Lipid composition of the normal human brain: gray matter, white matter, and myelin*

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SUMMARY Gray matter, white matter, and myelin were isolated from the frontal lobes of humans aged 10 months, 6 yr, 9 yr, and 55 yr and the lipid compositions of each were determined. Myelin had a much higher lipid content (78±1% of the dry weight) than white matter (49±6%) or gray matter (36±4%). Myelin contained much higher molar percentages of cerebroside and cerebroside sulfate, slightly higher molar percentages of cholesterol, and lower molar percentages of ethanolamine glycerophosphatides and choline glycerophosphatides than gray matter. The molar percentages of serine glycerophosphatides and sphingomyelin were about the same in each tissue.

The aldehyde content of glycerophosphatides, expressed as molar percentage of the total lipoidal residues in each lipid, were as follows: ethanolamine glycerophosphatides from myelin 40-50%; ethanolamine glycerophosphatides from gray matter 21-27%; serine glycerophosphatides from myelin 21-36%; serine glycerophosphatides from gray matter 0.3-3.8%. Choline glycerophosphatides from either tissue contained only traces of aldehydes.

The extra-myelin portion of white matter had a lipid composition that was very similar to that of myelin, but quite different from that of gray matter.

Assuming a molecular weight of 28,000 for myelin protein(s), it was calculated that for each protein molecule in human myelin there are 186 lipid molecules, 111 of which are polar lipids and 75 of which consist of cholesterol. The over-all molar ratios of the polar lipids are phosphatidyl ethanolamine:serine glycerophosphatides:choline glycerophosphatides:sphingomyelin:cerebroside:cerebroside sulfate:ceramide:uncharacterized lipids 25:9:20:9:29:7:3:9. It was calculated that the molar ratio of protein amino acids to polar lipids in human myelin is 2.38 to 1.

KEY WORDS brain - lipid - column chromatography - composition - gray matter - white matter - myelin - man - plasmalogen content - phospholipids - age

In order to interpret the chemical deviations from the normal which occur in diseases of the brain it is necessary to establish values in normal humans. Early investigations on brain lipids, especially those of Folch and co-workers (1) and Rossiter and co-workers (2-4) have established the nature and approximate concentrations of many of the major lipids in different areas of the brain. Brante (5) and Cumings and co-workers (6, 7) performed extensive analyses on a larger number of normal humans of varying ages. The methods used in these early studies often did not allow for complete fractionation and quantification of all the major lipids. Recently, Svennerholm and his co-workers (8) have used chromatographic techniques to achieve a more complete separation of the major lipid classes in the brain and they have applied these techniques to the analyses of cerebral gray and white matter of infants and adult humans. In their studies, each of the major sphingolipids was quantified separately, although the amino-glycerophosphatides were included in a "cephalin" fraction.

The present report is concerned with the column chromatographic procedures involved in the isolation and quantification of each of the major lipids, including cholesterol, cerebroside, cerebroside sulfate, sphingomyelin, EGP, SGP, and CGP in gray matter, white matter, and myelin from the frontal lobes of normal humans, ages 10 months, 6 yr, 9 yr, and 55 yr. Some of these data have recently been presented in a theoretical article (9), but it

Abbreviations: EGP, ethanolamine glycerophosphatides; SGP, serine glycerophosphatides; CGP, choline glycerophosphatides; C-M, chloroform-methanol; MeOH, methanol; NH4OH, concentrated ammonium hydroxide reagent (28-30% aqueous solution); DEAE, diethylaminoethyl; DMA, dimethyl acetals; GLC, gas-liquid chromatography.

was not possible to present there the details of the analytical procedures, the aldehyde content of each lipid, or the fatty aldehyde or fatty acid composition of each lipid. The present report deals with the chromatographic procedures involved in the isolation and quantification of these lipids and the accompanying report (p. 545) deals with the fatty acid and fatty aldehyde composition of each.

MATERIALS AND METHODS

The solvents used were all ACS reagent grade and were redistilled before use. Ethanol (1%) was added to the chloroform as a preservative. The nitrogen used contained less than 5 ppm of oxygen (high purity nitrogen, Linde Company). All solvent ratios given in the text are on a volume basis.

