Composition of myelin from peripheral and central nervous systems of the squirrel monkey

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ABSTRACT   Myelin was prepared from the brachial plexus and cervical spinal cord of adult squirrel monkeys (*Saimiri sciureus*). Brachial plexus myelin contained a larger amount of sphingomyelin and smaller amounts of cholesterol, lipid galactose, ethanolamine phosphoglyceride, choline phosphoglyceride, and alk-1-enyl ether than spinal cord myelin when compared as ratios to total lipid phosphorus. The peripheral nervous system myelin had a higher proportion of protein. All of these differences were statistically significant.

Thus peripheral nervous system myelin and central nervous system myelin differ in protein content and lipid composition in this subhuman primate.

KEY WORDS   myelin · nervous system · peripheral · central · squirrel monkey · electron microscopy · lipid composition · proteolipid protein

A SUBSTANTIAL AMOUNT of information is now available on the composition of CNS myelin from a number of vertebrate species. This information has been used for the construction of models of the molecular ultrastructure of the CNS myelin membrane (1–3). The composition of CNS myelin from subhuman primates has not been described, and only conflicting information is available for mammalian PNS myelin lipids. The lipid composition of PNS myelin has been reported for the rat and guinea pig by Evans and Finean (4) and for the ox by O'Brien, Sampson, and Stern (5). Since these authors did not include a direct comparison of CNS and PNS in the experimental design, they arrived at different conclusions regarding possible differences in lipid composition between CNS and PNS myelin. Differences between CNS and PNS myelin have been shown by physical studies on myelin ultrastructure using X-ray diffraction and electron microscopy (6) and by chemical studies on proteolipid proteins (7). Since CNS myelin is formed by oligodendroglia cells and PNS myelin is formed by Schwann's cells, the purpose of the present investigation was to determine if the oligodendroglia and the Schwann's cells produce myelin membranes with significant differences in composition in a subhuman primate.

METHODS

Tissue Fractionation and Extraction

The 18 squirrel monkeys (*Saimiri sciureus*) used as subjects for this experiment were maintained on Purina monkey chow for at least 4 months before sacrifice. Weights of the animals ranged from 320 to 790 g. The brachial plexus and samples of cervical spinal cord were removed within 5 min after decapitation. The tissues were stored at 4°C in the dispersion media until processed. Dispersion was accomplished with a Potter-Elvehjem tissue grinder equipped with a Teflon pestle. Four or five strokes were used to disperse spinal cord, but minced brachial plexus required twelve strokes and a substantial residue remained undispersed. A fraction with the properties of myelin was then isolated by one of the following three methods, in which a Spinco model L ultracentrifuge was used.

Method A

1.   The dispersion medium was 1.0 M sucrose–0.003 M Na_4EDTA. The dispersion was centrifuged for 2 hr at 40,000 g in a SW 25.1 swinging bucket rotor.[1]

Abbreviations: PNS, peripheral nervous system; CNS, central nervous system; TLC, thin-layer chromatography; Na_4EDTA, tetrasodium ethylenediamine tetraacetate; EPG, ethanolamine phosphoglycerides; CPG, choline phosphoglycerides; Sph, sphingomyelin; SPG, serine phosphoglycerides; IPG, inositol phosphoglycerides (including mono-, di-, and triphosphoinositides).

[1] Subsequent experiments have shown that flotation for 60 min in steps 1, 2, and 5 is sufficient. Also, sedimentation for 15 min at 13,500 g in steps 3, 4, and 6 produces a firm myelin pellet with a clear supernatant fluid.

2. After the tube had been sliced with a Spinco tube-slicer, the floating layer was removed by syringe, dispersed in 0.8 M sucrose–0.003 M Na₄EDTA, and recentrifuged as in step 1.

3. The floating layer was dispersed in distilled water and sedimented for 10 min at 100,000 g in a No. 40 rotor.[1]

4. The supernatant fluid was discarded and the pellet was dispersed and sedimented as in step 3.

5. The supernatant fluid was discarded and the pellet was dispersed in 0.8 M sucrose–0.003 M Na₄EDTA. The dispersion was again centrifuged for 2 hr at 40,000 g in the SW 25.1 swinging bucket rotor.

