Lipid and Basic Protein Interaction in Myelin

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(Received 25 March 1974)

1. Purified myelin labelled with [3H]myo-inositol or [1-14C]acetate was incubated with trypsin or acetylated trypsin at 37°C, pH 8.0 for 30 min. 2. After incubation and centrifugation analysis of the myelin pellet showed marked digestion of basic protein on polyacrylamide-gel electrophoresis. Proteolipid and Wolfgram proteins remained unchanged. 3. A loss of 15% of total protein and loss of all classes of lipids was also found. Most significant lipid losses were phosphoinositides, phosphatidylserine and sulphatide. 4. A low-density material containing more phospholipid than cholesterol and galactolipid was isolated from the supernatant obtained after centrifugation of trypsin-treated myelin. 5. Interaction of sulphatide and myelin basic protein was shown to take place in a biphasic system. Basic protein does not form any complex either with cerebroside or cholesterol in the same solvent system. 6. The release of acidic lipids from myelin suggests that they may be linked to basic protein by ionic forces and the neutral lipids may be by lipid–lipid interactions. 7. The relevance of these studies as a model of brain degeneration is discussed.

Myelin has proved a most convenient preparation for both physical and chemical studies leading to detailed concepts of the molecular organization of biological membranes (Schmitt et al., 1935; Fernandez-Moran, 1962; Blaurock, 1971; Casper & Kirschner, 1971). Earlier studies on membrane structure suggested ionic interactions between lipids and proteins, but more recent reports have emphasized the importance of non-polar lipid–protein associations (Singer & Nicolson, 1972). Although lipids in membranes may be associated directly with protein through both ionic and non-polar interactions substantial experimental evidence is needed to support this assessment. The relatively simple protein composition of myelin makes it easy to perform experiments which may demonstrate the associations of different classes of lipids with one of the major proteins of myelin. Since basic protein is a major component of myelin and has no α-helical or β-structure (Eylar, 1969) it is reasonable to assume that it can have maximum interactions with lipids for stabilizing the membrane in vivo. Reports on interactions of lipids and proteins in the myelin membrane have appeared (Palmer & Dawson, 1969; Mateu et al., 1973; London & Vossenberg, 1973; Wood & Dawson, 1974) and the results of digestion of isolated myelin by large amounts of trypsin was studied by Kies et al. (1965). To demonstrate which lipids may be linked to basic protein in myelin we incubated radioactively labelled myelin samples with small amounts of crystalline trypsin and measured the loss of protein from myelin together with the release of lipids into the supernatant after incubation.

We also demonstrated some specific interactions of the basic protein with certain classes of lipids in a biphasic system. A preliminary report of this work has appeared elsewhere (Banik & Davison, 1974).

Materials and Methods

Reagents

Trypsin (twice crystallized) and mono-, di- and tri-phosphoinositides were obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K. and acetylated trypsin from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K. Disodium hydrogen [32P]phosphate, [2-3H]myo-inositol and sodium [1-14C]acetate were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals were Analar grade (BDH Chemicals Ltd., Poole, Dorset, U.K.).

Animals

Wistar rats of either sex were used for all experiments. Rats (20–30 days-old) were injected intraperitoneally with 250 μCi of disodium hydrogen [32P]phosphate in 0.1 ml of 0.9% (w/v) NaCl. The rats were killed 2 days after injection. In another set of experiments 14-day-old rats were injected intracerebrally with 10 μCi of [3H]myo-inositol in 0.02 ml of 0.9% (w/v) NaCl. Myelin was prepared 2 days after injection. The same design of experiment was repeated by injecting 9-day-old rats intraperitoneally with 25 μCi of sodium [1-14C]acetate in 0.1 ml.
of 0.9% (w/v) NaCl. Myelin was prepared 6 weeks after injection.

Preparation of myelin

Young rats were decapitated and older rats were lightly anaesthetized with chloroform before exsanguination. Brains were quickly removed, weighed and homogenized in 0.32M-sucrose solution by using a Teflon homogenizer. Myelin was prepared by the method of Norton (1971). Purity of the myelin fraction was checked by electron microscopy and by enzyme studies as described by Banik & Davison (1969).

