Distribution and fatty acid composition of phosphoglycerides in normal human brain

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ABSTRACT A thin-layer chromatographic procedure for the isolation of tissue phospholipids and their subsequent analysis is described. The method has been applied to the determination of the fatty acids of phosphoglycerides in human brain from the early fetal stage to old age. The study shows changes in the distribution and fatty acid composition of each phosphoglyceride in normal brain, although they are quite small after early childhood.

A *lipid-specific* fatty acid pattern for each of the four major phosphoglycerides was found. Besides this, the pronounced differences between fatty acids of the lipids from the cerebral cortex and from the adjacent white matter justify speaking of a *tissue-specific* fatty acid pattern for brain phosphoglycerides. The phospholipids of cerebral white matter contained more monoenoic acid but much less polyunsaturated fatty acid than those of cerebral cortex. The brain phosphoglycerides also showed an *age-dependent* fatty acid pattern. With increasing age the concentration of the fatty acids of the linoleate family diminished while that of the linolenate family increased.

Brain inositol phosphoglycerides, the fatty acid composition of which has not been studied systematically before, were characterized by a large concentration of arachidonate which was nearly as high for white as for gray matter and showed only small changes with age.

KEY WORDS brain · man · cerebral cortex · white matter · phosphoglycerides · inositol · fatty acids · age · thin-layer chromatography

If deviations in brain lipid composition due to metabolic disorders are to be recognized and understood, it is necessary first to record with accuracy and completeness the composition of brain lipids from a large number of normal humans of various ages. The aim of the present work has been to develop a rapid, simple method for the analysis of a large number of brain samples. The procedure has been used to establish the amounts and fatty acid compositions of the phosphoglycerides in cerebral gray and white matter from normal humans from the early fetal stage to the ninth decade. A procedure for the analysis of brain phosphoglycerides with a sensitivity comparable to that achieved in the present study has been developed by Rouser and coworkers (1, 2), but it does not appear to have been utilized for routine analyses of tissue lipids.

Painstaking analyses of lipids from a large number of brain specimens from normal humans have been performed by Brante (3), Rossiter and coworkers (4–6), Folch and coworker (7), and Cumings and coworkers (8, 9). The methods available for these early studies did not allow for the fractionation and quantification of all of the major brain lipids. Subsequently, a more complete fractionation was achieved (10, 11) but without resolution of the cephalins, the latter being accomplished on columns of anion-exchange cellulose by Rouser, Baumann, Kritchevsky, Heller, and O'Brien (12). Lipid fractions have been separated by TLC and the amount of each fraction has been determined both by densitometry (13) and by chemical analysis (14). The separation methods developed by Rouser et al., (12) have been applied by O'Brien and coworkers (15, 16) to a representative but quite limited number of human brain specimens. O'Brien and Sampson have presented information on the fatty acid compositions of individual brain lipid fractions (17), and in previous reports (18,

Abbreviations: Fatty acids are deisgnated by chain length: number of double bonds; $n-6$ denotes that the first double bond from the methyl group occurs after the sixth carbon atom, the methyl group being counted as number 1. EPG, ethanolamine phosphoglycerides; SPG, serine phosphoglycerides; IPG, inositol phosphoglycerides; CPG, choline phosphoglycerides; Sph, sphingomyelins; CC, cerebral cortex; WM, white matter; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; C, chloroform; M, methanol; DEGS, diethylene glycol succinate polyester.

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19), we have given the fatty acid compositions of the brain sphingolipids. This report describes fatty acid compositions of the choline, ethanolamine, inositol, and serine phosphoglycerides from human brains ranging in age from fetal to 82 yr.

MATERIALS AND METHODS

All solvents used were of analytical grade quality and were redistilled before use. The light petroleum used for the extraction of the fatty acid methyl esters was shaken with concentrated sulfuric acid for 48 hr before distillation. All solvent ratios given in the text are on a volume basis.

Tissue Sources

11 brains were analyzed (Table 1). The brains of a 12 wk old fetus (FB 113) from a legal abortion for medico-social reasons, a 35 wk old premature fetus (FB 108) which died after cesarean section, and a full-term (FB 112) infant with unknown cause of death were used without microscopical examination. All other brain material was from subjects who showed no signs of malnutrition, had normal neurological and mental states prior to death, and had normal microscopical findings of the brain for the age. The ages and causes of death for these additional subjects were as follows: 1 month old female (CB 110) pyloric stenosis operata; 7 month old male (CB 111) hydrocephalus operata and pneumonia; 4 yr old female (CB 113) fire accident; 16 yr old male (AB 102) traffic accident; 26 yr old female (AB 112) acute pneumonia; 52 yr old female (AB 113) myoma uteri operata; 81 yr old male (AB 114) traffic accident; 82 yr old male (AB 115) myocardial infarction.

The autopsies were performed 12–48 hr after death. Sections were taken for microscopic examination and the rest of the brain was wrapped in polyethylene film which was sealed. The brains were frozen and stored at −20°C prior to lipid extraction. Cerebral cortex and white matter were separated from the frontal and precentral gyri after thawing at 2°C. The tissue samples were then immediately homogenized without addition of water or other solvent in a Potter–Elvehjem homogenizer with Teflon pestle.