6. The floating layer was dispersed in distilled water and sedimented three times as in step 3.

None of the myelin was lost if 1.0 M sucrose was used for dispersion. If 0.8 M sucrose was used for the first step, some of the myelin remained in the infranatant fluid and the floating layer was difficult to recover. For spinal cord material, steps 5 and 6 were probably not necessary, but step 5 for the PNS preparation gave a small pellet and a cloudy infranatant which were discarded.

Method B

1. The dispersion medium was 0.32 M sucrose–0.001 M Na₄EDTA–0.003 M Na₂HPO₄. The dispersion was centrifuged for 15 min at 13,500 g in a No. 40 rotor.

2. The supernatant suspension was discarded and the pellet was suspended by syringe in 25 ml of 0.8 M sucrose–0.001 M Na₄EDTA–0.006 M Na₂HPO₄. The latter suspension was centrifuged for 60 min at 40,000 g in an SW 25.1 swinging bucket rotor.

3. After the tube had been sliced with a Spinco tube-slicer, the floating layer was removed by syringe, diluted with distilled water, and sedimented as in step 1.

4. The supernatant fluid was discarded and the pellet was resuspended in distilled water. At this point the preparation was usually stored overnight, then sedimented as in step 1.

5. A continuous density gradient was formed in SW 25.1 centrifuge tubes by the admixture of 10-ml portions of 1.0 M sucrose and 0.32 M sucrose in a Buchler apparatus (Buchler Instruments, Inc., Fort Lee, N.J.). The pellet from step 4 was suspended in 5 ml of 0.32 M sucrose and layered over the gradient. The tubes were centrifuged as in step 2.

6. The clear supernatant fluid was removed and discarded. The tube was sliced and the single compact myelin layer (brachial plexus) or the light myelin layer (spinal cord) was removed. The tube slicing was repeated for removal of the heavy myelin layer (spinal cord). The layers were diluted with distilled water and the myelin was sedimented three times for 15 min at 13,500 g in a No. 40 rotor.

Method C

This method was a combination of steps 1–4 of Method B and steps 5 and 6 of Method A. Steps 1–4 of Method B were adapted from the myelin isolation procedure of Cuzner, Davison, and Gregson (8).

Electron Microscopy

Myelin suspensions were examined by both negative and positive staining procedures. For negative staining, equal volumes of the myelin suspension and 2% potassium phosphotungstate were mixed, a drop of the mixture was placed on a carbon-coated grid with a platinum loop, and the grid was dried on filter paper.

For positive staining, equal volumes of the myelin suspension and 8% glutaraldehyde buffered to pH 7.2 with 0.2 M cacodylate buffer were mixed and kept at 4°C for 3 hr before centrifugation in polyallomer tubes for 15 min at 13,500 g in a No. 40 rotor. The pellet was suspended in 1% osmium tetroxide in 0.2 M cacodylate buffer for 60 min, then centrifuged. Dehydration with 70%, 95%, and 100% ethanol was accomplished by suspending the pellet, centrifuging the mixture, and decanting the supernatant liquid. After dehydration, portions of the pellet were embedded in Maraglas (Polysciences, Inc., Rydal, Penn.) (9). Sections of 400–800 A thickness were obtained with an LKB (Stockholm, Sweden) ultratome, mounted on carbonized grids, and stained with lead citrate. All grids were examined with a Siemens (Karlsruhe, Germany) Elmiskop I electron microscope at 60 kv.

Extraction

The final white pellet was dispersed in distilled water to a volume of 1.0 ml and then mixed with 35 ml of chloroform–methanol 2:1. A clear or very slightly opalescent solution was obtained. 7 ml of 0.58% NaCl (10) was added to the tube, which was kept at 4°C overnight. The upper phase was removed and the lower phase was washed twice with an upper phase mixture (10). The washed lower phase was taken to dryness with additions of absolute ethanol and brought to a final volume of 10 ml in chloroform for analysis.

