THE IN VIVO AND ULTRASTRUCTURAL EFFECTS OF INJECTION OF LYSOPHOSPHATIDYL CHOLINE INTO MYELINATED PERIPHERAL NERVE FIBRES OF THE ADULT MOUSE

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SUMMARY

The action of phospholipase A and lysophosphatidyl choline (LPC) on mature, myelinated peripheral nerve fibres has been studied in vivo and electron microscopically, following subperineurial injection of these substances. Within 30 min, demyelination was observed in vivo along cylindrico-conical segments, spreading from Schmidt–Lanterman incisures and nodes of Ranvier. By 96 h, all traces of the myelin sheath had disappeared from the area of the lesion, and had been replaced by debris-laden cells lying in chains parallel to one another and the long axis of the fibre. During the next few weeks these cells gradually disappeared, and numerous finely myelinated axons, running between, and in continuity with, the normal fibres proximal and distal to the lesion were observed. If lower concentrations of LPC were used the number of fibres involved decreased, although the demyelinative changes followed the same time-course.

Ultrastructurally, demyelination involved progressive disruption and removal of the lamellar sheath, observed initially as a splitting of the intraperiod line within 30 min. Subsequent breakdown resulted in the formation of strands of 4–6 nm repeat material which was further degraded through quintuple- and triple-layered lamellar units to foam-like systems of disorganized lamellar fragments. The Schwann cell and axons appeared to be undamaged by phospholipase A and LPC, and retained their normal impermeability to exogenous ferritin.

The significance of the demyelinating capacity of LPC in vivo is discussed in terms of its known action on myelin in vitro, the rapidity and apparent specificity of its action demonstrated in this study, and its potential involvement in pathological demyelination.

INTRODUCTION

One view of the pathogenesis of the collectively grouped ‘demyelinating diseases’ has implicated the local action of some myelinolytic factor, or factors, possibly hydrolytic enzymes, or non-enzymic surface-active agents (Marburg, 1906; Thompson, 1961; Hallpike & Adams, 1969). Of interest in the latter category are the lysophosphatides, in particular lysophosphatidyl choline, the strongly detergent substance produced by the action of phospholipase A on phosphatidyl choline. It has been demonstrated that both phospholipase A and lysophosphatidyl choline can bring about demyelinating change in vitro in the CNS (Morrison & Zamecnik, 1950; Birkmayer & Neumayer, 1957); more recently Périer (1965) obtained demyelination and subsequent remyelination in vitro with cultured rat cerebellum treated with lysophosphatidyl choline. Moreover, lysophosphatidyl choline has been shown to produce
a rapid 'clearing' of brain homogenates in saline, this action being accompanied by the release into solution of a number of intracellular enzymes in an active state (G. T. Webster, 1957). It will also produce a complete solubilization of brain myelin (Gent, Gregson, Gammack & Raper, 1964).

However, in these situations the normal physico-chemical environment of the sheath has been grossly disturbed, with the possible resultant exposure of previously inaccessible binding sites; again, such traumatization may of itself initiate demyelinating changes so that the reaction to lysophosphatidyl choline is of secondary importance. It was decided to investigate the effects of varying doses of phospholipase A and lysophosphatidyl choline on peripheral myelinated nerve fibres, using a technique which allows observation of the fibres in vivo, in an attempt to determine the demyelinating capacity of these substances in relatively undisturbed tissue.

**MATERIALS AND METHODS**

*Preparation of lysophosphatidyl choline (LPC)*

LPC was prepared from egg lecithin following treatment with snake venom (*Ankistrodon p. piscivorus*) (W. L. G. Gent, N. A. Gregson & C. Lovelidge, in preparation). Solutions of LPC were made up with sterile isotonic saline, to give LPC concentrations of 20, 10, 5, and 2 mg/ml. Freshly prepared solutions were used for each batch of injections.

