BBA 55413

ALTERATIONS IN THE MOLECULAR SPECIES OF RAT LIVER LECITHIN BY CORN-OIL FEEDING TO ESSENTIAL FATTY ACID-DEFICIENT RATS AS A FUNCTION OF TIME

L. M. G. VAN GOLDE, W. A. PIETERSON AND L. L. M. VAN DEENEN
Department of Biochemistry, Laboratory of Organic Chemistry, The State University, Utrecht (The Netherlands)

(Received September 5th, 1967)

SUMMARY

The present paper describes, as a function of time, the qualitative and quantitative alterations in the molecular species pattern of rat liver lecithin which are observed when corn oil is fed to essential fatty acid-deficient rats. One of the most important changes observed was a very rapid replacement of (1-palmitoyl-2-eicosatrienoyl)- and (1-stearoyl-2-eicosatrienoyl)-lecithin by (1-palmitoyl-2-arachidonoyl)- and (1-stearoyl-2-arachidonoyl)-lecithin, respectively. Whereas this event occurred during a period of 9 days of corn oil feeding, the increase of (1-palmitoyl-2-linoleoyl)- and (1-stearoyl-2-linoleoyl)-lecithin had already reached the maximum level after 3 days. On the other hand a very rapid disappearance of the species (di-octadecenoyl)- and (1-hexadecenoyl-2-octadecenoyl)-lecithin was observed during this period. The decrease of the species (1-stearoyl-2-octadecenoyl)-lecithin was rather slow, whereas (1-palmitoyl-2-octadecenoyl)-lecithin started to diminish only 9 days after the change in diet. The results presented here suggest that different metabolic pathways may contribute, to a different extent, to the replacement of the individual molecular species of rat liver lecithin induced by a change of diet.

INTRODUCTION

In a previous paper we reported diet-induced differences in the composition of rat liver lecithins in terms of molecular species[1]. The results suggested that there is a tendency to preserve some of the physical properties of the phospholipids, although in rats grown on a diet depleted of essential fatty acids a number of membrane properties appear to be significantly affected. It is well documented that, *e.g.*, liver mitochondria of essential fatty acid-deficient rats exhibit an increased swelling compared with those from normal animals[2] (B. M. WAITE, unpublished results). It is not established whether this phenomenon is to be attributed to an inadequacy of the adapted molecular composition of membrane phospholipids or whether such an aberration is related to another function(s) of these fatty acids. Supplementation of essen-

tial fatty acids to deficient animals was found to induce rapid restoration of the swelling properties of mitochondria (B. M. WAITE, unpublished results). BRENNER AND NERVI[3] studied the kinetics of linoleic and arachidonic acid incorporation and eicosatrienoic acid depletion in the phospholipids of liver of fat-deficient rats after feeding of methyl linoleate and arachidonate. The present paper deals with the quantitative alterations occurring in lecithin molecules of liver when corn oil is fed to essential fatty acid-deficient rats.

EXPERIMENTAL

Dietary experiments

The essential fatty acid-deficient rats and diets were kindly donated by Dr. H. J. THOMASSON (Unilever Research Laboratory, Vlaardingen). 3 weeks after birth a group of 18 male white rats received, for a period of 3 months, a diet deficient in essential fatty acids, consisting of carbohydrates (72 cal %), proteins (23 cal %), hydrogenated coconut oil (5 cal %) and adequate amounts of salts and vitamins. After this time the essential fatty acid-deficient diet was replaced by a diet containing carbohydrates (50 cal %), proteins (16.8 cal %) and corn oil (33.3 cal %) as a source of the essential fatty acids, while the appropriate amounts of salts and vitamins were added to this diet. 60% of the overall fatty acids of corn oil turned out to be linoleic acid. Just before and 1, 3, 6, 9 and 15 days, respectively, after the change in diet, 3 rats were sacrificed. The livers were isolated quickly and, after pooling and rinsing thoroughly with distilled water, extracted immediately following the procedure of REED[4]. The lipids obtained were dissolved in pure chloroform and stored under N_2 atmosphere at $-20°$.

Isolation of phosphatidylcholine

Phosphatidylcholine was isolated from the total lipid mixture by preparative thin-layer chromatography on silica G (Merck-Darmstadt). The plates were run in chloroform–methanol–conc. ammonia–water (70:30:2:3, by vol.). After development the plates were sprayed with a 0.01% solution of Rhodamine 6G in water and phosphatidylcholine (lecithin) was removed from the plate after visualising under ultraviolet light. The lecithin was extracted quantitatively from the silica by exhaustive elution with methanol–chloroform (9:1, v/v). All manipulations were carried out in a CO_2 or N_2 atmosphere. The purity of the isolated lecithins was checked by thin-layer and paper chromatography using synthetic lecithins as reference substances. Alkaline hydrolysis of the lecithins revealed the presence of glycerophosphoryl choline only. The enzymatic breakdown of the lecithin with phospholipase A (EC 3.1.1.4) from *Crotalus adamanteus* venom was carried out as decribed in detail previously[1,5,6].