Analytical Methods

The protein content of portions of the aqueous myelin suspension was determined by a modification (11) of the method of Lowry, Rosebrough, Farr, and Randall (12) with bovine serum albumin as the standard. Phosphorus (13), galactose (14), cholesterol (15), alk-1-enyl ethers (16, 17), and the phospholipid class distribution were determined for portions of the lipid extract. The galactose values were corrected for charring by subtraction of a blank containing the same quantities of lipid but without anthrone. For cholesterol determinations, a portion of the

TABLE 1 COMPOSITION OF SQUIRREL MONKEY SPINAL CORD MYELIN PREPARED BY DIFFERENT METHODS

	Method of Preparation		
	A	B	C
Number of preparations	8	6	4
Protein (%)	19.8*	—	20.4 ± 1.6
Lipid P (µmoles/g tissue)	31.4 ± 3.3	40.1 ± 2.3	15.1 ± 1.3
	mole ratio, component to lipid P		
Cholesterol	1.30 ± 0.03	1.32 ± 0.02	1.40 ± 0.004
Lipid galactose	0.73 ± 0.04	0.56 ± 0.01†	0.68 ± 0.04‡
Alk-1-enyl ether	0.37 ± 0.02	—	0.37 ± 0.03
EPG	0.432 ± 0.007	0.438 ± 0.020	0.446 ± 0.012
CPG	0.187 ± 0.007	0.191 ± 0.012	0.186 ± 0.002
Sph	0.172 ± 0.006	0.162 ± 0.018	0.140 ± 0.005
SPG + IPG	0.176 ± 0.008	0.195 ± 0.014	0.196 ± 0.012

Values are the mean ± SEM.
* Mean of two samples.
† $P < 0.01$ for difference between A and B.
‡ $P < 0.05$ for difference between B and C.

lipid extract was taken to dryness under N_2, then dissolved in ethanol. For determinations of the phospholipid class distributions by TLC, a portion of the lipid extract containing 0.3–0.6 µmole of the phosphorus was applied to a 4 cm lane on a 0.5 mm layer of Silica Gel G. The plate was developed for 10 cm in chloroform–methanol–15 N ammonium hydroxide 65:25:4 (18). Lipids were detected by ninhydrin spray and iodine vapor (17). The entire lane was marked as indicated in Table 1 and areas were scraped into separate boiling tubes with the aid of a 1 inch plastic blade. The phosphorus of these areas and of blanks was assayed according to Gottfried (18a). The phospholipid composition was calculated from the corrected absorbance values. The lipid composition has been expressed in terms of mole ratios of components to total lipid phosphorus. Statistical comparisons of possible differences between CNS and PNS myelin lipid compositions were made by analysis of variance (19).

RESULTS

All of the myelin preparations were almost completely soluble in a mixture of 34 parts of chloroform–methanol 2:1 and one part of water. Negligible contamination of myelin fractions could be found upon examination of numerous electron micrographs. Occasionally, a strand of collagen was found in negatively stained preparations. Typical micrographs, selected only for sharpness of focus, are shown in Figs. 1–8. The myelin shown in Figs. 1–3 shows numerous multilamellar structures in various states of disintegration, probably due to relatively long storage at low osmolality. A comparison of Figs. 4 and 5 demonstrates that contamination by microsomes can be detected by the presence of small vesicles in negatively-stained preparations. No microsomes were present in myelin preparations (Figs. 3 and 4). Fig. 5 also shows that the "microsomal" fraction which was discarded contained numerous small myelin fragments. The PNS myelin illustrated in Figs. 6–8 is similar to that shown by O'Brien et al. (5).

All three methods were used to prepare myelin from samples of cervical spinal cord (Table 1). The amount of myelin lipid phosphorus obtained from 188–385 mg of spinal cord by Method A (six preparations) was 35.4 ± 2.8 µmoles/g (mean ± SEM). Preparations from 908–1713 mg of spinal cord gave myelin lipid phosphorus yields of 19, 40, and 15 µmoles/g for Methods A, B, and C respectively. Norton and Autilio (20) have reported a yield of 63 µmoles/g of bovine white matter. The results for myelin prepared by Method B are for the heavy and light fractions combined. The amounts of cholesterol and lipid galactose relative to lipid P in the heavy and light fractions were not significantly different as judged by a test for correlated samples. The lipid compositions by Methods A and C were the same, with the possible exception of sphingomyelin and serine plus inositol phosphoglycerides, but myelin prepared by Method B had a lower relative amount of lipid galactose. The differences were statistically significant.

