Quantification and fatty acid and fatty aldehyde composition of ethanolamine, choline, and serine glycerophosphatides in human cerebral grey and white matter*

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SUMMARY The quantities of ethanolamine glycerophosphatides (EGP), choline glycerophosphatides (CGP), and serine glycerophosphatides (SGP) were determined in the grey and white matter from three apparently normal adult human brains. In each locale the quantities decreased from EGP through CGP to SGP.

The quantities of aldehydes in these lipids were also determined. In grey matter the aldehyde content (expressed as per cent of fatty acids plus aldehydes) of EGP, CGP, and SGP was 22, 0.3, and 0.2% respectively; while in white matter the proportions were 49, 0.8, and 13%, respectively. Palmitaldehyde, stearaldehyde, and olealdehyde made up 90% of the aldehydes found. White matter glycerophosphatides contained much more olealdehyde than those from grey matter.

EGP and SGP contained much larger proportions of C_{20} and C_{22} polyenoic acids from grey matter than from white matter. CGP, on the other hand, had a similar fatty acid composition, comprised mainly of 16:0, 18:0, and 18:1 acids, in each locale. The differences in fatty acid composition of these three glycerophosphatides may be related to the higher myelin content of white matter.

ETHANOLAMINE, choline, and serine glycerophosphatides are the most abundant naturally occurring glycerophosphatides. Klenk and Bohm (1) and Debuch (2) reported that these phospholipids isolated from brain contain a variety of fatty acids, which were studied by low temperature crystallization and alkali isomerization. It was not possible to identify and quantify all the fatty acids present since these methods do not distinguish between certain fatty acids of similar structure nor give precise quantitative results. In the present study, these three phosphatide classes were isolated in pure form and in quantitative yield by column chromatography. Gas–liquid chromatography (GLC) was used to define for the first time the complete fatty acid and fatty aldehyde composition of these lipids in human cerebral grey and white matter.

MATERIALS AND METHODS

The solvents used were A.C.S. Reagent Grade and were redistilled prior to use. Methanol or ethanol (1%) was added to the chloroform as a preservative. The nitrogen used contained less than 5 parts per million of oxygen (High Purity Nitrogen–Linde Co.). All solvent ratios given in the text are on a volume basis.

Subjects

In none of the subjects studied was there evidence of cerebral pathology on gross examination or after sectioning of the cerebral cortex.

Subject 1. A 55 year old Caucasian male who had died suddenly from an acute myocardial infarction. There was no history of dietary inadequacy or malnutrition. Neurological status was clinically normal prior to death.

Subject 2. A 77 year old Caucasian male who died of a myocardial infarction. The patient had a past history of mild diabetes mellitus.

* Brain Lipids II. First paper in this series: J. S. O'Brien, D. L. Fillerup, and J. F. Mead. *J. Lipid Res.* 5: 109, 1964.

Subject 3. An 80 year old Caucasian male who died of congestive heart failure due to hypertensive cardiovascular disease. No significant past history was elicited.

Extraction of Tissues

The specimens were frozen after removal and stored at −20° prior to their extraction. Grey and white matter were separated by careful dissection in the cold (4°). In each case approximately equal portions of cerebral grey matter were taken from the frontal, parietal, temporal, and occipital lobes and pooled. White matter samples were obtained in the same manner, by pooling approximately equal portions from the four above-mentioned lobes. Grey matter obtained from these lobes (subject 1) was 1.2 times as much (by weight) as the white matter and contained 8.2% lipid, 9.6% nonlipid dry residue, and 82.3% water. White matter contained 16.9% lipid, 7.9% nonlipid dry residue, and 75.2% water. Visual inspection of grey and white matter showed an estimated contamination of one with the other of about 10%. These fractions were then extracted with chloroform–methanol (C–M) 2:1 under nitrogen as described previously (3).

Column Chromatography

The column chromatographic procedures used for isolation of ethanolamine, serine, and choline glycerophosphatides (EGP, SGP, and CGP)[1] have been outlined previously (3, 4). One scheme used for isolation of these lipids was as follows. An aliquot (300 mg) of the total lipid extracted from brain was applied to a 2.5 cm (i.d.) × 25 cm diethylaminoethyl (DEAE)-cellulose column (Standard Selectacel-DEAE, Schleicher and Schuell, Keene, N.H.) prepared as described by Rouser et al. (3, 4). The eluting solvents and the lipids contained in each eluate were as follows. C–M 8:1 (250 ml): cholesterol, ceramide, cerebroside, CGP, and sphingomyelin; C–M 3:2 (300 ml): EGP; methanol (300 ml): water-soluble nonlipid salts, amino acids, and sugars; glacial acetic acid (300 ml): free fatty acids, SGP, and gangliosides; methanol (300 ml) to wash out acetic acid, and C–M–NH_4OH 4:1:0.5 (300 ml): phosphatidic acid, polyglycerol phosphatides, cerebroside sulfate, and inositol glycerophosphatides. The C–M 8:1 eluate was subsequently fractionated into its components on a 2.5 × 20 cm silicic acid–silicate–water column (3) to obtain the choline phosphatides. This column was prepared as follows. The fine particles were removed from 200 g of silicic acid (Mallinckrodt Reagent Grade) by suspending the latter in 2 liters of distilled water, decanting the liquid after 30 min, and repeating the procedure. The silicic acid was washed on a sintered glass filter funnel with 2 liters of 6 N HCl followed by 10 liters of distilled water and dried at 120° overnight in a 3-neck flask in vacuo as described previously (5). The dry silicic acid was transferred quickly to a stoppered flask, weighed, and cooled, and methanol was added to a height of approximately 1 in. above the support. This preparation was designated as acid silicic acid. The addition of 10 ml of concentrated NH_4OH for each 50 g of acid silicic acid gave a preparation designated as silicic acid–silicate–water. These columns were packed to a height of 20 cm by pouring a slurry of each preparation in methanol into a 2.5 × 40 cm glass column equipped with a Teflon stopcock, using a fine glass wool plug to retain the adsorbent. Nitrogen pressure (2.0 psi) was employed to ensure tight packing and the final height was 20 cm. Methanol was washed out of the column with 300 ml of chloroform and the sample was applied in chloroform. A flow rate of 2.5 ml/min was maintained under nitrogen pressure during the run.