Incubation of myelin with trypsin

Purified myelin was suspended (250 mg fresh wt. of brain tissue/ml) in 0.1M-phosphate buffer, pH 8.0 (0.1M-Na2HPO4 adjusted to pH 8.0 with 0.1M-Na2HPO4): 3 ml of suspended myelin sample (equivalent to about 7 mg of myelin protein) was incubated at 37°C in a constant-shaking water bath with 10 or 250 μg of acetylated trypsin (non-acetylated trypsin was used in preliminary experiments only) for different time-intervals. Myelin without trypsin and also with added trypsin at zero time served as controls. The experimental and control tubes were quickly chilled in ice after the incubation and the samples adjusted to 0.32M-sucrose by the addition of 1.2M-sucrose solution, and then centrifuged in a 10 x 10 angle rotor (MSE A2469) at 12000 g for 10 min. After centrifugation a firm myelin pellet was obtained and a lipid-rich low-density material was found in the supernatant. Supernatants were decanted and the myelin pellet was resuspended in 3 ml of ice-cold water and centrifuged as described above. The supernatants obtained were combined and analysed. The low-density material was pelleted from the supernatants by centrifugation at 78000 g for 15 min. Thus three fractions were obtained: (1) washed trypsin-treated myelin residue, (2) released low-density material and (3) the final clear supernatant. The three preparations were analysed as a routine for lipid, proteins and enzyme activity.

Interaction of protein and lipids in a biphasic system

Interactions between the isolated myelin basic protein, prepared by the method of Banik & Davison (1973) with sulphadiazine, cerebrosides or cholesterol, were also studied in a biphasic system consisting of chloroform–methanol–water (8:4:3, by vol.) under the conditions described by Palmer & Dawson (1969). The lipids in 2.4 ml of chloroform–methanol (2:1, v/v) solution were added to the basic protein in 0.6 ml of water, mixed thoroughly and allowed to stand for 30–60 min at room temperature. The solution was centrifuged and the resulting upper and lower phases were analysed for protein and lipid.

Analytical methods

Determination of protein and enzyme activity. Protein content was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard. Adenosine 2',3'-cyclic nucleotide 3'-phosphohydrolase activity was measured by the method of Banik & Davison (1969) as modified by Agrawal et al. (1970).

Extraction of lipids. Lipids were extracted with 19 vol. of chloroform–methanol (2:1, v/v)/g fresh wet wt. of brain (Folch et al., 1957). Lipids were separated by t.l.c. for the determination of radioactivity (Cuzner & Davison, 1967). Total phosphoinositides were extracted from myelin samples by the method of Gonzalez-Sastre (1970).

Gel electrophoresis. Electrophoresis in a sodium dodecyl sulphate medium was carried out by the method of Weber & Osborn (1962) as described by Agrawal et al. (1972) and Bignami & Eng (1973); 30–40 μg of protein was used for electrophoresis and gels were run at 1 mA/gel increasing to 4 mA/gel. for 6–7 h (Banik et al., 1974). Samples were stained with Coomasie Blue, then de-stained by several changes with de-staining solutions (Agrawal et al., 1972) and finally kept in 7% (v/v) acetic acid. Gels were scanned in a Gilford u.v. spectrophotometer fitted with a scanner.

Electron microscopy. The pelleted fractions were fixed overnight in 4.0% (w/v) glutaraldehyde in 0.1 M-phosphate buffer, pH 7.4, (0.1M-Na2HPO4 adjusted to pH 7.4 with 0.1M-NaH2PO4), then washed three times in the same buffer and fixed in 1.0% (w/v) OsO4 for 2 h.

Determination of radioactivity. 14C and 3H radioactivity was determined in a Beckman liquid-scintillation counter. The scintillation fluid was as described by Banik & Davison (1971). 32P was counted in aqueous solution by the Čerenkov counting method (Haviland & Bieber, 1970).

