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EVOLUTION, IN MOUSE BRAIN MICROSOMES, OF LIPIDS AND THEIR CONSTITUENTS DURING MYELINATION

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SUMMARY

During myelination and in brain microsomes there is a transitory accumulation of galactolipids. These galactolipids will become constituents of mature myelin. The galactolipids are composed of very long-chain fatty acids already in microsomes; the meaning of the 'myelin-like' fraction is discussed. Very long-chain fatty acids, giving a particular stability to the structure of myelin, are synthesized in microsomes.

INTRODUCTION

Although brain microsomes contain only slight amounts of galactolipids[6,14–17,20,32,37,59], numerous biosynthetic studies indicate that they are the site of synthesis of myelin constituents. Immediately myelination begins, there should appear an increase of the content of galactolipids in brain microsomes. This accumulation should be transitory and cease at the end of the myelination period. Cerebrosides and sulphatides, particularly those composed of long-chain fatty acids (over 18 carbon atoms) are found in myelin in great quantity[45,53]. The overall effect of a preferential localization of these sphingolipids is to impart a higher degree of stability to this membrane structure[51,66]. The type of galactolipid synthesized in microsomes during myelination should obligatorily be the type found as myelin constituents, as these subparticles are also the site of long-chain fatty acid biosynthesis[9,10,47].

To verify these hypotheses, the lipid and fatty acid content of brain microsomes as a function of age was studied;

METHODS AND MATERIALS

Microsomal preparation

Brains from C57 black mice are extracted and washed in a medium containing 0.32 *M* saccharose and 0.9% NaCl. They are then homogenized in a glass homogenizer with a teflon pestle (1 ml medium per g wet brain). Mitochondria, tissue debris, nuclei, myelin and synaptosomes are sedimented by centrifuging the homogenate in an RC 2 Servall centrifuge (30 min at 17,500 × *g*)[20]. Microsomes are obtained by centrifuging the supernatant for 60 min at 104,000 × *g* (Spinco L2–50). They are washed and then sedimented under the same conditions. The pellet is resuspended in a minimal amount of distilled water and stored at —30 °C. The desired quantity, obtained after several preparations, is lyophilized.

Criteria of purity

Electron microscopy. The microsomal pellet is recovered with a spatula, washed with distilled water and fixed for 24 h in glutaraldehyde (2.5% in 0.1 *M* Sorensen buffer). After being washed with the same buffer, the pellet is post-fixed for 1 h with osmic acid (2% in the same buffer); then it is dehydrated and embedded in Epon. The ultra-thin preparations are contrasted with uranyl acetate and lead citrate, and studied with an electron microscope, Hitachi HU 11A.

Enzyme markers. Glucose-6-phosphate dehydrogenase, a soluble enzyme, is measured in the various fractions[39]. The incubation medium is made of Tris buffer (0.15 m*M*, pH 7.8), $MgCl_2$ (0.01 m*M*), NAD^+ (0.054 m*M*), glucose-6-phosphate (0.01 m*M*) and enzyme; it is made up to 2.5 ml with distilled water. Readings are taken at 340 nm at 1 min intervals, immediately after the addition of enzyme, with a PMQ II Zeiss spectrophotometer.

Malate-dehydrogenase, specific to the inner membrane and matrix of the mitochondrion, is also studied in all the fractions[24]. Crude preparations of mitochondria are obtained by sedimenting, at 1400 × *g*, the rehomogenized 17,500 × *g* pellet (1 ml of homogenization medium per g of pellet). The supernatant contains impure mitochondria. All fractions are sonicated at 0 °C during 2 min to break out membranes (by 30 sec periods) with a Branson apparatus (at the maximal power acceptable with the microprobe). The reaction mixture is composed of glycine–NaOH (0.27 m*M*, pH 10), malic acid (0.29 m*M* neutralized to pH 7.5) and NAD^+ (0.075 m*M*, pH 6.5) in a final volume of 2.5 ml. After the addition of 10–50 µl of enzyme solution, the rate of NADH formation is followed at 340 nm at 30°.

The plasma membrane enzyme, 5′-nucleotidase, is examined[21]. Brains are prepared in a medium containing Tris buffer (0.16 *M*, pH 7.5) and saccharose (0.32 *M*). Thus the incubation medium (1 ml) is composed of Tris buffer (0.05 m*M*), KCl (0.1 m*M*), $MgCl_2$ (0.005 m*M*), Triton X-100 (0.04%), saccharose (0.96 m*M*) and AMP (0.004 m*M* at pH 7.2). The reaction is stopped with 5 ml of 8% TCA. After sedimentation of the proteins, 1 ml of the supernatant is taken, and successively are

added 3.6 ml of 1.4 *N* H_2SO_4, 0.2 ml of 5% ammonium molybdate and 0.2 ml of Fiske and Subbarow reagent. After 7 min heating at 100 °C, phosphorus determinations are made at 830 nm (see ref. 3).

