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The Metabolism of Polyenoic Fatty Acids

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Abstract

Feeding experiments with C^{14}-labeled and unlabeled unsaturated fatty acids have been used to study the possible routes of formation of the C_{20}- and C_{22}-polyenoic fatty acids of rat liver phosphatides. The acids of the palmitoleate, oleate, linoleate, and linolenate types (considered on the basis of the position of the double bond closest to the methyl end) are apparently formed from the C_{16} and C_{18} unsaturated acids of the corresponding types. The results rule out possible transformations of the C_{20}- and C_{22}-polyenoic acids from one type to another, and demonstrate the exclusive introduction of new double bonds toward the carboxyl group. Isomers of linoleate or linolenate in which the double bonds were shifted by one carbon atom toward the carboxyl or methyl groups were incorporated into the phosphatides only to a negligible extent in the form of polyenoic acids.

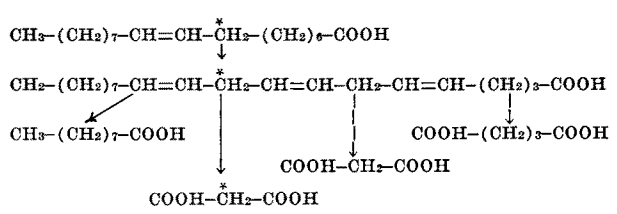
FIG. 1

$$\Delta^{9,12}\text{–}C_{18:2} \longrightarrow \Delta^{5,8,11,14}\text{–}_{20:4}$$

$$\Delta^{9,12,15}\text{–}C_{18:3} \longrightarrow \Delta^{5,8,11,14,17}\text{–}C_{20:5} \longrightarrow \Delta^{4,7,10,13,16,19}\text{–}C_{22:6}$$

$$\Delta^{9}\text{–}C_{18:1} \longrightarrow \Delta^{5,8,11}\text{–}C_{20:3}$$

FIG. 2



MOST OF THE C_{20}- and C_{22}-polyenoic fatty acids occurring in the phosphatides of the higher animals have their first double bond (counted from the terminal methyl group) in the 6 or 3 position. Linoleic acid, the C_{18}-diene, has its first double bond in the 6 position and linolenic acid, the C_{18}-triene, has its first double bond in the 3 position. Thus the larger polyenoic acids may be considered as being either linoleic or linoleic acid types (Fig. 1). The latter acids have been shown to be precursors of the longer acids, undergoing chain-elongation and introduction of new double bonds (1). The new double bonds are directed toward the carboxyl group and maintain the divinyl-methane rhythm. The enzyme system carrying out these reactions is found in the liver microsomal fraction, as shown by Stoffel (2).

Besides the long polyenoic acids of the above two types, we have found in phosphatides a smaller amt of $20:3^{5,8,11}$, with a structure of the oleic acid type, the first double bond being in the 9 position (3,4). It has been known for some time that a C_{20}-trienoic acid accumulates in liver lipids of rats on a fat-free diet. Mead and colleagues (5) have identified this acid as being $20:3^{5,8,11}$ and have given evidence for its synthesis from acetate (6). The reasonable assumption has been made that stearic and oleic acids are intermediates in the biosynthesis. We have given direct evidence for the transformation of oleic acid into this triene by feeding 8–C^{14}-oleate to fat-deficient rats (7). The labeled fatty acid was made by total synthesis. Both the C_{20}-triene and arachidonic acid were isolated from liver. The trienoic acid had a specific activity of 8730 dpm/mg and 96% of its radioactivity was located in the malonic acid obtained by oxidative ozonolysis. The nature of the reactions is illustrated in greater detail in Figure 2.

