Fatty acids of glycerophosphatides in developing chick embryonic brain and liver

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ABSTRACT Fatty acid compositions of glycerophosphatides of developing chick embryonic brain and liver were compared. In brain, ethanolamine and serine glycerophosphatides contained 30–40% polyunsaturated fatty acids, lecithin almost none (except for arachidonic). In the liver, these acids were equally distributed in the phospholipid fractions. The principal polyunsaturated fatty acids of the ethanolamine and serine glycerophosphatides in brain, liver, and yolk were 22:6, 20:4, and 18:2, respectively.

During embryonic development of brain from the 8th day of incubation to hatching, the fatty acid composition of individual glycerophosphatide fractions remained constant. Because of the relative increase of ethanolamine glycerophosphatides and decrease of lecithin, total glycerophosphatides showed a decrease in 16:0 and an increase in 18:0. Substantial amounts of palmitaldehyde and stearaldehyde were present on the 8th day of incubation in the brain ethanolamine glycerophosphatide fraction.

During the 3rd week of incubation, the liver showed a twofold increase in the relative amount of 18:2 in all glycerophosphatide fractions. A decrease of 16:0 in the lecithin fraction and consequently in total glycerophosphatides was also observed during this period.

No significant changes in glycerophosphatide fatty acids were observed in the yolk throughout incubation.

KEY WORDS chick embryo · brain · liver · yolk · development · glycerophosphatides · fatty acid composition · polyunsaturated · free · fatty acids · plasmalogens

Most chemical studies on the developing brain have been confined to mammals in which changes of lipids, enzymes, etc., have been reported to occur during the postnatal myelination (1, 2). In the chick it has been noted that an active increase of sphingolipids begins about the 17th or 18th day of embryonic development (3, 4). Postnatal changes in fatty acid composition have been reported in developing rat brain (5) and human brain (6). In mammalian brain (as in other organs) the glycerophosphatides, which are major components of biologically active membranes, contain large amounts of long-chain (C_{20} and C_{22}) polyunsaturated fatty acids (PUFA) (7–9). In brain the sphingolipids, which are stable components of membranous structures, e.g. myelin, contain very low amounts of PUFA (10, 8).

Since the lipid content of the diet influences the fatty acid composition of the brain (11) and liver (11, 12), the advantage of studying the chick embryo, which has the yolk as its sole nutrient source, is evident. Yolk cholesterol (13) and triglyceride (14) have been reported to be transported directly into the embryo. Yolk phospholipids may be hydrolyzed during their transport (14), but recently Siek and Newburgh (15) have reported that a part of phospholipids in the embryonic brain may be present there as a result of the transport of the intact molecule.

This report correlates brain development with the incorporation of fatty acids, especially PUFA, into glycerophosphatide fractions of the brain of the chick embryo. Comparative studies of embryonic liver and brain were performed at appropriate intervals to provide a basis for further studies, now in progress, for elucidating the biosynthetic pathway of PUFA in the chick embryo.

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Abbreviations: PUFA, polyunsaturated fatty acids; EGP, ethanolamine glycerophosphatides; SGP, serine glycerophosphatides; DMA, dimethyl acetal; GLC, gas–liquid chromatography; TLC, thin-layer chromatography. Fatty acids are designated number of carbon atoms: number of double bonds.

METHODS AND MATERIALS

Fertile eggs from White Leghorns (L. Sharp, Glen Ellyn, Ill.), fed Egg Popper (Honeggers' & Co., Inc., Fairbury, Ill.), were incubated under conditions of controlled temperature (38°C) and humidity (about 90%). The brain, excluding the cerebellum, and the liver, excluding the gall bladder, were analyzed after removal of excess fluid by blotting on a glass plate. Adult fowls were sacrificed immediately after they were purchased and also after they had been fed a Rockland mouse diet[1] (A. E. Staley Mfg. Co., Decatur, Ill.) for at least 1 week in the laboratory. About 0.3–1.0 g wet weight of each tissue was required for analyses. The homogenate was lyophilized in a lyophilizer purchased from the VirTis Co., Inc., Gardiner, N.Y.

