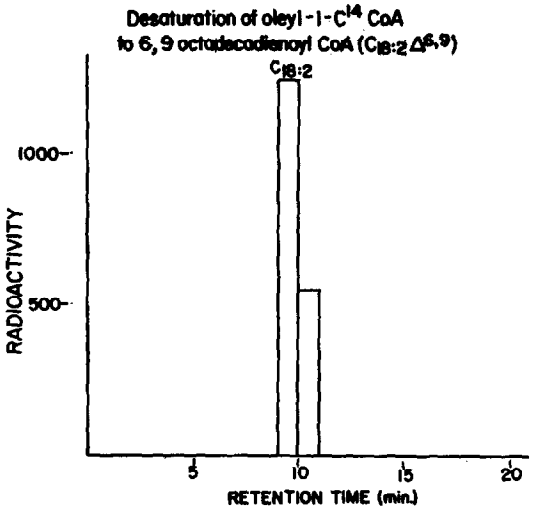

Fig. 1- Gas chromatographic analysis of the methyl ester of the dienoic acids after separation on thin layer chromatography. The bars represent the radioactivity (in cpm) of the effluent gas collected and counted during the time interval covered by the individual bars.

C^{14}-adipic acid, indicating that the product of desaturation of oleyl CoA was the $\Delta^{6,9}$ octadecadienoic acid. The overall desaturation reactions

of $C_{18:0}$ to $C_{18:2}$ in animal tissues can thus be written as follows:

$$CH_3(CH_2)_7CH_2CH_2(CH_2)_7COSCoA \xrightarrow{O_2,\ TPNH\ or\ DPNH} CH_3(CH_2)_7CH{=}CH(CH_2)_7COSCoA$$

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7COSCoA \xrightarrow{O_2,\ TPNH\ or\ DPNH} CH_3(CH_2)_7CH{=}CH{-}CH_2{-}CH{=}CH{-}(CH_2)_4{-}COSCoA$$

The fact that no radioactivity was found in the C_9 dicarboxylic acid after the periodate-permanganate oxidation of the $C_{18:2}$ acid indicated that no linoleic acid ($C_{18:2}$ $\Delta^{9,12}$) was formed from oleic acid. This *in vitro* observation is compatible with the overwhelming evidence obtained from *in vivo* studies that mammals do not synthesize linoleic acid possibly because of lack of the enzyme or enzymes necessary for the introduction of the double bond in the 12-13 position of the molecule.

REFERENCES

1. Harlan, W.R., Jr. and Wakil, S.J. Biochem. Biophys. Res. Comm. 8, 131 (1962).

2. Harlan, W.R., Jr. and Wakil, S.J. J. Biol. Chem. *in press*.

3. Bernhard, K., von Bulow-Koster, J. and Wagner, H. Helv. Chim. Acta 42, 152 (1959).

4. Marsh, J.B. and James, A.T. Biochim. Biophys. Acta 60, 320 (1962).

5. Stoffel, W. Biochem. Biophys. Res. Comm. 6, 270 (1961).

6. Nugteren, D.H. Biochim. Biophys. Acta 60, 656 (1962).

7. Bloomfield, D.K. and Bloch, K. J. Biol. Chem. 235, 337 (1960).

8. Morris, L.J. Chem. Ind. (London), 1238 (1962).

9. Farquhar, J.W., Insull, W., Jr., Rosen, P., Stoffel, W. and Ahrens, E.H., Jr. Nutrition Rev. 17, 8 (1959) (Suppl.).