Argentation method for fractionation of diglycerides derived from lecithin

After hydrolysis of the phosphatidylcholine with phospholipase C (EC 3.1.4.3) from *Bacillus cereus*, which was performed as described before[1,5,6], the resulting 1,2-diglycerides were fractionated on thin-layer plates impregnated with silver nitrate using chloroform–abs. ethanol (94:6, v/v) as a developer. After detection by Rhodamine 6G, the fractionated diglycerides were scraped from the plates and eluted quantitatively from the silica with a mixture of chloroform and methanol (9:1, v/v). The

relative amounts of these individual fractions were determined by adding heptadecanoic acid, which was not present in detectable amounts in our lecithin preparations, to each fraction as an internal standard. The hydrolysis of the fractionated diglycerides with pancreatic lipase (EC 3.1.1.3) (Calbiochem, U.S.A.) was performed as described in detail earlier[1,5,6].

Gas–liquid chromatography

The fatty acid constituents of the lipid samples were converted into the corresponding methyl esters by dissolving the sample in 18 ml of methanol–HCl (26 g of HCl per l) and subsequent transesterification during a period of 2 h in closed tubes under nitrogen pressure. The methyl esters were determined qualitatively and quantitatively by means of an F & M gas chromatograph using a flame ionisation detection system. The stationary phase of the column was 3% EGSS-X on chromosorb Q (Applied Science, U.S.A.) and the column was operating at 185°.

RESULTS

The effect of supplying a corn oil diet which contains a high amount of linoleic acid to essential fatty acid-deficient rats on the overall fatty acid composition of liver lecithin is demonstrated in Table I. The first column represents the fatty acids of lecithin from the livers of the essential fatty acid-deficient animals. This lecithin is characterized by a low level of linoleic acid and arachidonic acid, whereas high amounts

TABLE I

OVERALL FATTY ACID COMPOSITION OF LIVER LECITHIN FROM ESSENTIAL FATTY ACID-DEFICIENT RATS AFTER DIFFERENT PERIODS OF CORN OIL SUPPLY

Fatty acids	*Essential fatty acid-deficient*	*Period on corn oil*				
		24 h	*72 h*	*144 h*	*216 h*	*360 h*
14:0	0.2	+	+	+	+	+
16:0	19.4	19.5	19.3	20.3	18.6	17.1
16:1	3.5	2.0	1.8	0.7	0.4	+
18:0	23.5	25.0	24.8	25.3	25.4	26.0
18:1	21.3	16.2	12.7	12.2	11.2	7.4
18:2	1.7	6.9	14.4	14.2	14.6	15.8
20:3	23.7	19.2	9.9	2.0	+	−
20:4	6.9	11.2	17.1	25.1	29.6	33.7

of octadecenoic acid and eicosatrienoic acid are present, in agreement with the findings of several authors[3,7–11]. In the second column is given the fatty acid pattern of lecithin 24 h after the addition of corn oil to the diet. As can be seen the presence of linoleic acid in the corn oil is already reflected after 24 h in an increased level of this acid and of arachidonic acid, which is formed from the diet-supplied linoleic acid[12,13]. This increase of linoleic acid and arachidonic acid continues with prolonged feeding of corn oil. After 3 days' feeding of corn oil, the content of linoleic acid reached a maximum value, which was not found to be changed significantly by a further supply of corn oil. The level of arachidonic acid, however, continued to increase after 3 days' feeding of corn oil until, at about 12–15 days, a steady state was reached[1]. On the other hand, a decrease was noted in the content of eicosatrienoic acid and octade-

cenoic acid. The level of the latter fatty acid is lowered until, after 3 days of corn oil supply, it reaches a relatively constant level. This is similar to the observed increase of linoleic acid which stopped at about the same time. Eicosatrienoic acid, however, was found to decrease continuously at a very rapid rate: after 9 days nearly all of the eicosatrienoic acid had disappeared and after 15 days this fatty acid constituent was no longer detectable in rat liver lecithin. After hydrolysis with phospholipase A, which acts specifically on the fatty acid ester linkages at the 2-position of lecithin, it appeared that there was a positional interchangeability of the different polyunsaturated fatty acids at the various stages of this dietary experiment (Table II): both arachidonic and eicosatrienoic acid were located exclusively at the 2-position, in agreement with the findings of other investigators[3,11]. Linoleic acid appears to be linked, also almost exclusively, at the 2-position. Octadecenoic acid was found to be esterified at both ester positions, but after prolonged corn oil feeding a certain preference of this acid was noticed for the 1-position. As expected the 1-position was found to be occupied for the most part by the saturated fatty acids, palmitic acid and stearic acid. These differences in positional distribution have been studied in more detail by a determination of the individual molecular species of rat liver lecithin independence of the diet.

