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The lipid composition of brain and serum of young rats in relation to age and diet

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With 1 figure in 6 details and 5 tables

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As a consequence of the possible relationship between low intake of essential fatty acids and diseases such as multiple sclerosis (1, 2) and atherosclerosis (3), several reports have appeared on metabolism of the polyunsaturated fatty acids (4, 5, 6, 7). However, little is known about the effect of the intake of so-called non-essential fatty acids such as stearic and oleic acid, apart from their function as competitive inhibitors for the conversion of linoleic and linolenic acid to the longer chain fatty acids of the *w*6- and *w*3- series (8, 9).

In our laboratory, we have previously studied the fatty acid composition of the human foetal brain and found that the content of palmitic acid was higher and oleic acid lower as a percentage of the total fatty acids than the corresponding values for the adult brain (10). As a result of this observation we decided to study the change in fatty acid composition with age in the brain of rats receiving a fat-free diet compared to rats receiving the same diet supplemented with stearic, oleic and linoleic acid respectively. In addition, two groups of rats were raised on fat-free diets supplemented with cholesterol and lauric acid. The latter group was included because lauric acid is a main lipid constituent of many commercial baby food products (11).

Materials and methods

1. Diet.

Six groups, each consisting of 3 pregnant Wistar rats, were given the following diets 1–2 weeks before delivery:

A: Basal diet
B: Basal diet containing 5% stearic acid (w/w)
C: Basal diet containing 5% oleic acid (w/w)
D: Basal diet containing 5% linoleic acid (w/w)
E: Basal diet containing 5% lauric acid (w/w)
F: Basal diet containing 5% cholesterol (w/w)

where the basal diet had the following composition:

Casein (low vitamin)[1])	25.0% w/w
Sucrose	63.1%
Salt mixture[2])	0.6%
Vitamin mixture[3])	0.6%

The litters were kept with the mothers for the first 3 weeks after delivery, whereafter they were transferred to cages with screen bottoms. 1–2 animals from each litter were killed by decapitation at two week intervals for up to 3 months. Blood from the aorta was collected in capillary tubes. After coagulation, the serum was isolated by centrifugation of the sealed tubes. The brains were removed and all the samples were stored at −20° until analysis.

2. Lipid extraction, isolation and determinations.

The lipids were extracted from brain and serum with $CHCl_3$—CH_3OH (2 : 1) and washed three times with the theoretical upper phase (12). Phospholipids of brain tissue were separated and isolated by thin-layer chromatography in $CHCl_3$—CH_3OH-25% NH_3 (70 : 30 : 5) (13). 0.25 mm layer of Kieselgel H was used for estimation of phospholipids according to the method of PARKER et al. (14), and 0.5 mm layers of Kieselgel G for isolation of polar lipid zones for fatty acid analysis. The individual phopholipid fractions were visualized by 1 min exposure to iodine vapour. The fractions isolated were sphingomyelin (SF), phosphatidylserine (PS), phosphatidylcholine (PC), and phosphatidylethanolamine (PE). No attempt was made to separate phosphatidyl from phosphatidal glycerides.

3. Gas Chromatography.

Methanolysis was performed in sealed test tubes at 90° for 2 hours with 5% HCl in CH_3OH according to standard methods (15). The methylesters were dissolved in $CHCl_3$ and analysed by gas chromatography in a Perkin-Elmer Gas Chromatograph (model F7 or F11) using 2 m columns of diethyleneglycolsuccinate (15% on chromosorb W) or Apiezon L. The polyester columns were run at 170° or 190° and the Apiezon columns at 220° with N_2 as the carrier gas. The methylesters were identified by comparison with standard mixtures and when such were not available with the retention volumes as reported by JAMES (16) and by log-plotting. The last peaks appearing on the chromatograms included in the analysis were C 22 : 6 or C 24 : 1. The analytical error in the chromatographic analyses ranged from 5% for C 16 : 0 to 30% for C 22 : 6.

4. Chemicals.

Column packing materials were supplied by Applied Science Labs. (Pennsylvania) and reference mixtures for chromatography by the Hormel Institute (Minnesota) and Analytical Standards (Gothenburg). All other chemicals used were of the highest available purity from either Messrs. Merck or the British Drug House.