Lipid Extraction and Silicic Acid Column Chromatography

For analysis of individual glycerophosphatides, fresh tissues were homogenized in 15 volumes of cold acetone (w/v), allowed to stand at 4°C for 3 hr, and filtered through a sintered glass filter under suction. The acetone solution was used for the analysis of free fatty acids. The residue was extracted at 4°C overnight under nitrogen with its own volume of petroleum ether (bp 40–60°C)–absolute ethanol 3:1 (16), and the extract was evaporated at 40°C under a reduced pressure of nitrogen to yield the crude phospholipid fraction.

Further purification was carried out by silicic acid column chromatography, basically as described by Hanahan, Dittmer, and Warashina (17), on a short column, 1.0 × 30 cm, with a mixture of 5 g of silicic acid and 2.5 g of Hyflo Super-Cel. Neutral lipids were removed by elution with 100 ml of chloroform; ethanolamine glycerophosphatides (EGP) were eluted with 75 ml of methanol–chloroform 1:4, and a crude lecithin fraction with 70 ml of methanol–chloroform 4:1. Suction was applied to the bottom of the column during the elution. Serine glycerophosphatides (SGP) were separated from lecithin in the last fraction by TLC. No difference in the unsaturated fatty acid composition was observed among the extracts, with or without α-tocopherol added as an antioxidant.

For analysis of the *total* glycerophosphatides, fresh tissue (about 300 mg) was homogenized in 4 volumes (w/v) of saline and the crude phospholipids were extracted by a modified procedure of Bligh and Dyer (18), as follows: 1 part of the homogenate was extracted with 3 parts of chloroform 1:2 (v/v) and then 1 part of chloroform was added, the mixture being homogenized after each addition. The biphasic mixture obtained was allowed to stand at 4°C overnight and the lower layer was pipetted off, evaporated under reduced pressure at 40°C in a stream of nitrogen, and redissolved in petroleum ether. Total glycerophosphatides were obtained by elution with 70 ml of methanol–chloroform 4:1 from the silicic acid columns described above, after elution of neutral lipids with chloroform. Sphingomyelin remained on the column.

Petroleum ether–ethanol extraction avoided the formation of a petroleum ether-insoluble film which is found in chloroform–methanol extracts. However, chloroform–methanol was a more efficient solvent for extracting total phospholipids, as judged by the recovery of lipid phosphorus.

A longer column, 1.6 × 60 cm, was also used (reference 16) to verify the separations obtained by the standard procedures that employed the short column and TLC. The EGP obtained by the smaller column and the lecithin fraction obtained by TLC showed fatty acid compositions similar to those of the corresponding fractions obtained from the longer column.

Extraction of Free Fatty Acids

The acetone extract was evaporated under reduced pressure at 40°C under a stream of nitrogen which left some residual water; and this mixture was extracted three times with 3-ml portions of petroleum ether. Free fatty acids were extracted from the petroleum ether layer with 0.2 volume of 0.05 N aqueous KOH. The aqueous KOH layer was washed with petroleum ether and acidified with HCl and the fatty acids were reextracted with petroleum ether (19).

Thin-Layer Chromatography

Methods used for TLC were basically those of Wagner, Hörhammer, and Wolff (20). Silica Gel G or H (Brinkmann Instruments Inc., Westbury, N.Y.) was used at a thickness of 0.25 mm. Diisobutyl ketone–acetic acid–0.1 M $CaCl_2$ 40:25:5 was routinely used as developing solvent. Aminophosphatides were detected by spraying with 0.2% ninhydrin in *n*-butanol, and all lipids with either 10% phosphomolybdate in absolute ethanol (21) or 50% sulfuric acid in methanol (22). The TLC areas containing SGP and lecithin, obtained from the crude lecithin, were dried quickly under nitrogen, scraped from the plate, and subjected to methanolysis (23). The lecithin spot included some phosphatidyl inositol in the procedure used. Unsaturated fatty acids were not oxidized during this procedure since the fatty acid composition of the EGP fraction did not change.

