Organization and Structure in Central-Nerve Myelin

MARTIN G. RUMSBY

Department of Biology, University of York, Heslington, York YO1 5DD, U.K.

Degeneration of the myelin sheath in areas of the central nervous system is a characteristic feature of multiple sclerosis (Adams, 1977; Davison, 1978). The processes that cause this degradation of myelin during the course of the disease are not yet fully defined though the involvement of invading inflammatory cells is likely (Wisniewski, 1977). Any discussion on how the myelin sheath is attacked and broken down in situ during disease must be undertaken in the light of how the chemical constituents of the myelin membrane system are organized under normal conditions. An appreciation of the complex biosynthetic and metabolic interrelationships that exist between the myelin sheath and the myelin-synthesizing cell, and perhaps with the other cell types present in central nerve tissue, is also required.

Research over the last decade especially has provided a general picture of the properties and structural organization of lipids and proteins in natural membranes (Singer, 1974) and it is now possible to relate the structural principles established for membranes in general to individual membrane systems. This exercise will be attempted for the myelin sheath of central-nerve tissue in this review. A much more comprehensive examination of myelin structure has been presented elsewhere (Morell, 1977; Rumsby & Crang, 1977).

The compact myelin sheath of central-nerve tissue is characteristically revealed in electron micrographs as a multilamellar sequence of membrane (some 5–20 lamellae in number) that surrounds axons greater than about 1 μm in diameter (Fig. 1). The length of an individual internodal myelin sheath is related linearly to the diameter of the axon and is often 1.5–2 orders of magnitude larger. We tend not to realize how long the lengths of internodal myelin are relative to the size of the myelin-forming cell; the average internodal length for mixed fibres in central-nerve tissue is about 250 μm (Matthews & Duncan, 1971). The membrane of compact myelin is directly continuous with the plasma membrane of the oligodendrocyte, which is now considered to be the myelin-synthesizing and -sustaining cell in central-nerve tissue (Davison & Peters, 1970). There are now indications (e.g. Sabri & Davison, 1977) that neurons in the central nervous system do not participate in the synthesis of myelin components, as had once been suggested. Cytoplasm-filled processes extend from the body of each oligodendrocyte to form individual internodal myelin sheaths on closely adjacent axons (Fig. 1). In rat optic nerve each oligodendrocyte has between 30 and 50 processes (Peters & Vaughn, 1970). A single oligodendrocyte thus produces and sustains the membrane of many myelin sheaths. Norton & Poduslo (1973) have calculated that during the period of greatest membrane proliferation at myelination oligodendrocytes produce some 3 times their own weight of membrane every day. The synthetic capacity of these cells to produce the lipid and protein components of myelin is clearly prodigious. Now

Fig. 1. Perspective scale drawing of the interrelationships between the oligodendroglial cell, its processes and the myelin sheath in central-nerve tissue

A, node; B, paranodal region; C, perinodal region; D, internode.
that such cells can be isolated separately from other cell types in central-nerve tissue, they
should provide a good system in which to examine more closely how the components of
myelin are assembled (Poduslo & McKhann, 1977).

Our present understanding of how the spiral of compact myelin is formed during
myelination is scanty (Brady & Quarles, 1973). Clear chemical differences exist between
the plasma membrane of the oligodendrocyte and the compact myelin around axons
(Poduslo, 1975). A gradient of chemical composition varying in the proportions and
types of lipid and especially proteins would therefore seem to extend from the
oligodendrocyte along the processes to the compact myelin sheath. This is apparent
in some of the different subfractions of myelin that can be isolated (for review, see
Norton, 1977). How such differences are maintained in the membrane in view of the
probable mobility of lipid and protein components in the plane of the membrane system
(summarized in Rumsby & Crang, 1977) is hard to comprehend at present. It is also
noteworthy that the myelin sheath maintains an intimate apposition with the axolemma
membrane at the paranodal region where the two membranes are only some 2–3nm
apart; the gap between them appears to be bridged by intermembrane particles
(Livingstone et al., 1973), but is not sealed. In the perinodal region the myelin sheath and
axolemma are well separated by a distance of some 15nm or more.

Structural detail in the compact myelin sheath can be evaluated at two levels. On the
one hand techniques such as X-ray diffraction and electron microscopy can provide
accurate outline dimensions for the membrane system within which the lipid and
protein components of the structure must be arranged. Such techniques also give some
indication of other structural features of compact myelin, but fine detail about how the
individual lipid and protein species are arranged, or how they interact with each other,
cannot be obtained at present. This information can be gained from the application of
techniques in which myelin is probed and dissected chemically and enzymically. Further
insight can also come from a careful consideration of the properties of the individual
constituents of myelin in relation to what is now known about how lipids and proteins
are organized and associate with each other in other natural and artificial membrane
systems. For this approach a detailed knowledge of the chemistry of myelin and its
constituents is essential (Tables 1 and 2).

Table 1. Composition of compact myelin from a number of animal species compared with
the plasma membrane of oligodendrocytes from calf brain

The data are collected from a number of literature sources.

