Lipid composition of axolemma-enriched fractions from human brains

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Abstract The lipid composition was determined for axolemma-enriched fractions and myelin which were isolated via a preparation of purified myelinated axons. The myelin had a lipid composition which was compatible with that previously reported for myelin isolated by alternative procedures. The most dense axolemma-enriched fraction contained 25.3% cholesterol, 25.8% galactolipid (21.3% cerebrosides and 4.8% sulfatides), and 48.9% phospholipid. The major phospholipids were the ethanolamine phospholipid (19.8% of total lipid weight; 49.0% in the plasmalogen form) and choline phospholipids (18.7% of total lipid weight; 16.0% in the plasmalogen form) with lesser amounts of sphingomyelin, phosphatidylserine, and phosphatidylinositol also present; the ganglioside content was 13.9 μg of acetylneuraminic acid per mg protein. The less dense axolemma-enriched fraction had a lipid composition which was intermediate between that of myelin and the more dense axolemma-enriched fraction. On the average, less than 2.3% of the total protein in the axolemma-enriched fraction was myelin basic protein. Both axolemma-enriched fractions stained uniformly with Luxol fast blue and demonstrated specific saxitoxin-binding which was enriched 2- to 7-fold over that of the whole white matter homogenate from which the fractions were isolated. The choline and ethanolamine phospholipids in that most dense axolemma-enriched fractions contained a greater percentage of unsaturated fatty acids compared with the comparable phospholipids in myelin. The content of unsaturated fatty acids in these phospholipids of the axolemma-enriched fraction was not as great as that of human CNS synaptic plasma membranes. However, the chain length distribution of these phospholipid fatty acids was similar in myelin, synaptic plasma membrane, and the axolemma-enriched fraction. The distribution of aldehydes derived from the ethanolamine phospholipids of the more dense axolemma-enriched fraction closely resemble the distribution of the comparable aldehydes in the myelin fraction. The possible origin and function of the lipids in the axolemma-enriched fractions are discussed. —**DeVries, G. H., W. J. Zetusky, C. Zmachinski, and V. P. Calabrese.** Lipid composition of axolemma-enriched fractions from human brains. *J. Lipid Res.* 1981. **22:** 208–216.

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Although the lipid composition of a number of axolemmal preparations isolated from invertebrates or lower vertebrates has been described (1–5), the lipid composition of the mammalian axolemma has not been well documented. We have devised a method to isolate axolemma-enriched fractions via a preparation of CNS myelinated axons (6) and have recently reported the lipid composition of these fractions isolated from rat CNS (7). In this study, we report the lipid composition of axolemma-enriched fractions isolated from human brain, as well as that of human CNS myelin that is isolated simultaneously by the same procedure.

EXPERIMENTAL PROCEDURE

Preparation of subcellular fractions

White matter was obtained at autopsy (usually within 12 hr of death) from patients who had no evidence of any neuropathology. The exact age of the patients was not known although they were mostly in the range of 50 to 70 years. The tissue was immediately frozen at −80°C and used after having been frozen from 1 day to 8 months. Unless otherwise noted, all lipid analyses are the average of at least three different preparations. Each preparation was derived from six different brains. The axolemma-enriched fractions and myelin were isolated from white matter exactly as described previously (8). Briefly, the white matter from corpus callosum was minced, and a 2% (weight/volume) homogenate was prepared with a Dounce homogenizer in 0.85 M sucrose containing 0.15 M NaCl and 0.01 M TES buffer at pH 7.5. Following centrifugation, the floating layer of myelinated axons which was derived from 1 g of white matter was rehomogenized in 37 ml of the buffered salt-sucrose solution and further purified by recentrifugation. A total of three flotations was used to obtain the purified myelinated axons which

Abbreviations: AcNeu, acetylneuraminic acid; AXL, axolemma-enriched fraction; CNS, central nervous system.