Ribonucleoside 2',3'-cyclic phosphate diesterase is studied in all the fractions[52]. The assay mixture contains the following components: 0.1 ml of 0.1 *M* Tris–HCl (pH 7.5), 0.050 ml of adenosine 2',3'-cyclic phosphate solution (20 μmole/ml), 0.1 ml of *E. coli* type III alkaline phosphatase (0.3 mg/ml) plus 10 mg of sodium deoxycholate per ml, 50 μl of enzyme solution from the different fractions and water to a final volume of 0.25 ml. The tubes are incubated for 10 min at 37 °C. The reaction is stopped by the addition of 2 ml of 3% TCA. Of the supernatant obtained, 0.5 ml is assayed for inorganic phosphate[3].

Saccharose elimination

The definition of microsomes implies saccharose in the medium of preparation. This saccharose must be eliminated before galactolipids in the lipid extract can be measured. The orcinol method for galactose[55] also estimates saccharose in aqueous solution; no saccharose is detectable in the chloroform–methanol fraction, as saccharose is carried away in the upper Folch phase. For each preparation, it is verified that no saccharose comes from the medium in the lipid extract after Folch washing by adding 0.5 μCi of [^{14}C]saccharose to the lyophilized microsomes. After washing there is no radioactivity in the lipid fraction.

Lipid extraction

Lipids are extracted by chloroform–methanol (2:1, v/v)[27,64] with 20 ml solvent per g of lyophilized microsomes, by stirring whilst cold for 2 h[57]. The solution is then filtered; the washed residue is suspended in the same solvent. After another 2 h stirring under the same conditions, the solution is filtered. The two filtrates are mixed, dried, weighed and dissolved in chloroform–methanol (20 mg extract per ml of solvent). A Folch washing is made on this solution by adding 0.2 vol. water and then twice 0.15 vol. upper Folch phase (chloroform–methanol–water, 3:48:47)[28]; upper phases are eliminated. In this extract are measured galactolipids (cerebrosides + sulphatides)[55], cholesterol[59], phosphorus[3] and plasmalogens[29]. Lipids are possibly methylated[44]: fatty acid methyl esters thus obtained are directly analysed by gas liquid chromatography (SE 52 column)[34]. α-Hydroxylated and non-hydroxylated fatty acids, saturated plus unsaturated, can be separated by thin-layer chromatography on Silica Gel H, the solvent being light petroleum–ether (80:20, v/v) before being analysed[41]. On chromatograms, the peak areas are determined by weighing. Twelve determinations have been made on 3 different preparations. Proteins are measured in the lipid extract and in the various subcellular fractions[40]. Lipids are eventually visualized on thin-layer chromatograms after migration with chloroform–methanol–water, (70:30:4 (ref. 63) by Mangold reagent[42,58]. For two complete studies of the

different lipids, at least 300 mice were needed; at each age 3 or more preparations were made.

From the microsomal lipid extract, cerebrosides are eventually isolated[30,34]. However, after selective methanolysis, columns of Unisil were used instead of silicic acid (1.6 g in 7 mm diameter tube) each preparation supplying less than 40 mg of lipid extract. Thirty ml of chloroform separate cholesterol; 50 ml of chloroform–methanol (95:5) elute cerebrosides; sulphatides and sphingomyelins are eluted by 55 ml of chloroform–methanol (80:20) and by 30 ml of methanol, respectively. Fatty acid methyl esters are obtained as previously described[44]. Only one cerebroside preparation has been completed.

RESULTS

Microsomal purity

Electron micrographs at low and high magnifications (Figs. 1 and 2) show normal reticulum, without whole or fragmented mitochondria, nuclei, tissue debris; myelin and synaptosomes are totally absent.

