Neuronal perikarya and astroglia of rat brain: chemical composition during myelination

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ABSTRACT Cells isolated by a new technique from 10-, 20-, and 30-day-old rat brains have been analyzed for total lipid, cholesterol, galactolipid, individual phospholipids, gangliosides, DNA, and RNA. The lipid composition does not vary appreciably in either neurons or astrocytes during this period of rapid myelination. Moreover, the lipid compositions of the two cell types are surprisingly similar, both having very low galactolipid concentrations, high phospholipid content, and cholesterol concentrations lower than whole brain. Astrocytes have a higher ganglioside content than neuronal perikarya, a finding ascribed to the higher ratio of surface membrane to mass in the astrocytes, and considered as evidence that gangliosides are normal glial constituents. Compared with an average astrocyte, the individual neuron soma has less mass, a lower total lipid content, and a much higher RNA content.

SUPPLEMENTARY KEY WORDS glial cell · gangliosides · DNA · RNA · cholesterol · galactolipids · phospholipids

THE DEMONSTRATION by Rose (1) that it was apparently feasible to disrupt brain tissue without completely destroying the component cells, and that these cells could be separated into neuron- and glia-rich fractions, has stimulated considerable interest in the bulk isolation of neurons and glia. The ultimate perfection of such methods has widespread implications for neurobiology since they have the potential of furnishing the most satisfactory approach to the determination of the biochemical properties and chemical composition of the various cell types which make up the nervous system.

Abbreviations: DNA-P, DNA phosphorus; RNA-P, RNA phosphorus; NANA, *N*-acetylneuraminic acid; TLC, thin-layer chromatography.

Several biochemical investigations of bulk isolated cells have appeared (2–7), but few studies of the detailed lipid composition have been published (8–10). We (11) have recently published a new isolation method designed to produce high yields of cells suitable for compositional studies. This paper presents analytical data, some of an unexpected nature, on these cells isolated from brains of rats 10–30 days old, a period of rapid growth and maximum rate of myelination. This work has previously been presented in part (12, 13).

METHODS

Roughly equivalent numbers of male and female Sprague–Dawley rats were used for each experiment, since entire litters were killed at a time.

Cell Isolation

Cells were isolated from rat brains trimmed of cerebella exactly as described previously (11). A portion of each preparation was taken for cell counts, so that all data could be referred to a per cell basis. For this purpose a known aliquot of a cell suspension can be preserved with formalin and counted when convenient.

Analyses

Although lipid analyses can be referred to total lipid weight, lipid per cell, or DNA content, it is also essential to determine base-line values, such as dry weight per cell and percentage of lipid. Determination of these values presented a problem because of the presence of albumin and sugars in the medium in which the cells were pelleted. For these data (presented in Table 1) the cells were washed six times by suspending them in distilled water and centrifuging at 100,000 *g* for 1 hr. This procedure disrupted the cells and caused loss of soluble con-

TABLE 1 COMPOSITION OF NEURONS AND ASTROGLIA DETERMINED ON WASHED CELLS

	Neurons				Astrocytes			
Age, *days*	10	20	30		10	20	30	
Total no. of animals	84	86	84		84	86	84	
Total dry wt, *mg*	175.9	256.9	212.4		68.5	174.8	178.8	
				Average				Average
$CHCl_3$–CH_3OH insoluble residue*	68.5	71.0	73.8	71.1	51.6	52.8	56.0	53.5
Upper phase*	1.9	2.0	2.0	2.0	2.0	2.7	2.6	2.4
Proteolipid protein*	3.2	2.7	2.4	2.8	4.5	6.1	4.9	5.2
Total lipid*	26.3	24.3	21.7	24.1	41.9	38.4	36.5	38.9
Ganglioside NANA*	0.059	0.045	0.046	0.050	0.089	0.108	0.091	0.096

* Expressed as % of dry weight.

stituents; thus, recoveries were somewhat low. The washed pellets were then freeze-dried in tared tubes and weighed. Mechanical losses were prevented by inserting a plug of glass wool in the neck of the adapter connecting the tube to the lyophilizer. The dried cells were analyzed for $CHCl_3$–CH_3OH-insoluble residue, proteolipid protein, upper phase solids, and total lipid as previously described (14).