Duplicate samples were taken for the determination of tissue dry weight and the remaining homogenate was extracted with 10 volumes of chloroform–methanol (C–M) 1:1 at room temperature. The extract was filtered through a sintered glass filter and the residue was extracted twice with 5 volumes of C–M 1:1. After addition of 5 volumes of *n*-propanol to prevent foaming, the solvent was evaporated in a rotary evaporator to a small volume. The evaporation was repeated twice after addition of 5 volumes of C–M 2:1, the solution never being taken to dryness. The lipids were finally redissolved in 5 volumes of C–M 2:1 and transferred to a centrifuge tube. After centrifugation at 3000 rpm for 30 min the clear supernatant fraction was transferred to a graduated cylinder and the residue was resuspended in 2 volumes of C–M 2:1. After centrifugation the new

TABLE 1 DISTRIBUTION OF PHOSPHOLIPIDS IN CEREBRAL BRAIN TISSUE

Subject	Age	Tissue	Total Phospholipids	EPG	SPG	IPG	CPG	Sph
			g/100 g tissue dry weight	*molar percentage*				
Fetus FB 113	12 wk	T	12.6	28.6	13.2	2.9	50.8	4.6
Premature FB 108	35 wk	T	22.5	31.5	13.9	3.3	46.6	4.7
Full-term 112	38 wk	CC	21.5	28.0	14.4	3.4	46.7	7.4
		WM	20.4	31.0	14.2	4.3	45.0	5.5
Girl CB 110	1 month	CC	21.7	34.9	14.4	3.0	41.0	6.8
		WM	24.4	34.5	16.0	3.2	38.3	8.1
Boy CB 111	7 month	CC	23.8	34.4	14.2	2.8	39.2	9.4
		WM	26.6	33.7	16.0	2.6	34.5	13.2
Girl CB 113	4 yr	CC	23.0	35.4	13.6	2.2	37.9	10.9
		WM	30.7	35.9	20.5	1.9	25.6	16.1
Boy AB 102	16 yr	CC	24.6	36.1	13.3	2.6	37.1	10.9
		WM	29.7	35.2	21.1	2.3	24.6	16.9
Woman AB 112	26 yr	CC	21.7	33.6	11.4	4.4	39.7	10.9
		WM	31.3	33.2	19.6	3.1	25.8	19.6
Woman AB 113	52 yr	CC	26.1	35.6	13.9	3.2	36.9	10.4
		WM	30.8	33.9	20.3	2.4	25.1	18.3
Man AB 114	81 yr	CC	24.9	37.0	12.4	3.1	37.5	9.0
		WM	30.7	37.1	19.8	2.1	25.0	16.0
Man AB 115	82 yr	CC	24.8	36.8	12.7	4.1	35.5	10.9
		WM	28.7	36.4	19.3	2.7	25.3	16.3

T, total; FB, fetal brain; CB, child brain; AB, adult brain.

supernatant fraction was added to the cylinder and the volume adjusted to a convenient total.

A portion of the lipid extract, corresponding to 25 mg of fresh white matter and 100 mg of cerebral cortex, was transferred to a centrifuge tube and C–M 2:1 was added to give a total volume of 8 ml. 2 ml of 0.9% NaCl was added and after careful mixing the tube was centrifuged at 2000 rpm for 10 min. The upper phase was removed and discarded. The lower chloroform phase was rinsed twice with fresh upper phase, obtained by equilibrating 4 volumes of C–M 2:1 with 1 volume of 0.9% NaCl. The volume was finally made exactly 10 ml with C–M 2:1. Duplicates of 0.1 ml were taken for the assay of total lipid phosphorus by a modified Bartlett (20) procedure. Total phospholipids were calculted by multiplying the P value by 25.

Separation of Phospholipids by TLC

Quadruplicate portions of the lipid extract, equivalent to 10–20 μg of P, were evaporated under nitrogen in small conical tubes to about 50 μl and applied as 3 cm long bands to 0.25 mm layers of Silica Gel G activated overnight at 120°C. The tubes were rinsed with two portions of 25 μl of chloroform which were also applied to the same band. Use of the less polar chloroform for the rinsing resulted in no obvious widening of the lipid band. The plates were developed for 2 hr with C–M–13 N ammonia 70:30:5 at 21°C in tanks lined with filter paper. The developed plates were sprayed, when still slightly wet, with bromothymol blue reagent (21). The bands (Fig. 1) were scraped off, the gel being kept continually moistened. Duplicate samples were transferred to centrifuge tubes and their phosphorous contents assayed with standards containing gel scraped from the same plate. The gel blank corresponded to about 0.1 μg of P and never exceeded 0.2 μg.

Methanolysis of Phosphoglycerides

The bands from duplicate samples of each lipid extract were transferred to centrifuge tubes with ground stoppers. The tubes were placed over P_2O_5 in a vacuum desiccator which was promptly evacuated and kept overnight. At first we gassed the desiccator several times with nitrogen prior to final evacuation, but several tests showed no reduction in values for polyenoic acids when the nitrogen gassing was omitted. To the tubes containing the phosphoglycerides and silica gel 2.0 ml of 0.1 N sodium methoxide in dry methanol (E. Merck A.G., Darmstadt, Germany) was added and the tubes were shaken for 1 hr at room temperature. 0.2 ml of 1 N acetic acid in water was added and the methyl esters were extracted three times with 2 ml of light petroleum b.p. 45–50°C. The pooled petroleum extracts were rinsed twice with 1 ml of water, evaporated to a small volume on a water bath

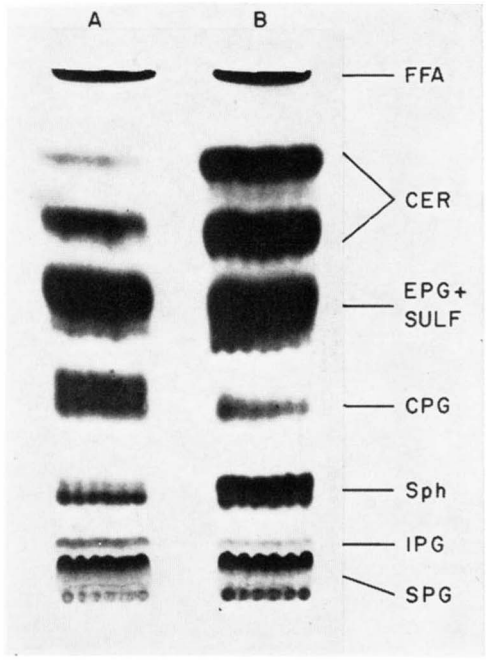

FIG 1. Thin-layer chromatogram of brain lipids of cerebral cortex (A) and white matter (B) in chloroform–methanol–13 N NH_4OH 70:30:5, 21°C, 2 hr. FFA, free fatty acids; CER, cerebrosides; SULF, sulfatides.

