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THE EFFECT OF DIETARY FAT ON THE MOLECULAR SPECIES OF LECITHIN FROM RAT LIVER

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SUMMARY

1. Lecithins from the liver of rats maintained on diets devoid of essential fatty acids or supplemented with coconut oil or corn oil revealed significant differences in fatty acid composition, whilst monomolecular films of these lecithin samples exhibited only limited differences in force-area characteristics.

2. The individual molecular species in the three lecithin samples were determined by means of the following techniques: (a) fractionation of the lecithin on silica impregnated with silver nitrate, followed by determination of the positional distribution of the fatty acids with snake-venom phospholipase A (EC 3.1.1.4); (b) hydrolysis of the lecithin with phospholipase C (EC 3.1.4.3) from *Bacillus cereus* and fractionation of the resultant diglycerides on silica impregnated with silver nitrate. Subsequently, the positions of the fatty acid constituents in the diglyceride fractions were determined by hydrolysis with pancreatic lipase (EC 3.1.1.3). The second approach gave the most detailed information and made it possible to recognize between 14 and 23 species. Quantitative determination of the major species accounted for 78.5 to 90.5% of the molecular composition.

3. Differences in diet induced significant variations in the proportions of the various lecithin species and also brought about qualitative differences. These shifts in the molecular composition of the lecithin samples may contribute to preserving the liquid–crystalline nature of these lipids in membrane structures.

INTRODUCTION

Although the fatty acid composition of phospholipids in animal membranes can be altered most significantly by dietary means, the physical properties of the lipids isolated remain fairly constant, indicating that nature tries to maintain the structural properties of cell membranes, at least to a certain extent[1,2]. In the light of these observations it became important to consider the phospholipids concerned in terms of individual molecular species. Fatty acids and glycerides have been subfractionated to a considerable extent by chromatography on silica impregnated with silver nitrate

(refs. 3–6). Recently several laboratories have also utilised this technique for the analysis of phospholipids[7–12]. In the present studies an attempt was made to determine the molecular composition of lecithin from the livers of rats fed on different diets by the following procedures: (a) subfractionation of the lecithins by thin-layer chromatography on silica impregnated with silver nitrate followed by determination of the fatty acid pattern in positions 1 and 2 of each of the separated lecithins with snake-venom phospholipase A (EC 3.1.1.4); (b) degradation of the phospholipids with phospholipase C from *Bacillus cereus* (EC 3.1.4.3) and subsequent subfractionation of the diglycerides. The positional distribution of fatty acids in the diglyceride fractions was ascertained by means of hydrolysis with pancreatic lipase (EC 3.1.1.3)*.

EXPERIMENTAL

Dietary experiments

Three groups of six male white rats were fed on different diets:

(A) One group received, for a period of two months commencing 3 weeks after birth, an essential fatty acid-deficient diet consisting of 72% calories as carbohydrates, 23% calories as proteins, 5% calories as hydrogenated coconut oil, and adequate amounts of salts and vitamins.

(B) A second group, 6 weeks old, was fed for 2 months on a diet containing 50% calories as carbohydrates, 16.8% calories as proteins and 33.3% calories as coconut oil supplemented with the required amounts of salts and vitamins**.

(C) A third group, also 6 weeks old, was given the same diet as Group B for two months except that in this group the coconut oil was replaced by the same caloric amount of corn oil.

Isolation of lecithin

After 2 months the animals were sacrificed by bleeding. The livers were removed quickly, rinsed thoroughly with distilled water and extracted immediately according to the method of BLIGH AND DYER[13]. The total liver lipids obtained in this manner were fractionated by means of silicic acid column chromatography using the procedure of HANAHAN[14] or with thin-layer chromatography on silica using chloroform–methanol–conc. ammonia–water (70:30:2:3, v/v) as a developer. In this way samples of chromatographically pure lecithins were obtained, as demonstrated by a comparison with synthetic lecithins. In accordance with previous investigations[15] the amounts of plasmalogens and glyceryl ether phospholipids were negligible.

Subfractionation of lecithin

The purified lecithins were subfractionated by thin-layer chromatography on silicic acid impregnated with silver nitrate. The plates (20 cm × 20 cm) were covered with a suspension of 20 g of silica gel G-Stahl (Merck, Darmstadt), 8.5 g of Hyflo and 3 g of calcium sulphate in 78 ml of a 12.5% solution of silver nitrate. The plates were

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* For a preliminary report see ref. 10.