Methods A and B were used to prepare myelin from brachial plexus (Table 2). The yield of myelin was much lower from this tissue, presumably because of difficulties in dispersing the tissue and because of the large amount of connective tissue. The relative amounts of cholesterol and lipid galactose were larger for Method B in this case but the differences were not significant. The alk-1-enyl ether values were not different.
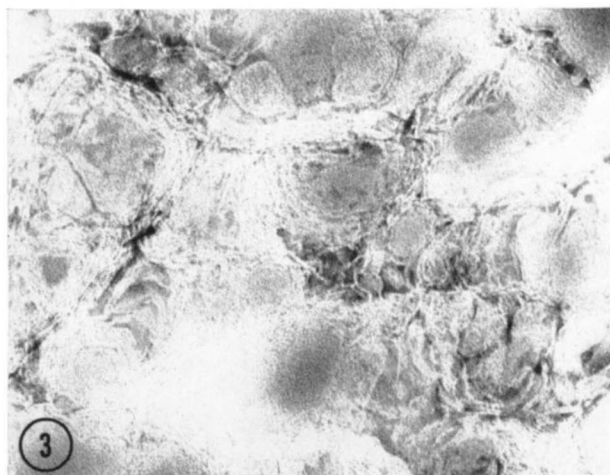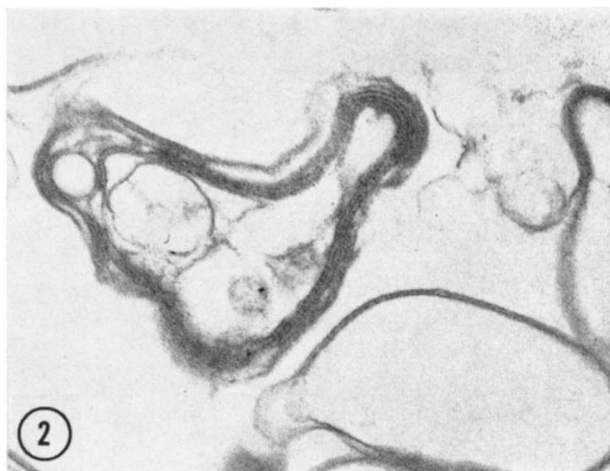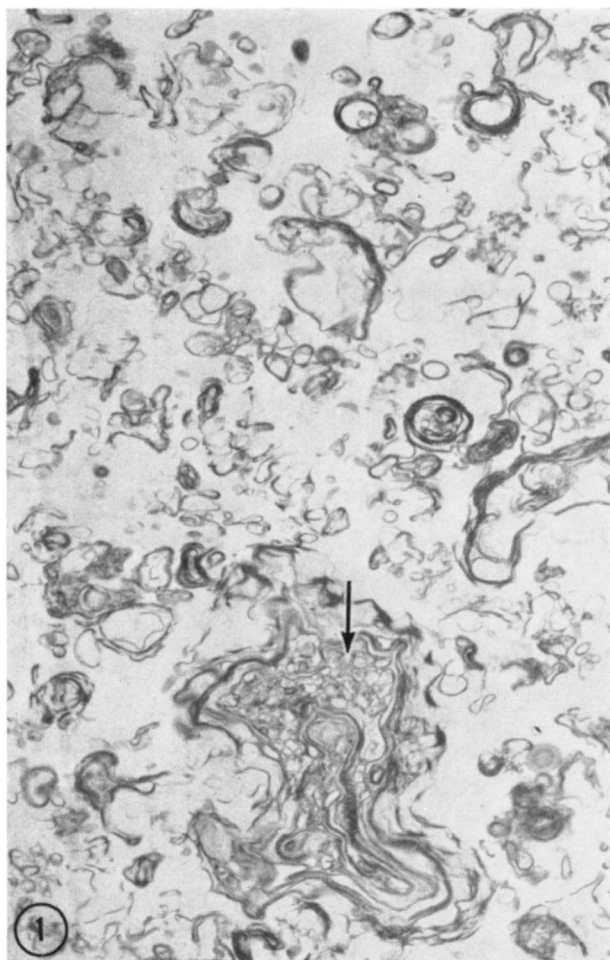
FIG. 1. Typical appearance of positively-stained myelin. Method C, spinal cord myelin, ×7500, lead citrate. The membranes within the large myelin sheath (arrow) can be traced to the sheath and do not represent contamination by axoplasm.
FIG. 2. Method C, spinal cord myelin, ×60,000, lead citrate.
FIG. 3. Method A, spinal cord myelin, ×60,000, potassium phosphotungstate.