Subjects

No abnormalities were noted in any subject on gross or microscopic examination of the cerebral cortex. The subjects showed no evidence of malnutrition on postmortem examination and their neurological states were clinically normal prior to death. All were Caucasian males who died suddenly. Their ages and the causes of death were as follows: subject 1, 10 months old, acute gastroenteritis; subject 2, 6-yr old, auto accident; subject 3, 9-yr old, acute glomerulonephritis; subject 4, 55-yr old, acute myocardial infarction.

Extraction of Tissues

Specimens were frozen after removal and stored at -20° until their extraction. The interval between death and freezing varied between 4 and 18 hr. If autolytic alteration of lipids occurred during this time period, it must have been negligible since hydrolysis products of lipids (including free fatty acids and lyso-derivatives) were present in the same concentrations in the postmortem tissue as in tissue obtained by surgical biopsy. Gray matter and white matter were separated from each frontal lobe by careful dissection at 4°. Visual inspection of the separated tissues showed an estimated contamination of one with the other of less than 10%. These tissues were then extracted with C-M 2 to 1 in a nitrogen atmosphere as described previously (10–12). In the extraction procedure and during all subsequent maneuvers, including chromatography, weighing, hydrolysis, and storage, the lipid fractions were kept in a nitrogen atmosphere to prevent oxidation of unsaturated fatty acids. The values for the lipid content of each tissue were obtained as follows. The C-M 2:1 extract of each tissue was evaporated to dryness on a rotary flash evaporator at 27°. The dried extract was weighed after thorough drying in vacuo for 48 hr in a vacuum desiccator over solid KOH. The extract contained a small amount of nonlipid material, mainly sodium chloride, potassium chloride, sugars, amino acids, purines, and pyrimidines, most of which was eluted from the DEAE-cellulose column with methanol in the third eluate from the column (see Fig. 1). The weight of this nonlipid fraction was subtracted from the original weight of the extract to give a value for the tissue lipid content. This procedure obviated washing the extract with water, which could lead to the selective loss of certain lipids [for example, lysolecithin (13)] and also permit the oxidation of unsaturated fatty acids.

Isolation of Myelin

Myelin was isolated from an aliquot of frozen white matter using the ultracentrifugation technique of Autillo, Norton, and Terry (14). These authors used fresh ox brain, but we found their procedure to be applicable to the isolation of myelin from frozen human white matter. The isolation of myelin was halted at the “crude myelin” step and no attempt was made to separate light and heavy myelin since these fractions did not differ morphologically (14). An aliquot of the purified myelin obtained from subjects 3 and 4 was examined by electron microscopy after embedding in Vestopan, staining with potassium permanganate, and counter-staining with uranyl acetate. The appearance of these myelin preparations was identical with that described by Autillo et al. (14). The preparations were composed of smooth membranes, often layered in lamellar fashion, and cellular organelles (mitochondria, microsomes, or granular debris) were not detected.

Human brain myelin, like beef brain myelin, dissolved in C-M 2:1 to give a slightly opalescent solution. The myelin lipid extract could be freed from nonlipid residue (mainly protein) by evaporating each extract to dryness in a container with a large surface (rotary evaporation flask), drying the extract for 24 hr in vacuo in a desiccator containing solid KOH, and extracting the lipid from the dried residue with C-M 2:1. Using this procedure a small amount (1–2% of the dry weight) of nonlipid material (mainly sodium chloride and potassium chloride) was also extracted, but the bulk of the nonlipid residue remained in the flask. Reextraction of the nonlipid residue yielded only traces of further lipid. No attempt was made to obtain quantitative extraction of inositol phosphatides by the use of acidified solvents, and the amount of these compounds was not determined.

Column Chromatography

The column chromatographic procedures for the isolation of cholesterol, ceramide, cerebroside, cerebroside
sulfate, sphingomyelin, EGP,2 SGP, and CGP employed a DEAE-cellulose column for fractionation of lipids on the basis of their ionic charge, followed by subsequent fractionation of groups of lipids on silicic acid or Florisil columns. In our previous work (11, 12) procedures were described for the isolation of several lipids, but our overall elution scheme was not given. Our present elution scheme for the fractionation of all the major brain lipids is modified from that of Rouser, Baumann, Kritchevsky, Heller, and O'Brien (15) and is given in Fig. 1.