** The results obtained with Diet B resembled most closely those observed on lecithin from animals fed on a laboratory chow. Since variations may occur in the fatty acid composition of lecithin from animals fed on different chows these results were not included.

activated by heating at 110° for 1.5 h immediately before use. About 20–30 mg of the lecithin samples were applied to the plates and chloroform–abs. ethanol–water (65:35:4, v/v) was used as developer. Spots were visualized under ultraviolet light after spraying with a 0.01% solution of Rhodamine 6 G in water. The subfractions were scraped off the glass plate and repeatedly extracted according to the method of BLIGH AND DYER; the final volume of each extract was 45 ml. All manipulations were carried out in a glove-box under a N_2 or CO_2 atmosphere.

Hydrolysis by phospholipase A

About 10 mg of lecithin were dissolved in 2 ml of ether; this solution was shaken with 0.5 ml of 0.1 M borate buffer (pH 7.0) containing calcium acetate (final concentration 0.0025 M) and 2 mg of *Crotalus adamanteus* venom. After an incubation period of 20–30 min the degradation was complete, this being checked by thin-layer chromatography on micro-slides coated with silica. The lysolecithins formed and fatty acids liberated were separated by thin-layer chromatography on silica plates (20 cm × 10 cm), using chloroform–methanol–water (65:35:4, v/v) as developer.

Hydrolysis by phospholipase C

About 80 mg of lecithin dissolved in 3 ml of ether were incubated with a solution of a crude enzyme preparation (containing 0.5 mg of protein) from *B. cereus*. After 1 h the hydrolysis was complete and the diglycerides were extracted[13]. There were no 1,3-diglycerides present after the incubation, as confirmed with thin-layer chromatography using synthetic 1,2- and 1,3-diglycerides as reference substances. The 1,2-diglycerides were then subfractionated by thin-layer chromatography on silicic acid impregnated with silver nitrate. The plates were prepared as described by BARRET, DALLAS AND BADLEY[4], using chloroform–abs. ethanol (93:7, v/v) as developer. After visualisation of the lipids under ultraviolet light with a solution of 0.01% Rhodamine 6 G in water, the fractions were removed from the plate and extracted immediately using the procedure of BLIGH AND DYER[13]. Again all manipulations were done in an atmosphere of N_2 or CO_2. The ratio of the subfractions was determined by adding to each subfraction, as an internal standard, a fatty acid not occurring in the lecithin preparation under investigation.

Hydrolysis with pancreatic lipase

To 5–10 mg of 1,2-diglycerides were added successively 9 mg of pancreatic lipase (Calbiochem), 1 ml of 1 M Tris buffer (pH 8), 0.1 ml of a 22% solution of $CaCl_2$ and 0.25 ml of a 0.1% solution of sodium deoxycholate. This mixture was first warmed for 1 min in a water bath at 40° and then shaken vigorously for 5 min at 40° in order to digest all the 1,2-diglycerides into 2-monoglycerides and free fatty acids. At the end of the incubation period 0.5 ml of 6 M HCl was added to stop the reaction and the monoglycerides and fatty acids were separated on thin-layer plates (20 cm × 10 cm) coated with a 0.5 mm thick layer of silica using light petroleum–ether–formic acid (60:40:1.6, v/v) as developer.

Gas–liquid chromatography

The fatty acids were converted into the corresponding methyl esters by transesterification for a period of 2 h at 70° with methanolic HCl containing 26 g of HCl

per l. The methyl esters were qualitatively and quantitatively analysed using an F & M instrument equipped with a flame ionisation detector and a 2-m column of 10% polyethylene glycol adipate. The working temperature of the column was 197°. The following fatty acids were used as standards: palmitic acid (Fluka A.G., Switzerland), stearic acid (Fluka A.G.), oleic acid (the Hormel Institute), linoleic acid (Light & Co., England), and arachidonic acid (the Hormel Institute). All-*cis*-methyl-5,8,11-eicosatrienoate and all-*cis*-methyl-7,10,13-eicosatrienoate prepared by chemical synthesis were kindly donated by Dr. D. A. VAN DORP and Dr. H. J. J. PABON (Unilever Research Laboratorium, Vlaardingen). Determination of the position of double bonds by these investigators revealed the absence of positional isomers.

RESULTS

The effect of diet on the fatty acid composition of lecithin from rat liver is demonstrated in Table I. The animals maintained on a diet depleted of essential fatty acids revealed a very low content of octadienoic acid and eicosatetraenoic acid. The values are in close agreement with the content of linoleic acid and arachidonic acid found by other investigators in liver lecithin from essential fatty acid-deficient rats [17–19]. The percentages of eicosatrienoic acid(s) and octadecaenoic acid are considerably increased in the lecithin of these animals, as compared with the values found for the lecithin of the rats receiving a regimen containing coconut oil or corn oil. An increase in the quantity of administered linoleic acid is clearly reflected by an increased level

TABLE I

FATTY ACID COMPOSITION OF LIVER LECITHIN OBTAINED FROM RATS FED ON DIFFERENT DIETS

The fatty acid composition in all tables is given in moles %. Diet A, essential fatty acid-deficient diet; Diet B, coconut-oil diet; Diet C, corn-oil diet.