**Table 1. Programme of injections and examinations**

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<th>Period following injection</th>
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* PHA<sub>1</sub> = phospholipase A<sub>1</sub>.  
† LPC = lysophosphatidyl choline.  
§ Two mice from each group received an additional injection of a 10% solution of ferritin (Calbiochem, Ltd.) sub-perineurially, into the LPC-treated nerve, 30 min before the nerve was excised for processing for electron microscopy. All nerve bundles were observed in vivo prior to excision and fixation for electron microscopy.
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Injections of LPC and phospholipase A₂ and in vivo observation

Adult mice were used throughout the experiment. The animal, anesthetized with Nembutal injected intraperitoneally, was placed on a cork block, the skin of the upper thigh shaved and incised, and the sciatic nerve was gently exposed between the bellies of vastus lateralis and biceps femoris. The animal was then positioned on the stage of a Meopta binocular dissecting microscope (final magnification x 22.4). Using a micromanipulator, the 1-μm tip of a freshly pulled hard-glass micropipette was introduced into the fibre bundle beneath the perineurium and the volume of solution contained within the tip (about 0.0002 ml) injected, by applying slight positive pressure to the syringe plunger. Fibre bundles were examined in vivo using the oblique incident light method (Williams & Hall, 1970). In 10 mice, the fibre bundles were examined immediately after injection, for periods up to 2 h. In the remainder, however, the wound was closed and the animals allowed to recover. Subsequently, injected fibre bundles in these mice were examined in vivo at intervals from 17 h to 75 days post-operatively. In 6 control mice, the sciatic bundle was injected with sterile isotonic saline, and the fibres examined in vivo after 30 min, 96 h, 6 and 14 days. In 2 further mice, the nerve bundle was excised 10 min after injection of LPC, placed unteased in a solution of LPC (10 mg/ml) and observed in vitro using oblique incident light for a period of 15 min. The numbers of experimental animals examined at the various dose levels are shown in Table 1.

Electron-microscopical preparation

Small pieces of excised, previously injected, nerve were immersed in 3 % glutaraldehyde in phosphate buffer, pH 7.3, for 90 min, washed overnight in buffer and postfixed in 1 % OsO₄ in Millonig’s phosphate buffer at pH 7.3 for 90 min. All fixation procedures were carried out at 4 °C. The specimens were dehydrated in a series of ethanols: 10 % (10 min), 70 % (20 min), and 100 % (3 changes of 20 min each) at room temperature, then immersed in propylene oxide (2 changes of 15 min each) and finally infiltrated and embedded in TAAB resin, polymerized at 60 °C for 48 h. Silver-grey sections were cut on an LKB ultramicrotome, stained with uranyl acetate and lead citrate (the ferritin-injected specimens were left unstained), and examined in RCA EMU3 and EMU4 electron microscopes.

RESULTS

In vivo observations following the injection of LPC at a concentration of 10 mg/ml

Within 1 h of the injection of a solution of LPC, 10 mg/ml, into the main sciatic bundle, changes occurred in the morphology of many of the myelinated fibres which were consistent with demyelination. The sheath along whole cylindrico-conical segments gradually thinned until only an attenuated band of material, optically similar to the original sheath, remained around the axon (Fig. 1). The Schmidt–Lanterman incisures were dilated and the paranodal myelin was retracted, the latter effect frequently being accompanied by a loss of the paranodal myelin profiles. Incisural dilatation was not as extreme as that seen immediately after local trauma (Williams & Hall, 1971) and ovoid formation was not seen. In some instances irregular unilateral convolutions of the total thickness of the sheath, particularly in relation to Schmidt–Lanterman incisures, were a feature of fibres within the field of view. In 2 cases, the nerve bundle was excised 10 min after injection of LPC, placed unteased in a solution of LPC (10 mg/ml) and observed using oblique incident light for a further 15 min in vitro. During this period, many fibres already demyelinating along cylindrico-conical segments lost their myelin sheaths along successive whole internodal lengths. Typically the axons appeared to be surrounded by somewhat swollen cylinders of pale
material which remained discontinuous at the nodes, with concomitant loss of para-
nodal asymmetry and myelin profiles. Fibre bundles were examined in vivo at various
intervals after injection of LPC, 10 mg/ml (2, 17, 22 and 96 h, 6, 14, 20, 25 and
35 days).