The C–M 8:1 fraction was separated into its components on a silicic acid–silicate–water column as described previously (3, 4) using chloroform, C–M 19:1, C–M 4:1 plus 0.5% water, C–M 4:1 plus 1.5% water, and methanol to elute cholesterol, ceramide, cerebroside, CGP, and sphingomyelin respectively. Ten-milliliter fractions were collected, and the solvent was changed when an aliquot of each fraction evaporated to dryness in a test tube yielded virtually no solid. It was often necessary to rechromatograph both the CGP fraction and the sphingomyelin fraction because they overlap.

The glacial acetic acid fraction was separated into its components on an acid silicic acid column to obtain the SGP fraction. Although 95 and 90% acetic acid have been used to hydrolyze aldehydogenic linkages in plasmalogens (2, 6), no detectable hydrolysis of phosphatidal serine occurred in the glacial acetic acid eluate, attested by the absence of free aldehydes and lysophosphatidyl serine as judged by thin-layer or paper chromatography. This confirmed earlier reports (3, 4). The column method used for the isolation of SGP from the glacial acetic acid fraction was as follows. Acid silicic acid was prepared as described and packed in a glass column (2.5 × 40 cm) equipped with a Teflon stopcock to a height of 20 cm using a glass wool plug to retain the adsorbent. The dried glacial acetic acid fraction from the DEAE column was dissolved in chloroform and applied to the column. Chloroform (210 ml) eluted free fatty acids; C–M 9:1 (200 ml), an unidentified lipid; C–M 4:1 (300 ml) SGP; and methanol (200 ml) gangliosides plus an unidentified lipid. Rouser et al. (4) have previously suggested using silicic acid columns to separate the mixture of ganglio-

[1] The column chromatographic methods described herein give fractions which are mixtures of diacyl glycerophosphatides and plasmalogens. The term *ethanolamine glycerophosphatides* (EGP) refers to the total fraction, comprising phosphatidyl and phosphatidal ethanolamine. *Serine glycerophosphatides* (SGP) and *choline glycerophosphatides* (CGP) have similar meanings.

sides and serine phosphatides in this fraction, but the details of this procedure were not given.

In all the column chromatographic procedures described above precautions were taken to prevent oxidation of unsaturated lipids. Rouser et al. (3, 4) have given many details for carrying out tissue extractions, column elutions, and solvent evaporations under nitrogen and these procedures were used throughout the present work. Using these three column procedures uncontaminated EGP, SGP, and CGP fractions were obtained.

Paper and Thin-Layer Chromatography

The purity of each phosphatide was determined by paper and thin-layer chromatography. Neutral silicic acid paper (7) was heated to 100° for 3 min just prior to use and kept dry under glass plates during spotting to obtain optimal separations. The papers were developed in the ascending manner using C–M–NH_4OH 4:1:0.5 as the solvent. The approximate R_F values were: ethanolamine glycerophosphatides, 0.8; CGP, 0.6; and SGP, 0.2. The chromatograms were stained with either Rhodamine 6G and viewed under ultraviolet light, or sprayed with ninhydrin, which gave a blue color with the aminophosphatides (5). The *p*-rosaniline method for nonspecific staining of lipids (5) gave inconstant results and was modified as follows. The reagent was prepared by dissolving 0.6 g of triaminodiphenyltolylcarbinol (*p*-rosaniline) and 5 g of sodium bisulfite in 1 liter of distilled water and adding 15 ml of concentrated HCl. After development, the silicic acid papers were dried overnight prior to staining and then dipped in the staining reagent in an enamel tray. The papers were rinsed with a wash solution prepared in the same manner as the staining solution except that the *p*-rosaniline was omitted. This procedure differs from that of Rouser, O'Brien, and Heller (5) in that the staining reagent is not decolorized with charcoal, it need not be freshly prepared but can be reused for months, no specific staining of plasmalogens occurs, and the silicic acid papers must be dried overnight to obtain optimal results.

Thin-layer chromatography was carried out using Silica Gel G coated plates developed with C–M–NH_4OH 87:13:0.3 and sprayed with bromophenol blue. R_F values for the phosphatides in this system were: EGP, 0.3; CGP, 0.15; and SGP, no migration. "Weakened plates" impregnated with $(NH_4)_2SO_4$ as described by Mangold (8) were also used to obtain separation of the amino-phosphatides using C–M–H_2O 85:25:2.5 as the solvent. R_F values in this system were: EGP, 0.6; SGP, 0.3; and lysoethanolamine glycerophosphatide, 0.2. Hydrolysis of phosphatidal ethanolamine occurred in this system, as evidenced by the appearance of lysophosphatidyl ethanolamine and fatty aldehyde, a behavior also noted by Horrocks (9). Hydrolysis could be prevented by adding NH_4OH to the solvent in small amounts, but separation of SGP and CGP was then not obtained.