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[1]) Fat-free (below 0.02% fat; extracted with CH_2Cl_2; polyenoic acids below 0.001% of total diet).

[2]) No. 2 US Pharmacopoiea XIII.

[3]) DL-Methionine 12 g, Vitamin A 150.000 i. u., Vitamin D_3 5000 i. u., Vitamin K (Menadione) 100 mg, α-tocopherol 1500 mg, Thiamine B_1 200 mg, Riboflavin 200 mg, Vitamin B_6 150 mg, Vitamin B_{12} 0.3 mg, Pantothenic acid (Ca) 500 mg, Niacin 500 mg, Choline HCl 10 g, Folic acid 100 mg, Biotin 3 mg, p-aminobenzoic acid 1 g, Inositol 1 g, Ascorbic acid 200 mg.

Results and Discussion

Tables 1–5 show the fatty acid composition of total brain, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and serum as a function of age and diet. It was found convenient to mention only the major fatty acids (above 1% of total fatty acids) undergoing significant changes during the experimental period.

As seen from Table 1 the majority of the groups show a fairly uniform pattern with a decrease in palmitic acid (C 16:0) between the second and the fourth week in all groups except group C, where C 16:0 remains fairly constant during the experiment. Among the remaining components shown, stearic acid (C 18:0) increases slightly, oleic acid (C 18:1) increases markedly, whereas arachidonic acid (C 20:4) decreases remarkably between the second and fourth week – thereafter remaining constant, and docosahexaenoic acid (C 22:6) decreases slowly in most groups. Eicosatrienoic acid (C 20:3), which is normally considered as an indicator of essential fatty acid deficiency, only appears in groups A, E, and F towards the end of the experiment.

Also in the phospholipid fractions, the greatest change in fatty acid composition occurs between the second and fourth week. The change in fatty acid composition in the PC fractions (Table 2) is almost the same in all groups. Two features which should be noted are the higher C 18:1 content in group C in the 8th week, and the fatty acid pattern in groups B and F. In these groups a decrease in C 16:0 and an increase in C 18:0 and C 18:1 is seen around the 8th week, followed by a reversal – an increase in the former and decrease in the latter – during the following weeks. At present we cannot find an explanation for this, but as this feature was observed in all the individual animals analysed[1]), we must consider the finding a significant one.

In the PE fractions (Table 3) we find a decrease in C 18:0 and an increase in C 18:1. This was most pronounced in group B. Small amounts of C 20:3 occur towards the end of the experiment, and C 20:4 and C 22:6 decline gradually in most groups. C 22:6 appears fairly constant, not only in the linoleic acid group (D), but also in groups A and C. In group C this finding might, however, possibly be attributed to polyunsaturated products formed by chain-elongation of C 18:1 and C 20:3 *w*9.

In the phosphatidylserine (PS) fraction (Table 4), the predominant feature is the decrease in C 18:0 and increase in C 18:1. As in the other phospholipid fractions, we find a slow decline in C 20:4 and in all groups except group C, a more rapid decline in C 22:6. In group B, around the 8th week we see a marked decrease in C 18:0, followed by a rise during the following weeks, as a parallel to the results for the PC fractions.

The difference between group D and the EFA-deficient groups is seen in Table 5. The oleic acid levels in group D remain lower than in the other groups and the linoleic acid (C 18:2) level, although declining during the experimental period, remains much higher than in the other groups. It is surprising that after 2 weeks C 20:4 is lower in group D than in the other groups, but on the other hand, the C 18:2 level is so high that the total amount of polyunsaturated

[1]) The ranges for C 16:0, C 18:0, and C 18:1 were 32.4–35.6, 19.0–20.2, and 35.6—38,1 for 4 animals in group B (8th week).