It was discovered that lipids prepared in this manner were unsuitable for detailed analysis because they suffered autoxidative breakdown even when BHT (2,6-di-*t*-butyl-*p*-cresol) was added. Thus, all further analyses were carried out on unwashed cells. One of the first signs of autoxidation is the appearance of "oxycholesterol" (probably 7-hydroxycholesterol) (15–17), which migrates behind cholesterol on TLC plates and stains bright blue when sprayed with 50% H_2SO_4 at room temperature. Other early changes are the lowering of phosphatidalethanolamine content and simultaneous formation of lysophosphatidylethanolamine. The latter compound cochromatographs with sphingomyelin in the $CHCl_3$–CH_3OH–NH_4OH 14:6:1 (v/v/v) system used for quantitative determination of phospholipids (18) and thus can cause falsely high values of sphingomyelin if one is not aware of the degradation. With further autoxidation, other lyso compounds and altered phospholipids are formed which create a diffuse, streaked TLC pattern.

Lipids were extracted by treating the unwashed pellets directly with $CHCl_3$–CH_3OH 2:1 (19). Either the pelleted cells were stored frozen and pooled for analysis when sufficient material (50–100 mg dry wt) had been collected or the lipid extracts were stored and pooled. The lipid extracts were partitioned with "pure solvents upper phase" (19) five times to ensure removal of all sucrose and hexose. The lipids were analyzed by methods described previously (18).

The upper phases were dialyzed 7 days against 15 changes of water before gangliosides were determined by the resorcinol method (20).

Nucleic acids were extracted from whole fractions of unwashed cells using conditions recommended by Munro and Fleck (21). The method in brief is as follows. Protein and nucleic acid were precipitated and washed with ice-cold 0.2 N $HClO_4$. RNA was hydrolyzed by treatment with 0.3 N KOH for 2.5 hr at 37°C and solubilized with $HClO_4$ at a final concentration of 0.2 N. DNA was extracted from the residue by treatment with two portions of 0.5 N $HClO_4$ for 30 min at 80°C. Complete UV spectra of both extracts were obtained and corrected for tissue breakdown products by a two wavelength method (22) using equations determined for brain under our conditions. RNA was determined from the corrected absorbance at 260 nm using an ϵ (P) of 9,500, and DNA was determined at 267 nm using an ϵ (P) of 10,700. ϵ (P) is the extinction of a solution having one gram atom of phosphorus per liter. Standards were yeast RNA and calf thymus DNA. The data, expressed as DNA and RNA phosphorus, were converted to nucleic acid weight using the theoretical values of 10.0% P for DNA and 9.6% P for RNA.

Whole rat brains were also analyzed by the same methods for comparison.

RESULTS

The data summarized in Table 1 were obtained on washed cells as described above. The recovery of weight is low because of the loss of soluble constituents. Each column represents the analysis of the pooled cells from the indicated number of brains, each pool consisting of cells from 6–8 separate collections of 11–14 animals each.

Although these data show apparent trends as a function of age, the trends are probably not significant, since subsequent experiments indicated that the lipid weight per cell does not vary significantly with age. The yield of neuronal perikarya is always higher than that of astroglial cells on a weight basis. The astrocytes have a higher percentage of total lipid and ganglioside than the neurons.

Tables 2 and 3 give data for lipid, nucleic acids, and gangliosides for several preparations of unwashed neurons and glia at each age studied. There is no significant difference in yield, amount of lipid, DNA, or RNA in either cell type throughout this period of 10–30 days. However, the gangliosides of both cell types apparently do increase between 10 and 20 days. The average yield of cells for all 13 experiments tabulated is $17.4 \pm 2.8 \times 10^6$ neurons/brain and $3.5 \pm 1.0 \times 10^6$ glia/brain. There is considerably more variation in the recoveries of glia than of neurons, but the variations in the values of constituents per cell are about the same for either cell type.

As will be discussed, the neurons are of higher purity than the glia. The cell counts reported in Tables 2 and 3 are, respectively, of whole, recognizable neuronal perikarya and of intact glial cells only. Differential counts of the neuronal preparation give a particle purity of approximately 90%. Estimations of the particle purity of the astroglial fraction are more difficult to make. The contamination in this fraction consists of particles smaller than astrocytes, mainly free processes and fragmented neurons. It is obvious from phase contrast studies that intact astroglia are by far the predominant element in these fractions. It should be borne in mind, however, that the data in Table 3 are maximal figures per cell.