Visualization of sites in a nerve that has previously been subjected to any surgical
procedure is made difficult by ensuing connective tissue hyperplasia around these
sites. While the observation of fibres proximal and distal to the injected area was per-
formed as easily as normal, it was found that careful focusing down through a rela-
tively dense cellular layer was necessary to permit description of fibre morphology
in the injected zone. During the first 96 h it became apparent that all the fibres in the
field of view within a well localized zone, approximately 0·2-0·5 cm in length, were
undergoing demyelination. Proximally and distally (in both common peroneal and
tibial divisions of the sciatic), the fibres appeared normal, i.e. with closed incisures of
Schmidt–Lanterman, small (less than 1 μm) nodal gaps, asymmetric paranodal bulbs,
with myelin profiles, and smooth external sheath contours. Very occasionally, a fibre
was seen which distally exhibited changes associated with Wallerian degeneration:
similar occasional distal degeneration was seen in saline-injected controls, and is a rare
feature of normal, previously undisturbed nerve bundles. By 96 h, all traces of the
myelin sheath had disappeared from the affected region, and the field was composed of
long, thin cellular elements, the majority apparently arranged in chains parallel to
each other and to the long axis of the fibre. These cells, which resembled those seen
in vivo during Wallerian degeneration (Williams & Hall, 1971), contained small,
optically homogenous globules, packed between large irregular amorphous masses
of material (Fig. 2). In some instances it was possible to detect surface indentations
on these masses, giving them a whorled appearance. These cells remained over the next
week, gradually disappearing thereafter. During the demyelinating phase it was not
possible, using oblique incident light, to determine the fate of the axons, and the demon-
stration of their persistence, inferred from the presence of normal fibres distal to
the demyelinating lesion, was dependent upon subsequent electron microscopy.

Observation in vivo of fibres treated with LPC 14–25 days previously revealed
numerous finely myelinated axons, running between, and in continuity with, the
normally myelinated fibres proximal and distal to the lesions, with a progressive
increase in sheath thickness with time (Figs. 3, 4). The external contour of these
remyelinating fibres exhibited a certain degree of undulation, of the type charac-
teristically observed in newly myelinating fibre populations in neo-natal mice, i.e.
unilateral outpushings involving the total thickness of the sheath, and deviations in
the course of the fibre as a whole in its passage through the reactive zone. Schmidt–
Lanterman incisures were frequently observed as small bilateral dark lines, crossing
the remyelinating sheath; on occasions moderately open incisures were present
(Williams & Hall, 1970). Nodes of Ranvier were seen within the remyelinating zone,
typically associated with myelin sheath infoldings, and at the proximal and distal
borders of the lesion, where nodes were composed of a hemi-node of thin myelin, and
a hemi-node of normal, much thicker myelin. There were no indications of short,
termed internodes (Lubinska, 1958; Berthold & Skoglund, 1968). During the
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14- to 20-day period there was an increase in the number of cells whose position (outside the longitudinal cellular chains) and shape were similar to the macrophages which invade the bundle in Wallerian degeneration, become lipid-laden, and gradually assume a sub-perineurial position. Such cells were less numerous by the end of the third post-operative week, when myelin debris was almost entirely removed from the injection site. Remaining debris, appearing as whorls and globules of material, was generally observed in close association with remyelinating fibres, lying in concavities of total sheath contour.

Effects of varying LPC concentration

A series of mice was injected with LPC solutions containing 20, 5 and 2 mg per ml, and examined at time intervals up to 25 days. The morphological changes were indistinguishable from those observed with a concentration of 10 mg/ml, and they followed the same time course. The only observable consequence of reducing the concentration was that the number of myelinated fibres involved decreased with the 2 lower dose levels; thus at 2 mg/ml, only 2–3 fibres could be demonstrated undergoing demyelination. Injection of phospholipase A solutions, 20 and 2 mg per ml, produced demyelinating changes similar to those described following LPC injection as regards time-course and graded response to concentration levels.