at 25°C under a gentle stream of nitrogen, and then transferred to a conical microtube with a glass stopper. The first tube was rinsed twice with hexane, which was also transferred to the microtube. The extract in the microtube was concentrated under gentle warming, the walls were rinsed with either hexane or chloroform, and the volume was finally adjusted to about 10 μl (5 μl for IPG). 1 μl was used for the gas-chromatographic analyses in each run. No difference was found when either hexane or chloroform was used for the storage and injection of the samples for GLC.

GLC of Methyl Esters

The methyl esters were analyzed by GLC in a Perkin–Elmer model 880 apparatus with flame ionization detector. A 200 cm stainless steel column of 3 mm I.D., packed with 15% diethylene glycol succinate polyester (DEGS) coated on 80–100 mesh Gas-Chrom Q was used. The flow rate was 25 ml of helium per min at an inlet pressure of 40 psi. The column temperature was 190°C; esters with retention times shorter than that of 18:2 were also chromatographed at 170°C. The quantitative recoveries from each column were determined with NIH fatty acid standards (Standards E and F), our own standards (8), and phospholipid fatty acid esters of bull testis (22). Bull testes were stored in physiological saline at −20°C and a sample of this material was regularly included in all the isolation steps. Quantitative results agreed with the stated composition data with a relative

error of less than 2% for major components and less than 5% for minor components.

The individual esters were identified with the aid of a diagram of log retention times and by comparison with internal standards wherever feasible. Representative samples of the phosphoglyceride fatty acid esters were also run on columns of 15% Apiezon L on 60–80 mesh Gas-Chrom P at 240–260°C, and of 3% EGSS-X (an ethylene glycol succinate polyester combined with a silicone) on 100–120 mesh Gas-Chrom Q at 220°C before and after hydrogenation. The methyl esters of total phosphoglycerides from FB 112 and AB 102 were analyzed by combined GLC and mass spectrometry and the mass number was determined for each individual peak. The peaks tentatively identified as 22:4(n − 6) and 18:3(n − 3) + 20:1(n − 9) were isolated by preparative GLC on the DEGS column and their mass numbers were determined by mass spectrometry. 22:4(n − 6) was homogeneous and gave the mass number for the methyl ester of 22:4. The other peak was a mixture of the methyl esters of 18:3 and 20:1. In FB 112 the percentage of 18:3 was larger than that of 20:1; but in AB 102 the peak consisted mainly of 20:1, and 18:3 constituted only about one-tenth of the total.

Quantification of the Fatty Acid Methyl Esters

Peaks were quantified by multiplying the height by the width at half height. The fatty acid composition is reported as a percentage of area.

The solvents used (reagent grade hydrocarbons, chloroform, and methanol obtained from several different manufacturers) contained minute amounts of impurities that gave peaks on GLC; they were not removed by distillation. 10 ml of hexane or light petroleum, evaporated to a small volume and injected into the gas chromatograph, gave peaks corresponding to about 0.1–0.2 μg of fatty acid methyl ester. Besides some irregularly occurring peaks before 16:0, two major peaks with carbon number 22.5 and 23.2 were regularly observed. Their concentration increased several-fold when polyenoic acid methyl esters were added to the solvent and stored at room temperature for 24 hr. These two major peaks of impurities interfered with the determination of 20:5(n−3) and 22:3(n−9) and no values are given for these acids. In the mass spectra esters with the correct mass number for 20:5 and 22:3 were identified; their concentration was estimated to be 0.1–0.5% for each. Impurities in solvents that might be identified as methyl esters have earlier been reported by Johnston and Roots (23).

We also had difficulties in the identification and quantification of 18:3(n−3) and 20:1(n−9). They were not separated from each other on the columns containing 15% DEGS; the difference in carbon number was less than 0.05. In gray matter there was in general as much 18:3 as 20:1 while in white matter of myelinated brains the percentage of 20:1 was about 10 times larger than that of 18:3 (as determined by GLC on Apiezon L).

Some further fatty acids have been identified but are not tabulated. 14:0, 15:0, and 17:0 have all been identified, but in general their concentration was less than 1%; in IPG and CPG, more 14:0 was sometimes present. With the GLC method used the values for 14:0 have shown large variations in the duplicate determinations. Because of the uncertainty in the quantitative figures and the presence of solvent impurities in this part of the chromatogram, these fatty acids have not been included in the tables. 20:2(n−6) has been identified in all samples and is of some interest as an intermediate in the formation of 20:4(n−6) from 18:2(n−6). Because its concentration was less than 0.2% it has not been tabulated.

RESULTS

Samples of cerebral gray and white matter from the cerebral hemispheres of two adults (AB 112 and AB 114) were dissected from four different areas: frontal, temporal, parietal, and occipital lobes. Except for slightly higher concentrations of SPG and Sph and slightly lower concentrations of polyenoic fatty acids in EPG and SPG of the occipital lobe, the variations between the different regions were within the error of the method. Because of these results, the lipid determinations were performed on gray and white matter from only one brain region, and we consider these results to be valid for comparison with pathological material taken from various regions of the cerebral hemispheres.