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were then subjected to osmotic shock in 0.01 M TES, pH 7.5. The shocked myelinated axons were separated on a discontinuous density gradient as previously described (8) to obtain a membrane fraction at the interface of the 0.8 M and 1.0 M sucrose (designated the 0.8/1.0 fraction) and a membrane fraction at the interface of the 1.0 M and 1.2 M sucrose (designated the 1.0/1.2 fraction). Each membrane fraction was reapplied to a second discontinuous density gradient to obtain final membrane fractions which were homogenous by the criterion of density. The myelin used in this study was obtained from the first discontinuous gradient and was used without further purification.

A 6-g preparation of gray matter obtained from 1-g portions of six separate brains was used for fatty acid analysis in this study. In addition, two separate preparations of grossly dissected gray matter from two different brains were used for fatty aldehyde analysis. Synaptosomal membranes were isolated by the procedure of Cruz and Gurd (9) from 6 g of gray matter obtained in equal proportion from three different brains. The morphology of the final preparation was examined by electron microscopy as previously described (10).

Lipid analysis

Lipids were extracted from all fractions by the procedure of Folch, Lees, and Sloane Stanley (11). The analytical procedures for the separation and quantitation of cholesterol, phospholipid, and galactolipid have been described (10). All solvents used in this study were redistilled. Sulfatides were quantitated by the procedure of Kean (12). Galactocerebroside content was determined by the difference between total galactolipid and sulfatide content. Gangliosides were extracted from the lipid extract as previously described (10). The ganglioside AcNeu content was quantitated by the gas–liquid chromatographic procedure of Yu and Ledeen (13). Ganglioside AcNeu was converted to ganglioside weight by assuming that one-third of ganglioside weight is ganglioside AcNeu.

Gas–liquid chromatographic analysis

Fatty acid and fatty aldehyde standards were obtained from Applied Science Laboratories (State College, PA). Column packings for gas–liquid chromatography were obtained from Supelco, Inc. (Bellefonte, PA). For fatty acid analysis, the extracted lipids were separated by thin-layer chromatography on silica gel G plates using the developing system $CHCl_3$–CH_3OH–H_2O 70:30:4 (v/v/v) to obtain a choline phospholipid and ethanolamine phospholipid fraction. Blank silica gel zones were also taken through the procedure to correct for non-phospholipid bound fatty acids. Free aldehydes were obtained from the ethanolamine phospholipid containing silica gel by acid hydrolysis with concentrated HCl using the method of Anderson et al. (14) which gives quantitative release of aldehydes. The aldehydes were extracted using diethyl ether and hexane and purified by thin-layer chromatography on silica gel G plates using benzene as the developing agent. The aldehydes were located on the plate using a standard aldehyde that was visualized with a 2,4 dinitrophenylhydrazine spray, while the sample lanes were covered with a glass plate. The sample aldehydes were then eluted from the silica gel with hexane and analyzed as the free aldehyde by gas–liquid chromatography using a 6-foot stainless steel column of 15% DEGS on 100/120 chromosorb P AW/DMCS or 12% stabilized DEGS on 100/120 Gaschrom Z. The identity of the fatty aldehydes was confirmed by comparison of the retention times with those of fatty aldehyde standards. A Beckman GC-72 Gas–Liquid Chromatograph equipped with a flame ionization detector and a Columbia Scientific Instrument Supergrator I was used in these studies. Hydrolysis, purification, and analysis were always carried out the same day to reduce the problem of degradation. Fatty acid methyl esters were prepared directly from the choline- and ethanolamine-containing silica gel zones by alkaline methanolysis using the method of Sun and Horrocks (1). The concentrated hexane extract was analyzed without further purification on a 6-foot stainless steel column of 10% SP 2340 on chromosorb P 100/120 AW/DMCS. The identity of the fatty acid methyl esters was confirmed by comparison of retention times with those of standard fatty acid methyl esters.

