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Chronic Wallerian degeneration – an *in vivo* and ultrastructural study

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INTRODUCTION

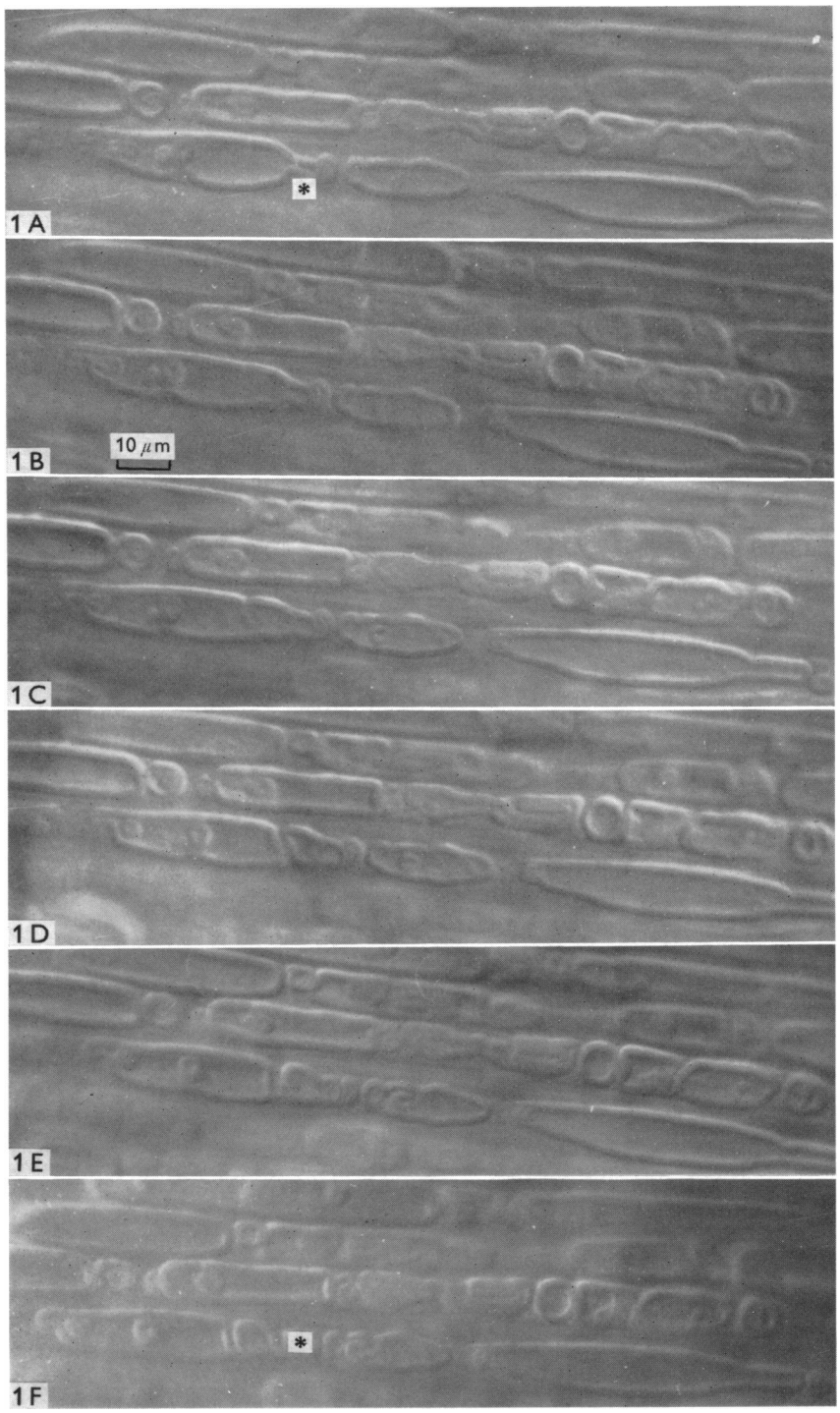
The rapid morphological responses of peripheral nerve fibres to a proximally situated crush *in vivo* were the subject of a previous communication (Williams & Hall, 1971). This paper describes the results of *in vivo* observation of the subsequent changes, particularly those of Wallerian degeneration, over a 60-day period. *In vivo* observation was also used to monitor various stages which were analysed ultrastructurally.

MATERIAL AND METHODS

The oblique incident-light method for the observation of mature myelinated peripheral nerve fibres *in vivo* has been described previously (Williams & Hall, 1970, 1971; Hall, 1970). Eighty-five adult mice, of either sex, were used in this study. Under Nembutal anaesthesia, the sciatic nerve was crushed unilaterally in the upper thigh with fine watchmaker's forceps for 5 sec, and the fibres in the exposed nerve bundle immediately distal to the crush were observed for varying periods of time *in vivo* to confirm the initiation of Wallerian change. The wound was subsequently closed and the animals allowed to recover. Fibres in the common peroneal, anterior tibial, or tibial branches were observed continuously *in vivo* for periods of 1–4 h, from the 2nd to the 60th day after crushing, at distances up to 4 cm distal to the crush. Three mice were examined each day until day 20, after which time three mice were examined every 5 days.