On the other hand, the C_{20}-tetraenoic acid of the linoleic acid type, arachidonic acid, had a specific activity of only 115 dpm/mg. Thus only the $20:3^{5,8,11}$

FIG. 3

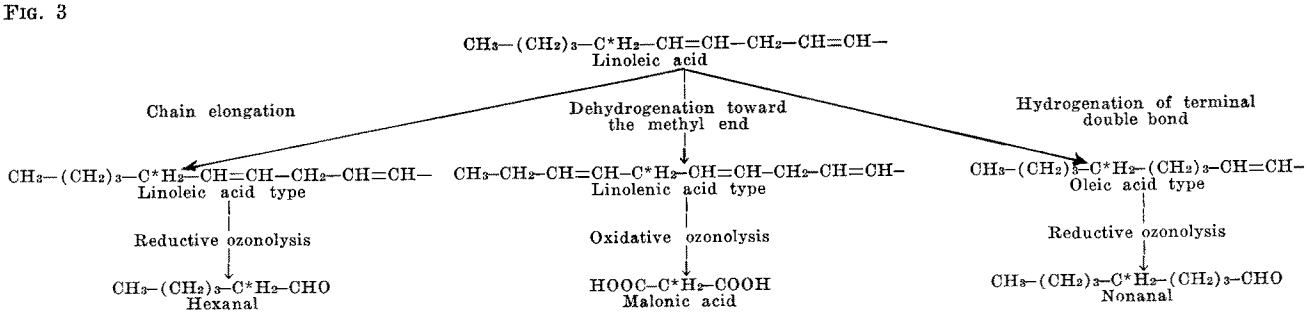


TABLE I

Polyunsaturated Fatty Acid Fraction (PFA) from Rat Liver after Feeding of (14-^{14}C) all-cis $\Delta^{9,12}$-$C_{18:2}$[a] · $C_{16:1}$ 20%; Spec. Activity 8420 dpm/mg. $C_{16:1}$ 14%; $C_{18:2}$ 10%; $C_{20:3}$ 24%; $C_{20:4}$ 28%; $C_{22:4}$ and 6 4%.

	Degradation by oxydative ozonolysis		Degradation by reductive ozonolysis		
	Yield in mole/mole PFA	Spec. activity dpm/mg	DNPH	Yield in mole/mole PFA	Spec. activity dpm/mg
Malonic acid	0.85	4	Nonanal	0.17	11[b]
			Heptanal	0.14	8[b]
			Hexanal	0.34	20 400
			Propanal	0.05	9[b]

[a] Fat-free diet + supplement of inactive linoleic acid.
[b] After repeated purification.

has its direct origin in oleic acid. Here again the positions of the newly introduced double bonds are determined by the position of the double bond nearest the terminal methyl group. Apparently the same enzyme system mentioned before (2) is active in introducing new double bonds and carbon atoms.

In further work we have investigated the possibility that dehydrogenation can take place towards the methyl group, that is, that the C_{20}-trienoic acid of the oleic acid type can be transformed into the C_{20}-tetraenoic acid of the linoleic acid type (arachidonic acid). This possibility is suggested by the observation that arachidonic acid is rigidly held by liver phosphatides even when linoleic acid is not supplied for its biosynthesis. Against this possibility is the result reported above with labeled oleate, for the ratio of specific activities in the two C_{20} acids was about 76:1 (triene:tetraene).

This point has been studied further by feeding experiments with linoleic and linolenic acids labeled with C^{14} in positions 14 and 17, respectively. These acids too were obtained by total synthesis. The principle of our proof is demonstrated with linoleate as an example (Fig. 3), in which two theoretically possible conversions—into polyenoic acids of the linolenate type or oleate type—are shown. If our labeled linoleate were to be simply elongated and transformed into acids of the linoleate type, the only radioactive aldehyde obtainable by reductive ozonolysis of the liver polyenoic acid mixture would be hexanal. If it were elongated and dehydrogenated to form acids of the linolenate type, oxidative ozonolysis would yield labeled malonate. Transformation into the oleate type would result in formation of radioactive nonanal on reductive ozonolysis. Table I shows that malonate and nonanal are both almost entirely devoid of radioactivity, so it may be concluded that fatty acids of the linoleic acid type are transformed into neither the linolenate nor oleate types. Half of the

TABLE II

Polyunsaturated Fatty Acid Fraction (PFA) from Rat Liver after Feeding of (17-^{14}C)-all cis $\Delta^{9,12,15}$-$C_{18:3}$[a] Fat-Free Diet + Supplement of Inactive Linolenic Acid; b) Normal Diet.