Differences in molecular species of rat liver lecithin by changing of diet

The molecular species of lecithin after different periods of corn oil supply have been determined by hydrolysis with phospholipase C from *B. cereus* and subsequent fractionation of the resulting 1,2-diglycerides by thin-layer chromatography on silica impregnated with silver nitrate. At present this approach gives a better basis for information about the molecular species pattern of lecithins than fractionation of the more polar lecithin molecules themselves on silver nitrate-impregnated silica. For appropriate fractionation of lecithins either a conversion into dimethyl phosphatidates[14] or a combination with reversed phase–chromatography appears to be necessary[15]. The fractionation of the apolar diglycerides on silver nitrate-impreg-

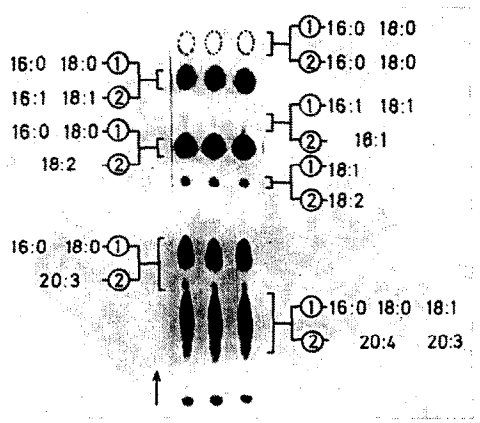


Fig. 1. Separation of 1,2-diglycerides derived from rat liver lecithin on silver nitrate-impregnated silica. The lecithins were isolated from the liver of essential fatty acid-deficient rats after feeding of corn oil for 24 h. The 1,2-diglycerides were obtained by degradation of the lecithins with phospholipase C. The plates were run in chloroform–abs. ethanol (94:6, v/v) and the spots of diglycerides were made visible by spraying the plates with a 20% aq. solution of $(NH_4)_2SO_4$ and subsequent heating in an oven for 2 h at 150°.

nated plates depends on: (1) differences in the total number of double bonds in the fatty acid chains, (2) a different distribution of the double bonds between the 1- and 2-ester positions and (3) a different location of the double bonds in the fatty acid chains[1,5,6] (Fig. 1). An excellent review on these argentation methods has recently been published by MORRIS[16]. Table III shows the fatty acid composition of the fractionated diglycerides derived from liver lecithin of essential fatty acid-deficient rats, and essential fatty acid-deficient rats after consumption of corn oil for 1, 3, 6, 9 and 15 days. These lecithins will be referred to in the following text as: lecithin-(deficient), lecithin-(1), lecithin-(3), lecithin-(6), lecithin-(9) and lecithin-(15), respectively. Fraction A represents the diglyceride spot with the lowest R_F value. It was found to

TABLE II

POSITIONAL DISTRIBUTION OF THE FATTY ACIDS OF LIVER LECITHIN FROM ESSENTIAL FATTY ACID-DEFICIENT RATS AFTER DIFFERENT PERIODS OF CORN OIL FEEDING

A complete phospholipase A degredation was carried out for all lecithins.

Fatty acid	*Essential fatty acid-deficient*		*Period on corn oil*			
			24 h		*72 h*	
	1-Position	*2-Position**	*1-Position*	*2-Position*	*1-Position*	*2-Position*
14:0	+	+	+	−	−	−
16:0	34.5	4.3	35.5	3.5	37.9	0.7
16:1	2.5	4.5	0.8	3.2	1.0	2.6
18:0	44.3	2.7	47.8	2.2	49.6	+
18:1	18.7	23.9	14.2	18.2	11.3	14.1
18:2	−	3.4	1.8	12.0	−	28.8
20:3	−	47.4	−	38.4	−	19.8
20:4	−	13.8	−	22.4	−	34.2
	144 h		*216 h*		*360 h*	
	1-Position	*2-Position*	*1-Position*	*2-Position*	*1-Position*	*2-Position*
14:0	+	−	−	−	−	−
16:0	38.6	2.0	36.1	1.1	34.0	+
16:1	0.4	1.0	0.4	0.4	+	+
18:0	50.5	0.1	50.8	+	52.3	−
18:1	10.1	14.3	12.3	10.1	11.8	3.0
18:2	−	28.4	+	29.2	2.0	29.6
20:3	−	4.0	−	+	−	−
20:4	−	50.2	−	59.2	−	67.4

* The fatty acid composition of the 2-position was calculated from the determined overall fatty acid composition and the fatty acid composition of the 1-position.