Table 1. The major fatty acids of the whole rat brain as a function of age. (% of total fatty acids)

Diet		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Group A	C 16 : 0	34.2	23.2	22.8	22.9	24.5	
	16 : 1	5.9	2.5	2.5	2.1	2.3	
− PUFA	18 : 0	20.4	26.7	26.6	25.7	23.9	
	18 : 1	26.2	23.6	24.5	24.6	23.4	
	20 : 3					1.1	
	20 : 4	6.6	8.4	6.9	6.7	7.7	
	22 : 6	6.0	5.7	4.3	1.9	1.9	
Group B	C 16 : 0	31.2	24.5	20.9	17.0	22.6	21.7
	16 : 1	2.3	2.5	2.3	2.1	2.5	3.0
− PUFA	18 : 0	19.0	21.4	24.9	22.7	21.2	21.4
	18 : 1	18.8	22.7	23.0	24.3	25.5	26.7
+ 5% stearic acid	20 : 3						
	20 : 4	10.6	9.2	8.1	7.5	8.5	7.2
	22 : 6	7.3	7.7	7.5	7.2	7.1	7.6
Group C	C 16 : 0	24.0	23.2	23.8	22.5	25.0	21.5
	16 : 1	3.0	2.6	2.5	2.7	2.5	1.4
− PUFA	18 : 0	17.0	27.1	23.4	23.1	23.3	23.1
	18 : 1	13.8	21.3	22.5	24.4	24.6	22.0
+ 5% oleic acid	20 : 3						
	20 : 4	11.0	5.4	6.9	7.9	7.0	7.9
	22 : 6	5.3	2.2	3.9	4.6	4.6	4.9
Group D	C 16 : 0	30.8	24.9	21.5	18.0	20.2	20.1
	16 : 1	2.5	2.5	1.9	1.3	1.4	1.8
− PUFA	18 : 0	19.2	21.3	20.4	19.5	20.7	21.9
	18 : 1	17.6	22.9	26.8	25.0	27.1	26.0
+ 5% linoleic acid	20 : 3						
	20 : 4	10.4	9.9	9.5	9.4	9.6	7.7
	22 : 6	6.9	5.3	6.9	7.4	6.7	7.1
Group E	C 16 : 0	38.7	23.7	22.4	20.1	20.0	
	16 : 1	2.3	2.4	3.0	2.9	3.1	
− PUFA	18 : 0	20.7	22.0	23.4	26.2	22.0	
	18 : 1	17.2	23.4	28.4	27.2	30.1	
+ 5% lauric acid	20 : 3					2.2	
	20 : 4	10.6	10.4	8.4	8.9	7.1	
	22 : 6	6.8	5.6	5.1	5.4	4.0	
Group F	C 16 : 0		23.4	20.4	21.3	22.7	23.5
	16 : 1		2.9	2.7	2.9		
− PUFA	18 : 0		22.5	23.1	23.4	22.8	24.3
	18 : 1		21.3	24.6	24.3	26.7	25.9
+ 5% cholesterol	20 : 3				1.0	2.0	2.6
	20 : 4		9.8	8.4	8.2	7.8	9.2
	22 : 6		7.1	5.9	6.4	4.3	2.7

Table 2. The major fatty acids of phosphatidylcholine as a function of age. (% of total fatty acids)

Diet		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Group A	C 16 : 0	56.3	50.3	45.2	45.0	44.3	
	16 : 1	4.8	4.5	3.2	3.2	4.1	
	18 : 0	10.2	11.1	15.5	14.8	14.1	
− PUFA	18 : 1	23.9	31.7	33.2	32.2	34.7	
	20 : 3						
	20 : 4	3.4	2.3	3.3	1.7	1.7	
	22 : 6						
Group B	C 16 : 0	63.1	55.9	49.4	33.9	45.4	46.2
	16 : 1	3.8	2.8	2.2	2.2	4.2	2.2
− PUFA	18 : 0	6.6	16.9	18.0	19.8	12.5	14.7
	18 : 1	17.0	26.0	32.9	37.6	31.3	30.0
+ 5% stearic acid	20 : 3						
	20 : 4	4.3	2.3	2.4	2.5	2.4	1.2
	22 : 6						
Group C	C 16 : 0	60.9	50.4	47.0	39.4		
	16 : 1	4.2	4.8	3.8	2.7		
− PUFA	18 : 0	9.2	13.7	13.7	12.6		
	18 : 1	19.5	28.0	32.8	44.1		
+ 5% oleic acid	20 : 3						
	20 : 4	3.2	2.3				
	22 : 6						
Group D	C 16 : 0	55.0	47.4	47.2	43.4	42.3	45.3
	16 : 1	3.2	3.1	2.5	2.5	2.5	3.7
− PUFA	18 : 0	7.8	10.5	11.9	13.5	14.4	14.0
	18 : 1	25.0	27.6	30.5	35.4	30.8	32.1
+ 5% linoleic acid	20 : 3						
	20 : 4		0.8	1.8	2.2	3.6	1.3
	22 : 6						
Group E	C 16 : 0	59.0	44.4	47.0	47.3	46.3	
	16 : 1	6.2	2.6	1.9	2.5	2.3	
+ 5% lauric acid	18 : 0	6.8	13.7	17.6	14.0	16.0	
	18 : 1	20.3	30.2	33.4	31.4	32.3	
	20 : 3						
	20 : 4	1.5	1.9	1.2	1.7	1.7	
	22 : 6						
Group F	C 16 : 0	55.1	44.7	37.9	37.5	50.5	52.1
	16 : 1	4.6	3.9	2.7	2.5	1.1	
− PUFA	18 : 0	9.4	12.8	16.8	15.3	14.5	14.7
+ 5% cholesterol	18 : 1	24.8	28.3	36.4	36.7	29.8	28.1
	20 : 3						
	20 : 4	3.4	2.0	2.7	2.1	1.3	1.3
	22 : 6						