Composition of the PFA-fractions											
	$C_{14:1}$	$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:3}$	$C_{20:4}$	$C_{20:5}$	$C_{22:5}$	$C_{22:6}$	Not indent.
a)	1	4	7	5	3	16	11	17	2	33	1
b)		1	2	28	1	1	45	1	2	16	2

Degradation by reductive ozonolysis				
	Yield in mole/mole PFA		Spec. activity dpm/mg	
DNPH	a	b	a	b
Nonanal	0.14	0.19	42	0[a]
Heptanal	0.06	0.13	29	14[a]
Hexanal	0.07	0.47	4	5[a]
Butanal	0.01	–	172	–
Propanal	0.44	0.19	8 670	12 300

[a] After repeated purification.

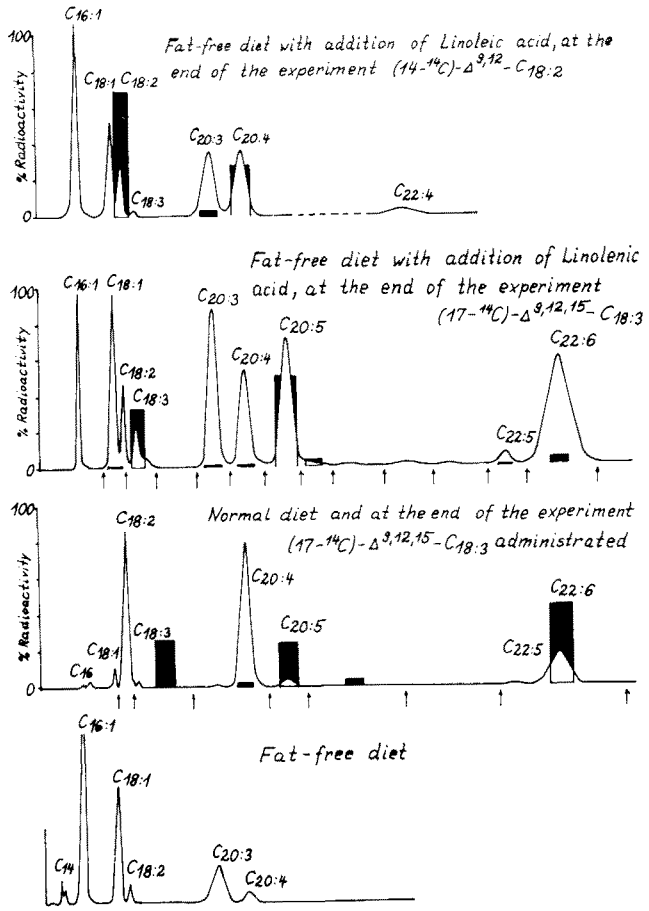


FIG. 4. Gas chromatograms and distribution of radioactivity in per cent of the PFA-ME of rat liver.

activity in the hexanal originated in the original linoleate present in the polyenoic acid fraction, and the rest from elongation products.

A similar result was obtained in a comparable experiment with 17-C^{14}-linolenic acid. Here the question was raised whether a transition into polyenoic acids of the linoleate or oleate types might occur by hydrogenation of the double bond nearest the methyl end. In this case, reductive ozonolysis would yield not only radioactive propanal (from linolenate type acids), but also labeled hexanal or nonanal. It is evident from Table II that both hexanal and nonanal have almost zero specific activity.

Thus it appears to me that an unambiguous proof is given for the biogenesis of the C_{20}- and C_{22}-polyunsaturated acids of the oleate, linoleate, and linolenate types from unsaturated C_{18} acids (possibly also from C_{16} acids) of the same type. Transition of one into the other type is excluded.

Further details of the transformations of the labeled linoleate and linolenate are shown in Figure 4. They confirm what is known from the earlier experiments of Mead and ourselves.