The differences between neurons and glia are best seen by examining Table 4, where the average figures for neurons and glia at all three ages are compared with values for whole brain. The figures expressed as pg/cell are obtained directly. The average total dry weight per cell is derived from the direct values of lipid per cell, using the average base-line figures of 24.1% lipid for neurons and 38.9% lipid for glia given in Table 1. All other percentage figures for the cells are calculated from the observed amount per cell and the derived cell weight.

The neurons have less lipid and the glia more lipid than whole brain at comparable ages. Neurons have considerably more DNA and RNA than glia on a percentage weight basis and, as expected, both cell types are much higher in nucleic acid than whole brain. The glia apparently have more DNA per cell than neurons, but this is an indicator of their lower degree of purity (see below). The most surprising finding is that neurons have a lower ganglioside concentration than glia and that in either cell it is lower than in whole brain.

In Table 5 the lipid composition of the two cell types is compared with that of whole brain for the three ages investigated. Two principal findings are apparent: there are no significant changes with age for either cell type during this period of rapid development, and the lipid compositions of the two cell types are remarkably similar. There are some consistent differences: glial lipids have more cholesterol, less phosphatidylinositol, and more phosphatidylserine than neuronal lipids. The other lipids do not differ significantly in amounts. Both cell types have less cholesterol, phosphatidylserine, and plasmalogen than whole brain lipids have at any age. The amount of galactolipid (cerebroside plus sulfatide) of either cell type is about equal to that found in 10-day-old, whole-brain lipids. These lipids increase rapidly with age in whole brain but they remain low in the isolated cells. A similar but reverse pattern is seen with total phospholipid and lecithin. Values for these components drop as myelination proceeds in whole brain, but remain high in the isolated cells; thus, neuronal and glial lipids from 20- and 30-day-old animal brains have more phospholipid and lecithin than whole brain lipids. The phosphatidylinositol concentration in each cell type is higher than that of whole brain lipids at any age.

TABLE 2 Composition of Isolated Neurons Determined on Unwashed Cells*

Age, *days*	10	20	30
Yield, *cells/brain*	$19.8 \pm 2.6 \times 10^6$ (6)	$15.9 \pm 1.3 \times 10^6$ (4)	$14.5 \pm 1.6 \times 10^6$ (3)
Total lipid, *pg/cell*	42.6, 43.1	46.2, 52.4	34.4, 44.1
DNA-P, *pg/cell*	0.754 ± 0.081 (4)	0.930	0.963
RNA-P, *pg/cell*	2.46 ± 0.13 (4)	2.31, 2.35	1.78
Ganglioside NANA, *pg/cell*	0.065, 0.071	0.151, 0.181	0.128, 0.143

* Mean values with average deviations. Where applicable, the number of experiments is in parentheses.

TABLE 3 Composition of Isolated Astrocytes Determined on Unwashed Cells*

Age, *days*	10	20	30
Yield, *cells/brain*	$3.3 \pm 0.7 \times 10^6$ (6)	$4.0 \pm 1.4 \times 10^6$ (4)	$3.0 \pm 0.6 \times 10^6$ (3)
Total lipid, *pg/cell*	177, 185	309, 255	272, 227
DNA-P, *pg/cell*	1.112 ± 0.105 (3)	1.26, 1.11	1.027
RNA-P, *pg/cell*	3.14 ± 0.31 (4)	2.48 ± 0.39 (3)	2.32
Ganglioside NANA, *pg/cell*	0.101, 0.184	1.64, 1.30	1.78, 1.38

* Mean values with average deviations. Where applicable, the number of experiments is in parentheses.

TABLE 4 COMPARISON OF THE COMPOSITION OF NEURONS, ASTROCYTES, AND WHOLE BRAIN

	Neurons		Astrocytes		Whole Brain		
Age, *days*	Avg 10–30		Avg 10–30		10	20	30
	pg/cell	*wt %*	*pg/cell*	*wt %*		*wt %*	
Total dry wt	178		591				
Total lipid	43.0 ± 3.9	24.1	230 ± 42	38.9	27.8	30.7	33.5
DNA	8.18 ± 1.18	4.6	11.2 ± 0.9	1.9		0.72	0.58
RNA	24.2 ± 2.1	13.6	29.1 ± 4.4	4.9		1.59	1.27
Ganglioside NANA	0.123 ± 0.037	0.069	1.06 ± 0.62	0.18		0.36	0.35
Protein	99.1*	55.7*	307*	51.9*	60.5	59.7	57.9

* Obtained by difference.