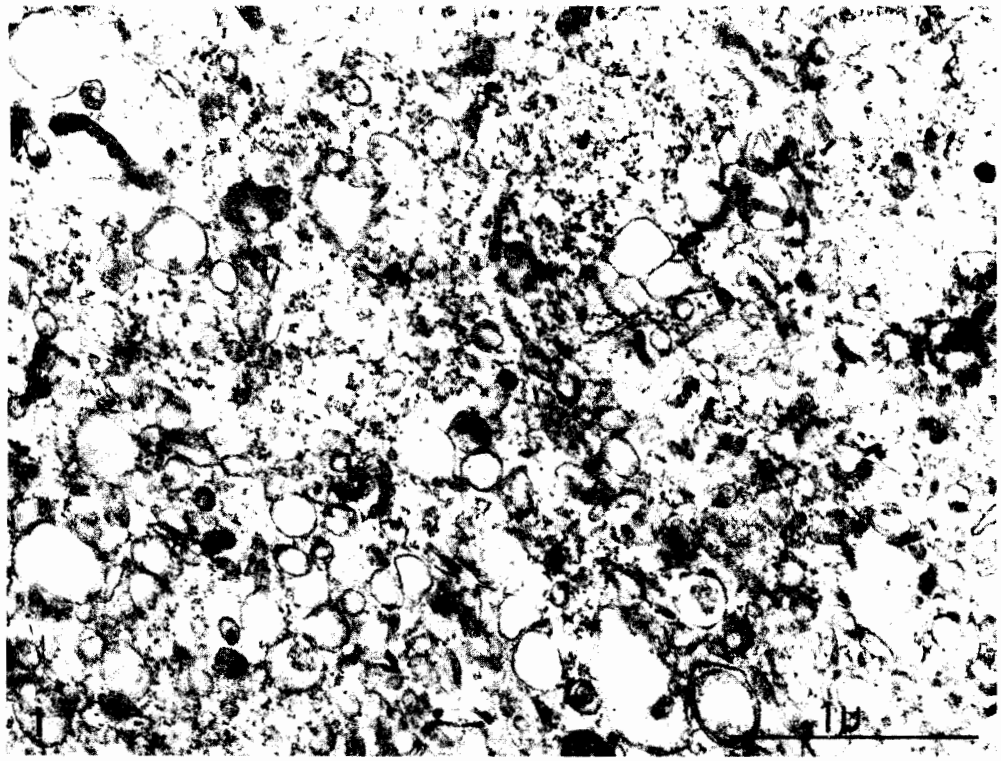
Enzymic determinations corroborate these results (Table I). The mitochondrial malate-dehydrogenase is virtually absent from microsomes (0.05% of total activity). Washing of microsomes is obligatory: 2% of the total malate-dehydrogenase activity

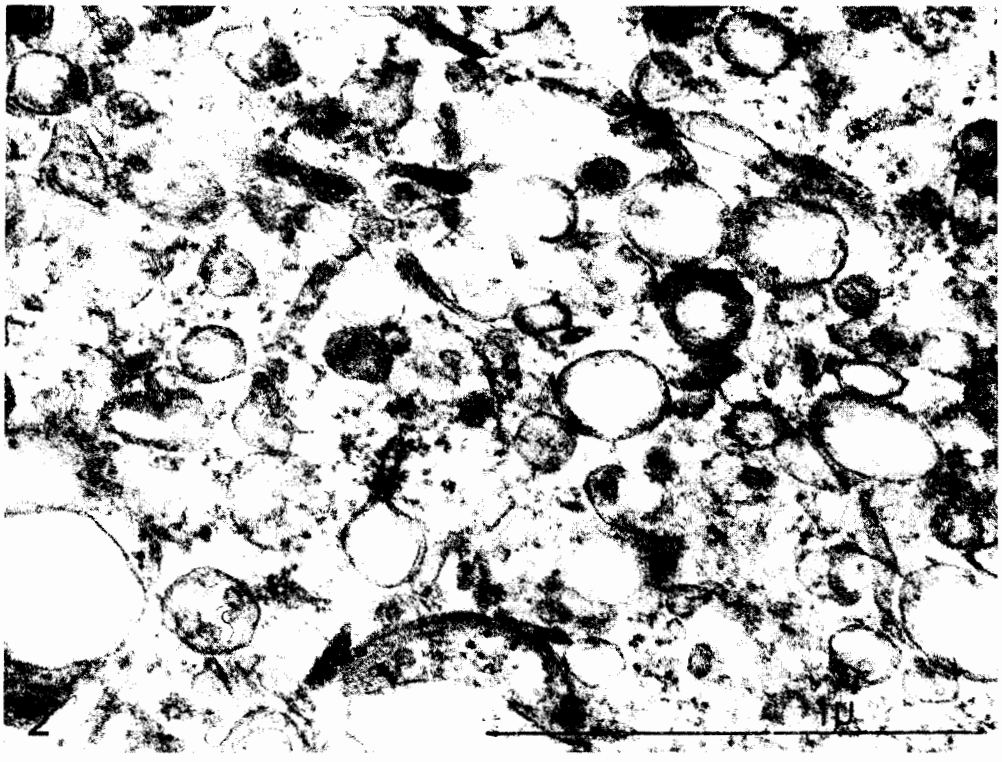
TABLE I

MICROSOMAL PURITY TESTED BY MEASURING THE ACTIVITY OF SOME SPECIFIC SUBCELLULAR ENZYMES

H, Homogenate; P_1, 17,500 × *g* pellet; P_2, 1400 × *g* pellet; M, mitochondria; S_1, 17,500 × *g* supernatant; S_2, 1st 104,000 × *g* supernatant; S_3, 2nd 104,000 × *g* supernatant; m, microsomes.