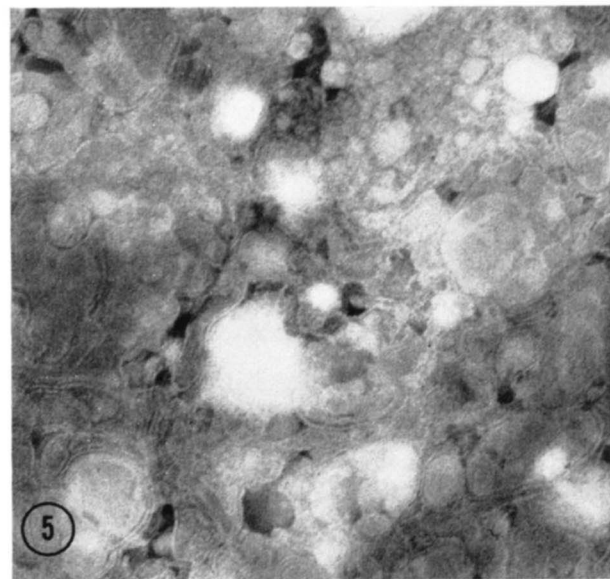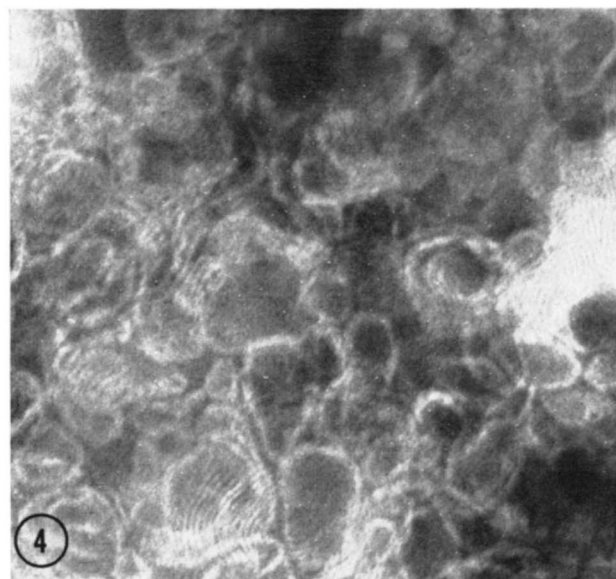FIG. 4. Method B, brachial plexus myelin, ×60,000, potassium phosphotungstate.
FIG. 5. Supernatant fluid from step 1 of Method B, brachial plexus microsomes and myelin, ×60,000, potassium phosphotungstate.