Fatty acids	*Diet A*			*Diet B*			*Diet C*		
	Overall composition	*1-position*	*2-position*	*Overall composition*	*1-position*	*2-position*	*Overall composition*	*1-position*	*2-position*
12:0	—	—	—	+	0.1	+	+	+	—
14:0	0.3	+	—	1.7	2.4	0.5	+	0.2	+
15:0	+	+	—	+	0.2	+	+	0.3	+
16:0	18.2	31.3	3.9	20.2	38.3	2.3	18.9	31.9	2.2
16:1	2.9	1.5	3.2	0.7	1.6	1.7	+	1.9	0.2
17:0	—	—	—	+	+	+	+	1.7	—
18:0	25.7	47.5	1.9	27.1	51.8	1.5	24.4	42.1	1.2
18:1	21.8	19.2	25.2	11.8	3.9	17.0	9.9	15.8	5.6
18:2	1.7	—	3.4	15.0	1.8	28.3	18.6	6.2	32.9
20:2	+	—	+	0.6	—	1.8	—	—	—
20:3	23.1	0.6	48.1	3.5	—	5.9	—	—	—
20:4	6.3	—	14.3	19.5	—	41.1	28.2	0.1	57.7

of this acid and particularly of arachidonic acid in the liver lecithin. Hydrolysis experiments with phospholipase A from snake venom confirm that the saturated fatty acid constituents are predominantly located at the 1-position, whereas the polyunsaturated fatty acids are esterified nearly exclusively at the 2-position. A comparison of the fatty acid distribution between lecithins A (essential fatty acid-deficient) and C (corn-oil diet) demonstrates the positional interchangeability of different

polyunsaturated fatty acids found in phospholipids of several origins[20,21]. The most abundant monounsaturated fatty acid(s) (18:1) is located preferentially at the 2-position of lecithin B; in lecithin A octadecaenoic acid(s) is more randomly distributed among the two ester positions, whereas in lecithin C there is a preference of 18:1 for the 1-position. As will be demonstrated in this paper these differences in the location of 18:1 are due to differences in the proportions of various molecular species among the lecithin preparations.

Molecular species of lecithin A

Subfractionation of lecithin. Chromatography of liver lecithin from rats deficient in essential fatty acids on silica plates impregnated with silver nitrate resulted in three spots. The spot with the lowest mobility (S_I), representing only a small fraction of the total lecithin, contained a considerable amount of 20:4 and 20:3 (Table II).

TABLE II

FATTY ACID COMPOSITION OF LIVER LECITHINS FROM ESSENTIAL FATTY ACID-DEFICIENT RATS (DIET A) AFTE SUBFRACTIONATION ON SILVER NITRATE-IMPREGNATED SILICA

Fatty acids	S_I			S_{II}			S_{III}		
	Overall composition	*1-position*	*2-position*	*Overall composition*	*1-position*	*2-position*	*Overall composition*	*1-position*	*2-positio*
14:0	+	—	—	+	—	—	+	—	—
15:0	—	—	—	—	—	—	+	—	—
16:0	20.0	40.7	+	23.2	39.9	3.2	20.9	38.1	5.6
16:1	+	—	—	3.2	—	1.6	8.4	12.1	4.3
18:0	23.6	51.3	+	24.2	48.4	2.0	21.6	45.5	+
18:1	4.5	7.9	+	23.1	11.7	30.9	45.4	4.7	90.1
18:2	+	—	2.3	+	—	4.0	3.5	—	+
20:2	—	—	+	—	—	—	+	—	—
20:3	30.6	—	60.0	23.7	—	52.9	—	—	—
20:4	21.2	—	37.7	2.5	—	5.4	—	—	—

A second fraction (S_{II}) appeared to contain only a small amount of 20:4, but 20:3 was abundant, whereas the content of 18:1 was higher as compared with S_I. Although both fractions were clearly separated in a number of experiments, this fractionation was not achieved. A third fraction (S_{III}) moving in all experiments much faster than S_I and S_{II} appeared to contain mainly monounsaturated and saturated fatty acids. These results are in contrast to those obtained by KAUFMANN, WESSELS AND BONDOPADHYAYA[7] who claimed a separation of natural lecithins into nine subfractions. Recently, ARVIDSON[11] modified KAUFMANN's procedure and obtained 2–6 subfractions, depending on the origin of the lecithin sample. The results of phospholipase A hydrolysis of the subfractions are in fair agreement with those obtained for the fatty acid distribution in the non-fractionated lecithin sample. The data obtained with Fraction S_I indicate the occurrence of the lecithin species: 16:0/20:3, 16:0/20:4, 18:0/20:3 and 18:0/20:4. (The first- and second-mentioned fatty acid occupying the 1- and 2-ester positions, respectively.) However, the data obtained do not permit any conclusions about the amount of these individual species. The presence of the species 16:0/20:3 and 18:0/20:3 is also suggested by the analytical data obtained on S_{II}. Therefore, it seems likely that S_I is contaminated on the plate with S_{II} which is quantitatively the more important fraction. Fraction S_{III} was found to have 90% of

18:1 at the 2-fatty acid ester position, allowing the conclusion that 16:0/18:1 is present. More quantitative information on these species could be obtained by the second approach.