The saline-injected controls appeared normal in vivo, with the exception of very occasional degenerating fibres, which may have been related to the injection or, more probably, were an incidental finding, since similar fibres are also seen in normal previously undisturbed fibres in vivo.

Electron-microscope findings

The initial response of the sheath to phospholipase A, 20 mg/ml, and all dose levels of LPC, was a splitting of the intraperiod line of compact myelin, and a widening of the incisural intraperiod line gap (Fig. 5). These changes occurred within 30 min of administration of the cytolytic agents. Subsequent breakdown, more rapid in the smaller fibres, was indicated by the jagged 'saw tooth' appearance of the outer aspect of the sheath at low magnification. This outline was attributable to the persistence of pegs of relatively compact myelin between strands of 4-nm repeat material. In the pegs, the intraperiod line was split, and the major dense line thickened, to give an overall myelin repeat period of 18–25 nm. Occasionally, apparent cross-linkages were observed along the intraperiod line gap, possibly indicative of a regular globular substructure, similar to that seen within isolated myelin preparations after treatment with LPC (W. L. G. Gent, N. A. Gregson & C. Lovelidge, in preparation). The 4-nm repeat material was arranged in strands of varying widths, some strands being only a few lamellae thick, while others were composed of 10–20 lamellae. This material was apparently formed from, and continuous with, the components of the myelin pegs: the 60–80% overall diminution in repeat period involved in its production being effected by the recombination of the intraperiod lines and a reduction in width and density of the major dense lines, at the outer border of the pegs.

Subsequent breakdown of the sheath was initially orderly, resulting from an
apparently sequential production of quintuple- and triple-layered lamellar units (Fig. 7). A widening of the intraperiod line gap, within a range of 25-65 nm was responsible for the appearance of the quintuple-layered units, which consisted, therefore, of 2 outer hemi-intraperiod lines, separated from a central major dense line by 2 pale interzones. These lamellar units were most often seen within 48 h, lying parallel to one another and to the long axis of the fibre, constituting either the whole, or part, of the remaining sheath. Formation of the triple-layered unit occurred either at the edges of reasonably compact myelin, or within the rows of quintuple-layered units, in both cases by separation of the major dense line, itself associated with previously split intraperiod lines. Rounding off of the triple-layered units produced circular profiles, sometimes arranged in orderly arrays, more frequently observed as foam-like reticular systems in which fragments of parallel lamellae and larger masses of recognizable myelin were dispersed (Figs. 6, 8).

Throughout the exposure to phospholipase A and LPC in vivo the axons remained normal, i.e. there was no evidence of any alteration in the morphology of their populations of mitochondria, neurofilaments, microtubules or components of the endoplasmic reticulum. It was more difficult to determine the state of the Schwann cell cytoplasm during the early stages of LPC activity. Generally, the Schwann cell cytoplasm was either not apparent, in which case the fragmented lamellae of the sheath were separated from the endoneurial space by the Schwann cell basement membrane; or it was represented by thin processes of characteristically granular tissue bounded by plasma membrane and lying internal to the basement membrane. Such processes became more numerous as demyelination progressed, so that each axon was surrounded by several layers of Schwann cytoplasm. Initially these narrow processes were widened only in the perinuclear regions. Because of the difficulty in determining the state of the Schwann cell during initial exposure to LPC, it was decided to inject the electron-dense marker ferritin sub-perineurally into LPC-treated fibre bundles, on the assumption that the free cytoplasmic distribution of ferritin was indicative of cell damage. The distribution of ferritin in normal peripheral nerve has been described recently; there is an absence of ferritin free or in vesicles in axoplasm, or free in myelin sheath and Schwann cytoplasm, but it is occasionally present in vesicles in Schwann cytoplasm (Hall & Williams, 1971). In the experiments with demyelinating fibres, no ferritin was found in axoplasm, nor was any observed free in debris-laden Schwann cytoplasm, or in the narrow fingers of Schwann cytoplasm. Occasional vesicles laden with the tracer, within Schwann cytoplasm, and invaginations of Schwann cell plasma membrane enclosing accumulations of ferritin were, however, observed. Ferritin was also seen, in much diminished concentration, along the periaxonal space of demyelinated fibres, within the mesaxonal space of incipient remyelinating fibres, and within the foam-like reticular systems of degenerating lamellae.