TABLE 5 LIPID COMPOSITION OF NEURONS AND ASTROCYTES COMPARED TO WHOLE BRAIN*

	Neurons							Astrocytes							Whole Brain		
Age, *days*	10		20		30		Average†	10		20		30		Average†	10	20	30
Experiment	1	2	3	4	5	6		1	2	3	4	5	6				
Cholesterol	10.0	10.1	10.3	11.2	10.5	11.5	10.6 ± 0.6	12.4	13.1	11.4	14.2	17.0	15.6	14.0 ± 1.9	18.8	21.1	21.5
Galactolipid	1.9	1.4	2.1	2.6	2.0	2.3	2.1 ± 0.4		1.2	2.2	1.5	1.9	2.0	1.8 ± 0.4	1.5	6.6	11.2
Total phospholipid	74.1	76.2	74.3	70.3	67.3	71.5	72.3 ± 2.9	68.0	71.5	73.3	69.3	70.1	73.3	70.9 ± 2.0	76.1	67.5	66.5
Phosphatidylcholine	41.2	44.2	40.3	38.9	36.1	38.8	39.9 ± 2.5	36.4	38.5	39.2	34.6	33.3	36.0	36.3 ± 2.1	37.8	28.3	26.3
Ethanolamine phosphatides	18.3	19.3	18.5	17.8	17.4	17.8	18.2 ± 0.6	16.9	19.3	19.6	21.3	22.0	21.6	20.1 ± 1.8	21.1	20.0	20.5
Phosphatidylinositol	5.1	4.4	5.6	5.1	4.6	4.5	4.9 ± 0.4	4.1	3.6	4.2	3.2	2.8	3.0	3.5 ± 0.5	2.7	2.2	2.5
Phosphatidylserine	4.1	3.4	4.0	3.7	3.9	4.3	3.9 ± 0.3	5.0	5.0	4.6	5.0	5.8	5.7	5.2 ± 0.4	9.8	7.3	7.8
Sphingomyelin	3.3	2.6	3.5	3.2	3.3	3.4	3.2 ± 0.3	3.4	3.0	3.2	3.5	4.3	4.6	3.7 ± 0.6	2.9	4.1	4.2
Unidentified	2.0	2.2	2.5	1.6	2.1	2.8	2.2 ± 0.4	2.3	2.2	2.5	1.7	2.0	2.5	2.2 ± 0.3	1.9	3.0	2.8
Plasmalogens	6.0	6.3	7.4	7.5	6.7	7.8	7.0 ± 0.7	6.4	6.7	7.0	7.9	8.9	8.5	7.6 ± 0.9	9.0	10.4	11.6

* All lipid figures expressed as weight % of total lipid.
† Average of the six results ± SD.

DISCUSSION

The criteria for identification of the cell types have been discussed previously (11). Although it seemed apparent that the glial fraction was composed predominantly of astrocytes, we were not sure how many oligodendroglia were in this preparation. Recently (23) we have been successful in isolating nearly pure fractions of oligodendroglia from bovine brain white matter. We have also studied these cells and the glia isolated from rat brain by electron microscopy (24). It is now clear that the glial fraction from rat brain is largely astrocytes. The oligodendrocytes have a different density on sucrose gradients during centrifugation, a completely different appearance in both phase and electron microscopy, and a different chemical composition.

Purity

The neurons are about 90% pure by particle count (11). This high degree of purity is confirmed by our calculated value of 7.36 pg of DNA per neuron. Previously obtained values of the amount of DNA per rat cell nucleus range from 6.0–7.4 pg. Neurons isolated by others were found to have 6.25 pg (10) and 6.1 pg (25) of DNA per cell. Thomson, Heagy, Hutchison, and Davidson (26) found values ranging from 6.3–7.4 pg of DNA per nucleus for various rat tissues, but they did not examine brain. Santen and Agranoff (22) found 6.4 pg of DNA for rat brain nuclei, and Brizzee, Vogt, and Kharetchko (27), in their determination of cell number in brain, use the value of 6.5 pg of DNA/cell found by Gray and De Luca (28). The variable with the largest error in our method is the cell count. A consistent underestimation of cell number may account for our figure being higher than the average obtained by others.