	H =	P_1		+ S_1			
		M	P_2	S_1 =	S_2 +	S_3 +	m
Malate-dehydrogenase							
Total activity of fractions (Δ OD/min)	1955	891	728	197	176	5.7	0.9
% of total activity		49	40	11	9.6	0.35	0.05
Specific activity (Δ OD/min/mg protein)	2.4	3.5	1.3	0.9	1.7	1.1	0.07
Glucose-6-*p*-dehydrogenase							
Total activity of fractions				11.05	9.27	0.19	0.01
% of total activity					97.8	2	0.1
Specific activity (Δ OD/min/mg protein)				0.085	0.09	0.038	0.0008
5′-nucleotidase							
% of total activity		89		11	7.5	0.5	2.5
Specific activity (μmole phosphorus/h/mg protein)		1.3		0.5	0.5	0.6	0.95





Figs. 1 and 2. Electron micrographs of microsomes at low and high magnifications.

is found in the first 104,000 × *g* supernatant. After this washing, microsomes are free of soluble enzymes: 0.1% of the total glucose-6-*p*-dehydrogenase activity.

5′-Nucleotidase, specific to plasma membrane, is present in microsomes (2.5% of total activity). Its specific activity is the same as in the whole homogenate: 1 μmole of phosphorus per h per mg protein; the 17,500 × *g* pellet in which plasma membranes are contaminated by cellular debris, nuclei, mitochondria and myelin, contains 89% of the total activity and presents the highest specific activity: 1.3 μmole of phosphorus per h per mg protein. For the whole homogenate, we found 105 μmole of phosphorus per h per mg wet brain; for microsomes the value was 3.3. These results are about the same as those found in the rat[2], taking into account that we used 20-day-old mice. However, the specific activity in microsomes is lower in our assays. The difference can be explained by a different microsomal definition. In rat, microsomes have been prepared by a 50,000 × *g* centrifugation for 1 h from a supernatant previously sedimented at 13,500 × *g*. In our study we sediment plasma membranes, with some other subcellular fractions, at 17,500 × *g*. This more important acceleration removes elements from the microsomal fraction. Moreover, a special fraction involved in lipid biosynthesis has been defined: a pellet obtained at 30,000 × *g* from a 12,000 × *g* supernatant[35]; this fraction contains few microsomes, the majority of the elements appearing to consist of membranous circular bodies. These bodies, which may have a 5′-nucleotidase activity, are mainly eliminated in our peparations.

Lipid measurements

The thin-layer chromatography in Fig. 3 shows the lipid extract pattern as a

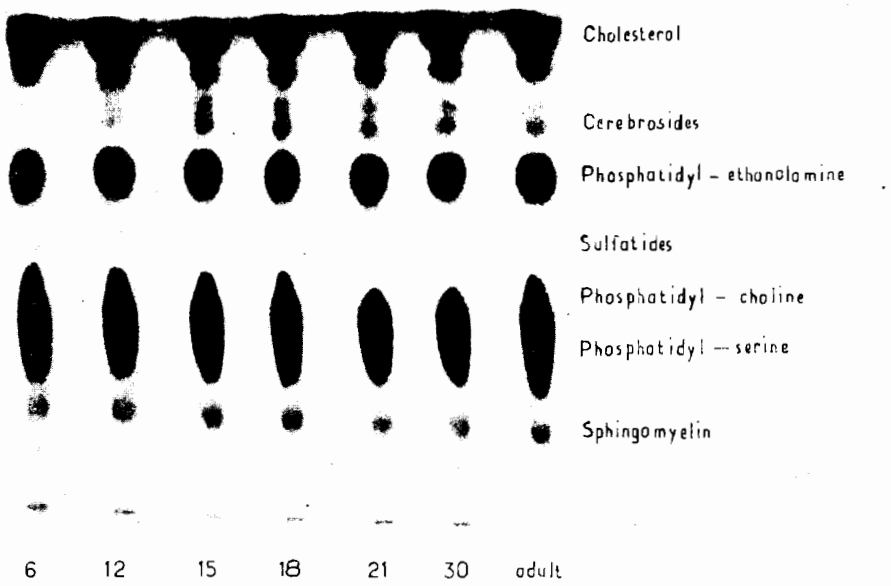


Fig. 3. Thin-layer chromatography of microsomal total lipid extracts as a function of age (in days).

TABLE II

VALUES OF DIFFERENT PARAMETERS AS A FUNCTION OF AGE

Molecular weights: phospholipids, 750; cholesterol, 387; galactolipids, 828. The values for mg phosphorus are multiplied by 26 obtain the phospholipid quantities. Each value is a mean of 6 determinations. The accuracy of the methods is 5% for cholesterol, 3% for phospholipids and 10% for galactolipids.