At the outer limits of the lesion, as indicated by half-demyelinated nodes of Ranvier, the nodal gap fingers appeared normal, with an apparent contribution from the demyelinated side, and the basement membrane was continuous across the nodal gap. By 48 h, most axons within the lesion produced with high-dose levels of LPC, and the few involved after injection of low-dose levels, were surrounded by the remnants
of their sheaths. Where more extensive areas of Schwann cell cytoplasm could be seen they were found to contain large myelin forms, crystalline bodies and homogenous globules, similar to those seen in Schwann cell cytoplasm during Wallerian degeneration. Within 6 days, much of the debris was removed from the fibre, and was observed either free in the endoneurial space, lying along the outer aspect of the basement membrane of the Schwann cell, or in the few macrophages which infiltrated the bundle. The remaining debris, although no longer in close association with axon, remained in the Schwann cell cytoplasm (Figs. 9–12).

Remyelination was well established in the 14-day samples (Fig. 14), with many axons surrounded by 2–12 lamellae of normally compacted myelin. Schmidt–Lanterman incisures and nodes of Ranvier were similar to those in the more thickly myelinated fibres in the zone proximal and distal to the lesion. Along the fibres, however, irregularities in myelin compaction were seen; these could generally be correlated with the outpushings described in vivo (see above), although occasionally only two or three wandering lamellae were involved, and were thus at or beyond the limit of resolution of the oblique incident-light optical system. Similar variations in mesaxonal position have been described by H. Webster (1969) in developing rat sciatic nerve. By the 40th day, it was difficult to determine the remyelinated fibres in produced lesions by high dosages, and impossible to find those that had been subjected to low doses of LPC.

DISCUSSION

The demyelinating capacity of phospholipase A and LPC, already described in CNS tissue in vitro (Morrison & Zamecnik, 1950; Birkmayer & Neumayer, 1957; Périer, 1965) has been demonstrated in mature, myelinated peripheral nerve fibres in vivo; we consider that the experimental results presented here raise 2 major points: (1) the action of LPC itself whether exogenous or endogenous, on the intact sheath in vivo; and (2) the tissue reaction during, and subsequent to, demyelination.

The action of LPC is interesting in that it is remarkably rapid and, as judged by the electron-microscope appearance of the tissue, restricted to the myelin sheath, unless very high concentrations are injected. Thus there is no apparent damage, on morphological criteria, to the Schwann cell cytoplasm or to the axon, unlike the axonal changes which have been associated with other experimental demyelinating models, such as diphtheritic polyneuritis (McDonald, 1967). Examination of the distribution of ferritin within the endoneurial space of fibre bundles previously injected with LPC confirmed the assumption that the Schwann cells and axons remained intact; no ferritin was observed free or in vesicles within any axons during the demyelination/remyelination sequence, nor was there evidence of free ferritin within Schwann cell cytoplasmic processes. It is interesting that ferritin was observed in the periaxonal space of completely demyelinated fibres within the foam-like debris bounded by the Schwann cell basement membrane, and in a few cases, was seen within the widened intraperiod line gap of the demyelinating sheath: such findings indicate access of these structures to the general extracellular space surrounding the fibre.