Labeled C_{16}-polyenoic acids of the linoleate and linolenate types behave in feeding experiments just like the corresponding C_{18} acids (8–10). Some of the

FIG. 5

All cis $\Delta^{7,10}$–$C_{16:2}$ ——— $\Delta^{5,8,11,14}$–$C_{20:4}$ (arachidonic acid)

All cis $\Delta^{7,10,13}$–$C_{16:3}$ ↘
All cis $\Delta^{4,7,10,13}$–$C_{16:4}$ ——— $\Delta^{5,8,11,14,17}$–$C_{20:5}$ ↓ $\Delta^{4,7,10,13,16,19}$–$_{22:6}$

All cis $\Delta^{6,9,12,15}$–$C_{16:4}$ No transformation into C_{20}- and C_{22}-polyenoic acids

TABLE III

Composition of the Polyunsaturated Fatty Acid Fraction of Liver Phosphatides of Rats, Fed a Fat-Free Diet

	Amt in %	Components of the components
$C_{16:1}$	13	Δ^{9}-
$C_{18:1}$	37	Δ^{9}- and Δ^{11}- (7:3)[a]
$C_{18:2}$	7	$\Delta^{8,11}$- and $\Delta^{9,12}$- (4:6)
$C_{20:1}$	Traces	Δ^{11}- and Δ^{13}- (7:3)
$C_{20:3}$	24	$\Delta^{5,8,11}$- and $\Delta^{7,10,13}$- (9:1)
$C_{20:4}$	13	$\Delta^{4,7,10,13}$- and $\Delta^{5,8,11,14}$-
$C_{22:4}$	3	Not investigated
$C_{22:6}$	3	

[a] The figures in brackets indicate the ratio of the two isomers.

TABLE IV

Feeding of All cis $\Delta^{8,11}$-$C_{18:2}$ to Rats on a Fat-Free Diet for a Period of 5 Weeks

Composition of liver PFA in %		Reductive ozonolysis of C_{20}-PFA from liver after application of (13-^{14}C)-all cis $\Delta^{8,11}$-$C_{18:2}$[a]		
		DNPH	Yield in mole/mole PFA	Spec. activity dpm/mg
$C_{16:1}$	12 (13)[b]	Nonanal	0,27 (0,32)[b]	20
$C_{18:1}$	42 (49)	Heptanal	0,15 (0,15)	220
$C_{18:2}$	6 (4)	Hexanal	0,30 (0,24)	0
$C_{20:3}$	26 (25)			
$C_{20:4}$	9 (7)			
Not identified	5 (2)			

[a] Spec. activities: $C_{18:2}$ 6900; C_{20}- and C_{22}-PFA 310 dpm/mg

[b] The figures in brackets are the corresponding comparative values for animals on a fat-free diet.

acids were obtained synthetically, some from algae and herring oil. Figure 5 summarizes the results.

Since a C_{20}-trienoic acid of the oleate type forms from oleate and accumulates in organ phosphatides in animals on a fat-free diet, we have sought to find (11) if by an analogous event polyenoic acids of the palmitoleate type are also formed. Such a conversion is to be expected particularly because rats on a fat-free diet contain appreciable amts of palmitoleate in the depot and liver fat (12). The results of this study are shown in the following table.

The mixtures of isomeric fatty acids listed in Table III were isolated in high purity, as proven by gas chromatography. The structures were determined by oxidative and reductive ozonolysis. In each isolated mixture, one of the two components had the structure of an acid of the palmitoleate type.

Palmitoleic acid, $16:1^{9}$, could be elongated and dehydrogenated to form $18:2^{8,11}$, a diene of the palmitoleate type. This is a positional isomer of linoleic acid in which the double bonds are shifted by one carbon atom toward the carboxyl group. Feeding such an acid might increase the body content of the C_{20}- and C_{22}-polyenoic acids of the palmitoleate type, particularly $20:4^{4,7,10,13}$, the isomer of arachidonic acid.