<table>
<thead>
<tr>
<th>Component</th>
<th>Composition in myelin (% of dry wt.)</th>
<th>Composition in oligodendrocyte plasma membrane (% of dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Man</td>
<td>Ox</td>
</tr>
<tr>
<td>Protein</td>
<td>21.3</td>
<td>22.3</td>
</tr>
<tr>
<td>Lipid</td>
<td>78.7</td>
<td>77.7</td>
</tr>
<tr>
<td>Ganglioside</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Composition in myelin (mol. % of total lipid)</th>
<th>Composition in oligodendrocyte plasma membrane (mol. % of total lipid)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Man</td>
<td>Ox</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>40.9</td>
<td>44.4</td>
</tr>
<tr>
<td>Cerebroside</td>
<td>15.6</td>
<td>17.3</td>
</tr>
<tr>
<td>Cerebroside sulphate</td>
<td>4.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>10.9</td>
<td>8.2</td>
</tr>
<tr>
<td>Ethanolamine phospholipids</td>
<td>13.6</td>
<td>15.1</td>
</tr>
<tr>
<td>Serine phospholipids</td>
<td>5.1</td>
<td>4.9</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>4.7</td>
<td>5.1</td>
</tr>
<tr>
<td>Other lipids*</td>
<td>5.1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Includes phosphatidylinositol.
Table 2. Characteristics of some of the better-defined proteins of central-nerve myelin

The data are collected from a number of literature sources. N.K., not known.

<table>
<thead>
<tr>
<th>Protein species</th>
<th>Molecular weight</th>
<th>Molecular size and shape</th>
<th>Myelin protein† (% of total)</th>
<th>Class of membrane protein</th>
<th>Interaction with lipid or other feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic protein</td>
<td>18400*</td>
<td>15 nm x 1.5 nm; prolate ellipsoid</td>
<td>30</td>
<td>Extrinsic</td>
<td>Phosphatidylserine, cerebroside sulphate, phosphatidylinositol and phosphatidylcholine</td>
</tr>
<tr>
<td>Pre-basic protein†</td>
<td>Basic protein+ 3000</td>
<td>N.K.</td>
<td>Low</td>
<td>N.K.</td>
<td></td>
</tr>
<tr>
<td>Proteolipid protein</td>
<td>23 500</td>
<td>3.8 nm; sphere</td>
<td>50</td>
<td>Intrinsic</td>
<td>Cholesterol, cerebroside and phosphatidylcholine</td>
</tr>
<tr>
<td>DM-20</td>
<td>20 540</td>
<td>N.K.</td>
<td>Low</td>
<td>Intrinsic</td>
<td></td>
</tr>
<tr>
<td>Higher-molecular-weight proteins</td>
<td>&gt;50 000</td>
<td>N.K.</td>
<td>20</td>
<td>Mostly intrinsic</td>
<td>Includes enzymes and glycoproteins</td>
</tr>
<tr>
<td>including:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W1</td>
<td>54 000</td>
<td>N.K.</td>
<td>N.K.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W2</td>
<td>62 000</td>
<td>N.K.</td>
<td>N.K.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total glycoprotein</td>
<td>&gt;60 000</td>
<td>N.K.</td>
<td>5</td>
<td>Intrinsic</td>
<td>Contain a variety of sugar residues including fucose, sialic acid, mannose, galactose and N-acetyl-glucosamine</td>
</tr>
<tr>
<td>including:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glycoprotein A</td>
<td>110 000</td>
<td>N.K.</td>
<td>0.4</td>
<td>Intrinsic</td>
<td></td>
</tr>
<tr>
<td>glycoprotein B</td>
<td>60 000</td>
<td>N.K.</td>
<td>Low</td>
<td>Intrinsic</td>
<td></td>
</tr>
</tbody>
</table>