Subfractionation of diglycerides. After complete conversion of the phospholipid sample with phospholipase C from *B. cereus* the 1,2-diglycerides were subjected to chromatography on silica plates containing silver nitrate. Lecithin preparation A resulted in seven spots, the separation depending on the number of double bonds, the location of the double bonds in the paraffinic chains and to some extent on the positional distribution of the fatty acids. Subfraction S_1 contained all of the 20:4 present in the diglyceride sample (Table III). This polyunsaturated fatty acid together with a small amount of 20:3 was found to occupy exclusively the 2-position,

TABLE III

FATTY ACID COMPOSITION OF DIGLYCERIDES OBTAINED AFTER PHOSPHOLIPASE C HYDROLYSIS OF LIVER LECITHIN FROM ESSENTIAL FATTY ACID-DEFICIENT RATS (DIET A) AND SUBSEQUENT FRACTIONATION ON SILVER NITRATE-IMPREGNATED SILICA

Fatty acids	*Fatty acid composition*						
	S_1	S_2	S_3	S_4	S_5	S_6	S_7
14:0	+	—	+	—	—	0.9	3.7
15:0	—	—	—	—	—	—	+
16:0	15.3	17.0	14.2	20.8	+	26.4	60.9
16:1	2.4	—	—	4.6	14.5	6.1	4.3
18:0	26.5	37.4	35.2	32.5	+	21.7	31.1
18:1	5.1	1.0	2.3	11.5	85.5	45.0	+
18:2	—	—	0.9	25.0	—	—	—
20:2	—	—	—	5.5	—	—	—
20:3	2.5	44.6	47.3	—	—	—	—
20:4	48.1		—	—	—	—	—
Per cent of total lecithin	16.4	13.3	22.7	7.2	11.5	20.5	8.3
	*Fatty acid composition of the 2-position**						
	S_1	S_2	S_3		S_5	S_6	S_7
14:0	—	—	—		—	—	+
15:0	—	—	—		—	—	+
16:0	+	+	+		—	6.5	80.0
16:1	—	—	—		4.5	3.2	7.3
18:0	+	+	+		—	—	12.7
18:1	—	—	—		95.5	90.3	+
18:2	—	—	—		—	—	—
20:2	—	—	—		—	—	—
20:3	6.1	100.0	100.0		—	—	—
20:4	94.0		—		—	—	—

* Hydrolysis with pancreatic lipase was used to determine this fatty acid composition. Fraction 4 was present in too small an amount for hydrolysis with pancreatic lipase.

as demonstrated by hydrolysis with pancreatic lipase. This enzyme is known to act preferentially at the 1-position of the diglycerides[22,23] and the results obtained in the present study are in good agreement with those from the phospholipase A hydrolysis of the lecithin preparations and their subfractions. Taking into account the very good separation between S_1 and S_2, it seems legitimate to make the assumption that all species present in fraction S_1 contain four double bonds. Assuming that 20:3 is combined with 18:1 and 16:1 (giving the lecithin species 18:1/20:3 and 16:1/20:3) it is

concluded that the saturated fatty acids present at the 1-position are combined with 20:4 located at the 2-position. The major molecular species present in this diglyceride subfraction are apparently 16:0/20:4 and 18:0/20:4 lecithins. The relative amounts of these individual species in the sample of lecithin A can be calculated from the observation that Subfraction S_1 represents 16.4% of the total glycerides (Table III). For example, the 18:0/20:4 species represents $53/100 \times 16.4$ or 8.7% of the total lecithin, 53 representing the percentage of the 1-position of Fraction S_1, occupied by stearic acid. Fractions S_2 and S_3 which represent two neighbouring spots (being, however, resolved to a fair degree) exhibited a similar fatty acid composition. In both subfrac-

TABLE IV

FATTY ACID COMPOSITION OF LIVER LECITHINS FROM COCONUT OIL-FED RATS (DIET B) AFTER SUBFRACTIONATION ON SILVER NITRATE-IMPREGNATED SILICA