A more quantitative idea of the purity of the glial fraction can be obtained from the DNA analysis than from the microscopic examination. This fraction has 11.2 pg of DNA/cell. If all cells are diploid, the difference in the DNA figures for glia and neurons (3.8 pg of DNA/cell) represents the cells and fragmented cells not recognized in the phase microscope as characteristic astrocytes. These figures give a cellular purity of 66% astrocytes in the glial fraction. The weight purity, however, will be considerably higher since the average nonastrocyte particle is considerably smaller than an astrocyte. Taking as a maximum figure that the weight of a fragmented cell is equal to that of a whole neuronal soma (178 pg), and that for every 34 of these particles there are 66 intact glia, we can calculate that the minimal weight of a single astrocyte is 499 pg. Therefore, the weight % purity is 499 × 100/591, or 84%, a figure comparable to the purity of the neuronal fraction. Correcting the values in Table 4 for this amount of contamination causes no significant change in any of the weight percentage values

other than those of nucleic acid. It should be noted that the separation method involves two low-speed (3300 *g*) centrifugations on density gradients (11). The astrocytes, in both steps, sediment through a layer equivalent in density to about 1.2 M sucrose, and are finally collected on a layer of specific gravity 1.21 (equivalent to 1.65 M sucrose). These steps ensure the absence of all small particulate debris such as microsomes, mitochondria, and synaptosomes. Such particulate matter is not seen in electron micrographs of either astrocytes or neurons (24).

Yield

This method furnishes very high yields of neurons compared with other techniques. Rose (1) obtained 7% and Satake and Abe (25) obtained 6–18% of our yield of 17.4×10^6 cells per rat brain. Recoveries of glia have not been reported by others. The recoveries of both neurons and astrocytes reported here are lower than those reported previously (11). Some sacrifice in yield occurs when the method is scaled up to provide enough material for analytical work. However, some of the data reported previously were inadvertently calculated with the higher cell counts, thus accounting for the discrepancies in figures per cell in the two reports.

It is difficult to calculate accurately the recoveries relative to the total number present in the brain sample. Recent studies suggest the total number of cells per g of fresh rat brain to be respectively, 112×10^6 (27), 340×10^6 (22), and 120×10^6 (29). Of this total number, the ratio of glia to neurons may be less than one in the age range of 10–30 days (27). Our own DNA analyses give a cell number closer to the latter figure. We find (recalculating the data in Table 4) 1.22–1.38 mg of DNA per g wet wt. This corresponds to 1.65 mg of DNA in our brain sample which consists of whole brain minus cerebellum. Using our calculated value of 7.36 pg of DNA per neuron, this gives a total number of 224×10^6 cells/brain sample or $165–187 \times 10^6$ cells/g. This figure will vary as cell density decreases with brain growth. The 224×10^6 cells includes not only neurons and glia, but epithelial and ependymal cells as well. A reasonable estimate is that there are 100×10^6 neurons and an equal number of glia in our brain sample. Our recoveries are thus in the order of 15–20% for neurons and 3.5% for glia. However, since the term "glia" includes astrocytes, oligodendrocytes, and microglia, the recoveries of astrocytes alone will be somewhat higher. The purified cell fractions together account for 11% of the DNA in the sample.

Weight and Nucleic Acid Content

With or without the correction discussed above, astrocytes are considerably heavier than neuronal somata, the glia/neuron weight ratio being 3.3. Freysz et al. (10) also found their glia to be considerably heavier than their neurons (the glia/neuron weight ratio being 3.56); however their figures for weight per cell are somewhat higher than ours. Rose and Sinha (5) now report a figure of 100–110 pg/cell for neurons. The earlier figure of 2300 pg/cell obviously was in error and inconsistent with his reported cell diameters of 8–25 μ, dimensions similar to those we reported (11). The neurons isolated by Satake and Abe (25) are of the same size range as ours, having 126 pg of protein/cell compared with our figure of 99 pg/cell. The high glia/neuron weight ratio must be explained by the fact that the astrocytes are more intact than the neurons.

As expected, neurons have much more RNA than glia, about three times more on a weight percentage basis. It is noteworthy how much of the neuron soma is nucleic acid. DNA and RNA together constitute the very high figure of 18.2% of the neuron dry weight, compared with about 2% of whole brain and 7% of glia. Thus, approximately half of the dry mass of the neuronal cell body may be composed of nucleic acid together with associated nucleoprotein.