Results

Effect of trypsin on an isolated myelin fraction

In a preliminary experiment young rats were injected with 32P-labeled myelin suspension (final concentration 700 mg wet wt. of brain in 3 ml of incubation media) which was incubated for 30–60 min with and without trypsin (non-acetylated). The myelin fraction was re-isolated by centrifugation and washed. The combined supernatant and wash contained an increasing amount of labelled phospholipid and there was a concomitant

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loss of $^{32}$P-labelled lipid from the myelin pellet (Table 1). The loss of total protein and lipid from myelin after 30 min incubation with trypsin was found to be about 18% and 15% respectively even with corrections for unavoidable losses of myelin on re-centrifugation and washing. All subsequent experiments were carried out with acetylated trypsin, for the non-acetylated enzyme was found to bind firmly to myelin. It was also difficult to assess the extent of digestion of proteolipid protein of myelin after incubation with non-acetylated trypsin since its electrophoretic mobility in the sodium dodecyl sulphate–polyacrylamide-gel system was similar to that of proteolipid protein (Raghavan et al., 1973; Wood & Dawson, 1974).

Effect of acetylated trypsin on isolated myelin

As controls for the procedure the isolated myelin fraction was first incubated without trypsin at 37°C for 30 min and as a second control acetylated trypsin was added to the myelin fraction without incubation. The treated fractions were diluted, centrifuged and washed (Table 2). There was a uniform loss of 15% of whole myelin by this procedure comparable with that described by other workers (Raghavan et al., 1973).

The loss of total protein from myelin owing to digestion by acetylated trypsin (10 μg/mg of myelin protein) is given in Table 2. The corresponding loss for total lipid from myelin was found to be about 10% (Table 4). With longer time of incubation there is an increased release of protein into the buffer. This can be recovered in part as a low-density floating fraction, which on dilution and fast sedimentation can be largely precipitated as an unorganized pellet.

Electron microscopic observation of the washed myelin residue after trypsin treatment did not reveal any significant structural difference compared with controls. The periodicity of the myelin lamellae in both cases remained the same, but splitting of the lamellae was observed at the intraperiod line in agreement with the observation of Masurovsky & Bunge (1971).

The electrophoretic pattern of myelin indicated that the 15% loss of myelin protein after trypsin treatment (10 μg/mg of myelin protein) for 30 min was primarily due to gradual digestion of basic protein with the concomitant appearance of new faster-moving

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Expts.</th>
<th>Supernatant</th>
<th>Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>1670</td>
<td>8643</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>2160</td>
<td>10482</td>
</tr>
<tr>
<td>60</td>
<td>4</td>
<td>2635</td>
<td>10295</td>
</tr>
</tbody>
</table>

Table 2. Loss of myelin protein and adenosine 2':3'-cyclic nucleotide 3-phosphohydrolase activity on incubation with acetylated trypsin

As a control purified rat myelin preparations were incubated for 30 min (Expt. 2) without trypsin or for zero time (Expt. 1) with trypsin, washed and sedimented as described in the text. In Expts. 3–6 samples were incubated with acetylated trypsin (equivalent to 10 μg of trypsin/mg of myelin protein) for 30–180 min at 37°C. Since the amount of initial myelin protein varied with age and the preparation of individual samples, results are all expressed in terms of the maximum yield obtained at the beginning of the experiment (9.51 mg of protein/g wet wt. of brain) with S.D. where applicable. Numbers in parentheses indicate numbers of experiments.

<table>
<thead>
<tr>
<th>Expts.</th>
<th>Incubation time (min)</th>
<th>Myelin residues</th>
<th>Loss of protein (% control value)</th>
<th>Low-density material</th>
<th>Phosphohydrolase activity (of control value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>9.51 (7)</td>
<td>0</td>
<td>0.209</td>
<td>100 (3)</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>9.54 ± 0.66 (3)</td>
<td>0</td>
<td>0.120</td>
<td>100 (3)</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>8.10 ± 0.18 (7)</td>
<td>14.8</td>
<td>0.340 ± 0.029 (3)</td>
<td>83 (3)</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>7.81 ± 0.21 (3)</td>
<td>17.9</td>
<td>0.481 (2)</td>
<td>78 (3)</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>7.47 ± 0.24 (3)</td>
<td>21.8</td>
<td>0.549 (5)</td>
<td>74 (2)</td>
</tr>
<tr>
<td>6</td>
<td>180</td>
<td>7.15 (2)</td>
<td>24.8</td>
<td>—</td>
<td>61 (2)</td>
</tr>
</tbody>
</table>