* Rat and mouse brain myelin also contain a small-molecular-weight basic protein (14 100 daltons).
† Total protein of myelin accounts for about 20–30% of the total membrane dry weight.
‡ Recently reported by Barbarese et al. (1977).
There is general agreement that the low resolution (1.7nm) X-ray-diffraction results for compact central-nerve myelin are best interpreted in terms of a bilayer arrangement (for review, see Rumsby & Crang, 1977). Electron-density profiles across the repeating unit of the myelin sheath show peaks and troughs that are interpreted as the hydrated polar groups of lipid with protein and the hydrocarbon chains of the lipid phase respectively. Bilayers are separated by layers of intermembrane fluid. The electron-density profiles for myelin are similar to those obtained from oriented lipid bilayers and the bilayer structure of lipids in other natural membranes (Levine, 1972; Shipley, 1973). The width of each bilayer in central-nerve myelin is 4.7nm, whereas the intermembrane channels are some 3.0nm in width. X-ray techniques produce a time-averaged result and the possibility that small regions of the lipid phase adopt some other structural form within the overall bilayer cannot be excluded. Freeze-fracture electron-microscopy studies, where the lamellar nature of the membrane system is clearly preserved, provide further support for the general bilayer arrangement in compact myelin. X-ray-diffraction studies also provide an indication that the chemical architecture of the two surfaces of each bilayer unit in myelin may be asymmetric. The electron-microscope image of myelin supports this view with the different staining intensities shown by the external surface apposition (intraperiod dense line) and the cytoplasmic surface apposition (main dense line). Asymmetry of protein, and probably of lipid, between the two surfaces of natural bilayer membrane systems occurs for functional reasons and is now an accepted feature of membrane organization (Rothman & Lenard, 1977). From the electron-density profiles for myelin it has been suggested that not more than 10% of the total myelin protein can be accommodated within the hydrocarbon phase of the bilayer structure (Caspur & Kirschner, 1971). It can be calculated, however, that sating all the proteolipid protein of myelin within the lipid phase of the membrane would only cause a 9% penetration of the bilayer (Rumsby & Crang, 1977). Both X-ray-diffraction and electron-microscopy results have shown how hypo-osmotic and non-ionic solutions can penetrate the multilamellar arrangement of compact myelin to cause a separation and swelling apart of lamellae, which occurs only at the external apposition region. A hydrophilic nature is indicated for the apposition, whereas the ease with which the adjacent surfaces separate indicates that only rather weak forces act between the opposing membrane surfaces at the external apposition. This is in marked contrast with the cytoplasmic apposition where adjacent surfaces do not readily separate, even under conditions of high stress (Hall & Williams, 1970), except at the Schmidt–Lanterman clefts. It must be concluded that the surfaces of the cytoplasmic apposition are much more tightly held together compared with the external apposition even though the width of each channel is about the same (see Fig. 2). Such differences must arise from the way in which the chemical components of myelin are specifically organized within and on either side of the bilayer structure of the system. Gent et al. (1970) have suggested that water in the intermembrane apposition channels may be highly structured.

The dimensions of bilayer width are too narrow to accommodate the long fatty-acyl chains of the sphingolipids in a rigid extended all-trans form (Lee, 1975). However, interdigitation of acyl chains between the two halves of the lipid bilayer may occur (O'Brien, 1970) to overcome this problem, although Clowes et al. (1971) did not observe interdigitation of acyl chains when cerebrosides were mixed with phospholipid. A second possibility, more likely in view of current knowledge of membrane structure, is that the bulk of the lipids in the myelin sheath are in a liquid-crystalline state. Transition from the rigid-crystalline to the liquid-crystalline state for lipids involves a decrease in bilayer width due to the decrease in the length of acyl chains as they adopt a more-distorted structural form (Lee, 1975).

The major chemical components of compact myelin are shown in Table 1 where they are compared with the composition of the plasma membrane of the oligodendrocyte. The lipid-rich nature of compact myelin is evident from the analyses. The low content of protein in myelin is entirely in keeping with the role of the membrane system as insulation around axons. Artificial lipid bilayers, devoid of protein, have a notably
higher resistance to ion movement (Tien, 1971) than natural membranes. The composition of myelin is specifically designed to suit its function in situ. Demyelination of nerve fibres, such as occurs in multiple sclerosis, results in significant changes in impulse-conduction velocity. The changes vary from complete failure of conduction in severely demyelinated areas to a slowing of the impulse due to decreased conduction velocity in less severely attacked regions of central-nerve tissue (Halliday & McDonald, 1977).

The lipids of myelin are like those of other membranes in their amphipathic nature, whereas the high concentration of cholesterol is a feature of animal plasma membranes. Myelin is notable for its high content of glycosphingolipids. Thus cerebrosides and sulphatides comprise some 20% of the total myelin lipid and are characterized by very-long-chain saturated and monoenoic fatty acids, some of which have an additional hydroxy group on C-2. No other animal membrane system has such high concentrations of these glycolipids and it must be presumed that they are concentrated in myelin for some structural purpose that may be related to the long acyl chains, the sphingosine moiety or the galactose of the lipid class, or all three. In compact myelin a high proportion of the ethanolamine phospholipids occur as the plasmalogenic form. 

At present there seems to be no clear reason why this should be so and it will be of interest to see if phosphatidylethanolamine predominates in the plasma membrane of the oligodendrocyte as well. The thermal properties of phosphatidal- and phosphatidy ethanolamine have not been contrasted (summarized by Rumsby & Crang, 1977) and may be different, for the alk-1-enyl linkage of the plasmalogens has a cis double bond that may give the lipid type a lower phase transition. The surface potential of plasmalogens is lower than the corresponding diester form, but the relevance of this finding to myelin is not clear. It has been pointed out that plasmalogens may be more resistant to attack by phospholipases than the corresponding diester form. However, specific plasmalogenase enzymes exist naturally to attack this lipid class. 

The molar ratio of the major myelin lipid classes is approx. 1.25:1:0.6 for cholesterol/phospholipid/glycolipid in a number of different animal species, including man, ox, guinea pig and rat (Table 1). The molar proportion of cholesterol to other lipids is thus about 1:1.25. Cholesterol cannot complex with other lipids in myelin in the preferred 1:1 molar ratio, therefore (Demel & de Kruyff, 1976), unless some complex lipid is removed from the bulk lipid phase by tight association with membrane protein as boundary lipid.