Other procedures

Myelin basic protein was quantitated by the radioimmunometric assay of Cohen, McKhann, and Guarnieri (16). Luxol fast blue staining of the human axolemma-enriched fractions was carried out as previously described for the bovine axolemma-enriched fractions (17). The binding of saxitoxin to the membrane fractions was carried out by the procedure of Weigele and Barchi (18) using a [^{3}H]saxitoxin concentration of 3.5×10^{-7} M and 50–100 μg of membrane protein. Labeled and unlabeled saxitoxin was obtained from Dr. P. Chen at the National Institutes of Health. The [^{3}H]saxitoxin was prepared as described by Ritchie (19) and had a specific activity of 9,100 dpm per picomole. Protein was determined by the procedure of Lowry et al. (20).

TABLE 1. Saxitoxin binding to subcellular fractions from human brain

Fraction	[^{3}H]Saxitoxin-Bound
	pmol/mg protein
Whole homogenate	1.18 (1.0)
Axolemma-enriched fractions	
0.8/1.0	2.04 (1.7)
1.0/1.2	8.28 (7.0)

All values represent the specific saxitoxin binding observed at a [^{3}H]saxitoxin concentration of 3.5×10^{-7} M. The numbers in parentheses indicate the enrichment in binding over that observed in the white matter whole homogenate from which the axolemma-enriched proteins were prepared.

RESULTS

The binding of the sodium channel probe saxitoxin to human CNS axolemma-enriched fractions and white matter whole homogenate from which the fractions are isolated is shown in **Table 1.** The binding observed at this concentration of saxitoxin is within the range observed for other axolemma fractions (3, 21) and is an indication of the enrichment of the sodium channel in these membrane fractions.

The general characteristics of the lipid composition of the axolemma-enriched fractions and the concomitantly isolated myelin are listed in **Table 2.** The lipid composition of myelin agrees quite closely with that previously reported for myelin isolated by another procedure (22). This indicates that, by the criterion of lipid composition, the myelin isolated concomitantly with the axolemma-enriched fractions is equivalent to the myelin isolated by the procedures designed to isolate myelin alone. An unexpected feature of the lipid composition of axolemma-enriched fractions is the high galactolipid content, with a cerebroside to sulfatide ratio similar to that of myelin. Several trends are evident when the lipid compositions of the myelin, intermediate density axolemma (0.8/1.0 fraction), and highest density axolemma (1.0/1.2 fraction) are compared. The more dense membrane fractions contain more cholesterol and galactolipid and less phospholipid. In addition, with increasing density of the membrane fraction, there is a decreasing content of sphingomyelin, phosphatidylserine, and sulfatide. Since the content of cerebroside stays about the same in all fractions, a lowered content of sulfatide accounts for the overall trend toward the lower amount of total galactolipid. The choline phospholipid and phosphatidylinositol content of the axolemma-enriched fractions are much higher than that of myelin. In all cases the predominant phospholipids are the ethanolamine phospholipids which are about 18% of the total lipid in each membrane fraction. However, both axolemma-enriched fractions contain significantly more phosphatidylethanolamine and less phosphatidalethanolamine than the myelin fraction. A distinguishing characteristic of the axolemma-enriched fractions is a ratio of ethanolamine to choline phospholipids of about one, compared with a ratio of almost two for myelin. The axolemma-enriched fractions are some-

TABLE 2. Overall lipid composition of human CNS axolemma-enriched fractions and myelin