Methanolysis Conditions

Each phosphatide fraction was heated in 5% methanolic HCl for 1 hr at 60°. The resulting fatty acid methyl esters and aldehyde dimethylacetals (DMA) were extracted into petroleum ether (bp 30–60°). The extract was washed with water, dried over $MgSO_4$, and evaporated to dryness. This mixture was analyzed by GLC. DMA were isolated from the methanolysate by saponification with 5% methanolic KOH (methanol–50% aqueous KOH 9:1) at 60° for a 2 hr period. Saponification of fatty acid esters was complete using this procedure, without detectable hydrolysis of DMA (10). The DMA were then extracted into petroleum ether and weighed after evaporation of the solvent to determine the aldehyde content of each lipid. DMA content was also determined by measuring the area of the DMA peaks obtained when the mixture of fatty esters and DMA obtained after methanolysis was chromatographed by GLC. The agreement between the weighing procedure and the GLC procedure for determination of DMA content was close; in 7 determinations the DMA content determined by the GLC method differed by ±5% from the value obtained by the weighing method. The aqueous phase containing fatty acid soaps was acidified, extracted with petroleum ether, and weighed after evaporation of the solvent. Fatty acids were methylated using boron trifluoride–methanol (11).

Analytical Data

Lipid hydrolysates were examined by paper chromatography using butanol–acetic acid–water 4:1:5 as solvent (12). The only bases detected in ethanolamine and serine phosphatide hydrolysates were ethanolamine and serine, respectively, after hydrolysis in 3 N HCl at 120° for 5 hr, a procedure similar to that used by Rouser, O'Brien, and Heller (5). These phosphatides were analyzed for phosphorus content and for the proportion of total fatty esters plus DMA released by each after methanolysis (Table 1).

GLC of Fatty Acids and Fatty Aldehydes

Fatty acid methyl esters and fatty aldehyde dimethylacetals were chromatographed on polar and nonpolar columns. The stationary phases used were 3% Apiezon L on silanized Chromosorb W (13) and diethyleneglycol succinate polyester. Purified fatty acids were used as reference standards for identification. Those fatty acids for which standards were unavailable were tentatively identified by carbon number (14), plotting log of retention time versus chain length, or degree of unsaturation. Phosphatide fatty acids were also chromatographed after hydrogenation to confirm identity of certain unsaturated

TABLE 1 ANALYTICAL DATA FOR PURIFIED GREY MATTER PHOSPHATIDES

	Phosphorus	Fatty Esters + DMA*
	%	%
Ethanolamine glycerophosphatides (EGP)	3.90 (3.93)†	76.1 (78.2)
Choline glycerophosphatides (CGP)	3.91 (4.03)‡	70.0 (70.5)
Serine glycerophosphatides (SGP)	3.81 (3.75)§	73.2 (72.5)

Theoretical values in parenthesis.

* By weighing fatty esters plus DMA after methanolysis.

† Calculated for a mixture with an average fatty acid chain length of 20.1 carbons and an average aldehyde chain length of 17.5 carbons in which the fatty acid content is 78%.

‡ Calculated for an average fatty acid chain length of 17.3 carbons.

§ Calculated for an average fatty acid chain length of 19.3 carbons.

fatty acids. The major phosphatide fatty acids could be identified with reasonable certainty. However, several peaks in the C_{20} and C_{22} polyene series could not be identified unequivocally. Aldehyde DMA were identified by comparing retention times with those of known aldehyde DMA.[2] Several minor DMA peaks were not identified. Since standards were unavailable for many of the unsaturated acids and aldehydes, no attempt was made to correct for variations in the detector response for each compound. Quantification of each fatty acid or aldehyde was made by measuring the triangular area of each peak and expressing the peak area as per cent of the total.

RESULTS

Characterization of Glycerophosphatides

The ethanolamine glycerophosphatide fraction isolated from adult human grey or white matter was a clear transparent colorless oily solid, soluble in hexane, cyclohexane, and chloroform. When left in the atmosphere it became pale yellow initially and later assumed a deep tan or orange color. As this fraction became more colored it became less soluble in nonpolar solvents, as noted previously (5). The fatty acid composition of the oxidized EGP fraction from grey matter was compared with that of the unaltered grey matter EGP fraction and it was found that C_{20} and C_{22} polyenoic acids, present originally in high proportions, were absent in the oxidized preparation. The serine glycerophosphatide fraction was a semitranslucent greyish solid, soluble in chloroform and insoluble in methanol. Although it became colored when exposed to the atmosphere this change did not appear to be as rapid as that of the EGP fraction. The choline glycerophosphatide fraction was a semitranslucent greyish solid, soluble in chloroform. It remained colorless for much longer periods of time when exposed to the atmosphere than did either the other two lipids. These lipids gave single spots when examined by paper and thin-layer chromatography (Fig. 1). As each lipid became oxidized additional spots appeared which migrated more slowly than the unaltered phosphatides, a behavior reported previously (5). Infrared spectra of EGP, CGP, and SGP resembled those reported previously for phosphatidyl ethanolamine (4, 15), phosphatidyl choline (4, 16–17), phosphatidal choline (18), and phosphatidyl serine (4, 19, 20).

