BIOSYNTHESIS OF POLYENOIC FATTY ACIDS

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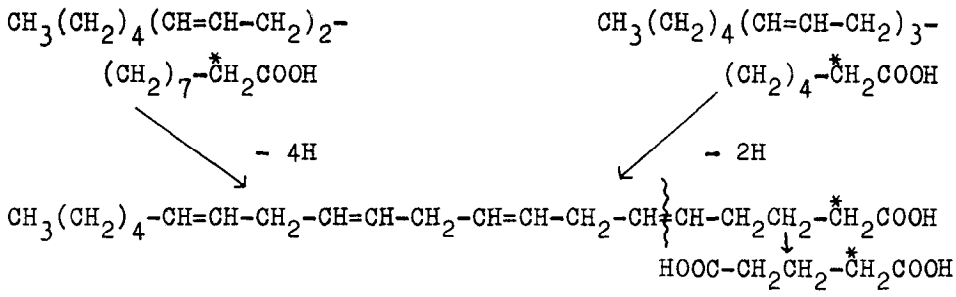
The highly unsaturated C_{20}- and C_{22} fatty acids are synthesized in the mammalian organism from exogenously supplied linoleic- ($\Delta^{9,12}$-octadecadienoic acid) and linolenic acid ($\Delta^{9,12,15}$-octadecatrienoic acid by chain elongation at the carboxylic acid group and by introduction of double bonds in the divinylmethane rhythm directed toward the carboxylic acid group.

Evidence for these mechanisms have been obtained from feeding experiments with non-labelled essential fatty acids (1), incubation experiments with C^{14}-acetate (2,3), in vivo experiments with 1-C^{14}-linoleic and 1-C^{14}-linolenic acid (4) and with different intermediates (5). The reaction mechanism of the chain extension and of the introduction of skipped double bonds however is still unknown.

In order to study the mechanism of the dehydrogenation in the biosynthesis of arachidonic acid ($\Delta^{5,8,11,14}$-eicosatetraenoic acid) in experiments in vitro 2-C^{14}-cis,cis-$\Delta^{11,14}$-eicosadienoic acid (spec. activity 0,3μC/μMol) and 2-C^{14}-all-cis-$\Delta^{8,11,14}$-eicosatrienoic acid (spec. activity 0,35μC/μMol) have been synthesized and the corresponding Coenzyme A derivatives prepared (R_F of the two CoA-acyl compounds 0,67 in the system pyridine-isopropanol-water 1:1:2 and 0,58 in the system 60% ethanol) (6).

If two double bonds arranged in the divinylmethane rhythm are introduced into the dienoic and one double bond into the trienoic acid one would expect radioactive $\Delta^{5,8,11,14}$-eicosatetraenoic acid, which on oxidative cleavage(7) would yield radioactive glutaric acid. The radioactivity of the glutaric acid would give information about the degree of the dehydrogenation.



Firstly the intracellular location of the polyenoic acid dehydrogenating enzyme was investigated. Table I summarizes the results of incubations with different cell fractions (8) from rat liver.

Table I

cell fraction	activity (c/min.) in dicarboxylic acids		
	$C_{11} - C_8$	C_5	C_3
mitochondria			
aerobic	26 800	395	40
anaerobic	25 100	180	25
microsomes			
aerobic	27 400	3 620	25
anaerobic	28 300	750	22
supernatant	28 400	650	15

Each incubation mixture contains: 15 mg protein, 100 μ M K-phosphate buffer pH 7,4; 0,01 μ Mol 2-C^{14}- $\Delta^{11,14}$-eicosadienoyl-CoA (33 000 c/min.); 10 μ M K-thioglycolate; total volume 1,5 ml; 60 min. 30°.

Additional important cofactors of the dehydrogenation are given in table II.

Since the column chromatography (7) does not permit the separation of C_{11}- and C_8-dicarboxylic acids, part of the dicarboxylic acid mixture has been separated by paperchromatography (5) and the radioactivity determined in the Tricarb-Liquid-Scintillation-Counter (9). 78% of the radioactivity were found in the C_{11}-dicarboxylic acid- 5% in the suberic acid- and 17% in the glutaric acid spot. Apparently the trienoic acid is rapidly transformed to the tetraenoic acid.

Table II

	activity (c/min.) of dicarboxylic acids		
	$C_{11} - C_8$	C_5	C_3
complete system	25 500	4 650	55
-CoA compound + NH_4-salt of 2-C^{14}-dienoic acid	29 300	1 445	0
-TPNH	27 400	1 620	22
-TPNH + DPNH	29 200	2 200	25
-TPNH + TPN	28 920	1 530	30
anaerobic	28 300	750	22

The complete system contains: 15 mg protein, 100 μMol K-phosphate buffer pH 7,4; 0,01 μMol 2-C^{14}-$\Delta^{11,14}$-eicosadienoyl-CoA (33 000 c/min.); TPNH 1,0 μMol; 10 μMol K-thioglycolate; total volume 1,5 ml; 60 min. 30°.

Under identical conditions 2-C^{14}-all-cis-$\Delta^{8,11,14}$-eicosatrienoyl-CoA is dehydrogenated by the microsomal enzymein about 22% yield to arachidonic acid as determindedby the radioactivity of the glutaric acid.

In summary: the polyenoic acid dehydrogenation enzyme is located in the microsomal fraction. The CoA-derivatives of the acids tested are the substrates for the enzyme. The dehydrogenation proceeds only under aerobic conditions and in the presence of reduced pyridine nucleotides. Whether TPNH or DPNH is the specific one can not yet be decided with certainty. The dehydrogenation proceeds only toward the carboxylic acid group and not toward the methylend of the acids. This could be demonstrated unambiguously by use of tritium labelled linolyl- ($T_2CH-CT_2-(CH_2)_3(CH=CH-CH_2)_2(CH_2)_6COSCoA$) and linolenyl-CoA ($T_2CH-CT_2-(CH_2)_3(CH=CH-CH_2)_3-CH_2)_3COSCoA$)(6). These results do not support a hypothetic scheme of the biosynthesis of polyunsaturated fatty acids presented previously (10).

The dehydrogenating enzyme or enzyme system of the microsomes is similar in its need of essential Co-factors to the one Bloch and coll.(11) obtained from yeast. This system was capable of dehydrogenating palmitoyl-CoA and stearyl-CoA to the corresponding Δ^9-monoenoic acids and furthermore oleic to linoleic acid.

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