Fatty acids	S_I			S_{II}		
	Overall composition	*1-position*	*2-position*	*Overall composition*	*1-position*	*2-position*
12:0	+	−	−	+	+	−
14:0	1.1	2.1	−	3.0	3.5	1.0
15:0	+	+	−	+	+	+
16:0	19.0	34.4	1.5	25.5	46.0	5.2
16:1	0.6	+	+	2.4	+	4.2
17:0	+	+	−	−	−	−
18:0	30.1	56.3	0.6	25.2	46.7	1.3
18:1	3.8	4.3	2.0	19.7	3.8	36.6
18:2	5.6	2.5	10.1	24.2	+	51.8
20:2	0.9	−	1.4	−	−	−
20:3	4.2	−	7.8	+	−	−
20:4	34.9	−	76.5	+	−	−

tions eicosatrienoic acid occupied over 99% of the 2-position of the diglycerides. However, the relative retention times measured during gas–chromatographic analysis suggested that Δ7,10,13-eicosatrienoic acid is present mainly in S_2, whereas Δ5,8,11-eicosatrienoic acid occurs predominantly in S_3*. For the calculation of the lecithin species no distinction has been made between the two isomeric fatty acid constituents (Table VIII). Fraction S_4 contained the dienoic acids 18:2 and 20:2; these fatty acids were not present in any other diglyceride fraction. The fatty acid distribution of this minor fraction has not been determined, but the analysis of the lecithin sample A (Table I) and its subfractions (Table II) indicates that these dienoic acids are located exclusively at the 2-position. The analytical data on this fraction available to date do not allow us to formulate any particular species, certainly not on a quantitative basis. Subfraction S_5 contained nearly exclusively the monounsaturated fatty acid constituents 16:1 and 18:1; the relative quantity of the latter at the 2-position exceeded that of the former. It is only possible to calculate minimal and maximal values for the four possible species *viz.* 18:1/18:1, 16:1/18:1, 16:1/16:1 and 18:1/16:1. The two first-mentioned species are quantitatively the most important ones and the analytical data make their presence unambiguous, but it cannot be stated with certainty

* This tentative conclusion was supported by experiments with the synthetic isomers provided by Dr. D. A. VAN DORP and Dr. H. J. J. PABON (Unilever Research Laboratory, Vlaardingen). These compounds revealed similar differences in retention times, this also being so when present as a mixture.

that the two minor species are both definitely present. Furthermore, it is worth noting that the species containing two unsaturated fatty acid constituents were separated from the species containing one diunsaturated fatty acid, a result also obtained by RENKONEN[12] in the analysis of diglyceride acetates derived from brain lecithin. On the other hand, it cannot be precluded that Fraction S_4 contained some species with two monounsaturated fatty acids. Subfraction S_6 contained about 49% saturated and 51% monounsaturated fatty acids; 18:1 and 18:0 were exclusively located at the 2- and 1-positions, respectively. On the other hand, 16:1 and 16:0 are located on both ester positions. Since the species containing saturated fatty acids only are recovered in a distinct fraction(s) it is reasonable to base the determination of the species present in S_6 on the fact that each contains one monounsaturated fatty acid. The major species which can be deduced are 16:0/18:1 and 18:0/18:1; in addition, 16:1/16:0, 16:0/16:1 and 18:0/16:1 can be envisaged as possible members. Depending on the amount of 16:0/16:1 (unknown; maximal value about 0.7% of the total lecithin) the quantity of 16:0/18:1 may vary between 8.8 and 9.5% of the lecithin preparations. Similarly the species 18:0/18:1 may represent between 8.2 and 8.9% of the lecithin. The species 16:1/16:0 may account maximally for about 1%

TABLE V

FATTY ACID COMPOSITION OF DIGLYCERIDES OBTAINED AFTER PHOSPHOLIPASE C HYDROLYSIS OF LIVER LECITHIN FROM RATS FED ON A COCONUT-OIL DIET AND SUBSEQUENT FRACTIONATION ON SILVER NITRATE-IMPREGNATED SILICA

Fatty acids	*Fatty acid composition*				
	S_1	S_2	S_3	S_4	S_5
12:0	0.4	+	0.7	+	+
14:0	1.5	3.7	1.8	2.9	9.0
15:0	0.5	0.7	+	0.5	1.5
16:0	16.9	14.6	15.4	32.7	53.4
16:1	1.0	0.6	2.3	5.0	3.3
16:2	+	—	+	+	+
18:0	27.4	25.1	24.9	14.1	16.0
18:1	2.1	2.8	4.7	44.8	16.8
18:2	0.2	2.6	50.1	+	—
20:2	—	+	—	—	—
20:3	—	50.0	—	—	—
20:4	49.8	—	—	—	—
Per cent of total lecithin	27.8	16.9	26.4	21.5	7.4
	*Fatty acid composition of the 2-position**				
	S_1	S_2	S_3	S_4	
12:0	—	—	—	—	
14:0	—	—	—	—	
15:0	—	—	—	—	
16:0	1.2	+	1.5	6.3	
16:1	—	—	—	—	
16:2	—	—	—	—	
18:0	0.9	+	1.1	3.0	
18:1	+	—	—	90.6	
18:2	—	—	97.3	—	
20:2	—	—	—	—	
20:3	—	100.0	—	—	
20:4	97.9	—	—	—	