Evidence from thermal studies (Ladbrooke et al., 1968), the mobility of concanavalin A receptors in the membrane (Matus et al., 1973; Matthieu et al., 1974) and the mobility of cholesterol (Rawlins, 1973) and phospholipids (Dawson & Gould, 1976) in compact myelin suggests that the lipid phase of the membrane system is in a liquid-crystalline arrangement. An appreciation of this conclusion is important for it means that lipid and protein components in compact myelin have mobility in the plane of the membrane unless other constraints operate to prevent it. This conclusion perhaps helps us to understand more easily how lipids and proteins are incorporated into compact myelin, how such molecules may turn over in such a tightly organized multilamellar system and even how the process of myelination may occur (see below). Fluidity in the lipid phase of compact myelin seems to be dependent on a high degree of hydration in the system and on the presence or cholesterol. With intact myelin, phase transitions begin to appear as the amount of water associated with the membrane is decreased. Hydrated myelin lipids free of cholesterol show marked endothermic phase transitions that are around 70°C for cerebrosides with their long acyl chains. A major function for cholesterol in myelin must be seen in terms of the maintenance of an intermediate fluid state in the lipid phase of the system. The mechanism of how this may be achieved is somewhat better understood now (Demel & de Kruyff, 1976), although how the long acyl chains of the glycosphingolipids are put into a liquid-crystalline state is not clear.
It may be through interaction with cholesterol (Oldfield & Chapman, 1972) or by association with other complex lipids in the membrane, such as phosphatidylcholine, that have shorter acyl chains (Clowes et al., 1971). Both mechanisms may operate.

The interaction of cholesterol with phospholipids indicates that the sterol preferentially interacts with sphingomyelin as opposed to glycerol-based phospholipids, regardless of the fluidity state (Demel et al., 1977). The reason for this is thought to be that the hydroxyl group of cholesterol is bound to water molecules by hydrogen-bonding at the surface of a lipid bilayer and sphingolipids, such as sphingomyelin, have more water associated at their hydrophilic-hydrophobic boundary than do glycerophospholipids. It will be of interest to see whether cholesterol also shows a preferential interaction with cerebrosides in myelin. This may be one way of ensuring that these complex lipids are maintained in a fluid condition. Pascher (1976) has noted that the hydrogen-bond donor as well as receptor properties of sphingolipids enable such molecules to form lateral networks of hydrogen bonds within the plane of the membrane. The effect would be to increase the stability of the membrane and to decrease permeability. It is interesting to speculate on this as a possible role for the high content of cerebroside in the myelin sheath. A decreased permeability to transmembrane ion movement would be in keeping with the required insulation properties of the myelin sheath around axons. The stability generated by a network of lateral hydrogen-bonds might be important in maintaining the integrity of the lipid-rich system. The extra stability provided in this way might account for the slow turnover rates observed for both lipid and protein components in myelin and for the fact that lipid is not readily transferred to myelin by phospholipid-exchange proteins (Miller & Dawson, 1972; Carey & Foster, 1977). The crystal structure of cerebrosides has recently been determined (Pascher & Sundell, 1977) and shows that the galactose head-group of the lipid is oriented parallel to the plane of the lipid bilayer. With the polar portion of the ceramide moiety the sugar head-group forms an extensive lateral system of hydrogen bonds. It should not be assumed that cerebrosides in myelin will necessarily adopt the same conformation. The absence of any phase transition from intact hydrated myelin suggests that cerebroside does not exist as a separate lateral phase in the membrane but is intermixed with other lipids to ensure an overall intermediate fluid state in the system. With their ester-linked fatty acids, glycerophospholipids only act as hydrogen-bond acceptors.

It is further noteworthy that although cerebrosides in myelin are amphipathic lipids that form stable bilayer arrangements like other myelin components, the polar headgroup carries no charge, yet can hydrogen-bond with water readily. Such molecules may be highly suitable for a multilamellar membrane system like myelin where the structuring of water and the close approach of adjacent membrane surfaces is required. The occurrence of galactose rather than glucose in the cerebrosides in myelin may perhaps be related to the hydrogen-bonding properties of the sugar residue with water or other lipid species and/or the adoption of a preferred orientation at the membrane surface. \( \text{Galactosyltransferase but not glucosyltransferase activity has been detected in isolated myelin samples (Nesovic et al., 1973; Costantino-Ceccarini & Suzuki, 1975). The addition of a sulphate group to C-3 of the galactose ring in cerebrosides, to form the sulphatides, gives such molecules a very strong negative charge at physiological pH (Abramson et al., 1967), which may be significant for ionic interaction with protein.} \)