Table 3. The major fatty acids of phosphatidylethanolamine. (% of total fatty acids)

Diet		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Group A	C 16:0	14.8	13.4	11.4	8.9	7.8	
	16:1	5.3	3.5	3.3	3.4	2.6	
− PUFA	18:0	23.9	23.6	22.4	21.9	20.0	
	18:1	15.1	20.1	20.2	21.5	21.1	
	20:3						
	20:4	16.5	13.5	10.2	11.1	11.7	
	22:6	12.4	12.1	12.4	12.0	13.2	
Group B	C 16:0	14.9	12.0	9.3	8.5	14.4	13.7
	16:1	1.9	1.8	1.5	2.3	2.4	3.6
− PUFA	18:0	27.2	25.6	26.1	22.4	20.0	20.1
	18:1	19.1	19.4	23.7	23.0	38.2	37.3
+ 5% stearic acid	20:3		1.3	1.3	2.9	4.9	2.5
	20:4	12.9	12.2	10.9	10.5	11.6	8.3
	22:6	13.5	11.7	9.2	7.1	4.2	4.6
Group C	C 16:0	17.0	11.2	9.0	8.7	8.1	8.1
	16:1	2.8	2.6	2.5	2.5	1.5	1.9
− PUFA	18:0	28.2	25.7	23.0	22.9	22.0	24.5
	18:1	17.6	20.4	22.1	24.4	24.5	22.3
+ 5% oleic acid	20:3					0.8	
	20:4	15.0	12.4	11.6	11.2	13.8	9.9
	22:6	11.0	11.8	13.4	14.2	11.9	12.2
Group D	C 16:0	15.5	13.2	9.6	9.1	10.4	11.5
	16:1	1.6	1.8	2.0	2.5	2.4	1.8
− PUFA	18:0	26.0	24.9	22.5	22.3	22.1	23.9
	18:1	15.8	21.7	24.2	23.6	27.0	26.4
+ 5% linoleic acid	20:3						
	20:4	12.4	10.5	11.0	9.0	10.4	8.8
	22:6	9.3	10.3	12.4	11.3	13.7	10.9
Group E	C 16:0	15.0	11.3	11.1	12.9	14.5	14.1
	16:1	4.3	3.7	4.7	2.7	2.7	3.4
− PUFA	18:0	29.7	23.2	24.2	28.4	21.5	21.3
	18:1	12.6	18.3	30.9	32.0	29.3	29.3
+ 5% lauric acid	20:3						1.7
	20:4	17.3	12.6	9.4	5.5	9.1	9.4
	22:6	10.6	10.4	10.8	6.4	3.7	3.2
Group F	C 16:0	13.4	12.7	12.7	11.6	13.4	14.6
	16:1	2.0	1.6	1.6	2.4	3.1	2.1
− PUFA	18:0	28.1	20.0	21.8	22.5	21.0	23.7
	18:1	16.4	18.2	24.6	23.0	26.1	27.8
+ 5% cholesterol	20:3			1.0	2.1	3.4	2.4
	20:4	16.6	13.2	8.4	8.5	8.4	7.2
	22:6	14.6	15.4	6.6	4.1	4.3	4.0