Lipids	Fraction		
		Axolemma-enriched	
	Myelin[a]	0.8/1.0[b]	1.0/1.2[c]
Lipid (as % dry wt)	68.9 ± 1.3	57.0 ± 1.2	47.3 ± 1.2
Cholesterol	27.1 ± 0.4	26.4 ± 0.5	25.3 ± 0.3
Total galactolipids	29.4 ± 0.7	28.2 ± 1.0	25.8 ± 1.1
Cerebrosides	21.4 ± 1.0	22.6 ± 1.5	21.3 ± 1.1
Sulfatides	8.0 ± 0.5	5.6 ± 0.2	4.8 ± 0.6
Total phospholipids	43.5 ± 0.9	45.4 ± 0.7	48.9 ± 1.3
Ethanolamine phospholipids	18.4 ± 1.1	18.2 ± 0.1	19.8 ± 0.2
Phosphatidylethanolamine	3.9 ± 0.2	7.5 ± 0.02	10.1 ± 0.1
Phosphatidalethanolamine	14.5 ± 0.9	10.7 ± 0.03	9.7 ± 0.1
Choline phospholipids	10.5 ± 0.7	16.1 ± 0.1	18.7 ± 0.2
Phosphatidylcholine	9.8 ± 0.6	15.0 ± 0.06	15.7 ± 0.2
Phosphatidalcholine	0.7 ± 0.04	1.1 ± 0.02	3.0 ± 0.03
Sphingomyelin	7.3 ± 0.4	4.2 ± 0.1	3.0 ± 0.1
Phosphatidylserine	6.3 ± 0.1	5.3 ± 0.1	3.0 ± 0.1
Phosphatidylinositol	1.0 ± 0.1	1.6 ± 0.1	4.4 ± 0.1
Ganglioside AcNeu (μg/mg protein)	8.8 ± 1.5	9.6 ± 1.0	13.9 ± 5.0

[a] Myelin isolated concomitantly with the axolemma-enriched fractions.
[b] Axolemma-enriched fraction collected at the interface of 0.8 M and 1.0 M sucrose.
[c] Axolemma-enriched fraction collected at the interface of 1.0 M and 1.2 M sucrose.
The values represent weight percent of total lipid and are the mean ± S.E. of triplicate determinations.

what enriched in ganglioside content relative to myelin (Table 2), especially the more dense axolemma-enriched fraction. The level of ganglioside in the human CNS axolemma-enriched fractions is similar to that of the rat CNS axolemma-enriched fractions (7) while the myelin fraction has a much greater ganglioside content.

The possibility of myelin contamination accounting for the galactolipid found in the axolemma-enriched fractions was investigated by assaying the fractions for myelin basic protein content. The 0.8/1.0 axolemma-enriched fraction contained 17.2 μg myelin basic protein per mg dry weight while the 1.0/1.2 axolemma-enriched fraction contained 6.3 μg myelin basic protein per mg dry weight. Based on 32.6% of the protein in the myelin fraction as myelin basic protein (8), each μg of myelin-basic protein has 2 μg of associated myelin galactolipid in the myelin fraction. These data allow us to calculate that by the criterion of myelin basic protein, 79.6% of the galactolipid in the 0.8/1.0 fraction and 89.7% of the galactolipid in the 1.0/1.2 fraction is not due to myelin contamination.

Galactolipid is believed to be responsible for a positive Luxol fast blue stain (23). The galactolipid in the axolemma-enriched fraction could be due to contamination with some myelin-derived membrane having a high galactolipid content. If this were the case, Luxol fast blue staining would reveal a limited number of positively stained vesicles. The results of Luxol fast blue staining of the human axolemma-enriched fractions were identical to those observed with the rat CNS axolemma-enriched fractions (17): both axolemma-enriched fractions were stained uniformly throughout the entire preparation (data not shown). This indicated that the galactolipid in each fraction did not arise from a select population of the vesicles but was uniformly distributed throughout the fraction.

The average fatty acid compositions of the two major phospholipids of the 1.0/1.2 axolemma-enriched fraction (AXL) and myelin are shown in **Table 3.** The standard error of the mean values of all values in the fatty acid and fatty aldehyde analyses were 10% or less of each reported average value; they have been left out of these tables for the sake of clarity. Oleic acid is the predominant fatty acid in all cases. In general, the distribution of ethanolamine phospholipid fatty acids in myelin and AXL is rather similar. However, the unsaturation index of the AXL ethanolamine phospholipids is significantly higher than that of myelin, largely by virtue of the increased content of 20:4 and 22:6 in the AXL ethanolamine phospholipids. The myelin and AXL choline phospholipids also show an overall similarity in their fatty acid compositions. However, AXL contains more 16:0 and less 18:1 relative to myelin. The unsaturation index of AXL choline phospholipids is higher than that of myelin due to the increased content of 22:6, 20:4, and 18:2. It should be noted that the fatty acid compositions of the myelin ethanolamine and choline phospholipids are consistent with those previously reported for human CNS myelin (24). Relative to myelin, the major AXL phospholipids tend to have increased unsaturation although the distribution of fatty acid chain lengths is rather similar.