A portion (300 mg) of the total lipid extracted from each tissue was applied to a 25 x 2.5 cm (i.d.) DEAE-cellulose column. The eluting solvents and the lipids obtained in each eluate were as follows: C-M 9:1 (250 ml), cholesterol, ceramide, cerebroside, CGP, sphingomyelin, and lysolecithin (when present); C-M 3:2 (300 ml), EGP; methanol (300 ml), water-soluble nonlipid material; glacial acetic acid (300 ml), free fatty acids, two unidentified acidic lipids, SGP, and gangliosides; methanol (300 ml) to wash out acetic acid; and C-M-NH4OH 4:1:0.5 (300 ml), phosphatidic acid, polyglycerol phosphatides, phosphatidyl glycerol, cerebroside sulfate and inositol glycerophosphatides.

The C-M 9:1 eluate from the DEAE-cellulose column was evaporated to dryness and its constituents were subsequently fractionated on a 20 x 2.5 cm silicic acid column prepared as described previously (12). Cholesterol was eluted from this column with chloroform–hexane 1:1 (200 ml); ceramide with C-M 97:3 (150 ml); cerebroside with C-M 4:1 (200 ml); and CGP, sphingomyelin, and any lysolecithin present with methanol (200 ml). In previous publications the use of silicic acid–silicate–water columns has been reported for the separation of sphingomyelin and CGP (12, 15). However, we found it necessary to rechromatograph both the CGP and sphingomyelin fractions if this procedure was used because these fractions overlapped. Therefore, a mild alkaline hydrolysis procedure similar to that of Rapport and Lerner (16) was devised to quantify CGP and sphingomyelin.

An aliquot of the CGP–sphingomyelin fraction from the silicic acid column was first chromatographed on silicic acid paper to determine whether lysolecithin was present. In no instance was this compound present in more than trace quantities, in agreement with previous results (12, 15), and no attempt was made to isolate it. The solution of CGP plus sphingomyelin was evaporated to dryness and a sufficient quantity of 0.2 N KOH in methanol was added to make a final concentration of 2 mg of lipid per ml in a glass-stoppered graduated cylinder. Saponification was allowed to proceed at room temperature for 17 hr after flushing the cylinder with nitrogen and tightly stoppering it. An equal volume of 2 N aqueous HCl was then added and the reaction mixture was extracted three times with three volumes of chloro-
form. The separated chloroform extract contained fatty acids (released from CGP) plus unhydrolyzed sphingomyelin. The completeness of saponification was determined by chromatographing an aliquot of the chloroform extract on silicic acid-impregnated paper in C-M 4:1 (12, 17). Neither CGP nor lyssolecithin was detected after the saponification procedure. The chloroform phase, after evaporation to dryness, was redissolved in chloroform and applied to the same silicic acid column used for the initial isolation of CGP and sphingomyelin. The column was washed with chloroform (200 ml) before application of the sample; fatty acids were eluted with 200 ml of C-M 4:1 and sphingomyelin with 150 ml of methanol. Each eluate was evaporated to dryness and weighed on an analytical balance. The quantity of sphingomyelin was determined from this weight and the quantity of CGP was estimated by multiplying the weight of the fatty acids released after saponification by 1.4. This factor was employed because the weight of fatty acids released after saponification of CGP is equivalent to 70% of its molecular weight, its average fatty acid chain length in brain being 17.2 carbons.

The glacial acetic acid fraction from the DEAE-cellulose column was separated into its components (12) on the same silicic acid column described above. It was evaporated to dryness and applied to the column in chloroform. Chloroform (210 ml) eluted free fatty acids; C-M 9:1 (60–80 ml), an unidentified yellow autofluorescent lipid; C-M 9:1 (200 ml), SGP; and methanol (200 ml), gangliosides, plus an unidentified acidic lipid.

