The Distribution of Molecular Species of Phosphatidylinositol in Ox Brain and its Subcellular Fractions

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1. The phosphatidylinositol content of white and grey matter of ox cerebral hemispheres did not differ. The phosphatidylinositol from grey matter was slightly enriched in palmitic acid and arachidonic acid, and that from white matter was enriched in eicosatrienoic ($C_{20:3}$) acid. These regional differences were apparently due to the greater content of myelin in the white matter, since the same tendencies were observed when combined myelinic and non-myelinic subcellular fractions prepared from the cerebral hemispheres were compared. 2. Purified phosphatidylinositol was converted into its triacetylated methylated derivative and resolved to its molecular species by t.l.c. on $AgNO_3$ -impregnated silica gel. The tetraenoic molecular species was predominant in phosphatidylinositol from ox cerebral hemispheres, and this feature characterized all the phosphatidylinositol samples extracted from its regions or subcellular fractions. The grey matter was more enriched in the tetraenoic species and the white matter in the trienoic species. 3. The molecular-species composition of phosphatidylinositol from the subcellular fractions of ox cerebral hemispheres was studied. The trienoic species constituted nearly one-fifth of the phosphatidylinositol from two myelinic fractions. 'Large myelin' was more enriched in this species than was 'small myelin'. Both fractions also contained greater concentrations of the dienoic species than the non-myelinic subcellular fractions. The latter fractions, one containing nuclei and the other nerve endings plus mitochondria, were enriched in the monoenoic and tetraenoic species of phosphatidylinositol. The post-mitochondrial supernatant exhibited a pattern of distribution of phosphatidylinositol species intermediate between the myelinic and non-myelinic fractions.

It is known that a given biological membrane possesses a relatively constant and specific phospholipid class composition (Rouser *et al.*, 1968; Korn, 1969). In some mammalian tissues, such as brain and lung, the fatty acid composition of phospholipid classes from a given membrane is also constant and specific (veer Kamp *et al.*, 1962). However, dietary alterations may influence this composition in other tissues such as liver and kidney (van Golde *et al.*, 1968). Micro-organisms are more susceptible in this respect to the influences of nutrient supply (van Golde & van Deenen, 1966), temperature of growth (Marr & Ingraham, 1962) or pH of the medium (Houtsmuller & van Deenen, 1965).

Examination of the fatty acids of a phospholipid class yields limited information on their pattern of combination in the individual molecules of that class. Analysis of molecular species, however, sheds more light on the specificities of phospholipid class composition. Many techniques have been developed for this purpose involving t.l.c. on $AgNO_3$ -impregnated silica gel. An intact phospholipid class

(Arvidson, 1967) or one of many non-polar derivatives of that class (Renkonen, 1965, 1968; O'Brien & Klopfenstein, 1971) may be used.

The application of these techniques to study the distribution of molecular species of phosphatidylcholine in the brain of many animals yielded some interesting observations. Although much of the dipalmitoyl species was present in the brain, by far the most predominant was the 1-palmitoyl-2-oleoyl phosphatidylcholine (Montfoort *et al.*, 1971). On subcellular fractionation of the brain, O'Brien & Geison (1971) demonstrated that the dipalmitoyl phosphatidylcholine was predominant in the nerve-ending fraction, whereas the 1-palmitoyl-2-oleoyl phosphatidylcholine was predominant in the myelin fraction. The 1-stearoyl-2-arachidonoyl phosphatidylcholine, although a minor species in the whole brain, was abundant in the mitochondrial fraction. Clearly, then, in organs where the composition of the membrane is resistant to change, one can distinguish a membrane-specific pattern of phospholipid molecular species.

We have developed in this laboratory methods for the analysis of molecular species of phosphatidylinositol that preserve both the polar and non-polar parts (Luthra & Sheltawy, 1972*b*). This allows concurrent studies of both the metabolism and the mass distribution of molecular species to be carried out. In the present paper we describe the application of these methods to the study of the distribution of phosphatidylinositol species in different regions and subcellular fractions of bovine cerebral hemispheres.