Investigations into the possible asymmetric organization of lipids in the myelin lamellae are only just in progress (Abu-Salah & Findlay, 1977; Crang & Rumsby, 1977; Gregson, 1977; Linington & Rumsby, 1977, 1978; Rumsby & Grainger, 1977). Such studies are complicated by the nature of the system that, in intact form, may not be truly representative of compact myelin (Poduslo & Braun, 1975), whereas in isolated form difficulties over probe penetration, surface accessibility, etc., with the multilamellar preparations arise. As for other plasma membranes in animal cells, zwitterionic lipids may be concentrated on the external surface of the myelin lamellae (intraperiod dense line), whereas negatively charged lipids may be oriented at the cytoplasmic face (main dense line) where ionic interaction with the basic protein can occur (see below). Ethanolamine phospholipids are accessible to small covalently reacting probe molecules
(Crang & Rumsby, 1977; Rumsby & Grainger, 1977), which indicates an external location for some of this lipid though the amount, from the microelectrophoretic results of Gregson (1977), may be low. The negative charge at the surface of the myelin membrane is due to the localization of ganglioside there (Gregson, 1977). About 50% of the total cerebrosides of isolated myelin preparations is accessible to galactose oxidase; both hydroxy and non-hydroxy species are equally attacked (Linington & Rumsby, 1978). Such results indicate that some cerebrosides are externally oriented in myelin and this is confirmed by Gregson (1977) who has noted the presence of large patches of non-ionogenic lipid on the external surface of myelin and that anti-cerebroside antisera aggregate isolated myelin preparations (Oxberry & Gregson, 1974). Glycosylation reactions involved in transferring carbohydrate residues to protein are externally oriented in the endoplasmic reticulum and Golgi apparatus in animal cells. It may be that the same is true for cerebrosides and that the bulk of this major lipid class is externally oriented in compact myelin for the structural reasons discussed above.

Cholesterol will be distributed between the two halves of the myelin bilayer to balance out any uneven distribution of the complex lipid species. The total lipid of the lamellae may not be equally distributed between the two halves of the lipid phase because of the possible penetration of hydrophobic residues of the basic protein into the cytoplasmic surface. Cholesterol will preferentially complex with sphingolipids. This may lead to an asymmetry of the sterol in the membrane system.

Proteins account for about 20–30% of the total dry weight of compact myelin (Table 1). Two proteins, the basic protein and the proteolipid protein, account together for about 80% of the total protein of the membrane system and the remaining 20%, recoverable as chloroform/methanol-insoluble material after extraction, comprises higher-molecular-weight proteins and glycoproteins (Table 2). Whether these minor proteins of myelin are true components of the compact lamellae is still a subject of debate (Zanetta et al., 1977). The continuity of myelin with the plasma membrane of the oligodendrocyte and the proximity of the sheath to the axolemma at the paranodal region make it unlikely that a completely homogeneous preparation of the membrane will be achieved. Indeed, even the lamellae in compact myelin may not be absolutely homogeneous with respect to composition. Certain proteins, such as the basic protein, the proteolipid protein and the Wolfgram proteins W1 and W2, for example, are localized and concentrated in compact myelin for specific structural and functional reasons. A fluid phase in the membrane (see above) will allow for the movement of proteins of the oligodendrocyte plasma membrane into compact myelin at low dilution unless special constraints operate to prevent it. The low enzymic activity of purified myelin relative to the plasma membrane, however, tends to indicate that some normal plasma-membrane proteins have been excluded or are present in very low concentration only.

The basic protein of compact myelin has been widely studied (for review, see Carnegie & Dunkley, 1975) because of its involvement with experimental allergic encephalomyelitis, a demyelinating condition. The basic protein can be recovered from myelin without the need to disrupt the lipid phase. It is thus classed as an extrinsic membrane protein that is located at the surface of the lipid phase in the membrane system.

In purified form the basic protein has a highly open, yet ordered, structure that renders it very susceptible to proteolytic attack. Tyrosine and tryptophan residues in the isolated protein are largely exposed to the solvent (Jones & Rumsby, 1977) and about eight of the 13 lysine residues along the polypeptide chain can be acetylated (Steck et al., 1976). A prolate ellipsoid structure (15nm x 1.5nm) containing little α- or β-structure has been described for the purified protein (Epand et al., 1974). A β-bend, perhaps provided by the triproline sequence (Brostoff & Eylar, 1971), is necessary to explain the axial ratio of 10:1. It has been found that intramolecular folding of the polypeptide chain occurs within a sequence of residues (81–118) in the centre of the protein (Chapman & Moore, 1976). The structural form of the isolated basic protein may be somewhat different from its state in situ. Thus the α-helical structure of the basic protein is increased in organic solvents (Liebes et al., 1969) and on interaction with anionic detergents and acidic lipids (Palmer & Dawson, 1969; Anthony & Moscarello, 1971).
The tryptophan residue of the isolated protein is in a less structured situation compared with when it is in situ in the membrane (Crang et al., 1974; Jones & Rumsby, 1977). This could arise by interaction of the protein with lipid.