Cerebroside sulfate was isolated from the C-M-\(\text{NH}_4\)-OH eluate from the DEAE-cellulose column by chromatography on a Florisil column as described previously (12). The dried C-M-\(\text{NH}_4\)-OH fraction was dialyzed in a cellophane dialysis bag (12) to remove ammonium acetate generated by the ion-exchange process. After dialysis the dried fraction was applied to a Florisil column (20 X 2.5 cm) in C-M 9:1; C-M 9:1 (200 ml) eluted an uncharacterized lipid, and C-M 2:1 (250 ml) eluted cerebroside sulfate. The remaining phosphatides, mainly inositol glycerocephosphatides, were not eluted from this column and were not quantified.

The recoveries of total lipids from the columns used in isolating these lipids were as follows: DEAE-cellulose columns: 104 ±3% (31 column runs); and silicic acid columns: 100±4% (75 column runs). The recoveries from the DEAE-cellulose column were slightly greater than 100% because of the ion-exchange process; sodium chloride and potassium chloride present in the lipid extract were recovered as acetate salts in the third eluate from the column. The recoveries from the Florisil column were less than 100% since some phosphatides, mainly inositol glycerocephosphatides, were not eluted from this column.

**Paper Chromatography**

The purity of each lipid fraction was determined by paper chromatography. Silicic acid-impregnated paper, prepared as described previously (10), was used for the separation of each lipid fraction. For analysis of the cholesterol fraction, the solvent used was ether–hexane 5:95 (17). This fraction contained traces of components which co-chromatographed with triglycerides and hydrocarbons, but cholesterol esters were not detected. Ceramide was chromatographed in chloroform–hexane 1:1, its \(R_f\) in this system being 0.5. Cerebroside and cerebroside sulfate were chromatographed in C-M 9:1 (17, 12) and traces of lipids migrating ahead of cerebroside were detected in most preparations. These lipids were thought to be minor cerebrosides (18) and galactosyl diglyceride described by Norton and Brotz (18) and Stein and Benson (19). Their concentrations were too small for routine isolation and they were included in the cerebroside fraction. EGP, SGP, CGP, and sphingomyelin were chromatographed in C-M 4:1 or in C-M-\(\text{NH}_4\)-OH 4:1:0.5 (17, 12). All paper chromatograms were stained with either Rhodamine 6G and viewed under ultraviolet light (10, 12) or stained with the modified p-rosaniline method (12) which gave brilliant purple spots. Prior to fatty acid analysis, each lipid preparation was established as chromatographically pure except for trace amounts of other galactolipids in the cerebroside fraction and traces of lyssolecithin in the CGP fraction.

**Analytical Data**

In most instances each lipid was quantified in duplicate or in triplicate. The average percentage deviation from the mean of duplicate determinations of the major constituents (>5% of the total lipids) ranged between 2 and 5% while that for minor constituents (<5% of the total lipids) ranged between 5 and 12%. Each lipid was quantified by evaporating the appropriate eluate to dryness in a preweighed, stoppered flask, flushing with nitrogen, stoppering it tightly and weighing it on an analytical balance. In this way it was possible to weigh the fractions in a nitrogen atmosphere in an attempt to prevent the oxidation of unsaturated fatty acids.

Individual lipids were also analyzed for their contents of phosphorus, hexose, sphingosine, sulfate, and fatty acids. The results of these analyses for cerebroside, cerebroside sulfate, EGP, SGP, and CGP corresponded closely to theoretical values, as reported previously using these methods (11, 12), indicating that contamination with adsorbents or with other lipids did not occur. Each lipid was also analyzed by infrared spectroscopy as a further check of purity (11, 12). The carbohydrate compositions of cerebroside and cerebroside sulfate were also analyzed after acetylation of their hexoses and gas–liquid chromatography of the hexose penta-acetates as de-
TABLE 1 Concentrations of Lipids in Gray Matter, White Matter, and Myelin of Human Brains