Quantification of Glycerophosphatides in Grey and White Matter

The concentrations of these glycerophosphatides from human cerebral grey and white matter are presented in Table 2. The average per cent deviation from the mean of 16 determinations of EGP from grey and white matter in these patients was ±2.7%; for SGP and CGP, deviations were ±8.5% and ±9% respectively. The recoveries of total lipids from the three columns used in isolating these lipids were as follows. DEAE columns: 100 ± 3 (12 column runs); silicic acid–silicate–water columns: 100 ± 3 (10 column runs); and silicic acid columns: 100 ± 5 (12 column runs).

In the grey and white matter of all three subjects ethanolamine glycerophosphatides were the most abundant glycerophosphatide, choline glycerophosphatides were present in smaller quantities, and serine glycerophosphatides were the least abundant. The concentrations of EGP and CGP were slightly greater in white than in grey matter, while the concentration of SGP in white was twice that in grey matter. The sum of these three glycerophosphatides accounted for approximately 40% of the total extractable grey matter lipid and 30% of the total extractable white matter lipid.

A fraction (not listed in Table 2) containing lysolecithin was also isolated from both grey and white matter.

TABLE 2 QUANTIFICATION OF PHOSPHATIDES IN GREY AND WHITE MATTER

		Grey			White		
Subject	Age	EGP*	CGP	SGP	EGP	CGP	SGP
		mg/g fresh tissue					
1	55†	16.0	16.5‡	5.3	21.3	19.9	10.3
2	77§	12.2	14.2‡	4.7	25.8	15.8‡	9.8
3	80§	10.5	12.7‡	3.5‡	26.7	15.1‡	8.2

* EGP, CGP, and SGP: ethanolamine, choline, and serine glycerophosphatides. See Footnote 1 in text.

† Analyses made in U.S.C. Laboratory.

‡ Single determination only.

§ Analyses made in U.C.L.A. Laboratory.

[2] A series of known aldehydes from 9:0 to 16:0 were kindly provided by Dr. Robert Stein, Department of Biochemistry, U.C.L.A. School of Medicine.

This lipid was identified by paper or thin-layer co-chromatography with authentic lysolecithin; almost every sample was seen to be contaminated with lecithin and sphingomyelin. Since the quantities of lysolecithin present were very small (less than 0.4 mg/g or 0.7 mg/g of grey and white matter respectively) no attempt was made to further purify, quantify, or characterize this fraction.

Paper and thin-layer chromatography of each fraction revealed complete absence of the lyso derivatives of ethanolamine and serine phosphatides. Rouser, O'Brien, and Heller (5) have reported the absence of these compounds in beef brain. When oxidized phosphatide preparations were examined, extraneous spots were observed which migrated very close to, but slightly ahead of, ethanolamine or serine lysophosphatides. These spots appeared to result from oxidation of unsaturated fatty acids since they were absent when oxygen was rigorously excluded, in keeping with previous findings (5). When "weakened" plates were used, other extraneous spots were obtained which migrated exactly with the lyso derivatives. These spots appeared to result from acid-catalyzed hydrolysis of phosphatidal ethanolamine and phosphatidal serine since they were absent when a small amount of NH_4OH was added to the system, in agreement with Horrocks (9). The presence of either oxidation or hydrolysis products which migrate close to lyso derivatives of these lipids could give the mistaken impression that lyso derivatives are present in vivo unless precautions are taken to prevent their artifactual occurrence.

Fatty Aldehyde Composition

The quantity of total aldehydes present in each phosphatide from grey and white matter in subject 1 was determined by weighing the dimethylacetals obtained after saponification of the lipid methanolysate and, after checking the value with that obtained by GLC analysis, expressing the value as follows:

$$\text{Per cent DMA in each phosphatide} = \frac{\text{mg DMA}}{\text{mg DMA} + \text{mg fatty esters}} \times 100.$$

Grey matter EGP, CGP, and SGP contained 22, 0.3, and 0.2% DMA, respectively, while in white matter these values were 49, 0.8, and 13% DMA, respectively (each figure is the average of four analysis). Clearly, in these tissues, "CGP" can be replaced by "phosphatidyl choline."

The quantitative fatty aldehyde composition of these phosphatides from grey and white matter is given in Table 3. The average deviation from the mean of triplicate determinations of each aldehyde from two samples of ethanolamine phosphatides from both grey and white matter was ±7%. The major aldehydes in each lipid were palmitaldehyde, stearaldehyde, and olealdehyde (Fig. 2). Small amounts of odd-numbered and unknown aldehydes (branched chain?) were present. The three