Holub *et al.* (1970) have described the composition of molecular species of phosphatidylinositol, di- and tri-phosphoinositide from whole bovine brain. Our results on phosphatidylinositol, although obtained by different techniques, confirm and further extend their findings.

Experimental

Collection of tissue

Bovine cerebral hemispheres were collected from the local slaughterhouse, usually within 5 min of the death of the animal. They were transported to the laboratory under ice within 30 min of their collection. Dissection of the grey and the white matter was begun immediately and usually lasted 15–30 min. Care was taken to avoid all areas where the demarcation of the two layers was indistinct.

Subcellular fractionation

The procedure used for the subcellular fractionation was similar to that used by Eichberg *et al.* (1964) for guinea-pig brain, modified for a large-scale preparation. The exact conditions were as follows. All steps were carried out at 4°C. A homogenate [10% (w/v) in 0.32M-sucrose] was prepared from 40–70 g of tissue. Six complete up-and-down strokes were applied in a homogenizer made of Viridia glass tubing and a Perspex pestle with an all-round clearance of 0.25 mm. The pestle was rotated at 900 rev./min. The crude nuclear fraction (P_1) was sedimented at 1000g for 11 min in an MSE model 18 centrifuge (8 × 50 ml rotor). In some experiments the pellet was washed by re-sedimentation from the same volume of 0.32M-sucrose under the same conditions. The supernatant from fraction P_1 was centrifuged at 17 500g for 2 h in an MSE model 18 centrifuge (either 6 × 100 ml or 8 × 50 ml rotor) giving a pellet (P_2). The supernatant from P_2 (P_2S , post-mitochondrial supernatant) was not fractionated further.

The crude pellets (P_1 and P_2) described above were further fractionated separately on discontinuous sucrose density gradients. These were formed either by layering 0.8M-sucrose over 1.2M-sucrose (12 ml each/tube; type 1) or by simply pipetting 24 ml of 0.8M-sucrose/tube (type 2). The pellets were re-

suspended in 0.32M-sucrose so that 1 ml of the suspension was equivalent to 0.66 g of original tissue. A portion of the suspension (5–7 ml/tube) was layered on the gradient and centrifuged at 53 000g for 2 h in a Spinco (model L1 or L2) centrifuge (SW 27 rotor). Bands located between 0.32M- and 0.8M-sucrose were found on examination by electron microscopy to be equivalent to the 'large myelin' (P_1A) and 'small myelin' (P_2A) fractions described by Eichberg *et al.* (1964). The intermediate layers from the type 1 gradients were found on examination by electron microscopy to be composed mainly of nuclei with little contamination by myelin and mitochondria (P_1B) or pinched-off nerve endings (P_2B). The pellets at the bottom of the gradients were found to be either cell debris (P_1C) or mitochondria (P_2C). In type 2 gradients the single pellets formed were designated P_1BC or P_2BC .

Osmotic shock of the myelin fractions

This was carried out by a modification of the method of Jungalwala & Dawson (1971). The 'large and small myelin' fractions (P_1A and P_2A respectively) in approx. 0.5M-sucrose were each diluted with 4 vol. of water and centrifuged at 36 000g for 1 h in an MSE model 18 centrifuge (6 × 100 ml rotor). This process was repeated twice and the final pellets were resuspended in 0.32M-sucrose so that 1 ml of the suspension was equivalent to 0.66 g of original tissue. The suspensions (5–7 ml/tube) were layered on 0.8M-sucrose (type 2 gradient) as described above and centrifuged in the same manner. The bands at the interface were collected as the osmotically shocked myelin fractions.