Phospholipid Distribution

Human brain material from fetal to senile stages of development was studied (Table 1). The most striking finding was the small individual variations found in the phospholipid distribution of mature brain. In fetal brain, CPG constituted about 50%, EPG nearly 30%, and the remaining three phospholipids only a little more than 20%. With increasing age the phospholipids of cerebral gray matter underwent some changes: a decrease of CPG to 35–40%, and an increase of EPG to 35–40% and of Sph from 5 to 10%. Otherwise there was no change. Cerebral white matter differed from gray matter by a relatively smaller proportion of CPG (about 25%) and larger portions of SPG and Sph. EPG constituted the same percentage as in gray matter.

Compositional Differences in Lipids of Gray and White Matter

Ethanolamine Phosphoglycerides. In cerebral cortex (Table 2) 18:0 is the major fatty acid at all ages and contributes

TABLE 2 FATTY ACID COMPOSITION OF ETHANOLAMINE PHOSPHOGLYCERIDES IN CEREBRAL GRAY MATTER (CORTEX)

	Fetus 12 wk	FB 108 35 wk	FB 112 38 wk	CB 110 1 month	CB 111 7 month	CB 113 4 yr	AB 102 16 yr	AB 112 26 yr	AB 113 52 yr	AB 114 81 yr	AB 115 82 yr
16:0	10.6	6.9	7.6	6.4	6.5	5.0	6.0	5.9	5.7	6.3	6.8
16:1	1.2	0.6	0.6	0.7	0.7	0.4	0.5	0.4	0.4	0.6	1.2
18:0	30.0	29.1	32.8	34.6	27.8	30.1	29.8	30.4	28.4	29.6	27.2
18:1	13.3	9.8	8.5	8.7	10.3	8.8	10.2	8.7	10.3	9.1	9.8
18:2(*n*–6)	0.2	0.2	0.3	tr.	0.2	0.4	0.4	0.5	0.3	0.4	0.3
18:3(*n*–6)	0.1	0.1	tr.	tr.	tr.	tr.	tr.	0.1	tr.	tr.	tr.
18:4(*n*–3)	0.3	0.2	0.2	0.3	0.5	0.4	0.2	0.2	0.4	0.2	0.2
20:1(*n*–9) + 18:3(*n*–3)	0.8	0.5	0.3	0.3	0.6	0.5	0.6	0.5	0.9	0.5	0.6
20:3(*n*–9)	1.6	0.9	0.8	0.6	0.7	0.5	0.2	0.3	0.5	0.3	0.4
20:3(*n*–6)	0.5	0.8	1.0	1.2	1.5	1.6	1.1	1.1	1.0	1.3	0.8
20:4(*n*–6)	17.3	17.8	14.9	16.5	16.4	16.7	13.0	13.2	11.2	9.9	10.3
22:4(*n*–6)	9.5	10.1	10.6	11.1	11.7	9.9	8.4	8.3	7.7	7.0	6.3
22:5(*n*–6)	2.8	4.1	4.4	2.7	5.0	2.4	1.7	1.5	1.2	0.8	0.9
22:5(*n*–3)	0.5	0.6	0.4	0.7	0.5	0.6	0.4	0.5	1.1	1.0	1.0
22:6(*n*–3)	10.8	18.3	17.1	16.1	16.9	22.3	27.0	28.6	30.5	31.4	33.9
24:4(*n*–6)	0.6	0.2	0.5	0.3	0.5	0.5	0.5	0.1	0.6	0.4	0.2
18–24(*n*–6)	31.0	33.3	31.7	31.8	35.3	31.5	25.1	24.8	22.0	19.9	18.8
18–22(*n*–3)	11.6	19.1	17.7	17.1	17.9	23.3	27.6	29.3	32.0	32.6	35.1

Values are weight percentages of methyl ethers.

TABLE 3 FATTY ACID COMPOSITION OF ETHANOLAMINE PHOSPHOGLYCERIDES IN CEREBRAL WHITE MATTER

	FB 112 38 wk	CB 110 1 month	CB 111 7 month	CB 113 4 yr	AB 102 16 yr	AB 112 26 yr	AB 113 52 yr	AB 114 81 yr	AB 115 82 yr
16:0	7.8	5.9	5.0	4.8	4.9	6.2	3.4	4.7	5.4
16:1	0.6	0.5	0.9	0.5	1.0	1.1	0.5	tr.	0.8
18:0	28.9	28.7	13.8	10.2	9.6	13.8	9.3	17.6	10.7
18:1	7.6	13.0	23.6	35.2	38.3	43.2	38.9	39.7	40.4
18:2(*n*–6)	0.2	0.1	0.4	0.7	0.5	0.5	0.5	tr.	0.3
18:3(*n*–6)	tr.	tr.	tr.	0.1	tr.	0.1	0.1	tr.	tr.
18:4(*n*–3)	0.2	0.7	1.9	1.9	1.5	1.3	1.3	0.7	1.2
20:1(*n*–9) + 18:3(*n*–3)	0.3	1.6	3.6	6.4	5.6	6.0	8.5	6.1	6.2
20:3(*n*–9)	0.7	0.7	1.3	0.7	0.5	0.4	0.9	0.4	0.8
20:3(*n*–6)	1.1	1.4	1.7	1.9	1.2	1.0	1.0	1.2	0.9
20:4(*n*–6)	16.2	15.1	13.4	9.5	8.4	7.9	8.3	7.7	9.2
22:4(*n*–6)	13.3	13.3	19.6	18.0	18.6	13.4	16.5	10.6	12.8
22:5(*n*–6)	4.8	2.3	3.5	1.4	1.2	0.5	0.7	0.7	0.6
22:5(*n*–3)	0.5	0.9	0.8	0.7	0.6	0.3	1.0	0.9	1.1
22:6(*n*–3)	17.7	15.2	8.7	5.7	5.7	3.0	7.5	8.6	8.3
24:4(*n*–6)	0.2	0.5	1.6	2.2	2.7	1.5	1.4	1.1	1.1
18–24(*n*–6)	35.8	32.7	40.2	33.8	32.6	24.9	28.5	21.3	24.9
18–22(*n*–3)	18.4	16.8	11.4	8.3	7.8	4.6	9.8	10.2	10.6

Values are weight percentages of methyl esters.

about 30%. In younger brains polyunsaturated fatty acids of the linoleate series predominate, and 20:4($n-6$) and 22:4($n-6$) together constitute about 25%. With increasing age the concentrations of the latter fatty acids diminish, and there is a corresponding increase of fatty acids of the linolenate series from about 20% in the fetal brain to 35% in the older brain.