be present in the diglycerides from all lecithins, though in different quantities. Feeding of corn oil causes a strong increase of the relative amounts of this Fraction A (14.6, 28.0, 37.5, 48.2, 59.3 and 68% in lecithin-(deficient), -(1), -(3), -(6), -(9) and -(15), respectively). Fatty acid analysis of Fraction A revealed that it consisted mainly of the saturated fatty acids, palmitic acid and stearic acid, and the polyunsaturated arachidonic acid. (The total number of double bonds being 4 in this fraction.) In agreement with the results obtained by a phospholipase A hydrolysis of the intact phospholipid molecule (Table II), it was found by hydrolysis of Fraction A with pancreatic lipase that the 2-position was occupied almost exclusively by arachidonic acid. This enabled us to conclude that (1-palmitoyl-2-arachidonoyl)- and (1-stearoyl-2-arachidonoyl)-lecithin were the major species present in Fraction A. The amounts of

these species can be calculated as follows: the percentage of palmitic acid in the 1-ester position of Fraction A in lecithin-(deficient), -(1), -(3), -(6), -(9) and -(15) is 36.0, 30.6, 37.0, 34.4, 34.4 and 33.0%, respectively. From the relative amounts of Fraction A in the different lecithins as mentioned above, it is possible to calculate that the amounts of (1-palmitoyl-2-arachidonoyl)-lecithin are: $36/100 \times 14.6$, $30.6/100 \times 28.0$, $37/100 \times 37.5$, $34.4/100 \times 48.2$, $34.4/100 \times 59.3$ and $33.0/100 \times 68.0\%$ in lecithin-(deficient), -(1), -(3), -(6), -(9), -(12) and -(15), respectively.

TABLE III

FATTY ACID COMPOSITION OF THE FRACTIONATED DIGLYCERIDES DERIVED FROM RAT LIVER LECITHIN AFTER DIFFERENT PERIODS OF CORN OIL FEEDING

A, B, C *etc.* refer to the different fractions of diglycerides obtained by thin-layer chromatography on silver nitrate-impregnated silica (see text).

Essential fatty acid-deficient	*A*	*B*	*C*	*D*	*E*	*F*	*G*
Weight %:	*14.6*	*43.5*	—	*6.0*	*9.9*	*19.5*	*6.2*
14:0	0.8	+		+	—	0.4	3.8
16:0	18.0	16.3		22.8	+	29.0	60.4
16:1	1.2	—		6.8	11.5	6.7	+
18:0	25.7	32.7		30.7	+	18.5	30.2
18:1	6.6	4.4		10.9	88.5	45.2	5.2
18:2	—	—		30.2	—	—	—
20:3	5.4	46.2		—	—	—	—
20:4	42.2	—		—	—	—	—
Weight %: 24 h	*28.0*	*30.1*	*3.3*	*16.9*	+	*19.3*	*2.6*
14:0	+	+				+	—
16:0	15.3	15.6	+	30.7		35.6	62.3
16:1	2.4	+	+	+		4.4	+
18:0	25.6	30.2	+	17.3		15.0	16.7
18:1	8.3	3.2	49.0	4.0		45.0	21.0
18:2	0.9	1.7	51.0	48.1		+	—
20:3	3.3	49.3	—	—		—	—
20:4	44.2	—	—	—		—	—
Weight %: 72 h	*37.5*	*14.5*	*3.7*	*22.2*	—	*18.1*	*3.8*
14:0	+	+	+	+		+	+
16:0	18.5	20.4	+	27.2		37.7	65.6
16:1	+	+	+	—		5.9	+
18:0	26.6	27.3	+	24.4		11.5	19.3
18:1	6.0	7.2	55.0	+		44.9	15.1
18:2	1.6	+	45.0	49.3		—	—
20:3	3.8	45.0	—	—		—	—
20:4	43.5	—	—	—		—	—
Weight %: 144 h	*48.2*	*6.7*	*4.1*	*19.6*	—	*21.4*	+
14:0	+	+	—	+		+	
16:0	17.2	20.6	+	26.6		37.3	
16:1	+	+	+	+		5.0	
18:0	31.8	39.4	+	21.3		12.2	
18:1	3.7	+	51.8	4.2		45.5	
18:2	—	5.3	48.2	47.5		—	
20:3	—	34.5	—	—		—	
20:4	47.4	—	—	—		—	

TABLE III *(continued)*

Weight %: 216 h	*59.3*	*1.5*	*3.0*	*20.8*	—	*16.0*	+
14:0	+	+	—	+		+	
16:0	17.2	20.8	+	28.4		41.2	
16:1	—	—	—	—		+	
18:0	32.0	31.0	+	21.0		8.6	
18:1	4.5	—	51.0	4.7		51.2	
18:2	—	—	49.0	46.0		—	
20:3	—	48.2	—	—		—	
20:4	46.3	—	—	—		—	
Weight %: 360 h	*68.0*	*—8*	*3.1*	*23.4*	—	*5.5*	+
14:0	+		—	+		+	
16:0	16.5		+	29.3		40.2	
16:1	—		+	—		+	
18:0	30.0		+	21.8		12.4	
18:1	4.5		51.7	+		47.6	
18:2	—		48.3	48.9		—	
20:3	—		—	—		—	
20:4	49.1		—	—		—	