	Age (days)						
	6	*12*	*15*	*18*	*21*	*30*	*Adult*
Mice, mean weight (g)	1.5–4	5.5–7.5	6.5–8.5	6–9.5	7.5–10	8.5–13.5	15–24
Brain, mean weight (mg)	193	332	357	368	375	386	401
Lyophilized microsomes, mean weight (mg per mouse)	1.40	1.95	2.85	2.62	2.68	2.76	2.45
(mg per g wet brain)	7.20	5.87	7.98	7.12	7.14	7.15	6.2
Total lipid extract (mg per mouse)	0.39	0.74	0.74	0.65	0.67	0.72	0.90
(mg per g wet brain)	2.02	2.22	2.07	1.77	1.78	1.85	2.24
Total lipid after Folch washing (μg per mouse)	93	163	192	268	256	329	349
(μg per g wet brain)	481	490	537	728	782	852	870
Galactolipids (μmole per 100 mg of lipids)	1.8	3.0	3.7	5.4	4.7	3.6	3.4
(nmole per g wet brain)	9	15	20	39	36	30	29
Phospholipids (μmole per 100 mg of lipids)	87	83	83	92	92	89	92
Cholesterol (μmole per 100 mg of lipids)	60	47	47	46	38	30	42
Plasmalogens (μmole per 100 mg of lipids)	5.8	6.1	7.0	7.5	7.8	8.0	8.5
Molar ratio, cholesterol/phospholipids/galactolipids	33/48/1	16/28/1	13/22/1	9/17/1	8/20/1	8/26/1	12/27/1

function of age in brain microsomes, after Folch washing; 400 μg of lipids are put down for each age. Cerebrosides are hardly detectable at 6 days: the spot intensity increases until the 15th to 21st days and then slightly diminishes. This effect is more visible on the upper spot which contains cerasines.

Table II shows the evolution of various lipid parameters with age: they all increase when expressed per brain. For a defined age at myelination period, we have shown[33] that, according to the animal weight, more or less galactolipids are found in total brain lipids. It looks as if during the myelination period two parameters are involved: the animal weight, which is to be eliminated as much possible from the calculations, and age, which is the variable parameter in this study. Within a given age group, the animal weight is widely spread, so we had to discard 20% of the mice.

In the same quantity of lipids, *i.e.* 100 mg of lipid extract after Folch washing which eliminates gangliosides, it is possible to show a relative stability of phospholipids, a slight decrease of cholesterol and a slight increase of plasmalogens. Expressed per g wet brain, all these values increase more or less. Galactolipids show a phase of

TABLE III

VARIATION IN THE MOLAR RATIO OF CHOLESTEROL/PHOSPHOLIPIDS/GALACTOLIPIDS ACCORDING TO CELLULAR TYPE AND AUTHOR

	Cholesterol	*Phospholipids*	*Galactolipids*
Microsomes			
from Table II	12	27	1
guinea-pig brain[17]	5.6	12	1
rat brain[13]	6.5	9	1
rat brain[14]	7	8	1
rat brain[58]	5	9	1
Myelin-like			
rat brain[1]	8	12	1
Astrocytes } rat brain[44]	17	45	1
Neurones }	11	38	1
Oligodendrocytes			
bovine white matter[24]	4	3.7	1

increase, maximal at 18 days; after this age they decrease. Therefore galactolipids are quantitatively transitorily increased in microsomes. The difference is significant, even taking into account the important error in galactolipid assay which, however, is always less than 20%. This evolution and the values found are different from those described for rat brain[16]. In rat brain, microsomes are differently prepared; they sediment at 30,000 × *g* with a 60-min centrifugation.