Table 4. The major fatty acids of phosphatidylserine.
(% of total fatty acids)

Diet		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Group A	C 16 : 0	5.2	5.5	6.6	8.1	8.8	
	16 : 1		0.7				
	18 : 0	54.4	47.6	48.7	42.0	42.4	
− PUFA	18 : 1	11.4	18.4	23.3	27.4	30.2	
	20 : 3						
	20 : 4	9.7	8.2	5.9	4.5	4.1	
	22 : 6	10.6	9.2	8.7	4.9	4.2	
Group B	C 16 : 0	7.1	6.7	6.6	8.3	5.0	5.5
	16 : 1						
− PUFA	18 : 0	55.9	53.9	52.9	34.2	49.5	48.4
	18 : 1	14.5	21.6	28.2	32.5	31.5	27.9
+ 5% stearic acid	20 : 3						
	20 : 4	9.5	8.1	7.0	5.9	4.6	4.3
	22 : 6	8.2	8.4	4.1	4.7	2.6	2.2
Group C	C 16 : 0	6.7	6.1	6.3	5.6	5.7	
	16 : 1						
− PUFA	18 : 0	51.0	49.2	47.6	46.7	46.5	
	18 : 1	11.0	15.6	18.0	23.0	24.0	
+ 5% oleic acid	20 : 3						
	20 : 4	8.4	7.4	7.0	5.9	5.7	
	22 : 6	9.7	10.3	10.5	7.9	6.3	
Group D	C 16 : 0	7.3	6.2	6.0	5.4	5.1	6.1
	16 : 1						
− PUFA	18 : 0	58.5	54.3	50.2	46.9	49.4	42.6
	18 : 1	13.7	17.2	29.8	32.9	33.0	35.0
+ 5% linoleic acid	20 : 3						
	20 : 4	8.7	7.7	7.2	6.0	7.6	5.2
	22 : 6	3.7	6.2	6.5	7.2	7.4	6.7
Group E	C 16 : 0	9.4	10.5	9.3	10.2	7.7	5.0
	16 : 1						
− PUFA	18 : 0	55.1	45.8	48.5	42.6	41.1	45.5
	18 : 1	8.1	25.7	27.8	31.0	34.7	37.9
+ 5% lauric acid	20 : 3					3.6	0.4
	20 : 4	11.7	4.4	3.6	4.9	1.9	1.7
	22 : 6	7.8	6.5	4.0	5.6	1.5	2.8
Group F	C 16 : 0	6.9	9.7	6.7	6.8	6.0	
	16 : 1						
− PUFA	18 : 0	59.8	54.3	52.6	44.0	45.0	
	18 : 1	14.7	20.8	33.1	35.9	33.3	
+ 5% cholesterol	20 : 3					1.0	
	20 : 4	6.6	3.9	3.9	2.7	2.7	
	22 : 6	12.8	4.6	1.8	2.1	3.6	

Table 5. The major fatty acids of serum.
(% of total fatty acids)