The observation that on decreasing the concentration of administered LPC from
10 to 2 mg/ml, i.e. from $2 \times 10^{-6}$ g to $0.4 \times 10^{-6}$ g, the number of affected fibres is greatly diminished, is taken to indicate that some of the LPC injected subperineurally is rapidly bound and rendered ineffective. This binding is presumably to extraneous protein and is compatible with the observation of Pérèr (1965) that LPC was effective at much lower concentrations when added to tissue culture in salt solutions than when added in normal protein-containing media. Certainly serum albumin is capable of binding LPC (Steim, Edner & Bargoot, 1968; Klopfenstein, 1969). Maintenance of some demyelination even with the low concentrations is taken to support the idea that there is a direct interaction between LPC and the sheath, and that some minimal amount must be absorbed to the sheath before the latter becomes sufficiently unstable for solubilization to occur.

The rapidity with which LPC appears to initiate demyelination is almost unique, obvious thinning of the sheath, observable with incident illumination light microscopically, occurring within 30 min of injection. It seems reasonable to consider that the demyelination demonstrated in this experimental study is produced by a primary attack on the myelin sheath, rather than as a consequence of a defunct satellite cell. The precocious degeneration of incisures and paranodes favours this assumption. The spread of demyelinating changes along cylindrico-conical segments suggests the rapid ingress of the cytolytic substances along incisural and paranodal extracellular space channels (Hall & Williams, 1971); the immediate accessibility of a large surface area of myelin membrane which these routes provide, effectively 'by-passing' the Schwann cell plasma membrane and cytoplasm, resulting in the initial uptake of relatively high levels of LPC in these regions. However, since LPC is a general lytic agent (Marple, Thompson & Webster, 1959) it is interesting to consider the apparent specificity of the LPC in attacking the sheath, rather than the other membranes within the fibre bundle. It is possible that peripheral myelin has a particularly high affinity for LPC, or, alternatively, that the lack of interaction between the LPC and other membranes is apparent rather than real. Systems have been described in which there is a deacylating-acylating cycle for phosphatidyl choline (van Deenan, 1965); recently Stein, Widnell & Stein (1968) have demonstrated that labelled phosphatidyl choline appears in liver plasma membrane 5 min after injection of labelled LPC. Neural tissues have been shown to possess the ability to acylate LPC quite rapidly (G. Webster & Alpern, 1964). Consequently, other cell membranes may well bind LPC, but then rapidly convert it to phosphatidyl choline, and thus circumvent its lytic action.

The ultrastructural consequence of the action of LPC on the myelin sheath was a progressive lamellar disruption of the sheath contained within the Schwann cell basement membrane, beginning with splitting of the intraperiod line and thickening of the major dense line (Fig. 7). The altered lamellar units observed in the early stages of sheath breakdown are similar to those described in X-irradiated cultures of rat dorsal root ganglion (Masurovsky, Bunge & Bunge, 1967), although the changes these authors report occur 1 or 2 weeks after exposure to radiation, after extensive degeneration of the associated Schwann cell. A splitting of the intraperiod line is the typical feature of myelin sheaths which have been subjected to experimental manipulation, whether osmotic, mechanical (Robertson, 1958; Millington & Finean, 1961; Elfvin,
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1961), chemical (Aleu, Katzman & Terry, 1963; Scheinberg, Taylor, Herzog & Mandell, 1966; Hirano, Zimmerman & Levine, 1965), or pathological, particularly in association with Wallerian degeneration (Glimstedt & Wohlfart, 1960; Williams & Hall, 1971), experimental allergic encephalitis* (Lampert, 1965) and diphtheritic polyneuritis in peripheral nerve myelin (H. Webster, 1964). Lamellar disruption at the major dense lines is a far less frequent feature of the damaged sheath, although it has apparently been described in cochlear nerve fibres undergoing demyelination (Engstrom & Wersall, 1958). However, the thickening of the major dense line produced by LPC seems to be unique to this demyelinating system. The splitting of the intraperiod lines is generally interpreted as indicating an increase in the hydration of the sheath at the intraperiod line. It may be, however, that this rather more extreme separation of the intraperiod line reflects an increase in the hydrophilic nature of the molecular components of the sheath attributable to the addition of LPC (Gent, Gregson, Lovelidge & Winder, 1971).