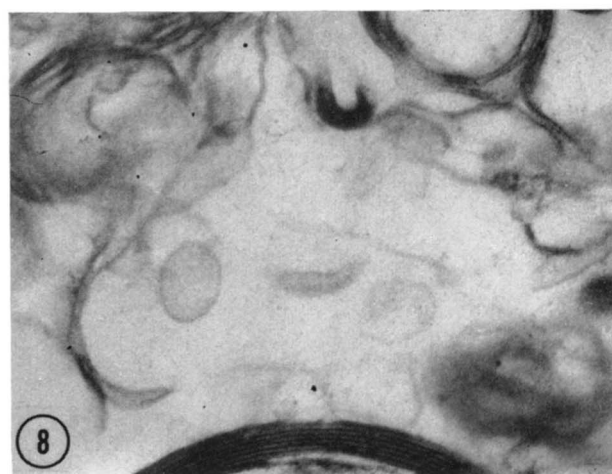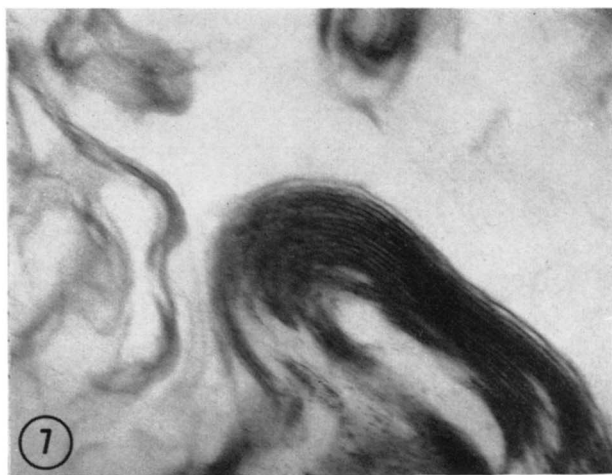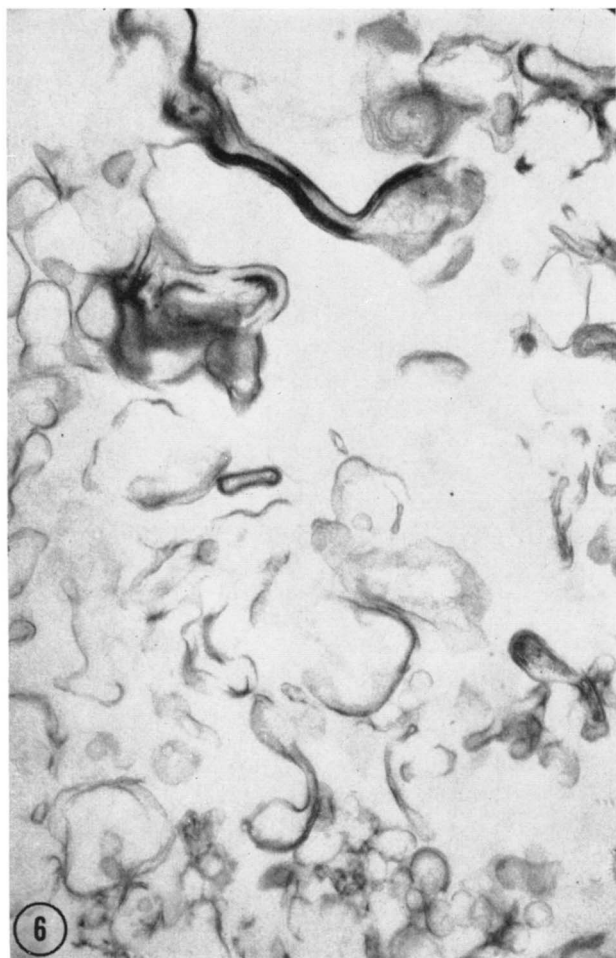
FIG. 6. Method C, brachial plexus myelin, ×21,600, lead citrate.
FIG. 7. Method C, brachial plexus myelin, ×60,000, lead citrate.
FIG. 8. Method A, brachial plexus myelin, ×60,000, lead citrate.