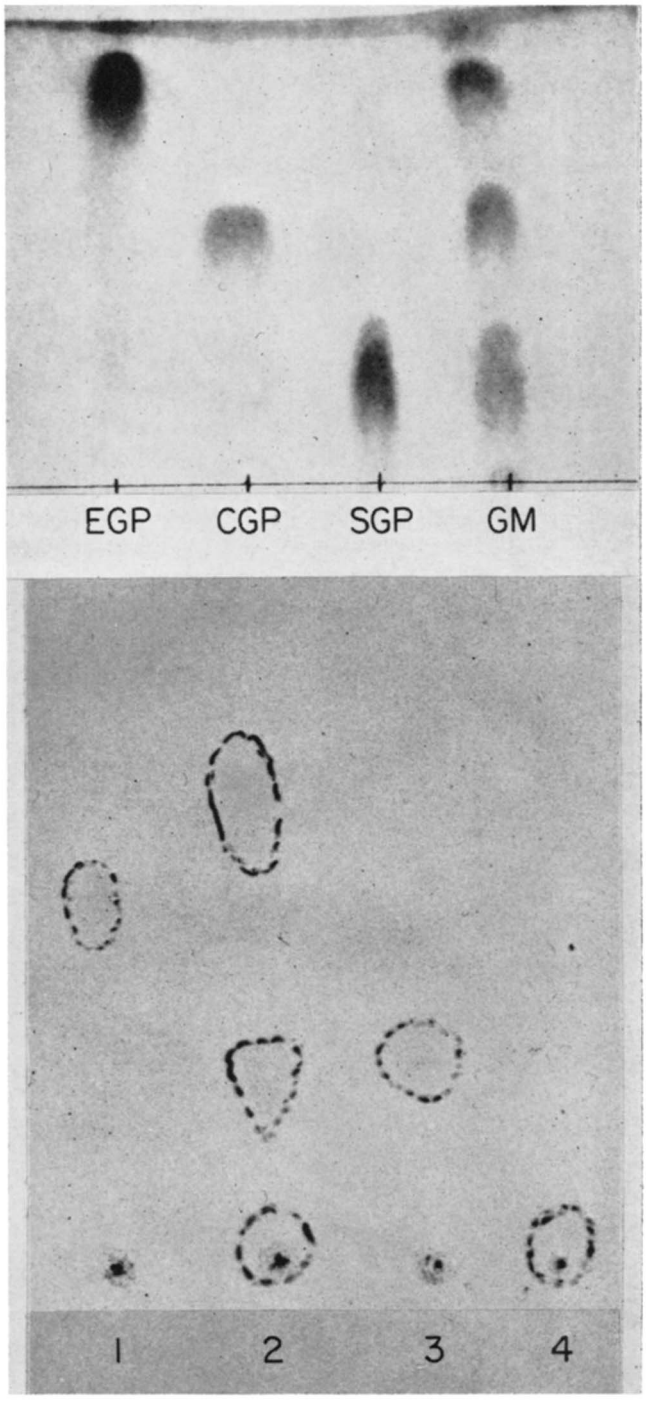

FIG. 1 *A*. Paper chromatography of phosphatides from grey matter (GM). Fifty micrograms of EGP, CGP, and SGP and 150 μg of grey matter total lipid chromatographed on silicic acid paper in C–M–NH_4OH, chromatogram stained with *p*-rosaniline. *B*. TLC of phosphatides from grey matter. Fifty micrograms of EGP, CGP, and SGP (1, 3, and 4 respectively) and a mixture (2) of the three phosphatides on Silica Gel G in C–M–NH_4OH. Sprayed with bromophenol blue; spots circled to improve photographic reproduction.

phosphatides were very similar to one another with respect to their aldehyde composition both in grey and white matter. However, each phosphatide from white matter contained much greater proportions of olealdehyde than from grey matter.

Fatty Acid Composition

The fatty acid compositions of these phosphatides in grey and white matter from subjects 1 and 3 are presented in Table 4. The average deviation from the mean of duplicate determinations of each fatty acid from two samples of each phosphatide from grey and white matter was ±8%. Comparison of results from two patients revealed close correspondence in qualitative and quantitative fatty acid composition of each phosphatide. When grey and white matter were compared, however, certain striking differences were observed. In grey matter, the major fatty acids of the EGP fraction were 16:0,[3] 18:0, 18:1, 20:4, 22:6, and an unknown C_{22} polyene [possibly 22:5ω6 (21, 22)] (Fig. 3). However, the EGP fraction from white matter was much more saturated than that from grey matter, containing increased proportions of 18:1 and 20:1, decreased 18:0 and 20:4, and markedly decreased 22:6. There was about 10 times as much 22:6, and one-fourth as much 18:1, in the EGP fraction from grey matter of each subject as in that from white matter (Table 4 and Fig. 3). Similarly, the SGP fraction showed a markedly greater proportion of 22:6 and somewhat lower percentage of 18:1 in grey matter. The CGP (phosphatidyl choline) fraction, on the other hand, had a similar fatty acid composition in each locale, containing predominantly 16:0, 18:0, and 18:1 acids.

Comparison of the fatty acid compositions of these three phosphatides reveals that EGP and SGP have similar compositions, both containing large proportions of C_{20} and C_{22} polyenoic acids. On the other hand, phosphatidyl cholines have a uniquely simple fatty acid composition comprised primarily of 16:0, 18:0, and 18:1 acids with only traces of polyenoic acids. Unlike the EGP and SGP, the fatty acid composition of phosphatidyl cholines from grey matter was almost identical with that from white matter.