Similarly, the content of (1-stearoyl-2-arachidonoyl)-lecithin can be calculated for all lecithins (Table IV). As a check the overall fatty acid composition of the total lecithins can be calculated from the tabulated data of the individual molecular species (Table IV). A good agreement is found between these values and those found by determination (Table I). From the data obtained on Fraction A there are, in addition, some minor species deducible *viz.* (1-octadecenoyl-2-eicosatrienoyl)-lecithin in lecithin-(deficient), -(1) and -(3) and (1-octadecenoyl-2-arachidonoyl)-lecithin in lecithin-(6), -(9), -(12) and -(15), respectively. This conclusion was possible because the small amount of eicosatrienoic acid in Fraction A was located exclusively at the 2-position.

TABLE IV

MOLECULAR SPECIES OF RAT LIVER LECITHIN AFTER DIFFERENT PERIODS OF CORN OIL SUPPLY

Units are expressed in percent.

1-Position	*2-Position*	*Essential fatty acid-deficient*	*Period on corn oil*				
			24 h	*72 h*	*144 h*	*216 h*	*360 h*
16:0	20:4	5.2	8.5	13.9	16.5	20.4	22.4
18:0	20:4	7.5	14.3	19.9	30.6	37.8	40.6
16:0	20:3	14.2	9.4	5.9	2.7	0.6	—
18:0	20:3	28.4	18.2	7.9	5.2	0.9	—
16:0	18:2	1.6	10.3	12.2	10.4	11.8	13.7
18:0	18:2	2.2	5.9	10.8	8.3	8.7	10.2
18:1	18:1	7.6	1.7	+	—	—	—
16:1	18:1	2.3	—	—	—	—	—
16:0	18:1	10.2	12.3	12.2	14.4	13.1	4.3
18:0	18:1	6.5	5.2	3.7	4.7	2.7	1.4
16:0	16:0	2.5	+	+	+	+	+
18:0	16:0	3.0	+	+	+	+	+
18:1	20:3	5.4	2.9	2.1	—	—	—
18:1	20:4	—	2.8	3.4	3.6	5.3	6.1
18:1	18:2	—	3.3	3.7	4.1	3.0	3.1

Fraction B (3 double bonds) turned out to be quantitatively the most important spot in lecithin-(deficient). Palmitic, stearic and eicosatrienoic acids were found to be the fatty acid constituents of this fraction. In most experiments this Fraction B appeared to be resolved into 2 spots having the same fatty acid composition but the position of the double bonds in the eicosatrienoic acid was found to be different in these 2 spots; Δ5,8,11-eicosatrienoic acid was found to be present in the spot with the highest mobility, whereas the Δ7,10,13 isomer was present in the other spot.* Because this subfractionation of Fraction B was not always achieved, these 2 subfractions were taken together for further analyses. The 2-position was exclusively occupied by eicosatrienoic acid as was demonstrated by hydrolysis of this fraction with pancreatic lipase. This result is again in complete agreement with the distribution of eicosatrienoic acid among both ester positions as revealed by phospholipase A hydrolysis of the lecithins. Similarly, as described for Fraction A, the amounts of (1-palmitoyl-2-eicosatrienoyl)- and (1-stearoyl-2-eicosatrienoyl)-lecithin could be calculated (Table IV). As could be expected already from the overall fatty acid pattern of the lecithins, the level of Fraction B decreased steadily after corn oil supply. In lecithin-(15) Fraction B was completely absent. Fraction C was also found to represent diglycerides containing a total number of 3 double bonds. In this case, however, two double bonds are present in the chain linked at the 2-position and one in the acyl-chain located at the 1-position. This species (1-octadecanoyl-2-linoleoyl)-lecithin could not be recovered in measurable amounts in lecithin from essential fatty acid-deficient rats, but appeared to be a minor though detectable fraction in the lecithins from the corn oil fed rats (Table IV). Fraction D (2 double bonds) was a minor fraction in lecithin-(deficient) as could be predicted. Feeding of corn oil to essential fatty acid-deficient rats causes a rapid increase of this Fraction D (Table III). After hydrolysis of Fraction D derived from lecithin-(1), -(3), -(6), -(9) and -(15) with pancreatic lipase, it appeared that the linoleic acid was linked in all these samples at the 2-position, and that palmitic and stearic acid were esterified to the 1-ester position. This result enabled us to quantify the species (1-palmitoyl-2-linoleoyl)- and (1-stearoyl-2-linoleoyl)-lecithin (Table IV). Fraction E also appeared to contain diglycerides with 2 double bonds but this fraction was clearly separated from Fraction D on the basis of a different distribution of the double bonds between the 1- and 2-positions. From the data obtained on Fraction E of lecithin-(deficient) (Table III) it was possible to derive the molecular species (di-octadecenoyl)-lecithin and in addition a very small amount of (1-hexadecenoyl-2-octadecenoyl)-lecithin, because hydrolysis with pancreatic lipase demonstrated that the 2-position of this fraction was occupied by octadecenoic acid. (Di-octadecenoyl)-lecithin, which is present in lecithin-(deficient) in an appreciable amount, was found to be reduced very rapidly after corn oil feeding. After only 3 days of corn oil feeding no distinct Fraction E could be observed on the thin-layer plates. A very small amount of this type of species was found to be present in Fraction D. Further feeding of corn oil causes a complete disappearance of this species. Fraction F appeared to represent diglycerides having 1 double bond, the main unsaturated