Basic-protein preparations show evidence of micro-heterogeneity, which is due to the loss of arginine residues from the C-terminal of the protein, phosphorylation and perhaps deamidation (Deibler et al., 1975). Only one serine and one threonine residue per basic protein molecule are available for phosphorylation. Loss of terminal-arginine residues may, to some extent, be an isolation artifact, for when the protein is purified from freeze-blown brain tissue, 75% or more of the polypeptide chain is unmodified in this region. On the other hand phosphorylation of the basic protein can be catalysed by a kinase enzyme present in myelin or added exogenously (for review, see Carnegie & Dunkley, 1975) and occurs both in vivo and in vitro. The significance of phosphorylation is not clear. Addition of one or two negatively charged phosphate groups to the polypeptide chain will decrease the number of available positive charges on the protein (14 at physiological pH), but the overall basic nature of the protein will remain. The question of how much modified basic protein exists in compact myelin is not known, but the studies by Deibler et al. (1975) show that in a standard preparation of the basic protein, 50% of the molecules are modified in some way. Cells possess many kinase enzymes for phosphorylating proteins and phosphatases for removing the phosphate residues are also active. The possibility that the myelin basic protein becomes phosphorylated simply as a consequence of its open structure, and not for any special structural reason, has to be considered. On the other hand Carnegie & Dunkley (1975) suggest that phosphorylation could induce changes in the conformation of the basic protein, which might influence myelin structure.

The preference of the basic protein for interaction with acidic lipid species can be accounted for in terms of the basic nature of the protein (for review, see Rumsby & Crang, 1977). Monolayer-protection studies indicate that sequences of the polypeptide chain along the N-terminal section of the protein interact hydrophobically with lipid and are thus protected against proteolytic attack (London et al., 1973; London & Vossenberg, 1973). Non-ionic forces may also be involved in the interaction of the basic protein with phosphatidylcholine liposomes (Smith, 1977). In a two-phase solvent system, sites for ionic interaction with lipid may be concentrated along the C-terminal section of the basic protein chain (Jones & Rumsby, 1977). The data suggest that sites for ionic and hydrophobic interactions with lipid are segregated along the protein chain. Mateu et al. (1973) have found that, mixed with acidic lipids and cerebroside sulphate, the basic protein orders the formation of a double-lamellar phase in which lipid species are asymmetrically distributed between the two lipid layers. Acyl-chain mobility is increased when the basic protein interacts with acidic lipid (Papahadjopoulos et al., 1975a), but no interaction was observed with the zwitterionic phosphatidylcholine. Very high protein/lipid weight ratios were used to observe these effects of the basic protein on lipid phase transitions, however.

Less information is available about the proteolipid protein of myelin (Table 2), largely because of the difficulties associated with extracting and handling the protein out of its normal membrane environment. The lipid phase has to be disrupted to recover the proteolipid protein, which defines it as an intrinsic membrane component. From the accepted molecular weight of about 23,500 (Nussbaum et al., 1974), the protein, if spherical, would have a diameter of some 3.8 nm (Rumsby & Crang, 1977). This size is just about sufficient for the proteolipid protein to span the lipid phase of the myelin lamellae. The protein has a high content of neutral and aromatic amino acid residues, in keeping with the hydrophobic nature of the molecule (Folch-Pi, 1973). The polypeptide chain of the proteolipid protein has a high degree of conformational flexibility and possesses considerable \( \alpha \)-helical content in organic solvents, which is lost when the protein is transferred to an aqueous phase. Progress is being made on the amino acid sequence of the protein (Jolles et al., 1977). Fatty acids covalently bound to the polypeptide chain of the protein increase the hydrophobic nature of the molecule and must aid in its interaction with the acyl groups of lipids in the lamellae. The proteolipid protein interacts
with charged, uncharged and zwitterionic lipids such as cholesterol, cerebroside and phospholipids. The protein shows a particular affinity for cholesterol where the 3β-hydroxy group of the sterol is important for interaction. Thermal studies reveal that the proteolipid protein binds acyl chains tightly, removing them from participation in the phase transition of the bulk lipid in the membrane (Papahadjopoulos et al., 1975).

The remaining proteins of myelin are a heterogeneous mixture of components of molecular weight higher than about 50000 (Table 2). Detailed characterizations of many

---

**Fig. 2. Diagrammatic representation of molecular organization in central-nerve myelin showing the arrangement of the basic protein and the proteolipid protein in the membrane system as suggested by current results (see the text)**

The basic protein (BP) is located exclusively at the cytoplasmic apposition (cyt) and has some hydrophobic sequences of polypeptide chain on the N-terminal section of the protein penetrating on the lipid surface. Ionic interactions with lipid occur predominately on the C-terminal section of the protein. BP molecules adopt two extremes of orientation at the cytoplasmic apposition (as shown), owing to the way the repeating unit of compact myelin is formed by compaction of two units of the oligodendrocyte plasma membrane (Crang & Rumsby, 1977). The proteolipid protein (PLP) is buried in the lipid phase with a slight exposure at the external apposition (ext). Dimensions for the repeating unit and the lipid phase and apposition regions are from X-ray-diffraction results. Other proteins are incorporated into the model and will include enzymes such as the 2',3'-cyclic nucleotide 3-phosphohydrolase and others. Such components are distributed between the external and cytoplasmic apposition regions. Carbohydrate-containing proteins and ganglioside (with tails) are exclusively located at the external apposition.
of these minor components are lacking though they will all probably be intrinsic membrane proteins. The W1 and W2 Wolfgram proteins have been isolated and their immunological properties studied (Nussbaum et al., 1977; Roussel et al., 1977). The enzyme activity associated with myelin will be located in some of the proteins in this fraction. Central-nerve myelin contains some glycoproteins and some idea of their molecular weights and carbohydrate content is known (Quarles et al., 1973; Quarles & Everly, 1977; Zanetta et al., 1977). A full characterization of these minor components of myelin (and of the oligodendrocyte plasma membrane), especially the glycoproteins, is vital for such molecules constitute the surface recognition, receptor and virus-binding sites of membranes and may be antigenic.