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protein bands for no alteration in the concentration of other proteins was discernible (Fig. 1). Under these same conditions a 17% loss of 2':3'-cyclic nucleotide 3'-phosphohydrolase activity was observed and most of this activity could be recovered in the pellet from the low-density floating material. When 250 µg of trypsin/mg of myelin protein was used for incubation, basic protein was completely digested; other proteins were also found to be partially hydrolysed. On electron microscopy the pelleted low-density material was found to contain predominantly membranous vesicular structures with occasional pinched-off myelin lamellae (Plate 1). Gel electrophoresis of the pelleted low-density material showed the presence of very faint protein bands, probably of high molecular weight. No protein bands for basic, proteolipid and Wolfgram proteins were observed in the electrophoretic pattern of these gels.

Release of labelled lipid from myelin treated with acetylated trypsin

[3H]myo-Inositol was given intracerebrally to young rats to label myelin polyphosphoinositides. After incubation of the myelin with and without acetylated trypsin phosphoinositides were extracted from the myelin residues and supernatants. Radioactive inositol phospholipid was released and a similar proportion of radioactivity disappeared from the washed myelin residues with increasing time of incubation (Table 3). The release of total phosphoinositides from the residue was found to be about 27% after 30 min of incubation. Results were expressed on the basis of total released polyphosphoinositide for rapid decomposition of individual polyphosphoinositides is well-recognized (Sheltawy & Dawson, 1969).

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Supernatant (1)</th>
<th>Residue (1)</th>
<th>Supernatant (2)</th>
<th>Residue (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>218</td>
<td>1807</td>
<td>252</td>
<td>2073</td>
</tr>
<tr>
<td>30</td>
<td>440</td>
<td>1280</td>
<td>564</td>
<td>1560</td>
</tr>
<tr>
<td>60</td>
<td>586</td>
<td>1248</td>
<td>732</td>
<td>1458</td>
</tr>
</tbody>
</table>

* Myelin incubated without trypsin.

Table 3. Release of total phosphoinositides from myelin treated with acetyltrypsin

Experimental procedures and extraction of polyphosphoinositides are described in the Methods section. Myelin labelled with [3H]myo-inositol was incubated with acetyltrypsin (10 µg/mg of protein).

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Supernatant</th>
<th>Low-density material</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>43266 ± 376</td>
<td>4400 (1)</td>
</tr>
<tr>
<td>30</td>
<td>39160 ± 773</td>
<td>9394 (1)</td>
</tr>
<tr>
<td>60</td>
<td>46000 (1)</td>
<td>3854 (1)</td>
</tr>
</tbody>
</table>

Radioactivity (c.p.m./g wet wt. of brain

Similar results were obtained for release of total lipid when [14C]-labelled myelin was incubated with acetyl trypsin (Table 4). Individual lipids released into the buffer and those remaining in the myelin residue for both controls and acetyltrypsin-incubated samples were separated by t.l.c. Radioactivity in each separated lipid was determined (Table 5). Trypsin treatment resulted in some loss of all the individual myelin lipids especially sulphatide, phosphatidylcholine and phosphoinositides. The smallest change was in sphingomyelin.
EXPLANATION OF PLATE I

Electron micrograph of low-density material (x87260)

Membranous vesicular structures resembling phospholipid micelles with occasional myelin lamellae can be seen.
Table 5. Release of different classes of lipid from acetyl-
trypsin-treated myelin

Experimental procedures and separation of lipids are
described in the text. Myelin was labelled with \([^{14}C]\)acetate
and incubated with acetyltrypsin for 30 min. Results are
expressed as percentage of lipid released from myelin.
Numbers in parentheses indicate numbers of experiments.
Polyphosphoinositide was calculated from experiments
with \([^{3}H]\)myo-inositol-labelled myelin. Results are the
means of two experiments.