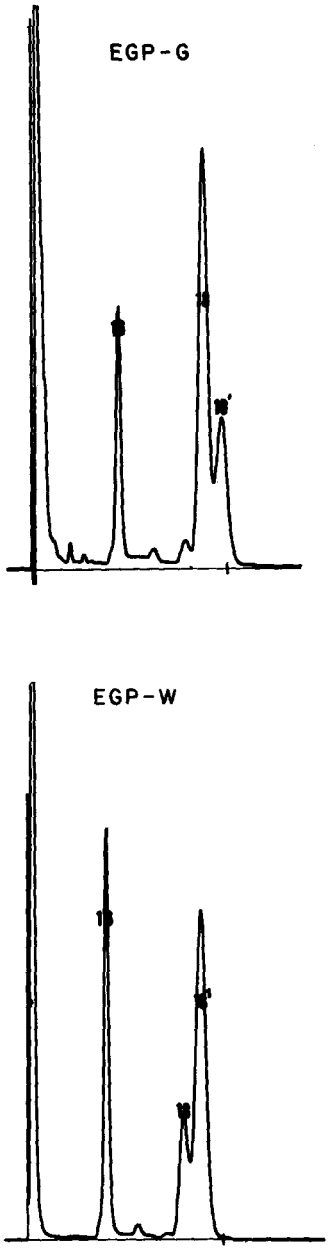

FIG. 2 GLC of EGP fatty aldehyde dimethyl acetals from grey matter (above) and white matter (below). Conditions: column 6 ft × 1/4 in. (i.d.), 10% diethyleneglycol succinate polyester on Chromosorb W, column temperature, 176°, flow rate, 150 ml of argon per min. Divisions at baseline are 10-min intervals.

TABLE 3 FATTY ALDEHYDE COMPOSITION OF PHOSPHATIDES (SUBJECT 1)
(EXPRESSED AS PER CENT OF TOTAL ALDEHYDE COMPOSITION)

	Grey Matter			White Matter		
Aldehyde	EGP	CGP	SGP	EGP	CGP	SGP
14:0	0.4	2.0	tr.	tr.	2.7	5.1
15:0	tr.	1.7	tr.	1.4	2.3	1.3
*	tr.	tr.	tr.	tr.	tr.	tr.
16:0	15.5	21.9	18.7	25.0	25.0	24.0
17:0	1.3	2.2	—	—	tr.	1.8
†	2.3	1.3	tr.	0.4	tr.	0.9
18:0	56.7	43.7	53.0	22.0	19.1	19.6
18:1	23.1	26.3	28.2	49.9	50.5	42.0

* Carbon number 15.7 on a polyester column (diethyleneglycol succinate).

† Carbon number 17.7 on a polyester column (diethyleneglycol succinate).

Each value is the average of duplicate determinations on two isolated samples.

[3] The chain length is given by the numeral before the colon and the number of double bonds by the numeral after it.