TABLE 3. Fatty acid composition of choline and ethanolamine phospholipids in a human CNS axolemma-enriched fraction and myelin

	Ethanolamine Phospholipids		Choline Phospholipids	
	Myelin[a]	AXL[b]	Myelin	AXL
16:0	2.8	3.2	19.2	25.8
16:1	0.8	0.9	1.3	—[c]
18:0	8.1	10.7	15.3	16.3
18:1	48.7	39.7	57.6	48.8
18:2	1.8	1.5	0.3	0.8
20:1	11.7	8.6	2.6	1.2
20:2	1.4	1.0	—	0.1
20:3	0.2	0.3	0.2	0.4
20:4	4.5	8.9	1.4	5.1
22:2	0.7	—	—	—
22:3	1.5	1.4	0.3	0.1
22:4	14.0	16.8	0.6	0.1
22:5	0.1	0.2	—	—
22:6	2.0	6.8	0.4	0.9
24:4	1.7	—	0.7	0.4
C_{16}–C_{18}[d]	62.2	56.0	93.7	91.7
Unsaturation index[e]	167.4	203.9	74.9	95.1

[a] Myelin isolated concomitantly with the axolemma-enriched fractions.

[b] Axolemma-enriched fraction collected at the interface of 1.0 M and 1.2 M sucrose.

[c] Dash (—) indicates less than 0.1% of the fatty acid methyl ester was detected.

[d] Weight percentage of total fatty acid methyl esters having a chain length from 16 to 18 carbons.

[e] Unsaturation index = $\sum$ (% of fatty acid methyl ester × number of double bonds).

Values are the average weight percentages of methyl esters from three separate preparations each containing three different brains, except for the AXL choline phospholipid fatty acids which are the average of two separate preparations of three brains each.

As shown in **Table 4**, aldehydes derived from myelin ethanolamine phospholipids are predominantly 18:1, while 78.2% of the comparable aldehydes in grey matter are stearic aldehyde. Clearly, the ethanolamine phospholipid-derived aldehydes closely resemble the myelin aldehydes in their chain length distribution (Table 4).

When examined by electron microscopy, the synaptic plasma membrane preparation isolated by the procedure of Cruz and Gurd (9) consisted mostly of unilamellar linear pieces of membrane. Mitochondria and multilamellar fragments of myelin were seldom

TABLE 4. Fatty aldehyde composition of ethanolamine phospholipids of human CNS membrane fractions[a]

Aldehyde	Myelin	Axolemma-enriched Fraction[b]	Grey Matter
16:0	21.6%	23.3%	10.9%
18:0	17.0%	19.0%	78.2%
18:1	61.4%	57.7%	10.9%

[a] Values are the average weight percentage from at least two separate preparations, each containing three different brains.
[b] Axolemma-enriched fraction collected at the interface of 1.0 M and 1.2 M sucrose.

observed. Most of the membrane appeared to be presynaptic in origin since postsynaptic densities were not observed in the preparation. The fatty acid compositions of the synaptic plasma membrane ethanolamine and choline phospholipids were quite similar to those of the analogous lipids in grey matter (**Table 5**). Synaptic plasma membranes contain 50% of their dry weight as lipid. Therefore, the lipids of synaptic plasma membrane make a major contribution to the lipid composition of grey matter and the close relationship in their fatty acid compositions is not unexpected.