Extraction and purification of phosphatidylinositol

Phospholipids were extracted from the subcellular fractions by the method of Eichberg *et al.* (1964) as follows. Each fraction was diluted with 4 vol. of methanol, and a few drops of 0.05M-MgCl₂ were added to flocculate the proteins. The suspension was centrifuged at 1000g for 15 min and the supernatant combined with subsequent lipid extracts. The residue was extracted twice with 4 vol. of chloroform-methanol (2:1, v/v) and the extracts were combined and purified in preparation for chromatography of the phospholipids on a column of alumina as described by Luthra & Sheltawy (1972*a*). After elution of the choline- and ethanolamine-containing phospholipids, a crude phosphatidylinositol fraction was eluted with the solvent system chloroform-ethanol-0.7M-NH₄NO₃ (18:25:7, by vol.). The phosphatidylinositol in this fraction was purified from cardiolipin by preparative t.l.c. on silica gel H in 'S-Chambers' maintained at 4°C (Sheltawy & Dawson, 1969*c*). The solvent

system used was chloroform-methanol-acetic acid-water (65:24:5:1, by vol).

Fractionation of phosphatidylinositol into molecular species

This was carried out by using method II of Luthra & Sheltawy (1972b). The triacetylated methylated phosphatidylinositol was prepared and purified as described by these authors. However, it was frequently observed that a few minor impurities migrating near the solvent front on t.l.c. on AgNO₃-impregnated silica gel obscured the separation of the disaturated and the monounsaturated species. This difficulty was overcome by using the following developing solvents in the order described: (1) acetone-distilled chloroform (1:9, v/v) was used to resolve the above-mentioned species (R_F 0.69 for the disaturated; R_F 0.64 for the monoenoic) clear of the impurities; (2) acetone-distilled chloroform (1:3, v/v) was used to resolve the dienoic and trienoic species; (3) acetone-distilled chloroform (1:1, v/v) was used to resolve the tetraenoic and pentaenoic species. Results were finally expressed as mol/100 mol of the recovered molecular species.

Since the tetraenoic species was found to predominate in brain phosphatidylinositol samples it readily masked the pentaenoic species. Our values for the latter species are therefore likely to be overestimates, and vice versa for the former. We have calculated, on the basis of g.l.c. analysis of the fatty acids (Luthra & Sheltawy, 1972b), that in the brain as much as 30-40% of the pentaenoic value could be due to the tetraenoic species.

Gas-liquid chromatography

The g.l.c. analysis of fatty acid composition was carried out as described previously (Luthra & Sheltawy, 1972b), with one modification. The stationary phase was changed to diethylene glycol succinate [15% (w/w) of the support material] and the fatty acids were identified by their carbon numbers (Hofstetler *et al.*, 1965). The peaks were measured quantitatively by using an electronic integrator (Varian model 477).

Results

Experimental approach

Phosphatidylinositol is a minor component of membrane phospholipids, and to study the subcellular distribution of its molecular species in the brain two compromises were necessary.

The first related to the selection of bovine cerebral

hemispheres as the source of relatively large amounts (40-70 g) of experimental material. Part of the triphosphoinositide of the brain hydrolyses rapidly after death, and although the material was kept under ice during transport (30 min) the process would have most likely been complete on arrival at the laboratory (Eichberg & Dawson, 1965; Sheltawy & Dawson, 1969a). Any attempt to check this process by adding EDTA to the homogenization medium (Sheltawy & Dawson, 1969b) may have therefore been ineffective at this stage.

Phosphatidylinositol is a product of this hydrolysis *post mortem* through the action of triphosphoinositide phosphomonoesterase. It is therefore necessary to determine the extent to which the produced phosphatidylinositol contributes to the recovered pool of this phospholipid. By necessity the following experiment on hydrolysis *post mortem* was carried out on rats (average body wt. 150 g) and the results of which were assumed to apply to the situation in ox brain (for the analytical methods used see Sheltawy & Dawson, 1969a). The cerebral hemispheres were removed 2 min (control group, four rats) and 25 min (experimental group, six rats) after death by decapitation. As much as 50% of triphosphoinositide P was lost *post mortem* in the period allowed (23 min). The phosphatidylinositol produced can be entirely accounted for by the rise in the experimental group phosphatidylinositol P (an increase of 11 μ g of P) over the control values (45 μ g of phosphatidylinositol P/g fresh wt.). The phosphatidylinositol produced may contribute therefore a maximum of 25% of the recovered phospholipid. Experiments by Hauser *et al.* (1971) have indicated that this rise was minimal in structures enriched with myelin.