Gas–Liquid Chromatography

A gas chromatograph (Model 600 System, Research Specialties Co., Richmond, Calif.) equipped with ioniza-

[1] This diet was used because its fatty acid composition had been determined.

tion detector and 50 mv recorder was used with a $^1/_8$ inch × 6 ft column packed with Chromosorb ((60–80 mesh) coated with either a polar (15% diethylene glycol succinate polyester, DEGS) or nonpolar liquid phase [5% SE-30 (silicone grease, General Electric) or 5% Apiezon L]. The column temperatures were 187°C (DEGS), 220°C (SE-30), and 173°C (Apiezon L): flow rate of argon was maintained at 100–150 ml/min. The peak areas were obtained by triangulation. Quantitative results with National Heart Institute Fatty Acid Standard Mixtures D and F agreed with the stated composition with a relative error of less than 5.2% for major components and less than 13.8% for minor components.

Methanolysis was carried out either by refluxing in super-dried methanol–HCl at 90°C for 3 hr (24) or by heating with 10% (w/v) sulfuric acid in anhydrous methanol at 90°C for 1 hr (23). The methods yielded similar results.

Glycerophosphatides were saponified in 0.5 N KOH in 90% methanol at 70°C for 1 hr (25). After removal of the unsaponifiable fraction with petroleum ether, the saponifiable fraction was obtained by acidifying and reextracting with petroleum ether.

Palmitaldehyde was synthesized from palmitonitrile by a method similar to that for making the hexanal (26). Since palmitic acid was detected by GLC as a by-product of this procedure, the aldehyde was separated as a dimethyl acetal after saponification.

Total phosphorus was determined by the method of Horecker, Ma, and Haas (27) and protein by the method of Lowry, Rosebrough, Farr, and Randall (28). For identification of the phospholipid classes partial alkaline hydrolysis was carried out (29). Standard fatty acids were obtained from Applied Science Laboratories Inc., State College, Pa., and from the National Institutes of Health.

Identification of the Fatty Acids

Fatty acids were identified by plotting the retention time against carbon number on semilogarithmic paper (30) on both polar and nonpolar columns, by mixed chromatography with various standard fatty acids, and by analyzing the sample after hydrogenolysis and bromination (30).

RESULTS

Changes in Weight, Protein, and Lipids of the Brain and Liver During Development

As shown in Tables 1 and 2, developmental changes of wet weight, lipid phosphorus, and protein content suggest rapid growth of the brain as compared to the liver in the early developmental stage. On the other hand, at later stages of development the rate of liver growth given by these parameters was higher than that of the brain. The values for the brain lipid phosphorus reported here are generally lower than those reported previously (4, 31). This may be because our results are for the cerebrum only.

In the brain, a relative decrease in lecithin and a relative increase in EGP were observed during embryonic development. Similar changes have been reported in human fetal brain (32), while no significant change in the distribution of phosphatidyl ethanolamine has been reported in the chick embryonic brain (31). In contrast, the liver showed no significant changes in the distribution of the glycerophosphatide fractions except for a slight increase in a cardiolipin-like fraction.

Identification of Aldehydes

The methanolyzed EGP fraction contained dimethyl acetals of saturated C_{14}, C_{16}, and C_{18} aldehydes, identified

TABLE 1 DEVELOPMENTAL CHANGES OF THE BRAIN

	Weight				Glycerophosphatide Fraction				
	Wet	Dry	Protein	Lipid P	CA (?)	EGP	SGP	PI	Lecithin
	g/organ	*% of wet wt*	*mg/organ*	*μmoles/organ*	*% of glycerophosphatides**				
Embryonic age (days)									
8	0.08	12.0		1.4	0.7	23.4	8.1	5.0	62.8
10	0.13	12.2	6.1	2.6					
13	0.29	13.0	16.4	7.0	1.2	24.6	11.1	4.9	58.4
17	0.52	13.0	27.8	15.3					
20	0.68	14.9	37.0	23.3	3.3	35.0	9.7	5.0	46.8
After hatching (weeks)									
2	0.9	17.3	59.3	43.2					
5	1.2								
25	2.4	19.7		133.0					

Average values are given for the brain, excluding the cerebellum. Dry weight is expressed as a percentage of the wet weight after lyophilization. Lipid phosphorus values for the brain represent the fraction extracted with chloroform–methanol 2:1 from lyophilized tissues.