EPG of white matter (Table 3) differs from that of cortex in several respects. 18:1 is the major fatty acid in childrens' and adult brains and comprises about 40%, while 18:0 occurs only to the extent of about 10%. The percentage of fatty acids of the linoleate series is about the same as in cortex but that for 22:4($n-6$) is larger than that of 20:4($n-6$). There is some decrease in the content of the linolenate series with age. Unlike in the cortex, 22:6($n-3$) is comparatively sparse in white matter after early childhood and does not increase with age.

Serine Phosphoglycerides (*Tables 4 and 5*). The major difference between EPG and SPG is the much lower concentration of fatty acids of the linoleic acid family and the higher concentration of 18:0 in SPG; otherwise, they have similar fatty acid compositions. SPG of adult white matter has a low concentration of polyunsaturated fatty acids, but 18:0 and 18:1 constitute about 80%. As

TABLE 4 FATTY ACID COMPOSITION OF SERINE PHOSPHOGLYCERIDES IN CEREBRAL GRAY MATTER (CORTEX)

	Fetus 12 wk	FB 108 35 wk	FB 112 38 wk	CB 110 1 month	CB 111 7 month	CB 113 4 yr	AB 102 16 yr	AB 112 26 yr	AB 113 52 yr	AB 114 81 yr	AB 115 82 yr
16:0	10.9	4.6	4.2	3.0	4.6	5.4	3.6	2.9	4.2	4.7	3.7
16:1	1.2	0.9	0.5	0.4	0.6	0.4	0.4	0.6	0.3	0.4	0.2
18:0	41.1	41.9	47.4	49.9	48.6	47.4	50.1	43.6	45.3	46.9	40.6
18:1	10.3	8.2	6.8	9.6	9.5	12.0	17.5	10.7	17.5	14.0	14.2
18:2(*n*–6)	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2
18:3(*n*–6)	0.3	0.3	0.1	0.3	0.2	0.2	0.2	0.1	0.2	0.2	0.2
18:4(*n*–3)	0.2	0.1	0.1	0.2	0.3	0.4	0.5	0.3	0.5	0.2	0.3
20:1(*n*–9) + 18:3(*n*–3)	1.0	0.6	0.5	0.5	0.4	0.5	0.8	0.7	0.9	0.7	0.9
20:3(*n*–9)	0.5	0.3	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.2
20:3(*n*–6)	0.8	1.1	1.1	1.3	1.4	1.4	0.7	0.8	0.9	1.0	0.7
20:4(*n*–6)	6.6	5.7	4.3	3.8	3.1	2.4	1.8	2.0	1.8	1.7	2.2
22:4(*n*–6)	9.4	9.5	10.7	7.7	6.3	4.6	3.5	5.0	3.0	3.1	3.2
22:5(*n*–6)	3.1	5.4	5.8	3.5	6.3	3.0	1.7	1.8	0.7	0.9	1.0
22:5(*n*–3)	0.7	0.8	tr.	0.5	0.2	0.2	0.1	0.3	0.5	0.5	0.9
22:6(*n*–3)	13.7	20.5	18.2	18.7	18.1	21.5	18.1	30.7	23.2	24.9	29.8
24:4(*n*–6)	0.3	0.2	0.1	0.3	0.5	0.5	0.5	0.4	0.5	0.8	1.7
18–24(*n*–6)	20.6	22.3	22.3	17.0	18.0	12.2	8.5	10.2	7.2	7.8	9.2
18–22(*n*–3)	14.6	21.4	18.3	19.4	18.6	22.1	18.7	31.3	24.2	25.6	31.0

Values are weight percentages of methyl esters.

TABLE 5 FATTY ACID COMPOSITION OF SERINE PHOSPHOGLYCERIDES IN CEREBRAL WHITE MATTER

Fatty acid	CB 112 38 wk	CB 110 1 month	CB 111 7 month	CB 113 4 yr	AB 102 16 yr	AB 112 26 yr	AB 113 52 yr	AB 114 81 yr	AB 115 82 yr
16:0	3.0	3.3	4.6	4.0	3.4	1.8	1.9	6.0	4.4
16:1	0.4	0.7	0.6	0.5	0.4	0.6	0.3	0.5	0.4
18:0	45.4	50.1	47.3	43.9	43.7	45.7	44.1	42.0	39.1
18:1	6.6	19.1	26.2	36.5	40.0	38.1	41.4	41.5	42.0
18:2(*n*–6)	0.1	0.2	0.1	0.3	0.3	0.2	0.4	0.4	0.4
18:3(*n*–6)	0.3	0.3	0.2	0.1	0.1	0.4	0.1	0.1	0.1
18:4(*n*–3)	0.2	0.7	1.3	1.6	1.1	1.3	1.1	0.8	1.0
20:1(*n*–9) + 18:3(*n*–3)	0.5	1.1	1.2	2.7	2.9	3.5	4.5	2.9	4.4
20:3(*n*–9)	tr.	0.3	0.8	0.4	0.4	0.3	0.4	0.3	0.3
20:3(*n*–6)	1.2	1.2	1.0	0.9	0.4	0.6	0.4	0.4	0.5
20:4(*n*–6)	4.5	4.3	3.2	2.1	1.6	1.4	1.2	1.1	1.8
22:4(*n*–6)	11.3	5.8	4.2	2.6	2.1	2.0	1.6	1.2	1.9
22:5(*n*–6)	6.7	1.8	2.5	0.5	0.4	0.1	0.1	0.1	0.2
22:5(*n*–3)	0.3	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.3
22:6(*n*–3)	19.3	10.3	6.1	2.5	1.3	0.9	1.3	1.3	1.9
24:4(*n*–6)	0.3	0.5	0.4	1.2	1.7	2.4	0.9	1.2	1.5
18–24(*n*–6)	24.4	14.1	11.6	7.7	6.6	7.1	4.7	4.5	6.4
18–22(*n*–3)	19.8	11.4	7.6	5.3	2.6	2.4	2.6	2.3	3.2

Values are weight percentages of methyl esters.

with EPG, the concentration of 18:1 is much larger in white than in gray matter.