The second compromise, equally inevitable, was to combine some of the subcellular fractions to increase the yield of workable material. This was carried out through the use of a simplified density gradient (see the Experimental section for details).

Composition of phosphatidylinositol species from grey and white matter

Grey and white matter obtained in the present work exhibited a pattern of total phospholipid distribution (Table 1) that agreed with previous observations on the regional distribution of lipids in the brain (see Bass & Hess, 1969). However, the total phospholipid content was slightly higher (5%) in the white matter and lower (20%) in the grey matter than observed previously (Davison & Wajda, 1962). This difference was perhaps due to the difficulty of defining exact regions of the two anatomical structures. No difference between the phosphatidylinositol content of grey and white matter was apparent, although, expectedly, the total phospholipids of the grey matter

Table 1. *Distribution of total phospholipids, phosphatidylinositol and phosphatidylinositol fatty acids in ox cerebral hemispheres*

The shorthand designation of fatty acids indicates their chain length (first number), and their degree of unsaturation (second number). The abbreviations of the subcellular fractions are described in the Experimental section.

		Cerebral hemispheres	Grey matter	White matter	Subcellular fractions			
					Non-myelinic		Myelinic	
Total phospholipids (μ g of P/g fresh wt.)	...	2120	1090	3120	P ₁ BC 360 P ₂ BC 190 P ₂ S 103	P ₁ A 440 P ₂ A 410		
Phosphatidylinositol (μ g of P/g fresh wt.)	...	38	38	39	16	6.1		
(% of total phospholipid P)	...	1.8	3.5	1.3	3.5	1.5		
Fatty acid distribution in phosphatidylinositol (mol/100mol)								
Fatty acids C _{16:0}		10.1	12.6	8.8	12.5	10.8		
C _{16:1}		Trace	Trace	Trace	1.6	0.5		
C _{17:0}		1.3	Trace	0.7	Trace	0.7		
C _{18:0}		38.6	41.5	39.4	34.5	38.1		
C _{18:1}		13.3	10.3	12.8	16.1	13.6		
C _{18:2}		Trace	Trace	Trace	1.1	1.1		
C _{18:3}		Trace	Trace	Trace	1.0	1.6		
C _{20:3}		0.6	Trace	4.8	0.7	3.1		
C _{20:3} *		Trace	Trace	2.6	0.7	2.3		
C _{20:4}		36.0	35.6	31.6	31.4	27.4		
C _{20:4} *		Trace	Trace	Trace	Trace	Trace		

* These acids were each isolated in two isomeric forms (see the text).

were proportionally more enriched in these phospholipids than those of the white (3.5 and 1.25% respectively; Table 1).

Analysis of the fatty acid composition of phosphatidylinositol from grey and white matter revealed only minor differences. The phosphatidylinositol from grey matter was slightly enriched in palmitic acid (C_{16:0}) and in arachidonic acid (C_{20:4}) (Table 1), whereas the phosphatidylinositol from the white matter was enriched in eicosatrienoic acid (C_{20:3}) (Table 1). In agreement with the findings of other workers (Svennerholm, 1968; Holub *et al.*, 1970), the latter acid was always recovered from brain material (see below) in two isomeric forms, which on g.l.c. analysis gave carbon numbers 21.7 and 22.2 (Hofstetter *et al.*, 1965). Similarly arachidonic (C_{20:4}) acid was recovered in two isomeric forms (carbon numbers 22.4 and 22.9; Hofstetter *et al.*, 1965). No further identification of these forms was carried out in the present work.