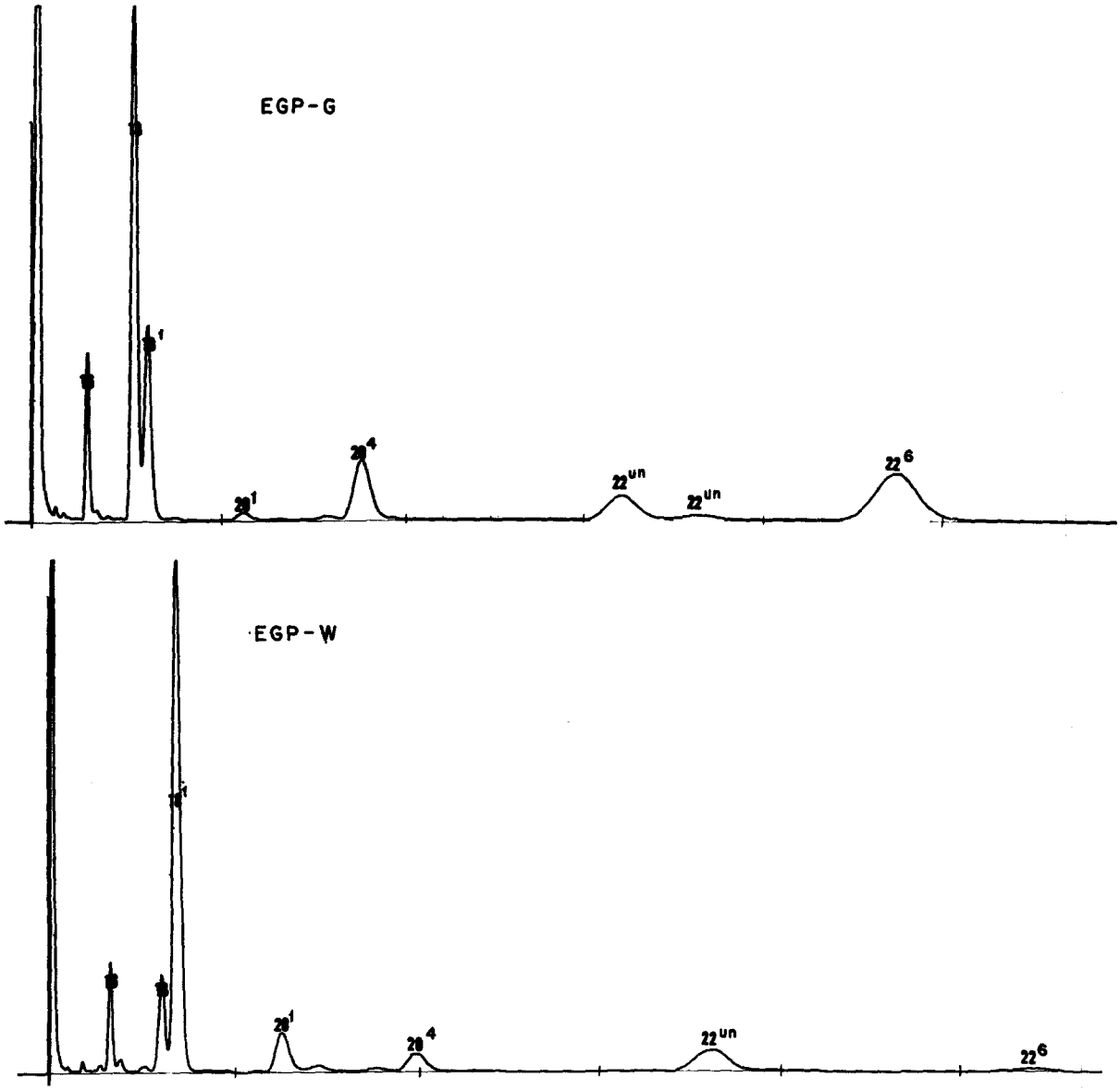

FIG. 3 GLC of EGP fatty acid methyl esters from grey matter (above) and white matter (below). Conditions as in Fig. 2 except for a column temperature of 187°.

DISCUSSION

The procedure employed in determining the quantities of these three glycerophosphatides and their fatty acid and fatty aldehyde compositions are thought to give accurate results for the following reasons. The column chromatographic isolation procedures gave reproducible quantitative results on repeated isolations. The recovery of lipids from these columns averaged 100 ± 5% of the applied sample. Each phosphatide contained only trace amounts of detectable contamination when examined by paper or thin-layer chromatography. Analytical data agreed closely with theory. Oxidative or hydrolytic alteration of these phosphatides did not occur during isolation or storage when appropriate precautions were taken in handling. Contamination of DMA with fatty acid esters or vice versa was not detectable after GLC of the purified esters or DMA. Quantification of DMA content by weighing or by GLC analysis gave values which agreed within 5%. GLC analyses of individual DMA or fatty acid esters agreed within 8%. With the exception of a 22-carbon unsaturated fatty acid (possibly 22:5ω6 (21, 22)), all the major fatty acids were identified. The fatty acid compositions of each phosphatide in grey and white matter gave reasonable agreement in both patients. For these reasons, it is felt that the fatty acid and fatty aldehyde compositions given for EGP, CGP, and SGP faithfully reflect in vivo compositions.

The quantities of the three glycerophosphatides in adult human cerebral grey and white matter agree in general with values reported previously in mammals (Table 5). It should be pointed out that in the other studies referred to, different methods of analysis were employed. Artom (23), Johnson et al. (24), and Folch and Lees (25) determined lipid-bound ethanolamine, choline, and serine while Rouser et al. (3), Rouser, O'Brien, and Heller (5), and the present authors isolated the phosphatides by column chromatography and weighed the product. The present values are the first reported for ethanolamine glycerophosphatides in grey and white matter and establish that white matter contains a higher concentration of these lipids than does grey matter.

The proportions of aldehydes derived from the three phosphatides in grey and white matter in subject 1 agree in general with those given in previous reports. The EGP fraction from white matter yielded 49% aldehydes and that from grey matter 22%, indicating that the EGP fraction from white matter is almost totally phosphatidal ethanolamine, while that from grey matter is about half phosphatidal ethanolamine. Webster (26) reported that 87–93% of the white matter cephalin and 48–52% of the grey matter cephalin is phosphatidal ethanolamine. Brante (27) also reported that the ethanolamine phosphatide fraction from white matter is almost totally phosphatidal ethanolamine

The SGP fraction yielded smaller quantities of aldehydes. Approximately one-third of the white matter serine phosphatide fraction was phosphatidal serine, while grey matter yielded only traces of phosphatidal serine. The choline phosphatide fraction in both grey and white matter gave only traces of aldehydes. Webster (26) also noted only traces of aldehydes in this lipid fraction from nervous tissue.

The quantitative and qualitative fatty aldehyde compositions of phosphatidal ethanolamine, phosphatidal choline, and phosphatidal serine were quite similar. Consistent with earlier observations (28–30) the major aldehydes found were palmitaldehyde, stearaldehyde, and olealdehyde (comprising approximately 90% of the total). Small amounts of odd-numbered and unknown (branched?) aldehydes were present, also noted by Debuch (30). The low proportions of branched chain aldehydes in grey and white matter is in contrast to extranervous tissue, where such components are present in larger proportions (31–33). An interesting difference was noted when the grey and white matter aldehyde compositions of each phosphatide were compared; olealdehyde was the major white matter aldehyde while stearaldehyde was the major grey matter aldehyde. The significance of this difference is not known.

There are several points of interest relating to the fatty acid composition of these phosphatides. One is concerned with the high proportions of polyenoic acids in EGP and SGP fractions, also found by others (1, 2). The choline glycerophosphatide fraction, however, contained small proportions of polyenoic acids both in grey and white matter, as previously reported (34). Although the *proportion* of unsaturated acids in the white matter EGP fraction, which is mainly phosphatidal ethanolamine, is 90%, the *degree* of unsaturation is less than in the EGP fraction

TABLE 4 Fatty Acid Composition of Glycerophosphatides
(Expressed as Per Cent of Total Fatty Acid Composition)