The protein values were calculated by dividing the amount of protein by the sum of the protein and the lipids [using the molecular weights calculated by Autilio, Norton, and Terry (21)] followed by multiplication by 100. The protein content of squirrel monkey myelin was 20% and 30%, respectively, for spinal cords and brachial plexus. These values were significantly different by the Mann-Whitney U test ($U = 0$; n = 6, 5; $P < 0.002$). Possible differences in the relative lipid compositions were tested by an analysis of variance between means for correlated samples (Table 3) using values for the same component from the same animal.

The TLC procedure produced discrete spots of Sph, CPG, and EPG. The area above EPG was also taken for phosphorus determination. The amount found, which varied from 0 to 6% of the total phosphorus, was included in the calculations of phospholipid but is not tabulated. Serine phosphoglycerides were the major components of the phospholipid fraction designated SPG + IPG. About 3–4% of the total phosphorus, which may represent polyphosphoinositides, were found at the origin on these TLC plates. Less than 1% of the total phosphorus, probably inositol phosphoglyceride, was in a spot just above the serine phosphoglycerides.

DISCUSSION

Isolation of Myelin

Three procedures for the isolation of myelin from the spinal cord (CNS) and two procedures for the isolation of myelin from the brachial plexus (PNS) of young adult squirrel monkeys were used in this investigation. As in all other studies that include the isolation of myelin fractions, myelin is defined as a lipid–protein complex which has a hydrated density lower than that of 0.8 M sucrose, occupies one or two narrow bands when at equilibrium in a sucrose density gradient, and has a microscopic appearance similar to that of the myelin sheath in tissues. Isolated myelin has been found to be rich in ethanolamine plasmalogen, galactolipids, and cholesterol, to

TABLE 2 COMPOSITION OF SQUIRREL MONKEY BRACHIAL PLEXUS MYELIN PREPARED BY DIFFERENT METHODS

	Method of Preparation	
	A	B
Number of preparations	9	5
Protein (%)	—	30.5 ± 2.1
Lipid P (μmoles/g tissue)	4.2 ± 0.7	3.4 ± 0.7
	mole ratio, component to lipid P	
Cholesterol	1.07 ± 0.03	1.13 ± 0.03*
Lipid galactose	0.43 ± 0.02	0.52 ± 0.04*
Alk-1-enyl ether	0.26 ± 0.02	0.28 ± 0.02*
EPG	0.376 ± 0.007	—
CPG	0.133 ± 0.008	—
Sph	0.278 ± 0.011	—
SPG + IPG	0.190 ± 0.010	—

Values are the mean ± SEM.
* Difference between A and B not significant.

TABLE 3 COMPARISON OF LIPID COMPOSITIONS OF MYELIN FROM SPINAL CORDS AND BRACHIAL PLEXUS OF SQUIRREL MONKEYS

	Spinal Cord	Brachial Plexus	t	df	P
	mole ratio, component to lipid P				
Cholesterol	1.30	1.08	4.14	11	<0.01
Lipid galactose	0.65	0.46	3.19	11	<0.01
Alk-1-enyl ether	0.35	0.28	3.48	11	<0.01
EPG	0.431	0.384	5.08	6	<0.01
CPG	0.188	0.135	3.47	6	<0.02
Sph	0.175	0.279	7.80	6	<0.001
CPG + Sph	0.363	0.414	4.11	6	<0.01
SPG + IPG	0.172	0.185	1.02	6	NS

have a rather low content of protein, lecithin, nucleic acid, and gangliosides, and to be almost completely soluble in a Folch solvent system.

In this study, the criteria for the purity of the myelin isolated from the CNS and the PNS were microscopic appearance, chemical composition, density gradient behavior, and solubility. The negative staining technique was useful for detection of membranous impurities and collagen. Since a uniform dispersion of the myelin preparation was used for negative staining, we concluded that only minor amounts of nonmyelin material could have been present in any preparation. This was confirmed by the results from a positive staining procedure. The uniformly high content of ethanolamine plasmalogen and the low content of lecithin is regarded as important evidence for the purity of the myelin. All preparations from both the CNS and the PNS could be defined as proteolipids because they contain lipid and protein and were almost completely soluble in chloroform–methanol. Other subcellular fractions give a flocculant precipitate under these conditions. In addition, the low protein content of the spinal cord myelin agrees well with the protein

values reported by Norton and Autilio (20) and Lowden, Moscarello, and Morecki (11) for bovine myelin. The protein content of the brachial plexus myelin is somewhat higher than the value found by O'Brien et al. (5) for bovine spinal root myelin.

In agreement with Norton and Autilio (20), no differences were detected in the lipid compositions of heavy and light CNS myelin. The PNS myelin formed a single band under the same centrifugation conditions. The myelin isolated from spinal cords by a density gradient procedure had a significantly lower relative lipid galactose content than the myelin isolated by methods A or C but an opposite trend was obtained for the PNS myelin from the same animals. This is most probably due to the variance between different samples of squirrel monkeys. We concluded that all of the preparations were substantially free from nonmyelin contamination.

CNS and PNS Differences

Most of the myelin preparations were made from both tissues from the same animals. Table 3 summarizes the results of tests of significance for differences between correlated means. Highly significant differences were found for all lipid components except for the serine plus inositol phosphoglyceride fraction. Thus, the oligodendroglia (CNS) and the Schwann's cells (PNS) of the squirrel monkey produce myelin with significant differences in protein content and lipid composition.