Table 2 shows the distribution of the molecular species of phosphatidylinositol in grey and white matter. Phosphatidylinositol from both regions was characterized by an enrichment of the tetraenoic species, the proportion being greater in grey than in white matter. Conversely, phosphatidylinositol from

white matter contained more of the trienoic species than that from the grey matter. A less-pronounced enrichment in the dienoic species in white matter was also observed (Table 2).

Composition of phosphatidylinositol species from subcellular fractions of ox cerebral hemispheres

Progressive losses of phospholipid P were observed during the subcellular-fraction procedure. For example, in a typical experiment recoveries from the first stage of differential centrifugation amounted to 88% of the value for the whole homogenate. On further fractionation on discontinuous sucrose density gradients the recovery amounted to 80% of the phospholipid P of the whole homogenate. After purification of the secondary fractions by washing or osmotic shock (see the Experimental section) only 40% of the whole-homogenate phospholipid P remained (Table 3). To a small extent these incomplete recoveries may be due to inevitable losses during the experimental handling, but they are more likely to be due to the solubilization of loosely bound membrane lipoproteins (maximum loss occurred during the osmotic shock). Significantly, when the recovery of phosphatidylinositol (% of total phospholipid P) was

Table 2. *Composition of phosphatidylinositol molecular species from ox cerebral hemispheres*

Experimental details are given in the text.

Molecular species (mol/100mol)	Cerebral hemispheres	Grey matter	White matter	Subcellular fractions	
				Non-myelinic	Myelinic
Monoenoic†	5.9	2.3	10.1	6.5	16.4
Dienoic ($\Delta 1+1$)*	5.6	8.2	6.3	9.4	11.0
($\Delta 0+2$)*	2.0	0.7	3.6	3.6	4.8
Trienoic ($\Delta 1+2$)*	1.4	Trace	1.5	1.5	4.7
($\Delta 2+1$)*	2.8	Trace	0.9	1.3	3.5
($\Delta 0+3$)*	3.3	4.2	13.8	5.3	14.0
Tetraenoic†	45.5	63.8	52.4	52.9	36.0
Pentaenoic†	33.6	20.8	11.4	19.4	9.9

* These molecular species were identified by g.l.c. examination of their fatty acid content. Two bands of the dienoic species were resolved: one containing two monoenoic ($C_{18:1}$) acids and the other containing one dienoic ($C_{18:2}$) acid. Similarly three bands of the trienoic species were resolved. The first two contained one monoenoic ($C_{18:1}$) acid and one dienoic ($C_{18:2}$) acid, presumably in different positions on the glycerol backbone. The third contained the trienoic ($C_{18:3}$ and $C_{20:3}$) acids.

† See the text for the explanation of the high values of the pentaenoic species.

‡ These values for the monoenoic species are high, owing to impurities migrating near the solvent front (see the Experimental section).

Table 3. *Molecular-species composition of phosphatidylinositol from subcellular fractions of ox cerebral hemispheres*

The abbreviations of the subcellular fractions are described in the Experimental section. The myelinic fractions P_1A and P_2A were both purified by osmotic shock, and fraction P_1BC was washed once by sedimentation from 0.32 M-sucrose at 1000g for 11 min (see the Experimental section).

	Non-myelinic fractions			Myelinic fractions	
	P_1BC	P_2BC	P_2S	P_1A	P_2A
Total phospholipids (μg of P/g fresh wt.)	88	204	85	280	230
Phosphatidylinositol (μg of P/g fresh wt.)	3.0	6.0	1.5	3.8	2.8
(% of total phospholipid P)	3.4	2.9	1.8	1.4	1.2
Molecular species of phosphatidylinositol (mol/100mol)					
Saturated	0.9	0.8	4.0	1.8	1.0
Monoenoic	7.5	5.3	7.4	3.7	1.4
Dienoic ($\Delta 1+1$)*	2.9	1.8	5.5	13.2	6.9
($\Delta 0+2$)*	2.5	2.1	5.3	7.6	4.7
Trienoic ($\Delta 1+2$)*	2.4	1.4	3.8	1.9	3.6
($\Delta 0+3$)*	9.0	6.7	9.8	17.9	19.3
Tetraenoic*	57.6	65.7	50.5	45.0	52.8
Pentaenoic*	17.2	16.1	13.6	8.9	10.3