<table>
<thead>
<tr>
<th></th>
<th>10-month old</th>
<th>6-yr old</th>
<th>9-yr old</th>
<th>55-yr old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>84.1</td>
<td>80.8</td>
<td>—</td>
<td>83.2</td>
</tr>
<tr>
<td>Total lipid</td>
<td>36.4</td>
<td>49.0</td>
<td>78.0</td>
<td>35.8</td>
</tr>
<tr>
<td>Nonlipid residue</td>
<td>63.6</td>
<td>51.0</td>
<td>22.0</td>
<td>64.2</td>
</tr>
<tr>
<td>Total glycerophosphatides*</td>
<td>20.3</td>
<td>20.3</td>
<td>31.7</td>
<td>22.5</td>
</tr>
<tr>
<td>Total sphingolipids†</td>
<td>5.1</td>
<td>14.3</td>
<td>24.7</td>
<td>3.8</td>
</tr>
<tr>
<td>Unidentified</td>
<td>3.0</td>
<td>2.9</td>
<td>3.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>7.9</td>
<td>11.5</td>
<td>18.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Ethanolamine glycerophosphatides</td>
<td>6.8</td>
<td>9.4</td>
<td>14.2</td>
<td>10.6</td>
</tr>
<tr>
<td>Serine glycerophosphatides</td>
<td>2.8</td>
<td>2.4</td>
<td>5.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Choline glycerophosphatides</td>
<td>10.8</td>
<td>8.6</td>
<td>12.1</td>
<td>8.3</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>1.8</td>
<td>2.1</td>
<td>4.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Cerebroside</td>
<td>1.8</td>
<td>8.5</td>
<td>13.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Cerebroside sulfate</td>
<td>0.7</td>
<td>2.5</td>
<td>5.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Ceramide</td>
<td>0.8</td>
<td>1.1</td>
<td>1.2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

All values, except water, are expressed as a percentage of the dry weight.

* Sum of EGP, SGP, and CGP.
† Excluding gangliosides.

scribed previously (11). In subjects 2 and 4, more than 99.5% of the hexose present in these two lipids (both from gray and white matter) was galactose.

Determination of Fatty Aldehydes

The fatty aldehyde content of each glycerophosphatide was determined by isolating the purified aldehyde dimethyl acetals by the method of Farquhar (20, 12). Fatty acids and fatty aldehydes were released from each glycerophosphatide by heating the lipid in 5% methanolic HCl for 1 hr at 60°. Fatty acid methyl esters and aldehyde DMA were extracted into petroleum ether. The DMA were selectively isolated after saponification of the fatty acid esters in methanolic KOH. The quantities of total DMA present in each glycerophosphatide were determined both by weighing the isolated DMA and by GLC. The GLC method for quantification of total DMA has been outlined previously (12).

The aldehyde content of each lipid was calculated using the expression:

\[ \% \text{aldehyde} = \frac{\text{mg DMA}}{\text{mg DMA} + \text{mg fatty esters}} \times 100. \]

This calculation gave the content of aldehydes as a weight percentage of the total lipoidal residues present in each glycerophosphatide. These values were then converted to molar percentages using the appropriate conversion factors, knowing that the average fatty aldehyde chain length was 17.5 carbons, and that the average fatty acid chain length was 19.0 carbons. It was also assumed that all the aldehydes were originally present in \( \alpha, \beta \)-unsaturated ether linkage.

RESULTS

Quantification of Each Lipid

The lipid content and composition of each tissue is given in Table 1. The sum of the major lipids was, on the average, 93% of the lipids present in each tissue. Lipids not listed in the table included inositol glycerophosphatides, gangliosides, free fatty acids, hydrocarbons, traces of cholesterol esters, triglycerides, galactosyl diglyceride, phosphatidylglycerol, polyglycerolphosphatides, and phosphatidic acid, all of which are included in the "uncharacterized" fraction.

Gray matter contained 36–40% lipid, white matter 49–66%, and myelin 78–81%. When these tissues were compared, the lipid composition of myelin was very close to that of white matter. In order to make direct comparisons between each tissue, the value for each lipid was calculated as a molar percentage of the total lipids present (Table 2). Although these molar percentages have been presented elsewhere recently (9), they are included here for comparative purposes. The molecular weight of each lipid was known since the fatty acid compositions of each were determined; these molecular weights are listed in Table 3 for reference. Gray matter contained higher molar percentages of EGP, CGP, and ceramide than myelin, while myelin contained slightly higher molar percentages of cholesterol, and much higher molar percentages of cerebroside and cerebroside sulfate than gray matter. The molar percentages of SGP and sphingomyelin were approximately equal in gray matter and in myelin.
The lipid content of gray matter and myelin did not vary significantly with age. However, the lipid content of white matter was lower in the youngest subject (49%) than in the three older subjects (58–66%). The smaller lipid content of white matter in the 10-month-old child probably reflects a smaller content of myelin in white matter at this age, since myelinization in the human is incomplete, as judged histologically, until approximately 2 yr of age (21).