This expansion of the myelin structure is also seen when LPC is added to CNS myelin in vitro. It is produced by the addition of approximately half the total amount of LPC required to solubilize the myelin completely, and its appearance is correlated with the loss of a component containing the principal part of the basic protein (W. L. G. Gent, N. A. Gregson & C. Lovelidge, in preparation). The second half of the solubilization process is marked by the collapse of the expanded structure to give a 4-nm repeating lamellar structure in which the bands of electron-dense material are narrow, and of identical density. This phase is also seen in peripheral nerve treated with LPC in vivo. No distinction between intraperiod and major dense lines can be made. In this state the myelin structure closely resembles that seen within the dilated pericentral spaces and paranodal regions immediately following nerve crush (Williams & Hall, 1971).

A similar myelin morphology has been reported by Dickinson, Jones, Aparicio & Lumsden (1970) as a consequence of post mortem change or the incubation of isolated myelin under conditions of low pH. These authors also find that the morphological change is associated with release of basic protein. They further conclude that the intraperiod line has been lost, hence that the basic portion may be associated with the intraperiod line. This is, however, a 'before' and 'after' comparison, and such conclusions are based on slender evidence. Indeed, Adams, Bayliss, Hallpike & Turner (1971) using electron-microscope histochemical techniques suggest the major dense line as the more likely site for association of the basic protein. In our own experiments and those on isolated myelin, it is obvious that the release of basic protein effected by LPC is accompanied by a major structural rearrangement of the myelin; under such circumstances the credence that may be placed on claims of the specific association of basic protein with either osmiophilic line is questionable.

Phospholipase A2 produced an effect on the preparation identical to that produced by LPC, even at the ultrastructural level. This suggests that phosphatidyl choline (or possibly phosphatidyl ethanolamine) is amenable to hydrolysis by this enzyme, and that the amount of LPC so released is sufficient to produce the demyelination. Since the other cellular elements do not appear to be damaged after injection of phospholi-
pase A₂, we must conclude that the phospholipid of the myelin sheath provides the substrate. Cooper & Webster (1970) have shown that phospholipase A₁ is present in human sciatic nerve, but not A₂. This latter enzyme would produce the 2-acyl lysophosphatidyl choline rather than the 1-acyl lysophosphatidyl choline used in this study. It is not known whether the 2-acyl lysophosphatidyl choline has lytic activity similar to that of the 1-acyl compound, because it readily (and rapidly) isomerizes into the 1-acyl form.

It is interesting that certain of the initial consequences of LPC action on the peripheral sheath resemble the changes observed in the early phases of Wallerian degeneration: incisural dilatation, splitting of the intraperiod line and lamellar collapse to 4–6 nm repeat material (Williams & Hall, 1971), whereas other agents, such as trypsin, produce quite different ultrastructural changes in the fibre (unpublished observations). Martin & Rosenberg (1968) have demonstrated the ability of phospholipase A to effect a marked breakdown of the Schwann sheath of excised squid giant axon. However, in the present study, Schwann cell death is rare, the apparent absence of Schwann cytoplasm during the early response to LPC in some fibres, and the subsequent re-appearance of thin cytoplasmic process around the denuded axons, most probably reflecting an initial retraction of the Schwann cell similar to that described in vitro in diphtheritic demyelination (Murray, 1965). Since axonal continuity and Schwann cell-axon contact are both apparently undisturbed, remyelination commences rapidly once the degraded myelin has been removed.