* See Table 2.

compared for the crude subcellular fractions (Table 1) and those purified by washing or osmotic shock (Table 3) no indication of any preferential loss of this phospholipid was observed.

The differences mentioned above between the grey

and the white matter could be due to the relative enrichment of myelin in the latter. Consequently the myelinic fraction (P_1A) and the combined non-myelinic ones (P_1B , P_1C , P_2B and P_2C) were compared. The difference in total phospholipid content

supported this hypothesis, although it was less in magnitude than that between the grey and the white matter (Table 1). There were, however, clear differences between the phosphatidylinositol content of the myelinic and non-myelinic fractions (Table 1), both when expressed as μg of P/g fresh wt. of tissue and as % of total phospholipids. This is in agreement with the findings of Eichberg *et al.* (1964), in spite of methodological differences in the analysis of phospholipids. As mentioned above, the grey and the white matter showed differences only when the phosphatidylinositol concentration was expressed as % of total phospholipids. Analysis of the fatty acid and molecular-species composition (Tables 1 and 2) confirmed the findings from grey and white matter. It is thus apparent that myelin is a membrane distinct in the composition of its phosphatidylinositol from other membranes in the cerebral hemispheres, and that this is reflected in the differences observed between grey and white matter.

These findings were extended by studying the composition of the molecular species of phosphatidylinositol from separate subcellular fractions (Table 3) isolated on a type 2 gradient and purified either by washing or osmotic shock. Confirming the observations described in Table 2, all the subcellular fractions exhibited a typical 'brain pattern' for the distribution of phosphatidylinositol molecular species. The disaturated phosphatidylinositol occurred in small concentrations and the tetraenoic species was predominant (Table 3). However, clear differences were observed. In the phosphatidylinositol from the two myelinic fractions (P_1A and P_2A), the trienoic species constituted nearly one-fifth of the total species. Moreover, the distribution of the dienoic species showed a clear enrichment in the two myelinic fractions over the non-myelinic ones (P_1BC and P_2BC). Interestingly, the 'large myelin' (P_1A) contained more of this species than the 'small myelin' (P_2A). The two non-myelinic fractions exhibited a greater enrichment in both the monoenoic and tetraenoic species.

The P_2S fraction (containing microsomes) showed a pattern of distribution of phosphatidylinositol molecular species intermediate between the myelinic and non-myelinic ones.

Discussion

Tissue specificity in the distribution of phosphatidylinositol molecular species

Our results indicate that all phosphatidylinositol samples derived from ox cerebral hemispheres or from its structures possess a typical 'brain pattern' in the distribution of their molecular species. They were greatly enriched in the tetraenoic species with only

traces of the disaturated species. These results are in agreement with a similar study on phosphatidylinositol from whole ox brain (Holub *et al.*, 1970) and with observations in this laboratory on phosphatidylinositol from guinea-pig brain (M. G. Luthra & A. Sheltawy, unpublished work). In contrast to brain, the molecular-species composition of phosphatidylinositol from the liver of lamb (Luthra & Sheltawy, 1972*b*) differs from that of rat (Akino & Shimojo, 1970; Holub & Kuksis, 1971). Montfoort *et al.* (1971) have demonstrated a similar tissue specificity in the distribution of phosphatidylcholine molecular species from brain and lung, but not in that from liver and kidney.