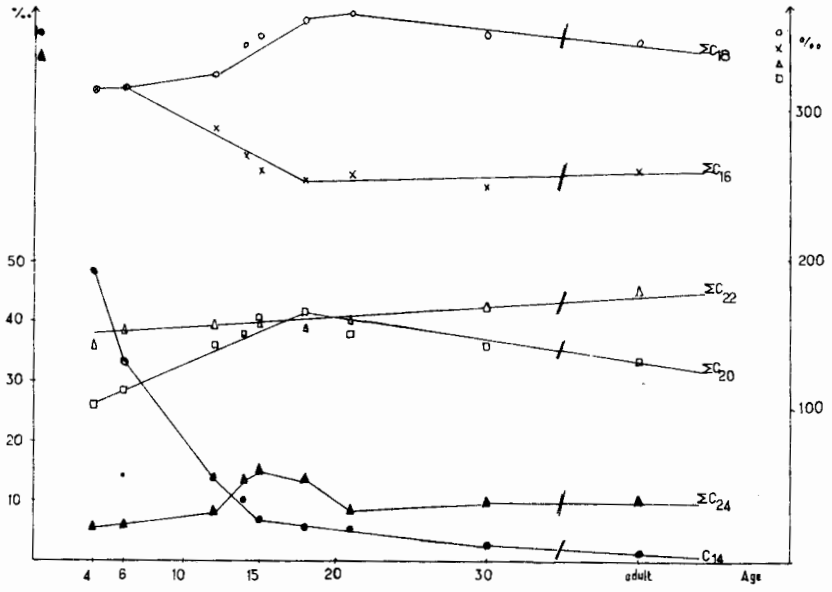


Fig. 4. Microsomal total fatty acids as a function of age (saturated, unsaturated, α-hydroxylated). The lower detection of α-hydroxylated fatty acids has not been taken into account. Age is expressed in days.

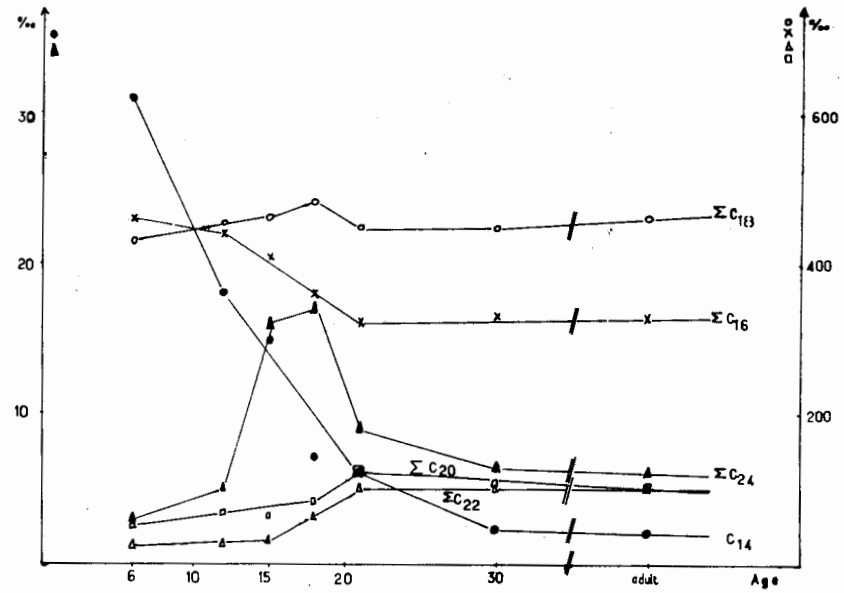


Fig. 5. Microsomal fatty acids as a function of age (saturated + unsaturated). Age is expressed in days.

Table III compares the different values found in the literature for adult animals. A different definition of microsomes (20,000 × *g*, 25 min)[15] gives a relative increase of galactose. A longer centrifugation with a greater acceleration sediments more phospholipids and cholesterol than galactolipids. The molar ratio of phospholipids/cholesterol is different according to some authors; we find a ratio of nearly 2; this is in agreement with some authors[20,60], at variance with others[15,16]. The molar ratio cholesterol/phospholipids/galactolipids in our hands is medial between the 'myelin-like' fraction[1] and astrocytes[46]. Oligodendrocytes[26] have a ratio nearer to the myelin ratio than to the rat 'myelin-like' fraction; however, those oligodendrocytes were prepared from white matter of bovine brain, and not from rat brain.

The curves in Fig. 4 shows the evolution of the various fatty acids, α-hydroxylated and non-hydroxylated, from the microsomal total lipid extract after Folch washing. The C_{14} shows an important diminution as a function of age, The $\Sigma\ C_{24}$ shows a particular profile which increases weakly but regularly with age although it presents a peak between 12 and 21 days. At 15 days the quantity of $\Sigma\ C_{24}$ present is twice the quantity seen in a regular evolution.

When α-hydroxylated fatty acids are eliminated, the same evolution is seen (Fig. 5). $\Sigma\ C_{16}$ and $\Sigma\ C_{18}$ vary in opposite direction until 21 days; $\Sigma\ C_{20}$ increases until 21 days and slightly decreases thereafter: it is stable after 30 days. $\Sigma\ C_{22}$ increases until the same age and is stable thereafter.