Diet		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Group A	C 16 : 0	28.1	33.2	31.8	32.1	26.6	
	16 : 1	13.6	3.0	6.7	7.8	8.6	
	18 : 0	5.4	2.5	11.6	10.6	7.7	
− PUFA	18 : 1	34.0	38.1	38.0	38.2	33.2	
	18 : 2	12.4	8.7	6.9	4.2	4.0	
	20 : 3		0.5	2.7	2.3	3.2	
	20 : 4	2.6	3.2		4.6	5.2	
Group B	C 16 : 0	26.4	25.4	23.5	17.0	21.4	22.1
	16 : 1	6.3	7.0	8.1	12.4	9.8	10.9
− PUFA	18 : 0	9.7	12.9	14.3	14.9	10.2	11.1
	18 : 1	31.8	33.2	35.4	36.3	38.9	41.6
+ 5% stearic acid	18 : 2	7.0	4.7	3.1	3.9	4.5	3.9
	20 : 3		5.8	4.8	3.7	4.0	3.5
	20 : 4	8.5	4.0	2.6	4.6	4.7	4.0
Group C	C 16 : 0	23.7	23.7	24.9	24.5	25.6	24.5
	16 : 1	5.5	5.3	7.6	5.3	5.8	5.3
− PUFA	18 : 0	14.2	13.7	16.7	14.8	15.3	14.6
	18 : 1	36.7	31.3	31.3	34.0	36.8	37.5
+ 5% oleic acid	18 : 2	7.2	7.8	6.8	6.5	6.5	5.1
	20 : 3						1.1
	20 : 4	11.3	4.9	4.2	3.6	3.0	4.1
Group D	C 16 : 0	23.9	22.1	25.0	27.2	24.1	
	16 : 1	3.8	5.1	7.8	7.1	6.3	
− PUFA	18 : 0	2.2	13.9	11.5	10.8	10.9	
	18 : 1	24.2	27.9	27.9	30.4	29.6	
+ 5% linoleic acid	18 : 2	20.6	17.6	16.1	14.0	14.1	
	20 : 3						
	20 : 4	4.9	2.8	4.1	5.1	6.0	
Group E	C 16 : 0	25.4	23.8	24.1	21.5	23.1	23.4
	16 : 1	3.1	7.0	6.5	9.3	7.9	9.8
− PUFA	18 : 0	11.4	11.3	13.7	13.4	11.5	12.6
	18 : 1	21.6	25.4	35.1	34.9	37.1	37.7
+ 5% lauric acid	18 : 2	8.3	8.9	5.6	4.1	5.1	5.5
	20 : 3					1.2	
	20 : 4	10.4	2.9	5.4	6.1	4.9	4.5
Group F	C 16 : 0	32.2	21.4	21.5	22.1	24.0	23.5
	16 : 1	5.7	8.1	8.2	10.9	10.4	10.6
− PUFA	18 : 0	9.3	8.4	8.6	10.1	9.3	11.2
	18 : 1	26.8	29.8	34.8	41.4	42.1	38.8
+ 5% cholesterol	18 : 2	9.5	6.0	6.2	5.2	5.4	4.5
	20 : 3						
	20 : 4	8.0	4.4	5.2	4.4	6.6	5.4

fatty acids is well above the total PUFA in the other groups. Palmitoleic acid (C 16:1) increases with age for most of the groups, but remains fairly constant in groups B and C, receiving stearic and oleic acid respectively. The latter observation could possibly be explained as inhibition of biosynthesis of fatty acids of intermediate chain length by the most common biological end product of this synthesis (17). C 20:3 appears in some of the groups after some weeks on the experimental diets (most marked in group B).

The changes in the individual phospholipids as percentage of the total phospholipids show an increase in phosphatidylethanolamine and sphingomyelin, and a decrease in phosphatidylcholine (Fig. 1) with age; this pattern

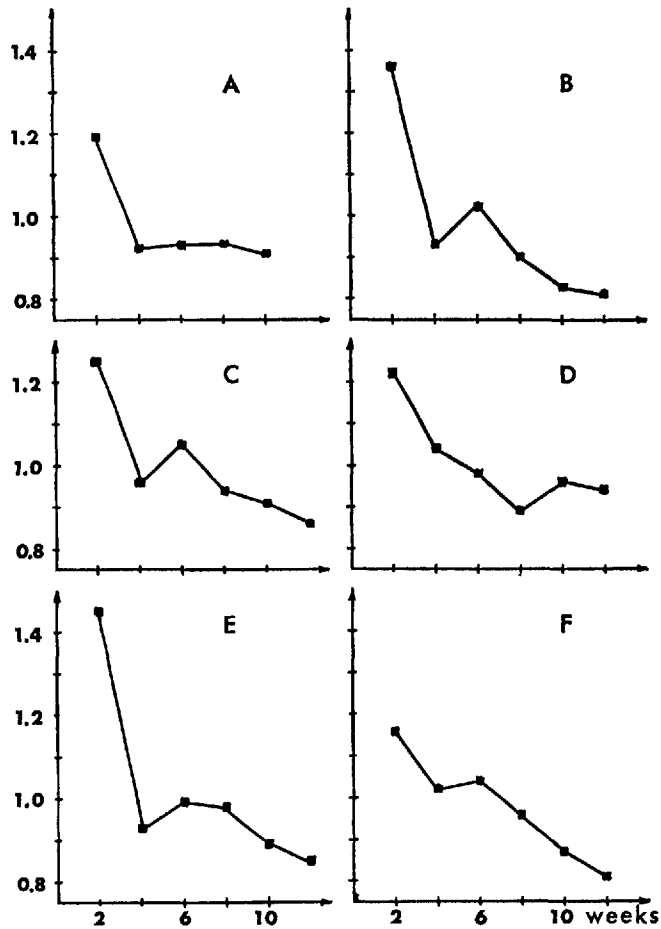

Fig. 1. Phosphatidylcholine/Phosphatidylethanolamine (PC/PE) ratio in the brain of young rats as a function of age.