Presumably this typical 'brain pattern' is dictated by the specificity of the biosynthetic process (Paulus & Kennedy, 1960), which in brain is located in the endoplasmic reticulum (Carter & Kennedy, 1966). There is evidence that phosphatidylinositol, like other phospholipids, is then exported from this site to other membranes (Miller & Dawson, 1972*a,b*) through an exchange process similar to that described for the liver (Wirtz & Zilversmit, 1968; McMurray & Dawson, 1969). It is of note that the molecular-species composition of phosphatidylinositol from the P_2S fraction (containing microsomes) is intermediate to that in the other subcellular fractions (Table 3), as has been observed for the molecular species of phosphatidylcholine in rat brain (O'Brien & Geison, 1971).

Membrane specificity in the distribution of phosphatidylinositol molecular species

Within the limitations of the above-mentioned 'tissue pattern', phosphatidylinositol samples from different subcellular fractions were observed to possess different molecular-species distribution (Table 3). In this respect, these results are similar to those of O'Brien & Geison (1971) for the subcellular distribution of phosphatidylcholine in rat brain (see the introduction). Even the 'large myelin' and the 'small myelin' fractions differed in the distribution of phosphatidylinositol molecular species (Table 3). These two fractions also differ in other aspects of their lipid composition (Eichberg *et al.*, 1964) and lipid metabolism (Ansell & Spanner, 1968).

Two possible explanations for the different molecular-species composition of phospholipids in cellular membranes may be provided. First, local modulations of the species of a phospholipid class may be catalysed through acyl-CoA-phospholipid acyltransferases of the type described by many authors (Hill & Lands, 1968; Waku & Lands, 1968; Webster, 1962, 1965; Keenan & Hokin, 1962, 1964). Although these enzymes are mostly of microsomal localization, some reports indicate their existence in other membranes (Turkki & Glenn, 1968). This possibility can only be finally confirmed when a com-

prehensive study of the turnover of phosphatidylinositol molecular species in the brain is carried out.

Secondly, these differences between the subcellular fractions may occur through specific interactions of the exported phospholipids with the target membrane proteins. Of all the membranes of the nervous system, the myelin proteins are most studied. Three main proteins have been recognized in myelin from the central nervous system: the proteolipid protein (Folch & Lees, 1951), the basic 'encephalitogenic' protein (Eng *et al.*, 1968) and the acidic proteolipid protein (Wolfgram, 1966). It is conceivable that one of these proteins, and in particular the first two, may provide sufficient specificity towards hydrophobic interactions to account for the specific distribution of molecular species of phospholipid classes in myelin.

Significance of membrane specificity in the distribution of phospholipid molecular species

The significance of membrane specificity in molecular-species distribution should be considered in view of their physicochemical characteristics. Early work on the packing of different phospholipid molecules at the air/water interface indicated that the more saturated are the fatty acid constituents, the more 'condensed' are the monolayers formed (de Haas *et al.*, 1962). These phospholipid monolayers can also accommodate cholesterol (Shah & Schulman, 1967) in a manner dependent on the nature of their fatty acids. Optimum 'condensation' occurs with phospholipids that contain monoenoic and dienoic fatty acids (Demel *et al.*, 1967), providing maximum hydrophobic interaction (Chapman & Penkett, 1966). The configuration of saturated phospholipids or those containing polyenoic fatty acids does not allow optimum interaction with cholesterol, and an increase in 'fluidity' is observed (Ladbrooke *et al.*, 1968; Joes,

1970). Significantly, systems (with or without cholesterol) that produce 'condensed' monolayers at the air/water interface can also form bilayer structures that are less permeable to glycerol (Haest *et al.*, 1969; de Gier *et al.*, 1969) or Na^+ (Moore *et al.*, 1969) than less-condensed ones.

van Deenen (1965) first advanced the concept that a characteristic optimum 'fluidity' is always maintained in a given biological membrane despite alterations in environmental conditions. Thus yeast cells grown anaerobically compensate for the absence of unsaturation in their membrane lipids by the increased incorporation of short-chain fatty acids. The resulting phospholipid mixture has physicochemical properties almost indistinguishable from the original one (Meyer & Bloch, 1963). Other instances from micro-organisms have been studied (Marr & Ingraham, 1962; Haest *et al.*, 1969), and this concept has been further borne out by studies on mammalian systems (Moore *et al.*, 1969).