* Hydrolysis with pancreatic lipase was used to determine this fatty acid composition. Fraction 5 was present in too small an amount for hydrolysis with pancreatic lipase.

of the lecithin. A relatively small fraction (S_7) appeared to contain more than 95% of saturated fatty acid constituents. Inasmuch as palmitic acid was the major constituent esterified to the 2-position, the species 16:0/16:0 and 18:0/16:0 must certainly be present. Taking into account the possible presence of 16:0/18:0 and 18:0/18:0, the values for 16:0/16:0 and 18:0/16:0 can be calculated to vary between 2.4–3.5% and 3.3–4.1% of the total lecithin, respectively.

Molecular species of lecithin B

Subfractionation of lecithin obtained from the liver of coconut oil-fed rats on silver nitrate-impregnated silica yielded only two spots. The fatty acid composition of these two subfractions S_I and S_{II} is recorded in Table IV together with the positional distribution of the fatty acids. The eicosatrienoic and eicosatetraenoic acids were all recovered in Subfraction S_I which had the lowest R_F value, whereas most of the octadecadienoic and octadecaenoic acids were recovered in Subfraction S_{II}. In view of the high percentages of palmitic acid and stearic acid at the 1-position of Subfraction S_I and the significant amount of eicosatetraenoic acid at the 2-ester position, it is clear that the species 16:0/20:4 and 18:0/20:4 are present in this lecithin. If similar reasoning is applied to the results obtained by hydrolysis of Subfraction S_{II} with phospholipase A, then 16:0/18:2 and 18:0/18:2 must exist. More detailed information was obtained by fractionation of the 1,2-diglycerides. Five fractions were obtained (Table V) containing predominantly tetraenoic, trienoic, dienoic, monoenoic and saturated fatty acid constituents. The unsaturated fatty acid constituents, which accounted for 45–50% of the total fatty acids, were mainly located at the 2-position. The analytical data on Subfraction S_1 allowed us to calculate the quantities of the species 16:0/20:4, 18:0/20:4 and 18:1/20:4 (Table VIII). Furthermore other species, *e.g.* 14:0/20:0, present in small quantities, are recovered in this fraction. Subfraction S_2 apparently contains 16:0/20:3 and 18:0/20:3 as major components, while in S_3 16:0/18:2 and 18:0/18:2 are the predominating species (Table VIII). No distinct subfraction containing two monounsaturated fatty acid constituents was obtained from this lecithin preparation. Subfraction S_4 revealed the presence of 16:0/18:1 and 18:0/18:1. If we assume that all species present in this fraction contain one monounsaturated fatty acid, then it is also possible to calculate the quantities of 16:1/16:0 and 16:1/18:0. Subfraction S_5 was not degraded with pancreatic lipase, but probably contains a number of species with two saturated fatty acids. It is not clear why this fraction contains a certain quantity of monounsaturated fatty acids. It may be of interest to investigate whether there is a difference between S_4 and S_5 with respect to the content of *trans* fatty acids.

Molecular species of lecithin C

Subfractionation of the intact phospholipid preparation gave quantitative information about the occurrence of only a few molecular species (Table VI). After chromatography of the diglycerides five subfractions were obtained (Table VII). The major fraction, S_1, consisted of a number of species containing 20:4 at the 2-position *viz.* 16:0/20:4, 18:0/20:4 and, remarkably, also 18:1/20:4. The lecithin C, in contrast to lecithins B and A, did not furnish a spot containing eicosatrienoic acid, although Subfraction S_2 contained a considerable quantity of diunsaturated and monounsaturated fatty acids. The majority of the species in this fraction consisted

TABLE VI

FATTY ACID COMPOSITION OF LIVER LECITHINS FROM CORN OIL-FED RATS (DIET C) AFTER SUBFRACTIONATION ON SILVER NITRATE-IMPREGNATED SILICA

Fatty acids	S_I			S_{II}		
	Overall composition	*1-position*	*2-position*	*Overall composition*	*1-position*	*2-position*
12:0	—	—	—	—	—	—
14:0	+	+	—	0.2	+	—
15:0	+	+	—	0.1	+	—
16:0	17.2	32.8	1.6	25.2	41.9	6.7
16:1	0.8	1.6	0.4	1.2	1.7	+
17:0	+	1.0	+	0.5	—	+
18:0	26.8	48.5	1.8	20.0	37.8	1.3
18:1	8.9	12.5	1.6	14.0	15.9	14.4
18:2	7.7	3.5	9.4	38.9	2.7	77.6
20:2	—	—	—	—	—	—
20:3	—	—	—	—	—	—
20:4	38.7	+	85.2	—	—	—