Tables 4 and 5 compare the present results for a subhuman primate with previous reports obtained by various methods and with fewer subjects. The composition of squirrel monkey spinal cord myelin is quite similar to that reported by Norton and Autilio (20) for bovine myelin. The compositions reported for human myelin are rather different, but in all instances the content of alk-1-

TABLE 4 COMPARISONS OF CNS MYELIN COMPOSITIONS FROM PRIMATES AND THE OX

	Man (22)	Man (23)	Squirrel Monkey	Ox (20)
	% of dry weight			
Protein	29.7	21.3	20.2	22.3
	mole ratio, component to lipid P			
Cholesterol	1.33	0.99	1.33	1.32
Lipid galactose	0.58	0.48	0.66	0.63
Alk-1-enyl ether	0.31	0.38	0.37	0.35
EPG	0.38	0.33	0.44	0.44
CPG	0.26	0.27	0.19	0.24
Sph	0.19	0.11	0.16	0.15
SPG + IPG	0.11	0.29	0.18	0.16
	mole ratio			
Eng-Smith ratio*	0.86	0.93	0.95	0.92

* (Lipid galactose + EPG + Sph)/Cholesterol.

TABLE 5 COMPARISON OF PNS COMPOSITIONS FROM MAMMALS

	Squirrel Monkey Myelin	Rhesus Monkey Sciatic Nerve (24)	Ox Myelin (5)	Ox Spinal Root (5)	Rat Myelin (4)	Rabbit Sciatic Nerve (24)	Guinea pig Myelin (4)
	% of dry weight						
Protein	30.5	—	24.2	33.3	—	—	—
	mole ratio, component to lipid P						
Cholesterol	1.09	1.48*	0.86	0.77	0.83	0.78	0.64
Lipid galactose	0.46	0.38	0.24	0.22	0.33	0.25	1.15
Alk-1-enyl ether	0.27	0.28	0.25	—	—	0.30	—
EPG	0.38	0.34	0.26	0.27	0.37	0.40	0.45
CPG	0.13	0.13	0.21	0.24	0.24	0.16	0.21
Sph	0.28	0.30	0.27	0.22	0.20	0.24	{0.34
SPG + IPG	0.19	0.22	0.26	0.27	0.19	0.19	
	mole ratio						
Eng-Smith ratio	1.03		0.90		1.08		>2

* A value of 0.83 ± 0.016 (*n* = 8) was found for sciatic nerve lipids from rhesus monkeys at 9 months of age (Horrocks, unpublished data).

enyl ethers is high and the EPG account for the largest fraction of the phospholipids.

PNS Myelin Composition

The phospholipid composition of PNS myelin from the squirrel monkey agrees closely (Table 5) with that reported for whole peripheral nerve of the rhesus monkey by Sheltawy and Dawson (24). The compositions of bovine PNS myelin and bovine whole nerve show similar agreement. The lipid composition of rat and guinea pig PNS myelin was reported by Evans and Finean (4) and can be compared with that of rabbit sciatic nerve. The data for rat PNS myelin and rabbit sciatic nerve agree well but the data for guinea pig PNS myelin are distinctly different from those for any other myelin. In most species, the cholesterol and lipid galactose values are consistent with a relatively greater content of cholesterol and cerebrosides in myelin than in other peripheral nerve membranes.

On the basis of metabolic data for rat brain myelin, Eng and Smith (25) have proposed a 1:1 molar relationship for the sum of ethanolamine phosphoglycerides and sphingolipids to cholesterol. With the exception of the guinea pig myelin, all of the PNS myelin lipid compositions agree well with the Eng-Smith concept, as do all of the CNS myelin lipid compositions (Table 4). The Eng-Smith ratios and the proteolipid nature of the PNS myelin suggest that PNS and CNS myelin are qualitatively similar in molecular ultrastructure (1–3) although they show significant quantitative differences in composition. Compared with CNS myelin from the same species, the PNS myelin has less cholesterol, galactolipid, and alk-1-enyl ether and more sphingomyelin.

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