* This conclusion was supported by comparison with the gas chromatographic behaviour of the synthetic Δ7,10,13 and Δ5,8,11 isomers provided by Dr. D. A. VAN DORP and Dr. H. J. J. PABON (Unilever Research Laboratory, Vlaardingen). These compounds revealed similar differences in relative retention times also when present in a mixture.

fatty acid being octadecenoic acid. By pancreatic lipase digestion of this fraction it was found that both octadecenoic and hexadecenoic acid were linked at the 2-position. Therefore, it is possible to conclude from these data that (1-palmitoyl-2-octadecenoyl)- and (1-stearoyl-2-octadecenoyl)-lecithin are the major species. The amounts of (1-palmitoyl-2-hexadecenoyl)- and (1-stearoyl-2-hexadecenoyl)-lecithin, which occur as minor species in this Fraction F, were not calculated. Fraction G, a very small fraction in lecithin-(deficient), represents mainly disaturated diglycerides, though a small amount of an unidentified octadecenoic acid was always found in this fraction. Because 80% of the 2-position was occupied by palmitic acid as found by hydrolysis with lipase, the amounts of (dipalmitoyl)- and (1-stearoyl-2-palmitoyl)-lecithin could be calculated. In the lecithins of the corn oil fed rats it was also possible to detect a fully saturated diglyceride Fraction G, but the amounts of this fraction were too small for accurate analysis.

DISCUSSION

The influence of supplying corn oil to essential fatty acid-deficient rats on the overall fatty acid composition of liver lecithin is represented graphically in Fig. 2. A significant increase of arachidonic acids is to be noted, particularly during the first 6 days of the experiments, after which this increase lessens. An accompanying decrease in eicosatrienoic acid is apparent and after 6–9 days of corn oil feeding this fatty acid constituent was found to be nearly absent from liver lecithin. A significant uptake of linoleic acid was observed but, in contrast to arachidonic acid, a maximal

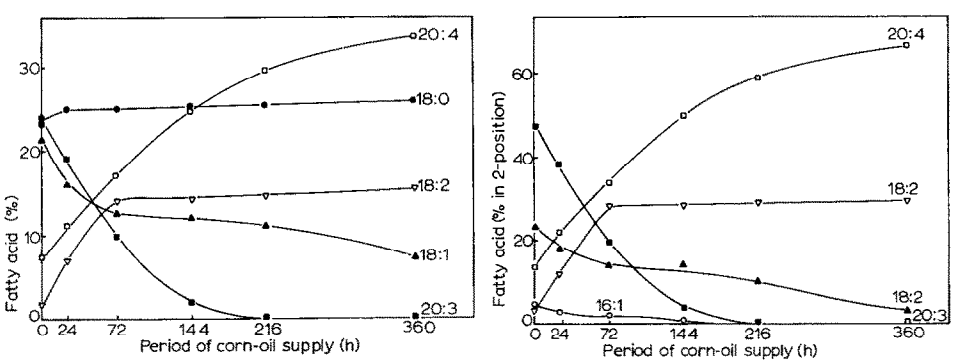
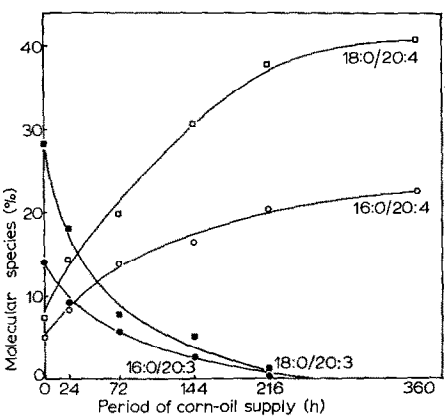


Fig. 2. Alterations in the fatty acid composition of liver lecithin after feeding of corn oil to essential fatty acid-deficient rats, as a function of time.