Several authors have suggested the possible involvement of intrinsic phospholipases, and their products, in the process of demyelination (Marburg, 1906; Thompson, 1961). While the present experiments suggest that LPC, whether exogenous or produced in situ from endogenous phosphatidyl choline, is associated with a rapid demyelination in peripheral nerve, certain differences exist between such experimental demyelination and that described in pathological states. The most obvious differences are the lack of involvement of other cellular elements, and the time scale for the demyelination/remyelination process. The purity of the demyelination produced on injection of LPC is of course independent of the mechanical dislocation accompanying primary glial or axonal damage, as in Wallerian degeneration, and of the immune system, as in experimental allergic encephalitis and experimental allergic neuritis where demyelination occurs only in tissue infiltrated by pleomorphic mononuclear cells, macrophages and small lymphocytes (Wiśniewski, Prineas & Raine, 1969; Lampert, 1969).

REFERENCES


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Figs. 1-4. Fields observed *in vivo* after injection of LPC, 10 mg/ml. Oblique incident light.

Fig. 1. 30 minutes after injection. Two fibres exhibit myelin loss (arrows); in the upper fibre the outlines of the paranodal bulbs are faintly discernible. Most incisures of Schmidt-Lanterman are dilating, and certain irregularities of sheath contour are apparent.

Fig. 2. 6 days after injection. The field is composed of debris-laden cells arranged in approximately parallel chains.

Fig. 3. 14 days after injection. Finely myelinated fibres are observed crossing the field. Schmidt-Lanterman incisures are frequent features of the ribbon-like sheaths. Note the reduction in debris-laden cells as compared with Fig. 2.

Fig. 4. 25 days after injection. Schmidt-Lanterman incisures and nodes of Ranvier are prominent. Some undulations of sheath contour may be observed, particularly near nodes of Ranvier.
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Figs. 5 and 6. For legend see opposite.
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Fig. 7. Variations in periodicity of myelin following treatment with LPC.
Fig. 7A, B are densitometer tracings of regions A and B in the micrograph of myelin sheath of an axon after the injection of 20 mg/ml LPC solution.

Fig. 5. LPC, 20 mg/ml; 30 min after injection. Strands of 4–6 nm repeat material are separated by pegs of relatively compact myelin in which the major dense line is thickened and the intraperiod line is split. Osmiophilic cross- striations give the intraperiod line gap an apparent globular substructure. × 96,000.

Fig. 6. LPC, 10 mg/ml; 48 h after injection. Most of the sheath has broken down to triple-layered units, here arranged as circular and elliptical profiles, in a relatively ordered array. × 96,000.
Fig. 8. Phospholipase A, 20 mg/ml; 48 h after injection. The sheath is predominantly altered; the lamellar pattern is broken down into 5-layered units which in places have rounded up, and in others have become further dispersed into areas of membranous disarray (•). \( \times 45,000 \).

Fig. 9. LPC, 10 mg/ml; 48 h after injection. Low-power view of Fig. 6 demonstrating the precocious degeneration of part of the sheath, most probably an incisure of Schmidt–Lanterman. \( \times 50,000 \).
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Image 8

Image 9
Fig. 10. LPC, 10 mg/ml; 72 h after injection. Various lamellar changes are apparent in the sheath. The axolemma, however, is continuous, and the axoplasmic neurofilaments appear normal. × 96,000.

Fig. 11. LPC, 10 mg/ml; 96 h after injection. A somewhat tortuous, but otherwise normal axon, surrounded by the remnants of the disrupted sheath, contained within the Schwann cell basement membrane. × 11,000.

Fig. 12. LPC, 10 mg/ml; 6 days after injection. Further removal of solubilized myelin has occurred. Schwann cell cytoplasm is apparent in the lower right corner. × 23,000.
Fig. 13. LPC, 10 mg/ml; 6 days. Completely demyelinated axon, surrounded by Schwann cytoplasm, containing numerous free RNP particles. × 57,000.

Fig. 14. LPC, 10 mg/ml; 14 days. Remyelinating axon, with Schmidt–Lanterman incisure. Schwann cytoplasm contains swollen endoplasmic reticulum, filaments and rounded mitochondria. × 30,000.
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