TABLE VII

FATTY ACID COMPOSITION OF DIGLYCERIDES OBTAINED AFTER PHOSPHOLIPASE C HYDROLYSIS OF LIVER LECITHIN FROM RATS FED ON A CORN-OIL DIET AND SUBSEQUENT SUBFRACTIONATION ON SILVER NITRATE-IMPREGNATED SILICA

Fatty acids	*Fatty acid composition*				
	S_1	S_2	S_3	S_4	S_5
12:0	—	—	+	—	—
14:0	+	+	+	+	+
15:0	+	+	+	—	+
16:0	14.4	9.0	23.6	33.2	64.7
16:1	+	1.7	+	1.3	+
16:2	0.6	2.0	+	+	+
18:0	29.8	8.6	22.9	14.2	20.8
18:1	5.0	34.8	2.8	45.6	14.5
18:2	+	43.9	50.7	5.6	—
18:3	—	—	—	—	—
20:2	—	—	—	—	—
20:3	—	—	—	—	—
20:4	50.2	—	—	—	—
Per cent of total lecithin	38.5	11.1	33.7	12.1	4.5
	*Fatty acid composition of the 2-position**				
12:0	—	—	—	—	
14:0	—	+	—	—	
15:0	—	+	—	—	
16:0	+	7.2	4.8	12.2	
16:1	—	7.0	+	+	
16:2	—	2.9	+	6.0	
18:0	+	1.8	5.8	4.5	
18:1	—	13.6	2.4	77.3	
18:2	—	67.5	87.0	—	
18:3	—	—	—	—	
20:2	—	—	—	—	
20:3	—	—	—	—	
20:4	100.0	—	—	—	

* Hydrolysis with pancreatic lipase was used to determine this fatty acid composition. Fraction 5 was present in too small an amount for hydrolysis with pancreatic lipase.

TABLE VIII

MAJOR MOLECULAR SPECIES OF LIVER LECITHIN FROM RATS FED ON DIFFERENT DIETS: ESSENTIAL FATTY ACID-DEFICIENT DIET (A), COCONUT-OIL DIET (B) AND CORN-OIL DIET (C)

1-position	*2-position*	*A**	*B*	*C*
16:0	16:0	3.5	**	**
18:0	16:0	4	**	**
16:0	18:1	9.5	12.5	6.5
18:0	18:1	9	5	3
16:0	18:2	+	7.5	14
18:0	18:2	+	13	13.5
16:0	20:3	11	5	—
18:0	20:3	26	8.5	—
16:0	20:4	5	9	11
18:0	20:4	8.5	15	23
16:1	18:1	3	—	—
18:1	18:1	8.5	—	—
18:1	18:2	—	2	4
18:1	20:3	2.5	—	—
18:1	20:4	—	1	4

* In some cases only the maximal value is given (see text).
** Not determined.

of 18:1/18:2 and perhaps 18:2/18:1, while this spot is believed to be contaminated to some extent with S_3, containing as major species 16:0/18:2 and 18:0/18:2 (Table VIII). It seems likely that small amounts of 18:2/16:0 and 18:2/18:0 are also present in this subfraction. Subfraction S_4 contains 16:0/18:1 and 18:0/18:1 as major components and probably also small quantities of species with a reversed distribution of fatty acids. As in the other lecithin preparation, Subfraction S_5 yielded a large quantity of saturated fatty acids as well as octadecaenoic acid.

DISCUSSION

Comparing the two procedures utilized for the determination of the molecular species of lecithin, analysis of the diglycerides was found to give much more information than direct subfractionation of the lecithin sample. The latter method was improved recently by ARVIDSON, but the results obtained in both studies on rat-liver lecithin demonstrated that, to date, the former procedure is still superior. Our results on the subfractionation of diglycerides formed enzymically from phospholipids are in good agreement with those of RENKONEN[9,12] who converted the phospholipids into diglyceride acetates. Rapid enzymic hydrolysis with phospholipase C may have certain advantages, and the present results indicate that the hydrolysis products can be utilized directly for subfractionation. Although it is possible to recognize the major molecular species by this technique, a complete quantitative analysis has not yet been achieved. Further improvement of the technique is necessary before a fully complete lecithin composition can be given with certainty.