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Myelin residue</th>
<th>Supernatant containing low-density material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>Cerebroside</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Ethanolamine–phospholipid</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>28</td>
<td>14</td>
</tr>
<tr>
<td>Sulphatide</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>Polyphosphoinositide</td>
<td>27</td>
<td>21</td>
</tr>
</tbody>
</table>

Fig. 2. Interactions of basic protein with various lipids in a
biphasic system

\(\text{■, Cholesterol; \(\square\), cerebroside; \(\bigcirc\), sulphatide. For details see the text.}\)

Interaction between basic protein and lipid

Palmer & Dawson (1969) demonstrated an
interaction between purified myelin basic protein
and polyphosphoinositides and other acidic phos-
pholipids. In view of our results we used the same
system to investigate possible binding of sulphatide
(as an acidic lipid) and cerebroside and cholesterol
(as neutral lipids). When sulphatide and basic protein
were mixed in a two-phase solvent system of
chloroform–methanol–water (8:4:3, by vol.), a
complex was formed. This complex was found to be
chloroform–methanol (lower phase)-soluble. The
gradual disappearance of basic protein from the
upper aqueous phase into the lower organic phase
in the presence of increasing amounts of sulphatide
is seen in Fig. 2. Almost all added sulphatide was
found in the lower phase and 75\% of the protein
was present as a complex in the lower phase. There
was no evidence of lipid–protein complex formation
in the biphasic system for cholesterol or cerebroside.
No significant loss of protein was found from the
upper phase and the lipids were found exclusively
in the lower phase (Fig. 2).

Discussion

Recent reviews of membrane structure have stressed
the importance of types of associations of lipid and
protein and their contribution to the arrangement
and stability of membranes (Finean, 1965; Korn,
1966; Green & Tzagoloff, 1966; Davison, 1968;
Lehninger, 1968; Wallach & Gordon, 1968). Myelin
is convenient to study since it can be easily purified
and contains well-defined acidic, basic and proteo-
lipid proteins all of which are associated with lipids.
In myelin one of the most important features is the
basic protein whose absence of \(\alpha\)-helical or \(\beta\)-struc-
ture allows maximum lipid–protein interaction
(Eylar, 1969). Acidic phospholipids and sulphatides
are thought to be especially important in reacting
ionically with the basic residues of the protein and
hence contributing to the stability of the membrane.
Thus formation of complexes between myelin basic
protein and acidic phospholipids (triphosphoinosi-
tide, phosphatidic acid and phosphatidylserine) has
been demonstrated in a biphasic system by Palmer
& Dawson (1969). Since the basic protein is readily
digested by trypsin we used incubation with this
enzyme in the acetylated forms as a means of evaluat-
ing the role of the trypsin-digestible protein in
membrane stability.

On incubation of isolated myelin with acetyl-
trypsin there is a 15\% loss of protein within the first
30 min (Table 1) due primarily to selective digestion
of basic protein as indicated by gel electrophoresis
(Fig. 1). From the electrophoresis scan (Fig. 1) it
can be calculated that about 30\% of the total myelin
basic protein is digested within 30 min. There is also
a parallel loss of about the same proportion of
sulphatide, phosphatidylcholine and inositol phos-
pholipid as a result of tryptic digestion. Other neutral
lipids were also found to be released. The loss of
sulphur-containing lipids and acidic lipids was
expected since these lipids have been found to be
associated with isolatedencephalitogenic basic
protein (Saito et al., 1972) and sulphatide, together

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Table 6. Lipid composition of low-density material obtained from myelin after incubation with acetyltrypsin (10μg/mg of protein)

Results are the mean of two experiments. When myelin was incubated with acetyl trypsin in Tris–HCl buffer by the method of Raghavan et al. (1973) a greater loss of myelin components and a higher yield of low-density material was observed.