Gangliosides

All of the present evidence suggests that gangliosides are primarily neuronal (probably mainly dendritic) constituents (30, 31), although they are undoubtedly present in myelin (32, 33), and there is some indirect evidence that they may be in glia as well (34). In view of these findings the ganglioside data in Tables 1–4 are surprising. In eight preparations neuron ganglioside neuraminic acid was from 403–900 μg/g dry wt and glia ganglioside neuraminic acid was from 406–2540 μg/g dry wt. The glia were always higher in gangliosides whether determined on washed (Table 1) or unwashed cells (Tables 2 and 3). Using the average value of 33.3% NANA in gangliosides, these figures represent 0.12–0.27% gangliosides in neurons and 0.12–0.76% in glia. Both cell fractions have an average ganglioside content between that found for human white matter (0.14%) and human gray matter (1.57%) (20), less than the 1.07% found by Derry and Wolfe (31) in dissected ox neurons, and less than the amount in whole rat brain of the same age (see Table 4).

We are not suggesting that these data indicate that astrocytes have more gangliosides than neurons *in situ*. It is probable that most of the neuronal ganglioside lies in the synaptic endings and dendritic membranes, both of which are shorn off in this preparation and remain in layer A (11) in the density gradient separation step. As stated earlier, the astrocytes are further purified on a second gradient which removes most small particulate contamination not excluded in the first centrifugation. Preliminary experiments have shown that the bulk of the whole brain ganglioside stays in layer A and is associated with a fraction which may be dendritic processes. Another possibility is that it resides in the exterior "fuzzy

coat" of the plasma membrane and is partly lost during tryptic digestion. However, all the cells are exposed to the trypsin and this would not account for the observed difference. The ganglioside in the glial fraction certainly cannot be due to neuronal contamination since the purer neuronal fraction has less ganglioside.

A reasonable explanation of these results is that gangliosides are normal constituents of both glial and neuronal plasma membranes, but constitute a much higher proportion of the neuronal membrane than the glial membrane. The isolated intact glia have, however, a much greater surface-to-volume ratio than the process-free neuronal somata. Thus gangliosides are higher in the glial fraction because it has a much higher cell membrane content. We must point out here that in contrast to reports on other preparations, our cells are isolated with the plasma membrane largely intact (23, 24). We consider these data to be the first direct evidence for the presence of gangliosides in glial cells.

Lipids

The remarkable similarity in lipid composition between neurons and glia was also found by Freysz et al. (10) with cells isolated by a very different technique. They also found that their glial preparation had a higher lipid content and the glial lipids had more cholesterol than the neurons. However, the phospholipid distributions of both of their cell types resemble whole brain phospholipids more than they resemble the phospholipids of either of our cell types.

Our glial cells are quite different from the cells isolated by Fewster and Mead (8) from bovine white matter. The bovine cells, identified as oligodendroglia, have less total lipid, but this lipid has considerably more galactolipid and cholesterol than the rat glial lipid. This is further evidence for our cells being predominantly astrocytes.

One of the most dramatic of the several relatively distinct maturational stages of the brain is the period of rapid myelination. In the rat, myelination begins in the brain at approximately 10 days of age and reaches a peak rate at 20 days (35). By 30 days of age the brain has accumulated 40% of the amount of myelin it will have at 6 months (32, 36). During this 20-day period the brain weight has increased 55%, the amount of lipid per brain has increased 200%, and the amount of the myelin-typical lipids, cerebroside and sulfatide, have increased 2100%. The later compounds are apparently true indicators of myelination, yet isolable myelin accounts for only 25% of the lipid increment and 40% of the galactolipid increment. Moreover, it has been calculated (18) as well as actually demonstrated (37) that myelin accounts for only about 65% of the total galactolipid of mature central nervous system white matter. These data indicate that other changes are taking place in the brain at this time, besides myelination and general cellular growth. It would be desirable to see which other structures are being modified *pari passu* with myelin deposition.

It is obvious that neurons and astrocytes do not undergo any dramatic compositional changes during this period, and that there is little if any increment in the low galactolipid content of either cell type (this low figure can also be taken as verification of the low level of myelin contamination in either preparation). Thus, these components of nerve tissue have an apparently static lipid composition and are not responsible for the accumulation of myelin lipids in the nonmyelin portion of the brain.

The most important question to be considered about these preparations is how closely the properties of these cells correspond to those of brain cells in the living tissue. Are they representative of the total in vivo population? Fuller answers to such questions are necessary if routine investigations of such preparations are to be meaningful.

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