In the 6th figure, one can see simultaneously: the evolution of the ΣC_{24} from Figs. 4 and 5, the evolution of the non-hydroxylated $\Sigma\ C_{24}$ expressed as mg per 100 mg total brain lipid extract (by adding an internal standard[33]), the variation of the galactolipid quantity in the microsomal total lipid extract after Folch washing (from Table II),

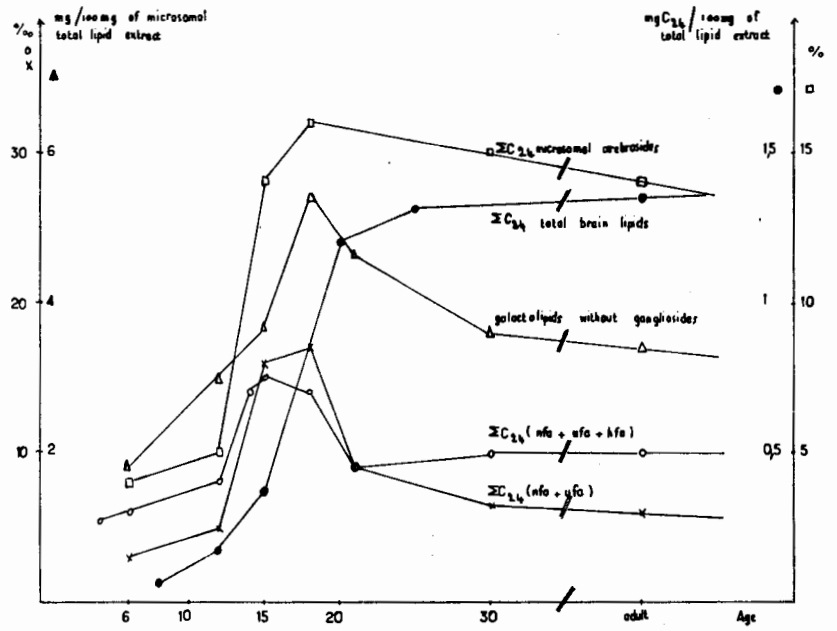


Fig. 6. Evolution of fatty acids and galactolipids as a function of age. Microsomal fatty acids with 24 carbon atoms from Fig. 4, ○; from Fig. 5, ×; nfa, normal saturated fatty acids; ufa, unsaturated fatty acids; hfa, α-hydroxylated fatty acids. Galactolipids (from Table II), Δ. Total brain fatty acids with 24 carbon atoms (Σ C_{24}) expressed as mg per 100 mg total brain lipid extract, ●. Fatty acids from microsomal cerebrosides (saturated, unsaturated, α-hydroxylated), □. The lower detection of α-hydroxylated fatty acids has not been taken into account.

and the increase of Σ C_{24} (hydroxylated + non-hydroxylated) from microsomal cerebrosides. The greatest quantity of galactolipids occurs at the same time as the maximum of very long chain fatty acids. This maximum corresponds to the inflexion point of the total C_{24} brain lipid extract curve. The pattern of ΣC_{24} from cerebrosides is similar to the latter. The evolution of total C_{24} as a function of age reflects the quantitative increase of microsomal glycolipids; when myelination occurs, this evolution is also characterized by a transitory increase in the percentage of long-chain fatty acids. During the myelination period some of the microsomal glycolipids have long-chain fatty acids; this new population either replaces or follows those containing medium-chain fatty acids. (The existence of two cerebroside populations has been described in total mouse brain, one built with medium-chain fatty acids preceding one with very long chain fatty acids[33].) The acquired cerebroside profile remains stable: the C_{24} acids from microsomal cerebrosides do not decrease after 18 days. Moreover, sulphatides and sphingomyelin from adult microsomes seem to contain minute amounts of fatty acids with 24 carbon atoms: 9.5 and 5% respectively; these last values are in agreement with the ones found in 16- to 24-day-old mice[49]. Cerebroside fatty acid values obtained are intermediary between those found by others[6,49].

DISCUSSION

The microsomal fraction is not contaminated; mitochondria, myelins and other subcellular fractions are not detected by electron microscopy or by marker enzymes. Moreover, the specific activity of 2′,3′-cyclic phosphate diesterase, an enzyme located in myelin[52], is 58 times less in microsomes than in myelin[4]. However, there is a low diesterase activity in our microsomes, but caution has been suggested in using this enzyme as a quantitative marker for myelin, because there is no quantitative relationship between diesterase activity and cerebroside content[19]. It is noteworthy that the specific activity of 5′-nucleotidase is the same in microsomes and homogenate; in the 17,500 × *g* pellet, which is rich in plasma membrane, it is slightly higher; 5′-nucleotidase may not be specific to plasma membrane because it is present in microsomes. Some authors have proposed that this enzyme resides almost exclusively in the plasma membrane of cells[21,22,62]; therefore the enzyme has been used as a plasma membrane marker enzyme[7,8]. However 5′-nucleotidase has been found in rat liver microsomes[62].