The fatty aldehyde content of each glycerophosphatide is given in Table 4 and the values are expressed as a molar percentage of the total lipoidal residues present in each lipid. The molar percentages of aldehydes in EGP from gray matter were 22–28% and in EGP from myelin 42–53%, indicating that EGP from gray matter consisted of about one-half phosphatidial ethanolamine and EGP from myelin was composed almost entirely of plasmalogens. The molar percentages of aldehydes in SGP from gray matter were 0.3–4.0% and in SGP from myelin 22–38%, indicating that SGP from gray matter were predominantly phosphatidyl serine, while SGP from myelin were about one-half phosphatidyl serine. In each tissue, CGP contained only traces of aldehydes, in accordance with previous results (22, 12).

DISCUSSION

Comparison With Previous Results

The values found in the present study for the major brain lipids of gray and white matter correspond in general with recent results reported by Svennerholm and his co-workers for humans (8). Although there were variations from individual to individual, these variations were smaller than those found in earlier studies. This may reflect the use of more accurate and precise methods, as Svennerholm has suggested (8). The occasional unexplained variations in our study, such as the low value for EGP in gray matter in subject 1, may result from either systemic diseases or drug therapy, since the values for EGP in gray matter of other subjects of the same age have been in the same range as in older subjects. The only major discrepancy between our values and those reported by Svennerholm (8) are the higher amounts of cerebroside in gray matter found by us. This difference could not be explained by contamination of cerebroside with other lipids, such as galactosyl diglyceride, since contaminants were detected in trace quantities only and it was possible to eliminate such contaminants by mild alkaline hydrolysis and rechromatography. When these contaminants were quantified they constituted less than 5% of the total cerebroside. Therefore, the reasons for the discrepancy between our results for cerebroside in gray matter and those reported by Svennerholm (8) are not apparent to us. Comparison of our values for total cerebrosides (cerebroside plus cerebroside sulfate) on a wet weight basis with those given by Davison and Wajda (23) gave close agreement; gray matter total cerebrosides in our subjects averaged 0.36% of the wet weight while these authors (23) gave values of 0.31 ± 0.02%.

Myelin in Different Mammals

The lipid composition of human brain myelin is similar to that recently reported for myelin from other species. In these species including ox (24), guinea pig (25, 26), and rat (27, 28), central myelin contains 70–80% lipid and 19–30% protein. In the recently reported study of ox myelin by Norton and Autilio (24), the molar ratios of the individual lipids in the “light myelin” fraction (fraction A) were cholesterol:EGP:SGP:CGP:sphingomyelin:cerebroside:cerebroside sulfate

10.0:2.8:1.5:1.6:1.3:3.7:0.7.

In human myelin the same ratios were

10.0:3.4:1.2:2.7:1.2:3.8:1.0.

Some differences exist between ox myelin and human myelin, especially their CGP content. These differences may represent species variations. However, the concen-

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trations of individual lipids varied from subject to subject both in Norton and Autilio's (24) ox myelin study and in our human myelin study, and the significance of the differences between ox myelin and human myelin cannot be fully assessed until a larger number of brains has been analyzed and the variability between individuals established. Nevertheless, one important point stands out when myelin is compared in these mammals: the lipid composition of myelin is surprisingly similar in each.

**Lipid Composition of the Extra-Myelin Portion of White Matter**

The major structures in white matter are oligodendroglial cells, axons, and myelin sheaths. Brante (5) has estimated that in the mature human, 50% of the dry weight of white matter is myelin. Autilio et al. (14) arrived at a value close to 40% for the myelin content of ox white matter. In the 55 yr old man (subject 4), assuming a myelin content of white matter of 50%, it can be calculated that 60% of the lipids in whole white matter are in myelin while 40% are in extra-myelin structures. Similarly, 64% of the cerebroside in white matter is in myelin and 36% is in extra-myelin structures. Autilio et al. (14) reported that 72% of the total galactolipids of ox brain white matter were in myelin and 28% were in extra-myelin structures. These observations refute the belief that cerebrosides are essentially confined to myelin.