Fig. 3. Alterations in the 2-position-linked fatty acid constituents of liver lecithin from essential fatty acid-deficient rats after supplying of corn oil, as a function of time.

level was reached 3 days after changing the diet. Similarly, the decrease of octadecenoic acid(s) was most pronounced during the first 3 days of the dietary experiment. Saturated fatty acid constituents such as stearic acid and palmitic acid did not undergo any notable alterations. The feeding of corn-oil to essential fatty acid-deficient rats caused alterations of the same trend as already observed by BRENNER AND NERVI[3], although some quantitative differences are to be noted. These investi-

gators fed methyl linoleate and methyl arachidonate to essential fatty acid-deficient rats and concluded that both dietary linoleate and arachidonate inhibited eicosatrienoate synthesis, but that only arachidonate replaced eicosatrienoate quantitatively in the 2-position of lecithin. Both dietary acids were found to displace some of the β-positioned oleate. BRENNER AND NERVI[3] observed that after methyl linoleate feeding the incorporation of synthesized arachidonic acid began after a delay of more than 24 h. In the present study an increase in arachidonic acid content was demonstrated 24 h after the feeding of corn oil and the high percentage attained after 15 days succeeded that observed after supply of methyl linoleate[3]. Moreover, the feeding of corn oil was found to induce a much more pronounced uptake of linoleate into lecithin, when compared with the data recorded by BRENNER AND NERVI[3]. Such a difference may be due to the differences in the dietary conditions. Actually, when this manuscript was in preparation, CATALA AND BRENNER[17] reported that administration of a higher dose of methyl linoleate induced a higher incorporation of linoleic acid into the phospholipids. The diet-induced alterations of fatty acid constituents at the 2-position of lecithin (Fig. 3) endorse the concept of positional interchangeability of poly-unsaturated fatty acids[3,11]. Under the conditions utilized, a reciprocity of the curves representing eicosatrienoic and arachidonic acid may be noticed. More details on this behaviour become apparent by a comparison of the data on the quantities of various molecular species of lecithin (Fig. 4). The curves of (1-palmitoyl-2-eicosatrienoyl)- and



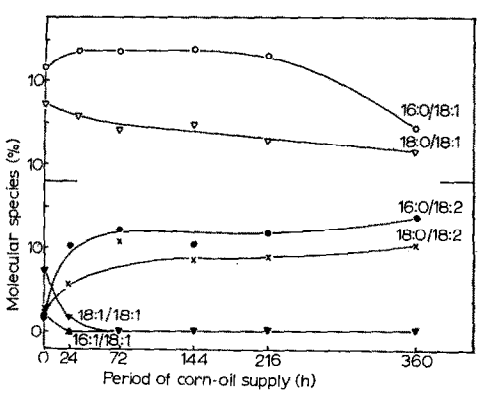


Fig. 4. A time study on the changes induced in the eicosatrienoic acid- and eicosatetraenoic acid-containing molecular species of liver lecithin from essential fatty acid-deficient rats after feeding of corn oil.

Fig. 5. Alterations induced in the mono- and diunsaturated molecular species of liver lecithin from essential fatty acid-deficient rats after feeding of corn oil, as a function of time.

(1-palmitoyl-2-arachidonoyl)-lecithin exhibit a strong reciprocity and the same holds true for (1-stearoyl-2-eicosatrienoyl)- and (1-stearoyl-2-arachidonoyl)-lecithin. For (1-palmitoyl-2-linoleoyl)- and (1-stearoyl-2-linoleoyl)-lecithin, which increased mainly during the first 3 days of the experiment it is more difficult to assign such counterparts (Fig. 5). The species (1-palmitoyl-2-octadecenoyl)-lecithin started to decrease 9 days after the change of diet, while the decrease of (stearoyl-2-octadecenoyl)-lecithin appeared to be rather gradual during the period studied. On the other hand,

(di-octadecenoyl)-lecithin and (1-hexadecenoyl-2-octadecenoyl)-lecithin responded very rapidly upon change of the diet.