Phospholipids and cholesterol are present in all subcellular fractions. Galactolipids are principally found in myelin[45,53], but because they have been detected in microsomes it has been postulated that microsomal cerebrosides are only built with α-hydroxylated fatty acids[37]. However, we have found non-hydroxylated fatty acids in appreciable quantity, as have others[6]. Only for galactolipids is it possible to measure a quantitative variation with age, starting from a small initial quantity. The transitory increase of microsomal galactolipids corresponds to a transitory accumulation of lipids which will be found in myelin. The myelin period completed, microsomes recover a normal amount of galactolipids. Microsomal galactolipids are present in maximal quantity when myelination is most active.

Fatty acid analysis shows an identical evolution for the fatty acids with 24 carbon atoms: between 12 and 21 days, there is a peak in the general profile of the evolutionary line during maturation. This peak is maximal at 15 days; it corresponds to the transitory presence of fatty acids with 24 carbon atoms in brain microsomes. We have shown[33] that $\Sigma\ C_{24}$ from total brain lipid suddenly increases at the 15th day; this increase ends at 21 days. The evolution is similar for C_{24} isolated from cerebrosides. All these effects are shown in Fig. 6. The important decrease of C_{14} with age is to be noted; $\Sigma\ C_{16}$ and $\Sigma\ C_{18}$ have opposite variations which may indicate a precursor–product relation.

Thus, brain microsomes are the site of synthesis of myelin constituents. Either they are in continuity with the plasma membrane which winds around the axon to build myelin, or they consist of protomembranes which have been detached from microsomes and transported to the appropriate site. Or, simply, they make complex lipoproteins which carry lipids from microsomes to their myelin site[31,36,54].

Though most membranes have different compositions[38,50,61], morphological data[12] indicate that the myelin sheath is derived from oligodendroglia in the central nervous system; its plasma membrane is sedimented, at least partially, in microsomes; and it has been suggested that lipids from the microsomal fraction, which would include the glial plasma membrane, are used in the formation of myelin[17]. Moreover,

there is a rapid turnover of microsomal sulphatides from intraperitoneally injected ^{35}S, and a delayed appearance of labelled myelin where the turnover is much longer; this can be explained if the myelin sheath is derived from glial cell membrane sedimenting with microsomes[18].

Additional evidence for the implication of the satellite cell plasma membrane itself in galactolipid biosynthesis comes from the myelin-deficient mutant in which there is a deficiency of cerebrosides preventing normal myelination[5,34,48]. A study of mutant microsomal lipids is in progress[49]. There is a slight increase in lipid content of the microsomal fraction during the 15–20 days post-partum in rat; this may be related to the active lipid synthesis that occurs within the central nervous system at this time of myelination[16] and not to an accumulation of lipids in the brain immediately before the onset of myelination, as has been suggested[65].

A finer analysis[37] shows that microsomes of white matter resemble both myelin and gray matter microsomes in composition. This is consistent with the concept that microsomes may contain two types of lipid: the first is characteristic of reticulum, a membrane synthesizing subcellular fractions (which is similar in microsomes from grey and white matters); and the second may be characteristic of recently synthesized protomembranes which have to be detached from the microsomes and transported to the appropriate myelin sites. Lamellar structures not connected to the myelin sheath have been discovered; these structures are occasionally seen within the Schwann cell in the peripheral nervous system; their presence probably reflects the rapid rate of membrane synthesis by Schwann cells during myelination because they are never seen in mature nerves[43].

In any case, galactolipids which would become myelin constituents already have very long chain fatty acids within microsomes; these are the only ones that increase transitorily during myelination.

Table III shows that the precursors of myelin, oligodendrocytes, have a molar ratio nearer to myelin than to the 'myelin-like' fraction. The meaning of the 'myelin-like' fraction is hard to define, because microsomes already contain myelin lipids built up with very long chain fatty acids. The 'myelin-like' fraction contains a small quantity of cerebrosides composed of medium-chain fatty acids. However, this fraction has been compared to the loose myelin which is seen by electron microscopy in young animals[1,2], and has been proposed to be an intermediate between glial cells and compact myelin[2]. But it is possible that a submicrosomal fraction has similar properties to that of the 'myelin-like' fraction, so this fraction may be a sub-microsomal contaminant present in high proportion in the developing brain[2] but not degraded myelin as suggested[23]. Enzymic studies show that the 'myelin-like' fraction is not typically microsomal[2], but microsomes are a highly heterogeneous mixture of membranes[25,56]. This work shows that lipids characteristic of myelin are present in microsomes with their very long chain fatty acids; these lipids will be found in myelin. So a premyelin intermediate built up with medium-chain fatty acids seems to be excluded. Besides lipid-synthesizing enzymes[11,13,35], long-chain fatty acid-synthesizing enzymes are found in microsomes[9].

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