Inositol Phosphoglycerides (*Tables 6 and 7*). IPG is characterized by a high concentration of 18:0 and 20:4 ($n-6$) and a moderately high concentration of 16:0 and 18:1, while all the other fatty acids constitute less than 20%. Unlike the other cephalins, IPG contains a very low concentration of fatty acids of the linolenic acid series. As in all other phospholipids, monoenoic acids constitute a larger proportion of the IPG of white matter than of gray. In contrast to the other lipid, the difference in concentration of 20:4($n-6$) between white matter and gray matter is comparatively small.

Choline Phosphoglycerides (*Tables 8 and 9*). Lecithin in brain tissue has a very low content of polyunsaturated fatty acids. The major fatty acids of CPG are 16:0 and 18:1; the concentration of the former is larger in gray matter and the latter in adult white matter.

DISCUSSION

The present study makes use of a simple, rapid, and reliable method for the quantification of phospholipids

TABLE 6 FATTY ACID COMPOSITION OF INOSITOL PHOSPHOGLYCERIDES IN CEREBRAL GRAY MATTER (CORTEX)

	Fetus 12 wk	FB 108 35 wk	FB 112 38 wk	CB 110 1 month	CB 111 7 month	CB 113 4 yr	AB 102 16 yr	AB 112 26 yr	AB 113 52 yr	AB 114 81 yr	AB 115 82 yr
16:0	18.3	15.9	18.1	14.0	11.4	10.4	15.9	14.0	9.0	14.3	8.1
16:1	2.3	2.8	2.3	1.6	1.0	1.3	1.0	1.8	0.2	1.0	0.3
18:0	33.1	33.5	33.7	40.2	41.5	39.7	37.8	34.9	39.1	37.2	38.0
18:1	11.9	10.3	9.9	8.2	8.6	8.0	12.7	12.6	13.0	9.9	8.9
18:2(*n*–6)	0.3	0.1	0.6	0.2	0.6	0.7	0.4	0.6	0.6	0.6	0.8
18:3(*n*–6)	0.2	0.1	0.3	0.3	0.2	0.2	0.2	0.1	0.3	0.1	0.3
18:4(*n*–3)	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.3	0.1	0.1
20:1(*n*–9) + 18:3(*n*–3)	0.4	0.2	0.3	0.1	0.2	0.3	0.4	0.3	0.5	0.4	0.2
20:3(*n*–9)	2.7	1.0	1.0	0.7	0.7	0.5	0.4	0.4	0.6	0.6	0.8
20:3(*n*–6)	0.6	0.6	1.0	0.6	1.2	1.3	0.9	1.4	1.3	1.9	1.4
20:4(*n*–6)	26.6	32.2	29.5	29.0	27.2	31.6	22.5	28.2	28.5	26.9	33.2
22:4(*n*–6)	1.5	1.2	1.3	1.4	1.7	1.2	1.4	1.5	1.6	1.1	1.7
22:5(*n*–6)	0.6	0.2	0.2	0.4	0.8	0.2	0.2	tr.	0.1	0.1	0.6
22:5(*n*–3)	0.2	tr.	tr.	0.2	tr.	tr.	tr.	tr.	0.2	0.3	0.4
22:6(*n*–3)	1.2	1.9	1.5	2.5	3.6	3.0	5.3	4.0	4.8	5.3	5.3
24:4(*n*–6)	tr.	tr.	tr.	tr.	0.1	tr.	0.4	tr.	0.1	0.2	0.1
18–24(*n*–6)	29.8	34.4	32.9	31.9	31.8	35.2	26.0	31.8	33.5	30.8	37.8
18–22(*n*–3)	1.6	2.1	1.7	2.9	3.8	3.1	5.4	4.1	5.3	5.7	5.8

Values are weight percentages of methyl esters.