Current data on the localization of the main proteins in compact myelin has been summarized recently (Rumsby & Crang, 1977). The proteolipid protein is located in the lipid phase, which suits its lipophilic character, though probe studies (Poduslo & Braun, 1975) suggest that the polypeptide chain is partially exposed at the external surface of the lamellae. It should be remembered that proteolipid proteins are not exclusively found in myelin and have been detected in other membrane systems where their lipid-binding properties may be important for stability in the system. The basic protein, on the other hand, is unique to the myelin system and the oligodendrocyte. As an extrinsic protein it could be located at either the external or cytoplasmic surface of the system. Knowledge of how membrane proteins are synthesized (Shore & Tata, 1977) and the absolute asymmetry of proteins in membranes (Rothman & Lenard, 1977) makes it unlikely that the basic protein is partially located at both surfaces in myelin. Current probe-labelling studies with pyridoxal phosphate, lactoperoxidase, dansyl chloride, etc. (summarized by Rumsby & Crang, 1977) provide the best available indication that the basic protein is located at the cytoplasmic apposition site. Other evidence supporting this conclusion comes from the application of immunological techniques to intact and isolated myelin systems (Herndon et al., 1973; Guarnieri et al., 1974; Oxberry & Gregson, 1974). The minor protein components of the system will be distributed between the cytoplasmic and external surfaces, whereas the glycoproteins will be located at the external surface of the lamellae, in keeping with their role in events on the outer surfaces of cells.

A model for molecular organization in compact myelin is included (Fig. 2) and the disposition of the basic protein, proteolipid protein and some minor components is shown together with the X-ray diffraction dimensions for the myelin repeating unit, the lipid phase and the cytoplasmic and external apposition regions. In the model the basic protein functions as a bridge between the opposing surfaces of the cytoplasmic apposition; the protein holds the surfaces together yet maintains an aqueous channel at the apposition, although water in the channel may be highly structured. It can be calculated that the basic protein occupies about 54% of the total cytoplasmic apposition surface, i.e. 27% of each individual opposing face (Rumsby & Crang, 1977). The basic protein is envisaged as interacting ionically with one surface at the apposition and via hydrophobic interactions at the other surface. Two extremes of orientation for the basic protein are shown. These arise because of the way the basic protein may be delivered to the site of compaction (Crang & Rumsby, 1977). The role of the basic protein in the compaction process was first discussed by Davison (1970), and becomes clear in the model by Crang & Rumsby (1977). In the model in Fig. 2 the basic protein and the proteolipid protein are shown forming a 1:1 molar complex in the membrane system. As yet there is no experimental data to support this speculative arrangement. In fact bifunctional probes capable of penetrating the lipid phase do not seem to cross-link the basic protein and proteolipid protein (J. B. C. Findlay, personal communication). However, such an arrangement of the basic protein with the proteolipid protein, taking into consideration the different lipid-binding features of both proteins, would be a good lipid-binding nucleus to provide long-range order and stability in the lipid-rich membrane system. This idea has been discussed elsewhere (Rumsby & Crang, 1977). In another model for myelin organisation, Moore et al. (1978) have also located the basic protein at the cytoplasmic apposition, but suggest that the protein may bridge
the adjacent surfaces in dimer form. The evidence for dimerization of the basic protein comes from nuclear-magnetic-resonance-spectroscopy studies on the isolated protein at pH 7 (Chapman & Moore, 1976). The tendency of the basic protein to aggregate at pH 7 and above is well known (Carnegie & Dunkley, 1975), but this is not in evidence at lower pH values.

Models of molecular organization in compact myelin (Fig. 2) provide a basis for interpreting how demyelination occurs in diseased states such as multiple sclerosis and experimental allergic encephalomyelitis, where some of the observed breakdown of the membrane is most probably caused by the action of invading inflammatory cells. One problem arising from the postulated location of the basic protein at the cytoplasmic surface is the inaccessibility of this component under normal circumstances to events occurring at the external surface. Thus invading cells sensitized to basic protein as antigen, as in experimental allergic encephalomyelitis, would not be able to locate the protein at its cytoplasmic apposition unless the membrane was damaged first. The action of lysosomal enzymes, notably proteinases and phospholipases, on myelin has been studied along with the effect of natural detergents such as lysophosphatidylcholine. The susceptibility of the isolated basic protein to proteolysis is well known, but studies of the action of trypsin and acetyltrypsin on isolated myelin have given rather conflicting results (for review, see Rumsby & Crang, 1977) ranging from no digestion of the basic protein to considerable breakdown of this component. The more recent observation of Banik et al. (1976) is of relevance in that it suggests that the joint action of phospholipase A2 and trypsin promotes degradation of the basic protein and, in addition, the proteolipid protein and some minor proteins are attacked. It is curious, however, that lysophosphatidylcholine added exogenously with trypsin did not induce an increased loss of protein as the action of lysophosphatidylcholine injected into myelinated peripheral nerves in vivo causes dissolution of the compact myelin around the injection site. Axons and Schwann cells are spared, presumably as they can metabolize the lysolipid (Gregson & Hall, 1973). Gent and colleagues have studied the solubilization of isolated myelin by lysophosphatidylcholine and observed that the basic protein is released early in the solubilization process. This would suggest that lysolipids generated by the action of phospholipases can radically alter the normal membrane structure so that the basic protein could become accessible to proteolytic enzymes on the external surface of the membrane. Our understanding of the action of hydrolytic enzymes and natural detergents on myelin needs to be clearly defined in the light of our appreciation of the molecular structure of the compact myelin system.

