THE FATTY ACIDS OF MYELIN PHOSPHOLIPIDS

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1. Introduction

The myelin sheath has been widely studied with regard to its morphology, biochemistry and ultrastructure [1–5].

Electron microscopic examination and X-ray diffraction studies have demonstrated that the myelin sheath has a trilaminated structure, chemically constitued by a lipid bilayer containing regular files of hydrocarbons chains, perpendicular to the phosphatidic groups and proteins. The myelin therefore is in many respects similar to other biological membranes (unit membrane).

Analysis of its physico-chemical characteristics is of interest not only for the study of the specific functions of the myelin, but also for the problems concerning biological membrane functions.

In previous research it was observed [6–8] that lipids constitute more than 80% dry weight of the myelin sheath, and that the phospholipids represent the prevalent fraction of structural lipids. We also studied both the type and distribution of fatty acids in lipid fractions of the myelin [9].

The phospholipids fractions seem to play an important role both in the electron transport and in the oxidative phosphorilation processes [10].

On the other hand, the biological features of phospholipids are closely correlated with the type of fatty acids present in their structure. In fact, the number of double bonds in fatty acids seems to be in close relationship with the biochemical reactivity and the membrane processes [11].

Thus we considered of interest to analyze systematically the type and distribution of fatty acids in the single phospholipid fractions of the myelin sheath.

2. Methods

The corpus callosum and the centrum semiovale of healthy adult Bos taurus was used, taken at 0–4°.

Purification of myelin was carried out by the method of Autilio et al. [12]. All centrifugation steps were done in a Phywe model U 50 L preparative ultracentrifuge.

Lipids were extracted and purified [13], then fractionated by means of TLC using a basic solvent [14]. Isolated phospholipids were visualized by means of iodine vapour and/or specific reactions; the plates were sprayed with 50% sulfuric acid containing methyl orange (5 mg%), and subsequently heated to 160° as previously described [15]. For the quantitative evaluation of the chromatograph, the densitometric procedure was used [16], employing a Joyce densitometer, and/or the method for phosphorus determination [17]. To examine fatty acid composition, phospholipid separation was performed as described above, adding hydrochinon (1 mg/100 ml). The spots were visualized with 2.7 dichlorofluorescein (0.2% in ethanol) under UV illumination, then scraped and eluted with chloroform-methanol (2:1, v/v). The extract was saponified [18] at 65° for 3 hr under N_2 reflux (18 hr for sphingomyelin); fatty acids were recovered and esterified [19].

Gas chromatography was carried out on a Fractovap model C Gas Chromatograph (Carlo Erba), as previously reported [20]. Peaks of methyl esters were identified by plotting the logarithm of the retention times versus chain length [21] and by comparison of retention times with known standards (Applied Science Laboratories). The areas of the peaks of the chromatograms were gauged by triangulation.