It was the purpose of the present study to account for the limited differences in the force-area characteristics measured in monolayer studies of the three lecithin preparations. Previous studies on synthetic phospholipid species containing defined fatty acid constituents demonstrated that the interfacial behaviour is governed mainly by the nature of the paraffinic side-chains, a decrease of chain-length or an increase of

unsaturation causing an expansion of the film[25]. As demonstrated in Fig. 1 the three liver lecithin preparations revealed liquid-expanded films with the average molecular area of the samples increasing in the order A, B and C. This is not surprising since in the same sequence there is an increase in unsaturation of these lecithin samples. A calculation of the total number of double bonds (by multiplying the percentages of monoenoic, dienoic, trienoic and tetraenoic fatty acids by 1, 2, 3 and 4, respectively) gives the following values: A 123, B 132 and C 160. From a comparison of the proportions of the major species in lecithins B and C it may be seen that the presence of corn oil in the diet causes a considerable increase of 16:0/18:2 and 18:0/20:4. No increase was observed for 18:0/18:2 and further investigations are required to confirm this result. On the other hand, the increase of these species is accompanied by a decrease of 16:0/18:1, 18:0/18:1 and the disappearance of 16:0/20:3 and 18:0/20:3. These shifts may to

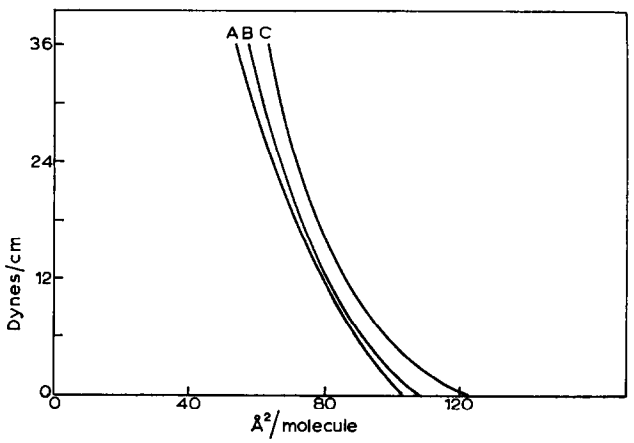

Fig. 1. Force-area characteristics of liver lecithin from rats fed on different diets: essential fatty acid-deficient diet (A), coconut-oil diet (B) and corn-oil diet (C). The experiments were carried out with a Langmuir–Adam trough at the air–water interphase at 22°. For each sample at least three experiments were carried out, the range being about 0.5 Å² per molecule.

some extent counteract the physical effects brought about by the increased quantities of 16:0/18:2 and 18:0/20:4. Furthermore, it is intriguing that the increase of 20:4 (located nearly exclusively at the 2-position) is accounted for by an increase of the species containing stearic acid at the 1-position rather than by an increase of the species containing palmitic acid. The results on lecithin A confirm and extend previous observations on the replacement of fatty acids of the linoleic acid family by those of the oleic acid family[16–19,24,26–28] and the positional interchangeability of these polyunsaturated fatty acids in essential fatty acid deficiency[20,21]. The relative quantities of 16:0/18:2, 18:0/18:2, 16:0/20:4, 18:0/20:4 and 18:1/20:4 are decreased and those of 16:0/18:1, 18:0/18:1, 16:0/20:3, 18:0/20:3 and 18:1/20:3 are increased as compared with lecithin C (and to a lesser extent with lecithin B). It is worth noting that 20:3 appears to exhibit the same preference as 20:4 for occurring with stearic acid. This result is in agreement with the recent studies of COLLINS[24], who analysed lecithins in the livers of rats deficient in essential fatty acids by means of countercurrent distribution. Furthermore, it can be noted that in lecithin A considerable

quantities of 18:1/18:1 and 16:1/18:1 were encountered; these species were not detected in lecithins B or C. The reverse is true for 18:1/18:2. The great similarity in the force-area characteristics of the different lecithins from rat liver, particularly lecithins A and B, may be traced back to the replacement of unsaturated fatty acids of different families and alterations in the proportions of the individual lecithin species under different dietary conditions. Apparently, there are several mechanisms operating which attempt to maintain the physico-chemical properties of the lecithin within certain limits. Although the force-area curve for the lecithin sample from essential fatty acid-deficient rats is nearly identical to that obtained from normal animals, it has to be realized that such measurements at the air–water interface are not necessarily applicable to natural membranes. On the other hand, the work of VAN DORP *et al.*[29] and BERGSTRÖM, DANIELSSON AND SAMUELSSON[30] on the biosynthesis of prostaglandins demonstrated that apart from a possible structural function in membranes the essential fatty acids also have other functions. A particular function of arachidonate in membrane phospholipids cannot be expected to be deducible from a simple lipid monolayer at the air–water interface. The present experiments demonstrate only that the liquid–crystalline state of the total lecithin from rat liver is preserved in essential fatty acid deficiency and that this result can be accounted for by the composition of the lecithin in terms of its molecular species.

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