TABLE 7 FATTY ACID COMPOSITION OF INOSITOL PHOSPHOGLYCERIDES IN CEREBRAL WHITE MATTER

	FB 112 38 wk	CB 110 1 month	CB 111 7 month	CB 113 4 yr	AB 102 16 yr	AB 112 26 yr	AB 113 52 yr	AB 114 81 yr	AB 115 82 yr
16:0	17.0	11.7	11.4	16.1	16.5	15.9	9.4	11.5	10.0
16:1	1.5	1.6	1.1	1.5	1.6	2.1	1.0	1.4	1.1
18:0	35.6	41.0	37.1	34.8	35.2	32.4	34.7	33.8	32.5
18:1	8.7	9.1	15.0	19.3	18.5	19.5	24.7	21.4	19.3
18:2(*n*–6)	0.2	0.4	0.4	0.4	0.5	0.5	0.3	0.5	0.4
18:3(*n*–6)	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.4
18:4(*n*–3)	0.1	0.1	0.4	0.3	0.6	1.2	0.5	0.7	0.7
20:1(*n*–9) + 18:3(*n*–3)	0.1	0.3	0.9	0.8	1.1	0.7	1.9	1.1	1.4
20:3(*n*–9)	0.7	0.9	1.1	0.5	0.8	1.6	1.1	1.6	1.7
20:3(*n*–6)	0.9	1.0	1.2	2.8	2.1	3.5	2.5	3.9	3.1
20:4(*n*–6)	32.8	29.8	24.4	19.2	17.1	17.3	18.7	18.0	21.4
22:4(*n*–6)	0.7	1.3	2.4	2.0	2.4	2.4	2.3	1.9	2.6
22:5(*n*–6)	0.2	0.2	0.8	0.5	0.7	tr.	tr.	0.1	0.3
22:5(*n*–3)	tr.	0.2	tr.	tr.	0.2	tr.	tr.	0.2	0.3
22:6(*n*–3)	1.3	2.1	3.0	0.9	1.9	1.1	1.5	3.3	3.8
24:4(*n*–6)	tr.	tr.	0.5	tr.	0.5	1.2	0.3	0.5	0.9
18–24(*n*–6)	35.0	32.9	29.7	25.2	23.6	25.2	24.4	25.1	29.1
18–22(*n*–3)	1.4	2.4	3.4	1.2	3.3	3.5	2.0	4.5	4.8

Values are weight percentages of methyl esters.

and their fatty acids in small tissue samples. Although the procedure was designed for tissue samples of 25–100 mg in the present study, it has routinely been used for biopsy samples of only 10–20 mg. The method applied to the determination of the molar percentage of individual phospholipids is similar to the procedure described by Norton and Autilio (14) and differs only in the analyses of phosphorus. It was not found necessary to use individual blanks for each spot nor to pool several samples for each analyses, because the blank figure never exceeded 0.2 μg of P. The recovery of phospholipids from the TLC plates was 98 ± 2%. The standard deviation, calculated from the duplicate analyses, was ±3% for EPG and CPG, ±5% for SPG and Sph, and ±8% for IPG.

It has generally been assumed that the polyenoic acids will undergo rapid autoxidation after the extraction from the tissue sample (cf. Holman, 22) especially in contact with air and water. In the present procedure, the spraying of the plates with an indicator dissolved in water, and after scraping, the drying of the moistened spots in a vacuum desiccator, seemed to us to be the

TABLE 8 FATTY ACID COMPOSITION OF CHOLINE PHOSPHOGLYCERIDES IN CEREBRAL GRAY MATTER (CORTEX)

	Fetus 12 wk	FB 108 35 wk	FB 112 38 wk	CB 110 month	CB 111 7 month	CB 113 4 yr	AB 102 16 yr	AB 112 26 yr	AB 113 52 yr	AB 114 81 yr	AB 115 82 yr
16:0	52.8	51.7	52.4	46.6	47.1	43.5	48.9	44.0	45.9	45.7	45.7
16:1	5.8	7.6	6.5	5.4	3.8	2.7	2.6	2.1	2.4	2.0	2.5
18:0	8.3	7.3	9.1	9.6	12.1	13.5	11.7	11.6	11.2	9.6	9.9
18:1	23.7	23.7	21.5	25.9	25.7	29.0	29.0	29.5	30.3	30.9	28.4
18:2(*n*–6)	0.5	0.5	0.4	0.4	0.5	0.5	0.5	0.7	0.4	0.5	0.5
18:3(*n*–6)	tr.	tr.	tr.	0.1	0.1	0.1	tr.	0.1	tr.	tr.	0.1
18:4(*n*–3)	0.3	0.2	0.3	0.2	0.2	0.3	0.1	0.1	0.2	0.1	0.1
20:1(*n*–9) + 18:3(*n*–3)	0.7	0.5	0.4	0.4	0.6	0.6	0.7	0.8	0.9	0.7	0.8
20:3(*n*–9)	0.3	0.1	0.2	0.1	0.1	0.1	tr.	0.1	0.2	0.1	0.1
20:3(*n*–6)	0.2	0.3	0.4	0.7	0.8	1.1	0.5	0.9	0.6	1.0	0.6
20:4(*n*–6)	3.0	3.0	4.2	6.1	5.0	5.2	3.8	5.5	3.6	4.5	4.7
22:4(*n*–6)	0.5	0.5	0.7	0.8	0.7	0.7	0.7	0.8	0.5	0.5	0.6
22:5(*n*–6)	0.1	0.1	0.2	0.1	0.3	0.1	tr.	0.1	tr.	tr.	tr.
22:5(*n*–3)	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	0.2
22:6(*n*–3)*	0.7	0.8	0.9	1.7	1.5	1.5	0.8	2.6	2.5	2.7	3.6
18–24(*n*–6)	4.3	4.4	5.9	8.3	7.5	7.8	5.6	8.2	5.2	6.7	6.6
18–22(*n*–3)	1.0	1.0	1.2	1.9	1.7	1.8	0.9	2.7	2.7	2.8	3.7

Values are weight percentages of methyl esters.
* 24:4(*n*–6) occurred only in trace quantities in all samples.