Apart from the effects of lipid constituents from the diet on the biosynthesis of fatty acids, *e.g.*, the inhibition of eicosatrienoic acid synthesis by dietary polyunsaturated fatty acids[18–20], the question of which mechanism transmits the alterations in the fatty acid pool to the phospholipids must be answered. At least two pathways may be involved, *viz* (a) a complete breakdown of phosphoglycerides and a replacement by molecules formed by a *de novo* synthesis as established by KENNEDY[21], and (b) a removal of one fatty acid constituent coupled with an acylation of the monoacyl phosphoglyceride intermediates. In liver a major catabolic pathway has been demonstrated by DAWSON[22] who found that enzymatic removal of both fatty acid constituents, resulting in the formation of phosphodiesters, occurs *in vivo* at a considerable rate. This enzymatic breakdown may proceed *via* monoacyl derivatives since the liver was found to contain an active lysophospholipase A activity[23]. Moreover, studies of VAN DEN BOSCH AND VAN DEENEN[24] indicated that liver homogenates exert phospholipase A-like activity acting both on the 1- and 2-fatty acid ester position of phosphoglycerides. Subsequent studies of SCHERPHOF, WAITE AND VAN DEENEN[25] demonstrated that these activities were more pronounced in the microsomal and mitochondrial fractions, respectively, whereas the lysophospholipase activity is well-known to be most abundant in the supernatant fraction. LANDS AND MERKL[26] previously showed that subcellular fractions of liver are able to convert the monoacyl phosphoglycerides into the diacyl analogues. SCHERPHOF AND VAN DEENEN[27] studied the simultaneous incorporation of 2-[^{3}H]glycerophosphate and [^{14}C]fatty acids into lecithin of rat liver *in vitro*. Their results indicated that the incorporation of fatty acid into lecithin exceeded that of glycerophosphate utilization, thus providing circumstantial evidence for an important role of acylation of lyso intermediates, at least under the conditions utilized. Although one is not able to extrapolate these results to the events occurring in the living animal, it is tempting to speculate that the alterations of some species, *e.g.*, replacement of (1-stearoyl-2-eicosatrienoyl)- by (1-stearoyl-2-arachidonoyl)-lecithin, are to a certain extent affected by a monoacyl phosphoglyceride acting as an intermediate. On the other hand the data presented in this paper on the increase of the lecithin species containing linoleic acid suggest an important contribution of a *de novo* synthesis and a complete removal of a number of species by the catabolic pathway. However, it is clear that extensive investigations are needed to verify such assumptions and to determine more directly the relative contributions of various pathways to the process of fatty acid renewal of molecular species of phospholipids *in vivo*.

ACKNOWLEDGEMENTS

The present investigations have been carried out under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.) and with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

The authors are greatly indebted to Mrs. H. I. P. VAN HUIS-FOKKINGA for technical assistance.

REFERENCES

1 L. M. G. VAN GOLDE AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 125 (1966) 496.
2 R. M. JOHNSON, *Exptl. Cell Res.*, 32 (1963) 118.
3 R. R. BRENNER AND A. M. NERVI, *J. Lipid Res.*, 6 (1965) 363.
4 C. F. REED, *J. Clin. Invest.*, 38 (1959) 1032.
5 L. M. G. VAN GOLDE AND L. L. M. VAN DEENEN, *Chem. Phys. Lipids*, 1 (1967) 157.
6 L. M. G. VAN GOLDE, V. TOMASI AND L. L. M. VAN DEENEN, *Chem. Phys. Lipids*, 1 (1967) 282.
7 F. D. COLLINS, *Biochem. J.*, 88 (1963) 319.
8 R. M. JOHNSON AND T. ITO, *J. Lipid Res.*, 6 (1965) 75.
9 K. TISCHER AND J. L. GLENN. *Biochim. Biophys. Acta*, 98 (1965) 502.
10 F. D. COLLINS, *Biochem. J.*, 99 (1966) 117.
11 D. B. MENZEL AND H. S. OLCOTT, *Biochim. Biophys. Acta*, 84 (1964) 133.
12 J. F. MEAD, *Federation Proc.*, 20 (1961) 952.
13 E. KLENK, *J. Am. Oil. Chemists' Soc.*, 42 (1965) 580.
14 C. F. WURSTER, JR. AND J. H. COPENHAVER, *Lipids*, 1 (1966) 422.
15 G. A. E. ARVIDSON, *J. Lipid. Res.*, 8 (1967) 155.
16 L. J. MORRIS, *J. Lipid Res.*, 7 (1966) 717.
17 A. CATALA AND R. R. BRENNER, *Lipids*, 2 (1967) 114.
18 R. T. HOLMAN, *Federation Proc.*, 23 (1964) 1062.
19 R. R. BRENNER AND R. O. PELUFFO, *J. Biol. Chem.*, 241 (1966) 5213.
20 E. KLENK, *J. Am. Oil. Chemists' Soc.*, 42 (1965) 580.
21 E. P. KENNEDY, *Federation Proc.*, 20 (1961) 934.
22 R. M. C. DAWSON, *Biochem. J.*, 62 (1956) 689.
23 E. A. MARPLES AND R. H. S. THOMPSON, *Biochem. J.*, 74 (1960) 123.
24 H. VAN DEN BOSCH AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 106 (1965) 326.
25 G. L. SCHERPHOF, B. M. WAITE AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 125 (1966) 406.
26 W. E. M. LANDS AND J. MERKL, *J. Biol. Chem.*, 238 (1963) 898.
27 G. L. SCHERPHOF AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 113 (1966) 417.