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Lipids (μmol/g wet wt. of brain)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol &amp; phospholipid</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.60</td>
<td>4.2</td>
</tr>
<tr>
<td>30</td>
<td>1.08</td>
<td>5.0</td>
</tr>
<tr>
<td>60</td>
<td>1.52</td>
<td>6.0</td>
</tr>
<tr>
<td>90</td>
<td>1.63</td>
<td>7.0</td>
</tr>
</tbody>
</table>

with the acidic phospholipids, readily forms complexes with purified basic protein (Palmer & Dawson, 1969, Fig. 2). Thus these acidic lipids are probably tightly bound to the basic protein by ionic forces; this applies especially to the polyphosphoinositides, which can only be extracted by acidified organic solvents. These results agree with those of Mateu et al. (1973), Demel et al. (1973) and London & Vossenberg (1973).

However, after incubation with trypsin there was also loss of cholesterol, cerebroside and phospholipid from the myelin preparation. Although a small part of the lipid may be directly associated in the membrane with basic protein it is probable that most is involved in lipid–lipid interaction through non-polar forces (Finean, 1953; Finean et al., 1968; Chapman, 1972). It has, for example, in model experiments been shown that there is interaction between cholesterol and cerebroside-containing α-hydroxy fatty acids (Mickel & Hill, 1972). Therefore on digestion of myelin basic protein, release of neutral lipid may be simply due to loss of the acidic lipid available for hydrophobic interaction. This may explain the massive loss of lipid found by Raghavan et al. (1973) when large amounts of trypsin were incubated with myelin. Although incubation of myelin with trypsin causes loss of myelin components the ultrastructure of myelin remains unchanged. No change is apparent in the periodicity between trypsin-treated and control myelin, and despite the partial removal of basic protein from myelin by trypsin the intraperiod line was still preserved suggesting that basic protein may not be exclusively localized in the intraperiod line as reported by Dickinson et al. (1970) or in the dense line as proposed by Wolman (1971). Recent evidence suggests that the basic protein may well be hidden inside the membrane and labelling studies also indicate that proteolipid protein is probably protected by a lipid milieu whereas high-molecular-weight proteins (e.g. Wolgram and glycoproteins) are exposed on the surface of the membrane (Poduslo & Braun, 1973). It may therefore be concluded that the membrane stability relies particularly on the integrity of the proteolipid protein and its covalently bound lipid (Folch–pi, 1971). Moreover, proteolipid protein (Davison, 1961; Sabri et al., 1974) and its associated tightly bound lipids (Uda & Nakazawa, 1973) have a high degree of metabolic stability. At least some of the basic protein is slowly catabolized and may also contribute to the relative inertness of myelin. Since the high-molecular-weight proteins can be removed from myelin fractions by repeated centrifugation (Waenholdt & Mandel, 1972) and are metabolized rapidly (Smith, 1972; Sabri et al., 1974) these proteins may not be involved in the stability of myelin.

As a result of trypsin treatment of myelin there is release of a lipid-rich low-density material composed of single membrane vesicles resembling phospholipid micelles (Plate 1, Table 6). Similar floating fractions can be isolated after centrifugation of degenerating nervous tissue (Norton et al., 1966; Gerstl et al., 1966; Smith, 1973; Ramsey et al., 1974), but high concentrations of cholesteryl esters are present in such low-density material. There is evidence to suggest (Einstein et al., 1972; Hallpike & Adams, 1969; Cuzner & Davison, 1973; Ramsey et al., 1974) that these changes are primarily the result of proteolytic digestion of the myelin basic encephalitogenic protein and our studies with trypsin therefore provide an experimental model for the degenerative process. Ramsey & Davison (1974) have demonstrated that cholesteryl esters are formed on incubation of nervous tissue with phospholipase. We are therefore looking for the co-operative effect of phospholipases on proteolysis of myelin to account for the dissolution of the membrane, loss of basic protein and synthesis of cholesteryl ester as occurs in demyelination.

We are grateful to Dr. D. Landon for electron microscopy, to Mr. Kishor Gohil for his invaluable assistance, to Mr. H. Goodwin for lipid analysis and to the Multiple Sclerosis Society of Great Britain and Northern Ireland for their support.

References


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