It therefore may not be a coincidence that in myelin, a highly compact membranous system, both cholesterol and the monoenoic species of phosphatidylcholine occur in high concentrations (see above). These together form 'condensed' artificial membrane systems. The functional significance of the enrichment in myelin of the trienoic species of phosphatidylinositol (Table 3) is yet to be similarly understood in physicochemical terms.

Metabolism of polyphosphoinositides in the nervous system

Results from the present work have important implications for the metabolism of polyphosphoinositides in the nervous system. Several lines of evidence (reviewed by Sheltawy & Dawson, 1969a;

Table 4. *Molecular-species composition of myelinic phosphatidylinositol and triphosphoinositide recovered post mortem*

Molecular species (mol/100 mol)	Phosphatidylinositol from myelinic fractions (present work)		Triphosphoinositide recovered <i>post mortem</i> (Holub <i>et al.</i> , 1970)	
	P ₁ A	P ₂ A		
Saturated	1.8	1.0		Trace
Monoenoic	3.7	1.4		6.4
Dienoic ($\Delta 1+1$)*	13.2	6.9	}	11.1
($\Delta 0+2$)*	7.6	4.7		
Trienoic ($\Delta 1+2$)*	1.9	3.6	}	22.6
($\Delta 0+3$)*	17.9	19.3		
Tetraenoic*	45.0	52.8		50.3
Pentaenoic*	8.9	10.3		9.6

* See Table 2.

Sheltawy *et al.*, 1972) indicate that polyphosphoinositides, although occurring in myelin in high concentrations, are also found in non-myelinic structures. Myelin is formed by the wrapping of the Schwann cell-surface membrane around an axon (Robertson, 1959), and it may be that polyphosphoinositides are characteristic of the cell-surface membrane, whether of Schwann cell, glial or neuronal origin.

This assumption is supported by the following evidence. (1) The turnover time (1h, average for more than one metabolic pool) of triphosphoinositide remains constant throughout the development of the rat's nervous system (Sheltawy & Dawson, 1969a). This suggested that the physiological role of this phospholipid may be divorced from the process of myelin deposition. (2) Many enzymes involved in the metabolism of polyphosphoinositides show a sub-cellular distribution in the nervous system indicative of localization in the cell-surface membrane (evidence reviewed by Sheltawy *et al.*, 1972). (3) The pools of phosphatidylinositol and triphosphoinositide in myelin (an easily isolated cell-surface membrane in the nervous system) may hold a precursor-product relationship. The triphosphoinositide recovered in the brain after hydrolysis *post mortem* is almost all located in the myelin (Eichberg & Dawson, 1965). In Table 4 the molecular-species composition of this triphosphoinositide from ox brain (Holub *et al.*, 1970) is compared with that of phosphatidylinositol from myelin obtained during the present work. The two compositions are remarkably similar. It has been demonstrated (see the Results section) that a maximum of 25% of the recovered phosphatidylinositol may be contributed by the hydrolysis of triphosphoinositide *post mortem*. This cannot account for all the above-mentioned similarity, and, further, the product phosphatidylinositol is likely to be located in the non-myelinic fractions (Hauser *et al.*, 1971).

Phosphatidylinositol may therefore be visualized to exist in the cell-surface membrane as part of a complex involving the phosphorylating and the hydrolysing enzymes. These together will catalyse the characteristically high metabolic turnover of the phosphoinositides (Eichberg & Dawson, 1965; Sheltawy & Dawson, 1969a). In view of evidence (3) above, it is reasonable to conclude that none of these enzymes is likely to have any specificity towards particular molecular species of these lipids.

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