DISCUSSION

The lipid composition of the axolemmal membrane may be expected to be closely related to that of neuronal membranes in particular (as in synaptic plasma membrane) and grey matter in general. However, it is also possible that the lipid composition of neuronal membrane could be modified in regions where it interacts with the myelin sheath. For example, Haley and Ledeen (25) have presented evidence for the equilibration of certain lipid precursors between the myelin, axolemmal, and axonal compartments in the rabbit optic system. **Table 6** summarizes the lipid composition of the major brain constituents in the human CNS. The characteristics of the lipid composition of white matter which distinguish it from grey matter can be clearly seen: a high galactolipid content, a ratio of choline phospholipids to ethanolamine phospholipids of less than one, a high plasmalogen content, and a low ganglioside content. The human AXL resembles gray matter in its choline phospholipid to ethanolamine phospholipid ratio, its content of ganglioside, and its phosphatidylinositol content. In addition, the choline phospholipid unsaturation index of AXL (95.1) is closer to that of synaptic plasma membranes (100.9) than to that of myelin (74.9), while the unsaturation index of AXL ethanolamine phospholipids (203.9) is intermediate between that of the ethanolamine phospholipids in myelin (167.4) and that of synaptic plasma membranes (276.1). However, the AXL shares with white matter and myelin a high galactolipid content and high plasmalogen content.

It is of interest to compare the fatty acid composition of comparable phospholipids from the AXL fraction and synaptic plasma membrane fraction since the latter represents a specialized extension of the axonal plasma membrane. Although the unsaturation indexes of the choline phospholipids of the two fractions are similar (see Table 3 and Table 5), a closer

TABLE 5. Fatty acid composition of ethanolamine and choline phospholipids from human CNS gray matter and synaptic plasma membranes

	Ethanolamine Phospholipids		Choline Phospholipids	
	Gray Matter	Synaptic Plasma Membranes	Gray Matter	Synaptic Plasma Membranes
16:0	5.2	4.0	40.2	34.1
18:0	20.6	22.3	12.4	14.4
18:1	19.8	18.3	33.0	36.7
20:1	1.6	1.9	0.5	0.8
20:3	0.8	1.0	0.8	0.6
20:4	13.6	15.8	6.3	7.6
20:5	0.0	—[a]	—	—
22:2	—	0.4	—	—
22:3	—	0.1	—	0.6
22:4	14.0	14.4	1.1	0.8
22:5	0.6	0.4	0.2	0.2
22:6	22.9	21.5	5.6	4.2
C_{16}–C_{18}[b]	45.6	44.6	85.6	85.2
Unsaturation index[c]	275.6	276.1	101.1	100.9

[a] Dash (—) indicates that less than 0.1% of the fatty acid methyl ester was deleted.
[b] Weight percentage of total fatty acid methyl esters having a chain length from 16 to 18 carbons.
[c] Unsaturation index = $\sum$ (% of fatty acid methyl ester × number of double bonds).
Values are the average weight percentage of methyl esters obtained from a lipid extract from either the gray matter or synaptic plasma membranes from six separate brains.

TABLE 6. Lipid composition of CNS brain constituents

Lipid	Fraction			
	Human Gray Matter[a]	Human AXL[b]	Human White Matter[a]	Human CNS Myelin[c]
Cholesterol	22.0	25.3	27.5	27.5
Total galactolipid	7.3	25.8	26.4	27.5
Cerebrosides	5.4	21.3	19.8	22.7
Sulfatides	1.7	4.8	5.4	3.8
Total phospholipid	69.5	48.9	45.9	43.1
Ethanolamine phospholipids	22.7	19.8	14.9	15.6
Choline phospholipids	26.7	18.7	12.8	11.2
Sphingomyelin	6.9	3.0	7.7	7.9
Phosphatidylserine	8.7	3.0	7.9	4.8
Phosphatidylinositol	2.7	4.4	0.9	0.6
Total plasmalogen	8.8	12.7	26.4	12.3
Total ganglioside (μg/mg dry wt)	17.0	24.1	3.0[d]	2.0[d]