* Values for glycerophosphatide fractions are expressed as a percentage of the phosphorus value recovered from TLC on which the glycerophosphatide fraction obtained from the short silicic column was applied. The following abbreviations are used: CA, cardiolipin-like fraction; EGP, ethanolamine glycerophosphatides; SGP, serine glycerophosphatides; PI, phosphatidyl inositol.

TABLE 2 DEVELOPMENTAL CHANGES OF THE LIVER

	Weight				Glycerophosphatide Fraction				
	Wet	Dry	Protein	Lipid P	CA (?)	EGP	SGP	PI	Lecithin
	g/organ	*% of wet wt*	*mg/organ*	*μmoles/organ*	*% of glycerophosphatides*				
Embryonic age (days)									
8	0.01			0.3	5.3	27.0	1.7	15.0	51.1
10	0.02	24.2	1.7	0.7					
13	0.10	23.8	10.1	3.4	6.5	25.6	3.1	15.8	49.1
17	0.40	25.9	37.8	13.8					
20	0.61	28.4	56.4	20.6	7.9	25.6	2.8	16.1	47.3
After hatching (weeks)									
2	3.5	28.0	320.0	128.1					
5	11.0								
25	34.5	28.8							

Values are given for the liver, excluding the gall bladder. Other experimental conditions and abbreviations are the same as in Table 1.

TABLE 3 FATTY ACID AND ALDEHYDE COMPOSITION OF THE GLYCEROPHOSPHATIDES IN DEVELOPING CHICK EMBRYO BRAIN, COMPARED TO ADULT

	Total Glycerophosphatides				Ethanolamine Glycerophosphatides				Serine Glycerophosphatides				Lecithin			
	Embryo (Days)				Embryo (Days)			Adult	Embryo (Days)			Adult	Embryo (Days)			Adult
	8	10	13	20	8	13	20	6 Mo.	8	13	20	6 Mo.	8	13	20	6 Mo.
14:0 DMA	1.9	1.3	0.9	2.1	0.7	1.1	1.6	0.7	—	—	—	—	—	—	—	—
14:0	2.1	2.0	1.5	1.0	1.0	0.8	1.1	0.9	tr.	tr.	tr.	tr.	0.9	0.5	1.0	0.7
16:0 DMA	1.0	0.9	0.7	1.7	7.9	8.0	7.7	5.2	—	—	—	—	—	—	—	—
16:0	49.0	44.0	41.5	37.2	16.6	11.3	10.8	12.7	13.0	13.4	11.1	11.2	45.0	48.5	49.5	51.0
16:1	3.8	3.3	2.8	1.6	1.9	1.7	0.8	1.1	tr.	tr.	tr.	tr.	2.5	3.2	2.4	tr.
Unident.								1.8								
18:0 DMA	tr.	tr.	tr.	tr.	5.1	4.7	4.2	1.6	—	—	—	—	—	—	—	—
18:0	11.6	13.0	16.7	21.3	19.6	20.5	22.9	26.9	35.0	41.5	40.0	41.9	16.0	11.9	13.1	16.5
18:1	14.9	17.3	17.5	14.8	10.3	10.2	8.2	12.4	7.9	4.8	5.1	9.3	25.0	25.6	26.1	26.3
18:2	1.0	1.4	1.8	1.1	2.3	1.5	1.1	0.4	2.7	2.5	1.5	1.9	1.6	2.8	1.1	0.7
20:4	6.7	7.2	8.2	7.7	12.0	12.0	13.5	12.6	5.9	2.1	4.1	6.7	8.5	5.7	3.8	3.9
22:4	1.7	1.6	1.7	1.4	4.6	3.9	3.9	4.4	6.3	4.3	5.6	2.7	0.8	0.6	0.7	tr.
22:5	1.7	1.9	1.7	1.7	2.2	2.8	3.9	2.0	3.3	4.0	5.6	4.0	0.3	tr.	0.7	0.4
22:6	4.8	4.8	5.7	7.3	16.4	21.6	19.8	17.5	24.7	27.6	26.2	23.4	0.9	1.5	1.1	0.6