To test this possibility, we synthesized cis,cis-$18:2^{8,11}$ containing C^{14} in the 13 position and administered it

TABLE V

Application of (16-^{14}C)-all cis$\Delta^{8,11,14}$-$C_{18:3}$ (I) and of (15-^{14}C-$\Delta^{10,13}$-$C_{18:2}$ (II) to Rats; Fat-Free Diet and Supplement of the Corresponding Inactive Acid

	Composition of liver PFA in %[a]		Specific activities in dpm/mg	
	I	II	I	II
$C_{16:1}$	18 (15)	12 (34)		
$C_{18:1}$	50 (59)	43 (22)		380
$C_{18:2}$	3 (2)	4 (6)	8 040	90 000
$C_{18:3}$	2 (–)			–
$C_{20:2}$	– –	0,2 (–)		42 000
$C_{20:3}$	11 (10)	20 (23)		340
$C_{20:4}$	11 (7)	11 (8)	4 680	250
$C_{20:5}$	1 (–)	– –		–
$C_{22:4\ a.6}$	3 (3)	3 (2)	2 110	290

[a] The figures in brackets are the corresponding comparative values for animals on a fat-free diet.

TABLE VI

Reductive Ozonolysis of C_{20}-PFA from Liver after Application of (16-^{14}C)-all cis $\Delta^{8,11,14}$-$C_{18:3}$ (I) and of (15-^{14}C)-all cis $\Delta^{10,13}$-$C_{18:2}$ (II); Fat-Free Diet + Supplement of the corresponding Inactive Acids

DNPH	Yield in mole/mole PFA				Specific activities in dpm/mg	
	I	II			I	II
	$C_{20:3-5}$	$C_{20:2}$	$C_{20:3}$	$C_{20:4}$	$C_{20:3-5}$	$C_{20:2}$
Nonanal[a]	0,39	0,28[a]	0,63	0,03	170	300
Heptanal	0,08	0,14	0,02	0,13	520	300
Hexanal	0,31	0,06	0,03	0,47	200	1 000
Pentanal	–	0,15	–	–	–	158 600
Butanal	0,04	–	–	–	108 000	–
Propanal	0,01	–	–	–	2 400	–

[a] Contains some decanal and octanal.

to rats held on a fat-free diet. The results are summarized in Table IV. It can be seen that the content of C_{20}-tetraenoic acid was not raised by the feeding of the diene, nor was the proportion of the $20:4^{4,7,10,13}$. Moreover, the heptanal obtained from this fraction by reductive ozonolysis had only a low specific activity. Despite the lack of arachidonate and its precursor from the diet, the liver arachidonate could not be displaced by the isomer of the palmitoleate type.

Having obtained these curious results, we then investigated the behavior of two other C_{18}-polyenoic acids the all-cis-$18:3^{8,11,14}$ (an isomer of linolenate) and the all-cis-$18:2^{10,13}$ (an isomer of linoleate). In the former acid, the double bonds are shifted by one atom toward the carboxyl end; in the latter, toward the methyl end. Table V shows that feeding these acids had no significant effect on the composition of the liver polyenoic acids. The acids are almost entirely of the oleate and linoleate types (Table VI). The same acids, labeled in position 16 or 15, respectively, were administered on the last day of the feeding experiments. The results are shown in Table VI. It can be seen that the C_{20}-polyenoic acids contained some isomers of very high specific activities, but only in very small amts. In the case of the rats fed labeled C_{18}-triene, there was slight conversion to $20:3^{10,13,16}$ and $20:5^{4,7,10,13,16}$, both of which yielded highly labeled butanal. In the case of the rats fed labeled C_{18}-diene, activity was found only in the C_{20}-dienoic acid fraction, yielding pentanal of high specific activity on reductive ozonolysis.

Thus we may conclude that only the C_{20}- and C_{22}-polyenoic acids of the oleate, linoleate, and linolenate types are built in higher amts into organ phosphatides. It cannot yet be decided whether this is due to the specificity of the chain elongating and dehydrogenating enzyme system, or to a particular selective principle during the formation of the lipoidal cell structures. Possibly both factors are operating. There may also be a relation to the still unknown function of these polyenoic acids as essential fatty acids, which depends on a particular chemical constitution.

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