TABLE 9 FATTY ACID COMPOSITION OF CHOLINE PHOSPHOGLYCERIDES IN CEREBRAL WHITE MATTER

	FB 112 38 wk	CB 110 1 month	CB 111 7 month	CB 113 4 yr	AB 102 16 yr	AB 112 26 yr	AB 113 52 yr	AB 114 81 yr	AB 115 82 yr
16:0	52.8	41.9	37.3	30.9	30.4	30.8	30.2	30.0	31.4
16:1	6.1	4.7	4.2	2.2	2.8	1.8	2.3	3.0	2.5
18:0	9.1	11.9	14.5	14.5	15.2	15.3	12.9	12.8	12.2
18:1(*n*–9)	21.5	30.1	34.6	44.5	45.8	44.0	47.1	46.3	46.7
18:2(*n*–6)	0.3	0.5	0.4	0.2	0.4	0.3	0.4	0.4	0.4
18:3(*n*–6)	tr.	0.1	0.1	0.2	tr.	0.1	0.1	tr.	tr.
18:4(*n*–3)	0.3	0.2	0.3	0.3	0.1	0.2	0.2	0.1	0.1
20:1(*n*–9) + 18:3(*n*–3)	0.3	0.6	1.1	1.4	1.1	1.2	1.8	1.6	1.5
20:3(*n*–9)	0.1	0.1	0.1	tr.	tr.	0.1	0.1	0.1	tr.
20:3(*n*–6)	0.5	0.6	0.5	0.5	0.2	0.5	0.3	0.5	0.4
20:4(*n*–6)	5.0	5.0	3.2	2.1	2.0	2.0	1.9	2.0	2.1
22:4(*n*–6)	0.8	0.4	0.7	0.8	0.7	0.9	0.8	0.7	0.6
22:5(*n*–6)	0.2	0.6	0.2	tr.	tr.	0.1	0.1	0.1	tr.
22:5(*n*–3)	tr.	tr.	0.1	tr.	tr.	0.1	0.2	0.1	0.1
22:6(*n*–3)	1.0	1.5	0.6	0.5	0.2	0.4	0.3	0.6	0.6
24:4(*n*–6)	tr.	tr.	0.4	0.8	0.1	0.7	0.2	0.7	0.3
18–24(*n*–6)	6.8	7.3	5.5	4.6	3.4	4.6	3.8	4.5	3.8
18–22(*n*–3)	1.3	1.7	1.0	0.8	0.3	0.7	0.7	0.8	0.8

Values are weight percentages of methyl esters.

steps in which destruction of the most highly unsaturated fatty acids would occur. Several other procedures in which these steps were omitted were tried, but they gave results which were similar to these obtained with the present TLC method. Fatty acid composition of human brain phosphoglycerides, separated by a column-chromatographic procedure in a nitrogen atmosphere, also showed results for polyenoic acids similar to those in this study (15, 17). Thus, it was not possible to show any measurable loss of polyenoic acids with the present method.

The present values for the fatty acid composition are similar to those reported earlier by O'Brien and co-workers (15, 17). Each of the four major phosphoglycerides has its characteristic fatty acid composition. Although the brain phospholipids show a lipid-specific fatty acid pattern, each lipid class has a strikingly different fatty acid composition in gray or white matter, which has been shown earlier by O'Brien and Sampson (17). The white matter differs from the gray in having a much higher concentration of monoenoic acids (18:1 and 20:1) and lower concentrations of saturated and poly-

enoic acids. EPG differs from the other lipids by a somewhat higher concentration of fatty acids of the linoleate series in white than in gray matter; the concentration of 20:4(n − 6) is lower but the concentration of 22:4(n − 6) higher. The latter fatty acid was tentatively identified by O'Brien and Sampson (17) as 22:5(n − 6) but isolation and mass spectrometric analyses shows it to be 22:4.

The fatty acid composition of human brain IPG has not been determined earlier. In cerebral cortex the three major fatty acids are 18:0, 20:4(n − 6), and 16:0. Arachidonate comprises about 30%, which is more than in any of the other phosphoglycerides. White matter does not differ much from gray; arachidonate comprises about 20%, and 18:1 has increased from 10 to 20%.

The polyenoic acids of human brain belong to the linoleate and the linolenate families. Klenk and Montag (24) determined the structure and composition of the C_{22}-polyenoic fatty acids of human brain lipids. They found that the C_{22} fraction consisted of 43% hexaenoic, 6% pentaenoic, 38% tetraenoic, 8% trienoic, and 5% dienoic acids. Our figures are similar, but we have not been able to determine trienoic and dienoic acids. Klenk and Montag (24) found that 22:4 belonged to the linoleate family, 22:6 to the linolenate family, and the major isomer of 22:5 to the linoleate family. All these findings have been confirmed in the present study. The 22:3 found by Klenk and Montag (24) was stated to be of the oleate family [22:3(n − 9)]. We were unable to determine this acid because of the impurities in the solvents, but the amount they found could hardly have been present in our samples.

The transport of fatty acids into adult mammalian brain is presumed to be restricted by the blood–brain barrier (25, 26). On the other hand Mohrhauer and Holman (27) suggested that at least some members of the linoleate and linolenate series could pass the barrier in rats. Because these two acids cannot be synthesized de novo in organs of mammals (28), including man (29), a relative increase of these two acids of human brain during maturation would suggest a transport through the barrier. It would not be easy, however, to determine the increase of linoleate in brain because of its low rate of deposition, but dietary linoleate has been shown to be converted into arachidonate (30) and docosapentaenoate (27). These two fatty acids, together with docosatetraenoate, constitute a large portion of the phosphoglyceride fatty acids in brain. During the first 2 yr of life brain phosphoglycerides increase four to five times in amount and the concentrations of these acids of the linoleate series are rather constant during this period. This means a 4- to 5-fold increase of the fatty acids of the linoleate series, which can be interpreted as indicating transport of these acids through the blood–brain barrier. 20:4, 22:4, and 22:5 might have been formed in extraneural organs from lineoleate and then passed the barrier, or linoleate may have been transported to the brain and then converted by enzyme systems of the brain to the more unsaturated fatty acids of the linoleate series. After adulthood has been reached these fatty acids of brain phosphoglycerides steadily decrease in amount, which may be an indication of their reduced passage through the blood–brain barrier.

The same explanation for the accumulation of acids of the linoleate series with maturation can also be used for fatty acids of the linolenate series. There is also an important difference between the two series with aging; the fatty acids of the linolenate family show a steady increase in cerebral cortex. This may be explained by a larger affinity of brain for acids of this family or by a slower metabolism of acids of this family.

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