Values are expressed as percentage of total area obtained from GLC tracing and indicate the average of samples from 2 to 9 different pools of embryo and hemispheres of adult. Values of different samples were within a variation of ±3.5%. 18:3, 20:1, 20:2, and 22:5ω3, traces in all samples.

by their retention times on Apiezon L (25, 33), their appearance in the unsaponifiable fraction after saponification, their persistence in the GLC patterns after hydrogenation, and the R_f value on TLC of the dinitrophenylhydrazones (34, 35) after acid hydrolysis (36) of the EGP fraction compared with the R_f of the same derivative of palmitaldehyde. In the EGP fraction of the brains of 13- and 20-day embryos (Table 3), dimethyl acetals of C_{16} and C_{18} aldehydes constituted 66 and 26% of the total dimethyl acetals.

Fatty Acid Composition of the Glycerophosphatides in Brain

The EGP and SGP fractions contained a high proportion of PUFA, mainly 22:6 (Table 3). In contrast, the lecithin fraction contained about 65% saturated fatty acids and 25% 18:1. As previously noted, the methanolyzed EGP fraction contained substantial amounts of aldehydes from the 8th day of incubation and perhaps earlier.

The fatty acid composition of the individual glycerophosphatides remained relatively constant from the 8th day of incubation to hatching. Because of the relative increase of EGP and decrease of lecithin (Table 1), fatty acids of the total glycerophosphatides showed a decrease in 16:0, an increase in 18:0, and a slight increase in 22:6 during embryonic development.

Fatty Acid Composition of Glycerophosphatides in Liver

Until the end of the 2nd week of incubation, the major PUFA in the EGP of the embryonic liver was 20:4 (Table

TABLE 4 FATTY ACID AND ALDEHYDE COMPOSITION OF THE GLYCEROPHOSPHATIDES IN DEVELOPING CHICK EMBRYO LIVER, COMPARED TO ADULT

	Total Glycerophosphatides						Ethanolamine Glycerophosphatides					Lecithin				
	Embryo (Days)				Adult		Embryo (Days)			Adult		Embryo (Days)			Adult	
	8	10	13	20	6 Mo.*	6 Mo.†	8	13	20	6 Mo.*	6 Mo.†	8	13	20	6 Mo.*	6 Mo.†
14:0 DMA	—	—	—	—	—	—	tr.	tr.	tr.	tr.	tr.	—	—	—	—	—
14:0	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
16:0 DMA	tr.	tr.	tr.	tr.	0.3	0.7	1.5	0.9	0.5	1.1	1.2	—	—	—	—	—
16:0	31.9	32.5	31.8	21.2	26.6	26.5	14.9	13.6	11.7	17.0	13.1	37.8	38.2	27.1	32.7	33.0
16:1	tr.	tr.	tr.	tr.	1.3	0.8	1.2	0.3	0.3	1.1	tr.	tr.	tr.	tr.	2.0	tr.
18:0 DMA	—	—	—	—	—	—	1.2	0.4	0.5	tr.	tr.	—	—	—	—	—
18:0	31.9	30.8	32.2	34.3	20.5	27.0	38.0	37.8	32.8	28.2	31.0	28.8	29.0	33.7	17.1	21.8
18:1	6.2	5.2	5.0	7.8	25.0	14.0	7.7	6.2	7.8	17.3	9.8	5.6	4.6	6.9	29.8	23.2
18:2	5.2	6.5	6.9	16.0	13.7	11.0	10.2	11.8	20.5	14.4	12.8	4.0	4.7	13.6	11.2	9.3
20:4	17.2	19.9	19.1	15.6	8.7	13.8	16.4	22.2	20.2	14.6	19.8	16.1	18.7	14.7	3.3	7.5
22:4	0.5	0.5	0.3	0.3	0.2	0.3	0.7	0.4	0.4	0.6	1.7	0.7	0.2	0.2	0.7	tr.
22:5	2.2	1.3	1.0	1.0	0.7	0.1	2.3	1.5	1.5	1.1	0.5	1.7	1.6	1.1	0.3	0.7
22:6	4.8	3.4	2.9	2.7	2.2	4.6	3.8	4.3	3.1	3.6	9.3	4.7	2.4	2.3	1.3	4.5