A: Polyunsaturated fatty acids (PUFA)
B: PUFA + 5% stearic acid
C: PUFA + 5% oleic acid
D: PUFA + 5% linoleic acid
E: PUFA + 5% lauric acid
F: PUFA + 5% cholesterol.

agrees with the observation made by other authors in experiments with young rats (18, 19).

In conclusion, our findings give the overall impression that the fatty acid composition of the brain tissue appears to be rather independent of the diet, in spite of the differences in PUFA-content between the serum of rats receiving a dietary supply of linoleic acid and EFA-deficient rats. This is in agreement with the assumption that brain tissue is less influenced by diet than most other tissues, but on the other hand, RATHBONE (19) has reported differences in brain fatty acid composition of EFA-deficient rats and rats fed linoleic acid (in the form of safflower oil). In our experiment, one would have expected such changes to occur since the experimental period covers both the foetal state and the neonatal period, which is characterized by rapid myelin formation from the 10th to the 20th day (20). The only differences we find among the various groups, however, are seen in C 22:6, and since the analytical error involved in the analysis of long chain fatty is considerable, qualitatively (21) as well as quantitatively (22), we would not like to draw definite conclusions from our results in this respect. We would, however, like to draw attention to the fact that the most rapid decline in polyunsaturated fatty acids was found in the PE- and PS- fractions of the cholesterol group (F) – even at a time when the concentrations of these acids in total brain lipid were identical with the values found in the other groups. These observations indicate that the cholesterol preferentially forms esters with the long-chain unsaturated fatty acids, and consequently drains other lipid fractions of these constituents.

The only example of direct diet dependence among our results seems to be in the lecithin figures from the 8th neonatal week; here we find a high content of stearic acid in group B and a high oleic acid content in group C. This is in accordance with the observation made by ENG & SMITH (22), who found a significantly shorter half-life for brain-lecithin from young rats than for the other phospholipid fractions examined here. It is strange, however, that the following weeks show a decrease in PC–C 18:0 in group B.

Apparent EFA-deficiency symptoms, as can be judged physically by reduced growth and appearance of skin lesions, and biochemically by presence of C 20:3, seemed to be absent in most groups during most of the experimental period. Dermatitis of the tail started to appear in some animals after the 10th week, but as far as growth is concerned, the EFA-deficient groups grew faster than the rats in group D. A possible explanation for this is that the litters were not absolutely EFA-deficient as long as they suckled their mothers; furthermore, OSTWALD et al. (24) reported that deficiency symptoms did not appear in rats during the first 4–6 weeks on an EFA-deficient diet – which would mean that deficiency symptoms should begin to occur only towards the very end of our experiment. It should also be borne in mind that skin lesions only develop slowly under humid conditions, according to observations made by AAES-JØRGENSEN (27), and therefore might have been delayed by the high humidity in our animal house. As an attempt to explain the late occurrence of eicosatrienoic acid, one might suggest that this be due to immaturity of the enzyme system converting C 18:1 to C 20:3 during the first weeks of life. We hope to investigate this problem further in future experiments. That, on the other hand the animals did not grow faster on the

linoleic acid diet might be explained by the data of PUDELKEWICS et al. (25) who found that dietary linoleic acid only seemed to be beneficial within a very narrow range, and that the growth rate in rats was reduced when the linoleic acid concentration exceeded 1.32% of the total calories. In our experiment the linoleic acid constituted 8% of the total calories, and we may therefore have approached a state corresponding to a hypervitaminosis.