The lipid composition of the extra-myelin portion of white matter closely resembled that of myelin but differed greatly from that of gray matter (Table 3). This was true not only for the 55-yr old man but also for the 10-month old baby. Although the lipid content of the extra-myelin portion of white matter was lower than that of myelin in all subjects, the extra-myelin portion of white matter contained nearly the same proportions of cerebroside, cerebroside sulfate, and cholesterol as myelin did. In the following study (p. 545) it was found that the lipids in the extra-myelin structures of white matter have fatty acid compositions which are very similar to those of myelin lipids but differ from those of gray matter lipids.

**Molar Ratios of Myelin Constituents**

Gent, Gregson, Gammach, and Raper (28) have recently reported the average molecular weight of rat brain myelin protein(s) as 28,000. The over-all molar composition of human myelin can be calculated from our data if the same average protein(s) molecular weight

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>MOLECULAR WeIGHTS OF MAJOR BRAIN LIPIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10-month old</td>
</tr>
<tr>
<td>EGP</td>
<td>777.4</td>
</tr>
<tr>
<td>SGP</td>
<td>843.4</td>
</tr>
<tr>
<td>CGP</td>
<td>763.2</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>730.9</td>
</tr>
<tr>
<td>Cerebroside</td>
<td>749.0</td>
</tr>
<tr>
<td>Cerebroside sulfate</td>
<td>837.5</td>
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<tr>
<td>Ceramide</td>
<td>564.5</td>
</tr>
</tbody>
</table>

The molecular weights of each lipid were calculated using the average fatty acid and fatty aldehyde chain length given in the following report. Sphingosine was assumed to be the common molecular weights of lipids in these two tissues will be nearly the same.

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>FATTY ALDEHYDE CONTENT OF GLYCEROPHOSPHATIDES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10-month old</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>24.8</td>
</tr>
<tr>
<td>Serine</td>
<td>2.6</td>
</tr>
<tr>
<td>Choline</td>
<td>tr.</td>
</tr>
</tbody>
</table>

Aldehyde contents of each lipid are given as molar percentages of the total lipoidal residues present. It was assumed that all fatty aldehydes were present in α,β-unsaturated eitha linkage.

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is assumed, and knowing that the protein(s) comprises 19–20% of the dry weight of human myelin and the lipids 78–80%. For each molecule of myelin protein there are 186 lipid molecules, of which 111 are polar lipids and 75 are cholesterol. Vandenheuvel (29) has calculated the average molecular weight of the amino acids of myelin protein(s) as 106 from the data of Hulcher (30) on beef myelin protein. Using this value, each molecule of myelin protein would contain 264 amino acids and the ratio of amino acids to polar lipids for human myelin would be 264:111, a ratio of 2.38 amino acids for each polar lipid. This value is close to that given by Vandenheuvel (29) from the data on ox myelin of Norton and Autilio (24), in which the ratio of amino acids to polar lipids was calculated as 19.45 to 7.02, or 2.78 to 1. Cholesterol is excluded from these calculations since it is assumed that each cholesterol molecule interacts with a glycerophosphatidyl molecule or a sphingolipid molecule and not directly with myelin protein amino acids as suggested by Finean (31, 32) and Vandenheuvel (33). If in human myelin there are 75 molecules of cholesterol and 111 of polar lipids, 67.5% of the polar lipids in myelin could be present as glycerophosphatidyl–cholesterol complexes or sphingolipid–cholesterol complexes. For each molecule of human myelin protein(s) the molar ratios of the individual lipids are cholesterol:phosphatidyl ethanolamine:SGP:CGP:sphingomyelin: cerebroside:cerebroside sulfate:ceramide:uncharacterized lipids (mainly phosphatidyl inositol) 75:25:9:20:9:29:7:3:9. We wish to emphasize that the ratios of individual lipids to one another are accurate, but the molar ratios of these lipids to the protein are correct only if the assumed average molecular weight (28,000) of the myelin protein is correct.

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