The presence of a liquid-crystalline (fluid) lipid phase in myelin perhaps provides an answer to several outstanding problems related to the mechanism of myelin formation in central-nerve tissue. Mechanisms for the synthesis and movement of lipids and proteins to the plasma membrane of the oligodendrocyte will almost certainly follow the general principles established for the flow of membrane material from the endoplasmic reticulum and Golgi apparatus to the surface membrane (Palade, 1975). Thus it can be envisaged that lipid (and protein) is incorporated into the oligodendrocyte plasma membrane from sites of synthesis (Fig. 3) as vesicles that then fuse with the membrane to supply membrane components oriented in their correct disposition (Rothman & Lenard, 1977). The correct asymmetry of protein is maintained by the mechanism of synthesis on the rough endoplasmic reticulum (Shore & Tata, 1977). Lipid synthesis is constantly in progress in cells (Dawson, 1973) and phospholipid-exchange proteins may also play an important role in ensuring a constant supply of lipid on the cytoplasmic side of the membrane system (Wirtz, 1974). Such a mechanism for lipid synthesis explains why the lipid composition of the oligodendrocyte is quite similar to that of compact myelin in that glycosphingolipids are present (Poduslo, 1975). Some protein duplication may occur too. The same will also apply to the surface membrane of the oligodendrocyte processes.

At myelination a massive synthesis of the lipids and proteins required for compact myelin is 'turned on' by a process that has not yet been identified. The formation of the membrane process from the oligodendrocyte and the extension of this process to contact
Fig. 3. Oligodendrocyte and processes showing the movement of lipid and protein vesicles from sites of synthesis on the endoplasmic reticulum and Golgi apparatus in the body of the cell to sites of fusion with the surface membrane of the process.

The continuous flow of lipid vesicles into the surface membrane near the sheath provides the driving force for myelination as described in the text. The myelin sheath expands along the axon as well as around it.

an axon occurs and it is likely that some specific recognition mechanism between the two membranes may be involved when contact is first made. It could be asked whether such recognition events eventually form the particles of the axon–glial junction region? The subsequent spiraling of membrane around the axon has been studied in some detail (Peters & Vaughn, 1970; Caley & Butler, 1974) and seems to involve the formation of membrane loosely around the internal mesaxon, which is more closely associated with the axolemma. Compaction at the cytoplasmic apposition occurs and subsequently the multilamellar system tightens up as more lamellae are formed. Rotation of the Schwann cell has been suggested to account for the formation of compact myelin in the peripheral nervous system, but this cannot be the case in the central nervous system, because of the processes of the oligodendrocyte, which would immediately impede such a movement.
Similarly it is difficult to see how rotation of the axon could occur. The process can, however, be explained by the proposal that the driving force for myelination and the formation of the multilamellar system is provided by the continuous flow of lipid (as vesicles, Fig. 3) into the process surface membrane near the point of contact with an axon. If the process tip were located on the axolemma, flow of lipid would subsequently lead to an extension of the process around the axon. This will continue as more and more lipid (with associated protein) is fed into the membrane. Subsequently the loose membrane begins to tighten as more lamellae form and as the axon diameter increases through natural growth. One essential proviso for such a scheme is that the bulk lipid phase of the axolemma is also in a fluid state.

A liquid-crystalline state for the bulk lipids of myelin and the axolemma readily explains how expansion of the myelin sheath during axon growth can occur. Hirano & Dembitzer (1967) have suggested that sheath expansion can occur by lamellar slippage. Such a system would require a fluid lipid phase and in such a condition there is little problem in envisaging how lateral expansion of lamellae occurs when there is a constant supply of lipid provided to the membrane system from the site of synthesis in the glial cell (Fig. 3). In this situation the expansion of the axon will provide the force and this will occur along with formation of new lamellae around the axon. It should also be remembered that the sheath will be extending along the length of the axon at the same time. The enzymology of some of the events associated with this process have been discussed previously by Brady & Quarles (1973).

Financial support for this work from The Wellcome Trust, The Multiple Sclerosis Society of Great Britain and Northern Ireland, the Medical Research Council and the Science Research Council is gratefully acknowledged. I thank my colleagues, especially Dr. A. J. Crang, for many comments and discussions on some of the points raised in this review.

Costantino-Ceccarini, E. & Suzuki, K. (1975) Brain Res. 93, 358–362