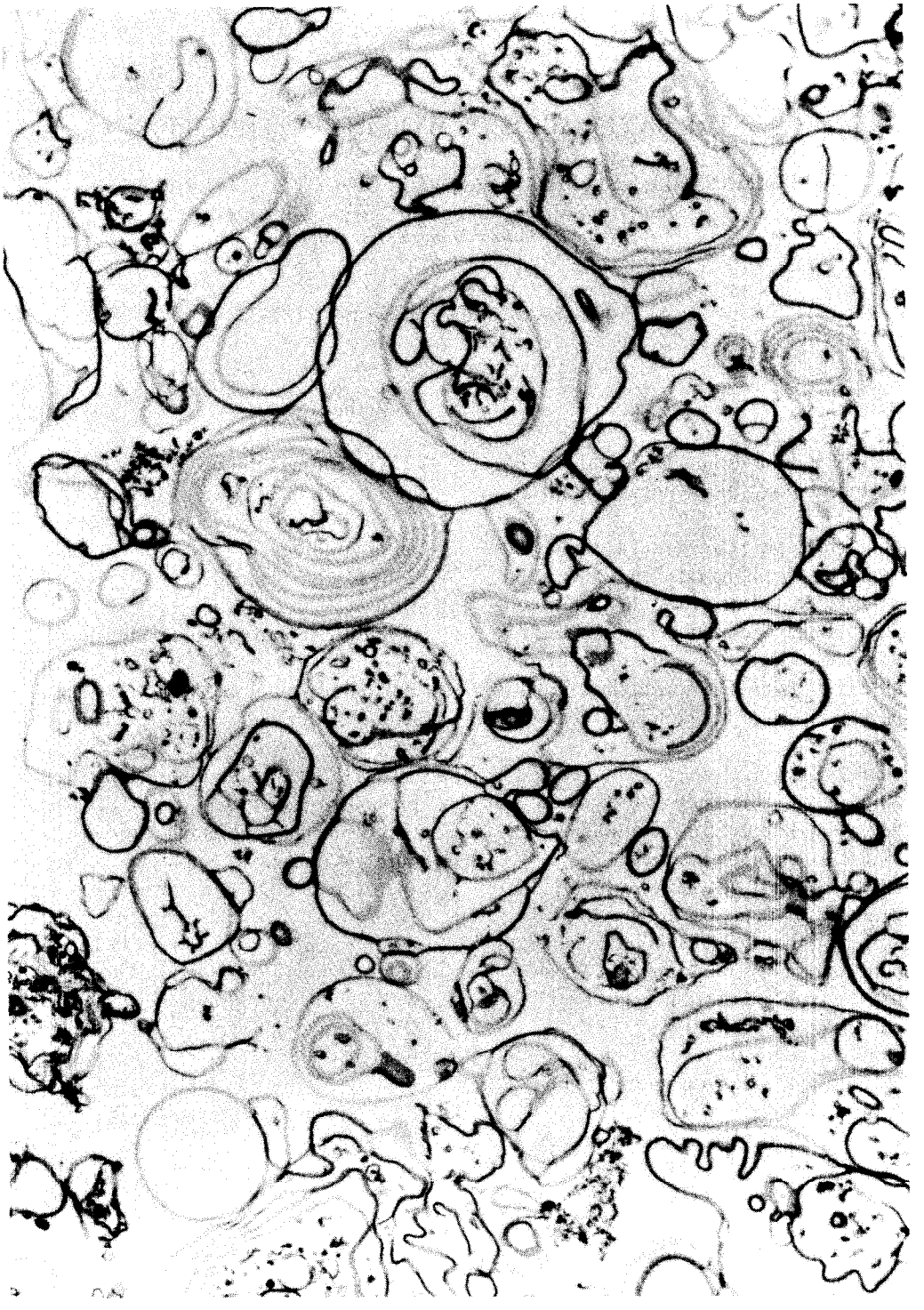

Fig. 1. Electron micrograph of myelin. Fiss. OsO_4 2% × 10,500.

3. Results

We obtained myelin highly pure as demonstrated by electron microscopy: condensed, concentric membranes having a typical trilaminated structure; absence of ergastoplasm, ribosomes and residual mitochondria (fig. 1). The myelin purity was also tested by the solubility in chloroform-methanol (2:1) [22].

Table 1 shows the percentage content in phosphorus of the various phospholipid fractions isolated from the purified myelin. The most conspicuous phospholipid fraction was phosphatidylethanolamine, which represents about 34% of the total phospholipids, then

Table 1
Phospholipids in myelin.

	% Total lipid phosphorus	
	Exp. 1	Exp. 2
Phosphatidylethanolamine	34.3	33.4
Phosphatidylcholine	23.4	22.6
Sphingomyelin	17.2	16.2
Phosphatidylserine	15.5	16.4
Phosphatidylinositol	4.7	5.3
Phosphatidic acids	4.6	5.9

Table 2
Fatty acid composition of individual phospholipids in myelin.

Fatty acids	Phosphatidyl-ethanolamine	Phosphatidyl-choline	Sphingo-myelin	Phosphati-dylserine	Phosphatidyl-inositol	Phosphatidic acids
12:0	–*	–	–	–	–	–
14:0	1.6	1.7	1.1	1.0	–	–
15:0	–		1.4	1.2	–	
16:0	16.1	40.7	9.7	17.1	15.2	6.1
16:1	2.6	3.2	2.3	1.4	2.6	1.0
17:0	1.4	1.3	2.4	1.1	2.0	–
18:0 iso	3.8	1.2	1.1	1.2	2.0	–
18:0	13.5	16.9	24.3	18.2	16.8	35.6
18:1	42.7	28.1	27.5	45.9	27.9	40.2
18:2	1.7	1.0		1.3	3.0	2.3
20:0		–	4.4		4.9	–
20:1	2.2	–	3.7	1.7	6.1	–
20:4	3.8	1.1	5.3	3.1	7.3	9.1
22:4	1.5	–	2.0	1.2	1.6	
22:6	6.2		2.3	2.3	4.2	1.7
23:0	1.0	–	1.4	2.3	5.3	–
24:1		–	10.2	–	–	
Saturated	37.4	61.8	45.8	42.1	46.2	41.7
Monounsaturated	47.5	31.3	43.7	49.0	36.6	41.2
Polyunsaturated	13.2	2.1	9.6	7.9	16.1	13.1
$\frac{\text{Saturated}}{\text{Unsaturated}}$	0.61	1.85	0.85	0.73	0.87	0.75

* The values are expressed as a percentage of the total fatty acids, and reported only when major than 1%.

phosphatidylcholine, sphingomyelin and phosphatidylserine. Phosphatidylinositol and phosphatidic acids were present in smaller quantities.

Table 2 presents the fatty acid composition of the various phospholipids of myelin sheaths.

The saturated/unsaturated fatty acid ratio demonstrates that phosphatidylcholine is characterized by a high content of saturated fatty acids (1.85), while, in the other fractions, the percentage values varied from 0.61 (phosphatidylethanolamine) to 0.87 (phosphatidylinositol).

4. Discussion

Our results concerning the qualitative and quantitative phospholipid composition of myelin sheath agree with those of Mandel and Nussbaum [23], Norton and Autilio [24], Soto et al. [25]; in particular our data confirm that a higher percentage of phosphatidylethanolamine (lipid of low turnover) [26] is present than phosphatidylcholine, as also observed by Gerstl et al. [27], Horrocks [28], and O'Brien et al. [29]. An analogous finding was not observed in the other subcellular fractions [20, 30–31].

The qualitative composition in fatty acids is similar for all phospholipid fractions of the myelin, except for the sphingomyelin in which $C_{24:1}$ was also present.

However, the percent content of single fatty acids is different and typical for each phospholipid fraction. The myelin phospholipids, unlike those of the other biological membranes [32–33], contain a high percentage of saturated and monounsaturated fatty acids (palmitic, stearic, and oleic acid), while polyunsaturated acids are present in lower quantities.

The cohesion of lipid molecules is stronger between the chains of saturated fatty acids than in those containing polyunsaturated fatty acids. Thus myelin sheath may be considered as a stable biological membrane [11, 34].

A comparison of our results with those of O'Brien et al. [29] concerning the posterior spinal roots of Bos taurus demonstrates that notable differences cannot be observed in the fatty acids content of myelin phosphophilipid fractions in the CNS and in PNS, although myelin sheaths have, in the two systems, a different origin and a dissimilar content of single phospholipid fractions.

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