[a] Data from Suzuki (26).
[b] This study, 1.0/1.2 fraction.
[c] Data from Norton (22).
[d] Data from Ledeen, Yu, and Eng (27).
All figures are expressed as weight percent of total lipid except as noted.

inspection of the fatty acid composition shows that AXL choline phospholipids contain a higher proportion of 18:1 and a lower proportion of 22:6 and 20:4 when compared with the choline phospholipids of synaptic plasma membrane. Comparison of the ethanolamine phospholipids shows a similar tendency toward an increased preparation of 18:1 and decreased preparation of 22:6 in AXL compared with the analogous values for synaptic plasma membrane. These differences are largely responsible for the lower unsaturation index and higher proportion of $C_{16}-C_{18}$ observed in the AXL fraction. The relative proportion of 18:1 relative to 18:0 in AXL ethanolamine phospholipids is more similar to that found in myelin than it is to that found in synaptic plasma membrane. Although the unsaturation index for choline phospholipids is similar to those of AXL and synaptic plasma membrane, there are subtle distinguishing differences in the fatty acid composition. These differences are more pronounced in the ethanolamine phospholipid fraction leading to a clear difference in the unsaturation indexes of these two membrane fractions. The AXL shares with the synaptic membrane a tendency toward a greater degree of unsaturation when compared with the myelin fraction, but tends to have an 18:0/18:1 ratio closer to myelin than that of the synaptic plasma membrane.

Therefore, the AXL fraction appears to have characteristics in its lipid composition that are typical of the neuronal membrane-enriched gray matter or neuronal membranes themselves, such as synaptic plasma membranes. At the same time, other characteristics in the AXL lipid composition resemble those of myelin-enriched white matter or myelin itself. If lipids such as cerebroside and sulfatide are indeed found in the axonal plasma membrane, they may be important for neuronal–glial relationships in the myelinated axon.

As shown in **Table 7**, the galactolipid of the mammalian axon plasma membrane preparations distinguishes its lipid composition from that of other preparations that have been characterized to date. The high galactolipid content of the mammalian preparations is accompanied by a somewhat lowered phospholipid content relative to the other preparations. The molar ratio of cholesterol to phospholipid is rather similar in all axolemmal preparations except for the higher value of human AXL which, due to its high galactolipid, has a lowered phospholipid content. The choline and ethanolamine phospholipids are clearly the major phospholipids in all cases, leading to a molar ratio of these two classes of lipids which approaches one in most cases. The unsaturation indexes of the major phospholipids of the human AXL are not as high as the corresponding values for the cold-blooded animals such as the squid or garfish. This can be rationalized by the lower degree of unsaturation in the lipids required by warm-blooded animals to achieve a membrane fluidity comparable to that of lipids of cold-blooded animals with a greater degree of unsaturation. It should be noted that the rat and human AXL are the only preparations isolated from well-myelinated nerve tracts, and they are also the only preparations to contain significant levels of galactolipid, usually considered to be a myelin-specific component. It is also interesting to note that the human AXL fractions that are isolated from white matter only have a higher level of galactolipid than the AXL iso-

TABLE 7. Lipid composition of axon plasma membranes isolated from different sources

	Human[a]	Rat[b]	Crab[c]	Squid[d]	Garfish[e]
Lipid (% dry wt)	47.3	51.5	67.8	45.4	66.0
Cholesterol	25.3	21.3	28.8	25.0	26.0
Galactolipid	25.8	11.3	0	0	0
Total phospholipid	48.9	67.4	71.2	75.0	74.0
Cholesterol/phospholipid	1.04	0.71	0.79	0.67	0.71
Choline phospholipids Ethanolamine phospholipids	0.8	1.0	0.8	0.67	0.71
Unsaturation index[f]					
Choline phospholipids	95.1	—[g]	—	210.8	130.3
Ethanolamine phospholipids	203.9	—	—	354.8	344.3