Fatty Acid	Ethanolamine Glycerophosphatides				Choline Glycerophosphatides				Serine Glycerophosphatides			
	Grey Matter		White Matter		Grey Matter		White Matter		Grey Matter		White Matter	
14:0	0.2*	—†	0.5*	—†	2.9*	—†	1.3*	—†	0.3*	—†	0.3*	—†
16:0	7.0	8.7	6.7	8.8	45.0	58.8	34.3	36.2	2.3	2.1	1.7	0.5
16:1	0.4	tr.	1.4	—	3.1	tr.	1.0	2.0	0.3	—	0.4	—
18:0	27.7	24.4	9.0	1.7	9.3	8.8	13.4	14.2	25.4	52.3	35.8	47.6
18:1	12.3	13.9	42.4	57.5	31.4	29.2	45.2	45.4	21.5	21.8	37.7	43.5
18:2	tr.	0.3	tr.	—	0.4	—	0.4	—	tr.	—	0.3	—
18:3	—	0.4	tr.	tr.	—	—	—	—	0.6	0.3	0.5	2.0
20:1	1.5	1.7	7.9	8.5	0.7	0.2	1.1	1.3	1.0	1.1	5.3	5.8
20:2	tr.	—	2.4	1.1	—	—	—	—	tr.	—	1.4	—
20:3	0.5	0.8	1.6	0.9	tr.	tr.	—	—	0.7	0.4	0.6	—
20:4	14.3	12.7	6.4	7.2	4.1	2.6	1.3	—	1.6	1.8	2.0	1.2
20:5	—	—	0.4	tr.	—	—	—	—	—	3.1	0.7	—
22 un‡	2.4	—	1.3	tr.	—	—	—	—	5.0	—	0.6	—
22 un§	12.0	10.7	13.7	12.5	—	—	0.3	—	3.3	—	4.2	—
22:5	—	0.6	0.5	tr.	—	—	—	—	—	1.3	0.9	—
22:6	25.2	24.0	3.4	1.9	3.1	tr.	0.1	—	36.6	15.7	5.6	—

Each analysis is the average of single or duplicate determinations on three isolated samples.
* Analyses made in U.S.C. laboratory on subject 1 (55 years).
† Analyses made in U.C.L.A. laboratory on subject 3 (80 years).
‡ Possibly 22:3, but identified by carbon number only.
§ Possibly 22:5ω6, since its carbon number (25.1 on a polyester column) and relative proportion of the total ester-linked acids is close to that given for this acid by Mohrhauer and Holman (21, 22). These authors (21) identified this acid by isolation and structural determination.

TABLE 5 COMPARISON OF BRAIN PHOSPHATIDE VALUES

Constituent	Grey Matter	White Matter	Whole Brain	Author
		mg/g fresh tissue		
"Phosphatidyl ethanolamine"			23–26*	Artom (23)
"Phosphatidyl ethanolamine"			23.6–26.7†	Rouser, O'Brien, and Heller (5)
EGP	10.5–16.0	21.3–26.7	18.6–23.3‡	Present study
"Phosphatidyl choline"	6.1, 11.6§	9.0, 14.9§		Johnson et al. (24)
"Phosphatidyl choline"			16.5†	Rouser et al. (3)
CGP	12.7–16.5	15.1–19.9	17.8–20.4‡	Present study
"Phosphatidyl serine"	6.0	14.0	13–17*	Artom (23) and Folch and Lees (25)
"Phosphatidyl serine"			9.3–10.0†	Rouser, O'Brien, and Heller (5)
SGP	3.5–5.3	8.2–10.3	6.0–10.3‡	Present study

* Rat brain.
† Beef brain.
‡ Calculated from grey and white matter values.
§ Two human brains.

from grey matter (where the aldehyde content is lower), mainly because of the replacement of 20:4 and 22:6 by 18:1. These results are in agreement with the work of Debuch (2), in which the fatty acids of the phosphatidal ethanolamine from whole brain were found to be exclusively unsaturated ones. The data also indicate that phosphatidal ethanolamine (and, by similar reasoning, phosphatidal serine) in brain do not contain large proportions of polyenoic C_{20} and C_{22} fatty acids.

The lower proportions of polyenoic acids in aminophosphatides from white matter than in those from grey matter may be a reflection of the higher myelin content of white matter. Since the introduction of polyenoic acids into a membrane results in a decrease in its stability because of a diminution in carbon–carbon interactions (35–38), and since myelin is a very stable membrane (39–41), it is tempting to explain the low proportions of polyenoic acids in white matter on this basis. The same hypothesis holds for the low content of branched-chain aldehydes in white matter, since it has been shown that the introduction of a branched methyl group into the chain also results in a marked diminution of van der Waals forces and decreased cohesion between adjacent hydrocarbon chains (36).

There is another significant point relating to the predominance of polyenoic acids in the grey matter aminophosphatides. In the brain, the primary site of lipochrome accumulation accompanying aging or in pathological states is grey matter. The formation of lipochrome results primarily from autoxidation of unsaturated fatty acids (42). It appears likely that ethanolamine and serine phosphatides in grey matter are prime candidates for lipochrome formation in vivo since they oxidize so readily in vitro and because they contain the highest proportion of polyenoic acids in any brain phospholipid, including those not reported here.[4]

[4] J. S. O'Brien, D. L. Fillerup, and J. F. Mead. Unpublished data.

Also revealing is a comparison of fatty acid composition of these three phosphatides with one another. As pointed out, EGP and SGP resemble each other in fatty acid composition (although they are not identical), while CGP resemble neither. If each of these phosphatides arises from a common pool of 1,2-diglycerides (43), in order to account for their differences in fatty acid composition one must propose either that selective incorporation of specific 1,2-diglycerides occurs into each phosphatide (44), that reacylation occurs after synthesis (45–47), or that both mechanisms exist.

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