Experimental conditions and symbols as in Table 3. SGP was not examined because of its small quantity.
* This hen was found to be ovulated and was assayed immediately after purchase.
† This fowl was fed a mouse diet for at least 1 week in the laboratory after purchase. Values for the adult liver represent an analysis for each fowl.

TABLE 5 FATTY ACID COMPOSITION OF THE YOLK AND DIETS

	Yolk				Diet	
	Glycerophosphatides		Free Fatty Acids		Egg Popper Ration (4.15% fat)	Rockland Mouse Diet (3% fat)
	Before Incubation	After Hatching (21 days)	Before Incubation	After Hatching (21 days)		
14:0	tr.	tr.	tr.	tr.	tr.	0.9
16:0	32.0	29.1	28.6	23.7	15.7	26.9
16:1	1.0	0.9	1.6	1.4	0.3	1.9
18:0	19.3	19.1	8.5	7.6	2.1	6.9
18:1	29.6	34.0	34.2	42.1	32.2	37.4
18:2	11.8	15.4	16.9	18.6	48.3	24.6
18:3	—	—	—	—	1.0	1.2
20:4	3.6	3.7	3.8	3.7	—	—
22:4	0.2	0.2	tr.	0.5	—	—
22:5	0.9	0.2	0.9	0.3	—	—
22:6	1.3	0.1	4.2	1.0	—	—
Unident.	—	—	1.0	0.6	—	—

Units and symbols as in Table 3. The fatty acids of the diets represent the total saponifiable fatty acids from the chloroform–methanol 2:1 extracts.

4). With a more than twofold increase during the 3rd week of embryonic development, 18:2 reached the same level as 20:4 at hatching. A decrease in 16:0 was observed during the corresponding period in the lecithin fraction, and consequently also in the total glycerophosphatides (Table 4).

In the adult liver, wide variations were found. The values for a commercially purchased hen differed markedly from those for a fowl maintained on a Rockland mouse diet for 1 week, especially in the proportions of 18:1 and PUFA (Table 4). The fatty acid composition of the diet used is shown in Table 5.

Fatty Acid Composition of the Yolk Phospholipids

As shown in Table 5, in the yolk phospholipids 18:2 was the major PUFA with a small portion of 20:4 (about 3.5%) and 22:6 (about 1.5%). No significant changes in the distribution of fatty acids of the yolk phospholipids were found during incubation of eggs.

Free Fatty Acids in Brain and Liver

The free fatty acids of the embryonic brain remained relatively constant throughout development (Table 6),

TABLE 6 FREE FATTY ACID COMPOSITION OF THE EMBRYONIC BRAIN AND LIVER

	Embryonic Brain (Days)			Embryonic Liver (Days)		
	8	13	20	8	13	20
14:0 DMA	0.3	0.7	0.3	tr.	tr.	tr.
14:0	1.7	1.7	1.6	tr.	tr.	tr.
16:0	2.5	2.5	1.6	tr.	tr.	tr.
16:0	37.9	35.7	37.7	32.3	24.9	17.1
16:1	4.2	3.6	2.5	tr.	tr.	0.9
18:0 DMA	1.1	0.6	1.1	—	—	—
18:0	9.4	10.0	12.2	18.3	14.4	11.8
18:1	19.1	16.5	17.5	14.6	23.4	42.6
18:2	1.8	2.3	1.3	4.6	8.1	14.1
20:4	8.1	8.9	9.0	14.2	16.7	7.6
22:4	2.3	2.2	2.6	1.6	tr.	tr.
22:5	1.2	0.8	1.5	2.6	1.7	tr.
22:6	9.9	12.2	8.9	11.9	9.3	5.0
Unident.	1.2	1.7	3.6	tr.	1.2	1.1