The conclusion to be drawn from our results must be that the fatty acid composition of brain tissue in the rat, even during the period of rapid myelination, is influenced very little by the presence or absence of dietary supply of linoleic acid as long as the animals suckle their mothers during the first weeks of life. This is the case even when the mothers themselves are kept on EFA-deficient diets. In all groups we find a much more pronounced age- than diet-dependence. In this connection, attention should be drawn to the increasing C 18:1 concentration in all brain fractions in all the groups. This increase parallels what we (10) and others (26) have found for the human brain. Of course an increasing C 18:1 concentration can always be regarded as a sign of EFA-deficiency, but judging from our results this deficiency should then occur within the brain and be independent of the dietary influenced serum-concentration of linoleic acid.

In order to elucidate the problem regarding a possible formation of an EFA-deficient brain, our studies should be followed up by a study of rats fed on definitely EFA-deficient diets during the period from birth to the 10th day, when rapid myelination is supposed to set in. For this purpose it would, however, be necessary to isolate and feed the young rats by stomach tube immediately after birth – or at least from the age of 3–4 days – which would be rather difficult from a practical point of view.

Summary

Young rats were raised on diets containing stearic acid, oleic acid, linoleic acid, lauric acid and cholesterol, respectively. The fatty acid and phospholipid composition of brain and serum were examined during the first three months of life.

Serum from the animals fed on a linoleic acid-containing diet had a higher content of linoleic acid and a lower content of oleic acid than serum from animals fed on essential fatty acid-deficient diets.

Whole brain fatty acids as well as the fatty acids in the individual phospholipid fractions showed only minor differences among the different dietary groups. A direct relationship to the fat component of the diet was found only in the phosphatidylcholine fraction from animals fed on stearic acid and oleic acid respectively, as they had the highest content of stearic acid and oleic acid, respectively, in their phosphatidylcholine fractions.

In most of the groups, the brain content of the polyunsaturated fatty acids declined during the experiment – docosahexaenoic acid faster than arachidonic acid. The content of polyunsaturated fatty acids in the phosphatidylethanolamine and phosphatidylserine fractions declined faster in the groups receiving cholesterol than in any of the other groups.

The brain content of palmitic, stearic and oleic acid showed a pattern related to age rather than to diet, with an increase in oleic acid and a decrease in palmitic acid and/or stearic acid depending on the individual brain lipid fraction. The increase of the phosphatidylethanolamine/phosphatidylcholine ratio with age also seemed to follow a fixed pattern, independent of the diet.

Zusammenfassung

Junge Ratten erhielten Nahrungsmischungen, die entweder Stearinsäure, Ölsäure, Linolsäure, Laurinsäure oder Cholesterin als Fettkomponente hatten. Die Fettsäuren- und Phosphatidzusammensetzung im Gehirn und Serum wurden während der ersten drei Lebensmonate untersucht.

Serum von den Ratten, die Linolsäure erhalten hatten, zeigte höhere Linolsäureprozente und niedrigere Ölsäureprozente als Serum von den Ratten der anderen Gruppen.

Die Fettsäurezusammensetzung sowohl im Gesamtgehirn als in der isolierten Phosphatidfraktionen zeigte nur kleinere Unterschiede zwischen den einzelnen Gruppen. Nur in den Lecithinfraktionen von Ratten, die Stearinsäure oder Ölsäure erhalten hatten, konnte eine direkte Beziehung zu der Nahrung erwiesen werden, da diese Ratten eine höhere Stearinsäure- bzw. Ölsäurekonzentration in ihrem Gehirn-Lecithin als Ratten aus den anderen Gruppen hatten.

Die Konzentration der mehrfach ungesättigten Fettsäuren im Gehirn wurde während des Versuches erniedrigt – C 22 : 6 schneller als C 20 : 4. Der Gehalt an mehrfach ungesättigten Fettsäuren in den Colaminphosphatid- und Serinphosphatid-Fraktionen fiel am schnellsten in den Ratten, die Cholesterin erhalten hatten.

Der Gehalt an Palmitin-, Stearin- und Ölsäure im Gehirn zeigte einen Verlauf, der mehr vom Alter als von der Nahrung bestimmt war. Es wurde eine prozentuale Steigerung in der Ölsäurekonzentration und einen Fall in der Palmitin- und/oder Stearinsäurekonzentration beobachtet. Auch die Steigerung in das Colaminphosphatid/Lecithin-Verhältnis erwies sich als unabhängig von der Nahrung, vom Alter dagegen abhängig.

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