[a] This study, 1.0/1.2 fraction.
[b] DeVries and Zmachinski (22).
[c] Balerna et al. (3).
[d] Fischer et al. (1).
[e] Chacko et al. (5).
[f] Unsaturation index = $\sum$ (% of fatty acid methyl ester × number of double bonds).
[g] Dash (—) indicates that fatty acid composition has not been determined.
The values are expressed as percentage of total lipid weight; all ratios are mole ratios.

lated from rat brainstem, which is a mixture of white and gray matter. Myelin-free axons isolated from bovine white matter contain 20.1% cerebroside (28) while the comparable fraction isolated from rat brain stem contains 23.7% cerebroside (10). The reason for this variation in cerebroside content is not known although similarity in the galactolipid content of the concomitantly isolated myelin fraction and myelin isolated by an alternate procedure argues against a gross loss of galactolipid from myelin to another neuronal fraction.

The possibility that a select population of glial-derived membranes contaminates the AXL fraction and is responsible for the presence of myelin-like lipids in the preparation must be considered. The plasma membrane of the astroglial cell and the plasma membrane of the oligodendroglial cell are candidates for such contamination. Presently there are no unique chemical markers for these membranes so it is not possible to evaluate their presence in the AXL fractions. However we think it likely that most of this membrane would be separated out as a pellet during the flotation of the myelinated axons and preliminary evidence in this regard has been reviewed recently (6). Our primary assumption is that the low levels of myelin basic protein in the AXL rule out myelin-related membranes as the source for galactolipid. Ansari et al. (29) have shown that incubation of previously frozen tissue at room temperature leads to a rapid breakdown of myelin basic protein. Although frozen tissue was used as the source in these preparations, we do not think it is likely that the low levels of myelin basic protein in the AXL are a consequence of breakdown during the isolation procedure, since after thawing to 4°C the tissue was never at room temperature. We have also shown that the basic protein content of the myelin obtained by this procedure is 32.4% of the total protein (8), a value that is within normal limits for human CNS myelin (22). A myelin-derived contaminant that would be present in the AXL fractions would likely be derived from the most dense myelin subfractions. Such subfractions have been shown to be enriched in 2,3′ cyclic nucleotide phosphohydrolase activity and deficient in galactolipid relative to the whole myelin fractions (30, 31). The low galactolipid content of those myelin subfractions does not fit the characteristics required of a myelin-derived contaminant in the AXL fractions that should have a galactolipid content higher than that of myelin as a whole. Based on previous data characterizing these axolemma-enriched fractions (8), if the specific activity of 2′3′ cyclic nucleotidase in myelin is used as an indicator of myelin related membrane, then about one-third to one-fourth of the AXL fraction must be considered to be myelin. This level of myelin contamination cannot be reconciled with the observed low content of myelin basic protein in AXL even taking into account that the most dense myelin subfractions may have only one-third the normal content of myelin basic protein. The uniform distribution of Luxol fast blue staining in the AXL fractions is an indication that, for the case of galactolipid, contamination by a select population of membranes is not a possibility. It is also possible that an intimate mixture of membranes of different types could also generate this uniform staining pattern. However, with this limitation of the staining procedure in mind, by the criterion of the uniform Luxol fast blue staining, the cerebroside in these fractions appears to be intrinsic to the fractions as a whole.

The previously demonstrated enrichment of sur-

face marker enzymes (8) coupled with the present enrichment of saxitoxin binding and the increased unsaturation index of the fatty acid composition of the major AXL lipids are characteristics which support the axonal plasma membrane origin of these AXL fractions. Other evidence to support this view has been reviewed recently (6).

If the lipids in the human AXL are uniformly distributed throughout the fraction, the axolemmal membrane beneath the myelinated axon could be a mosaic of myelin and neuronal lipids. This suggests a close metabolic relationship between the neuron and myelin as other recent studies have also suggested (32–35). Since changes in the axolemma can preceed demyelination (36), future investigations of the molecular architecture of the axolemmal membrane should be fruitful in understanding the mechanisms of demyelinating disease.

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