Units and symbols as in Table 3.

but in the liver the free fatty acids showed increases in 18:1 and 18:2 during the incubation and decreases in 16:0, 18:0, 20:4, and 22:6.

DISCUSSION

It has been demonstrated that lipids of a given class may have different fatty acid composition in different parts of the brain (7). The present study indicates that the principal PUFA of the EGP and SGP fractions in chick embryo brain, chick embryo liver, and yolk are 22:6, 20:4, and 18:2, respectively.

The fatty acid composition of the total glycerophosphatides of the chick brain is similar to that in adult mammals (5, 7, 9, 37). The high degree of unsaturation of SGP and EGP of chick embryonic brain has also been noted in mammalian brain (7, 9). O'Brien, Fillerup, and Mead (7) have observed that the highest proportion of PUFA is in the EGP and SGP fractions of the gray matter in human brain, and our values of 16–28% for 22:6 in these fractions are comparable to that in the human gray matter EGP and SGP reported by these workers. The rapid increase in PUFA of the cephalin fraction in developing rat brain demonstrated by Pritchard (2) could be explained if, in a manner similar to chick brain, all phospholipids increase in amount, especially EGP. The present study has also shown that EGP and SGP already contain a high percentage of PUFA at an early stage of development.

Substantial amounts of aldehydes are found in the EGP fraction of the embryonic brain prior to the onset of myelination, even though plasmalogens are considered to be characteristically myelin lipids (1, 38). The calculated plasmalogen content of the EGP is about 25–30% (DMA per cent × 2, in Table 3). This is lower than for adult mammalian brain (7, 9, 39, 40), in which about two-thirds of the EGP fraction is phosphatidal ethanolamine. O'Brien, Fillerup, and Mead (7) have found higher amounts of stearaldehyde and olealdehyde than palmitaldehyde in human brain, while Thannhauser, Boncoddo, and Schmidt (41) have found only saturated aldehydes (C_{16}, C_{18}) in bovine brain. Olealdehyde, described by Leupold (42), seems to be present in the chick embryonic brain only in small amounts, if at all. Debuch (39) has observed that lysophosphatide derived from the phosphatidal ethanolamine of ox brain by mild acid treatment includes C_{18}, C_{20}, and C_{22} unsaturated fatty acids only.

In the liver, marked inverse relationships exist between 18:2 and 16:0 in the fatty acid composition of glycerophosphatides during the 3rd week of embryonic development. Since a pathway from 16:0 to 18:2 has not been postulated in higher animals according to the present metabolic scheme (43, 44), these two events are probably independent. In addition, the growth rate of the mass of the embryonic liver is also marked during the corresponding period. Thus, the changes in fatty acid composition may be related to the structural and functional maturation of this organ. The values for the adult fowl liver indicate a pattern similar to that of the mammalian liver (45, 46), except for the PUFA distribution in the lecithin fraction of mouse liver (46). However, comparisons of liver composition are uninformative, unless the dietary conditions are controlled.

The finding that 22:6 and 20:4 acids are incorporated into the EGP and SGP fractions at an early phase of the development of the brain and liver suggests an active mechanism for the synthesis of these acids in the embryo at this stage. On the other hand, the transfer of the PUFA from the yolk phospholipids into the developing embryo cannot be eliminated as a possible mechanism, though no significant developmental changes in fatty acid composition are found in the yolk. Further studies with ^{14}C-labeled fatty acids are underway to evaluate the relative contributions of synthesis and transfer.

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