In four mice the perineurium was carefully removed under a dissecting microscope 20 days after the crush, immersed in isotonic saline on a slide and examined *in vitro*, using both oblique incident light and transmitted light. The denuded sciatic bundles from these mice were examined *in vivo* under oblique incident light, and were then excised and teased gently in isotonic saline to allow transillumination. They were then examined with both Köhler illumination and with plane polarized light.

Representative samples were prepared for electron microscopy from 25 mice. Each sample was removed rapidly from the body and placed immediately in 3% glutaraldehyde (pH 7·3) in phosphate buffer for 90 min. Samples were washed overnight in buffer, and post-fixed in 1% OsO_4 in Millonig's phosphate buffer at pH 7·4 for 90 min. All fixation and washing was carried out at 4 °C. Tissues were dehydrated in ethanol: 10% (10 min), 70% (20 min), 100% (60 min – 3 × 20 min), and then placed in propylene oxide for 30 min (2 × 15 min). Specimens were embedded in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TAAB resin and polymerized at 60 °C for 48 h. Sections (silver grey) were cut on an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined in an RCA EMU 4 electron microscope.

RESULTS

Light microscope findings

The earliest visible responses to crush confirmed the observations communicated in a previous paper (Williams & Hall, 1971); they occurred within 2 min over distances of at least 5 mm distal to the point of crush along fibres of all calibres. The changes involved dilatation of the incisures, alterations in the character of the node, with folding and retraction of paranodal myelin and widening of the nodal gap, and the development of undulations in the myelin sheath. Some small calibre fibres (4–6 μm diameter) showed a reaction as far as 2 cm distal to the crush during the first hour, but here and more distally the majority of fibres exhibited no change in the morphology of their incisures, nodes or myelin sheaths for 24 h. By 36 h, however, almost all fibres 2–4 cm distal to the crush were exhibiting the changes that had earlier involved their more proximal Schwann cell territories.

Extreme dilatation of most of the Schmidt-Lanterman incisures and splitting and retraction of paranodal myelin were succeeded by progressive constriction and final cutting-off of the axon at these points, resulting in the formation of primary ellipsoids. Thus, the majority of the primary ellipsoids corresponded to single enclosed cylindrico-conical and initial (Hiscoe, 1947) segments. However, some primary ellipsoids were longer, and included more than one cylindrico-conical segment, so that they contained closed incisures, or those in the early stages of change, in their walls. Small calibre fibres (4–6 μm diameter) often reacted with great rapidity, exhibiting the incisural and nodal changes described above; in addition, many developed an unduloid outline of alternating fusiform dilatations and constrictions, similar to the 'beaded' fibres described by Ochs (1965). The walls of these dilatations frequently contained closed Schmidt-Lanterman incisures, which subsequently dilated.

With the passage of time, the patterns of breakdown became more complex, and the primary ellipsoids became progressively shorter, so that by the fourth postoperative day few remained. Shortening followed three main events: (i) dilatation of the remaining incisures within the walls of the longer ellipsoids, followed by fusion of the infolding incisural material; (ii) the appearance of transverse striae in the rounded ends of the primary ellipsoids, the development of spaces between these striae, and subsequent peeling-back of strands of material into the interrupted axonal space; (iii) the appearance of sheath thickenings and infoldings, both unilateral and bilateral, which grew across the axonal space of the ellipsoid until they met and

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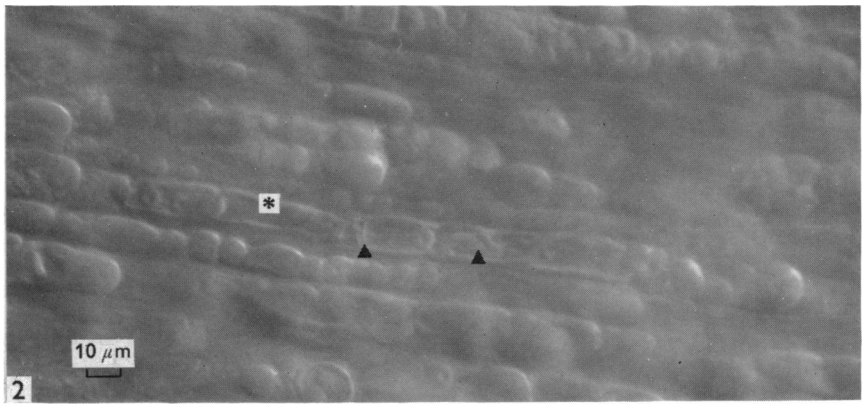
Figs. 1–10 are all from adult mouse common peroneal nerves observed *in vivo* using oblique incident illumination.

Fig. 1. A field of view 2 cm distal to a crush lesion, from a series of photomicrographs recorded during a 2 h period, 72–74 h after crushing. Note the changes resulting in the progressive subdivision of the end of a primary ellipsoid at *. A, at 72 h; B, 72½ h; C, 73 h; D, 73½ h; E, 73¾ h; F, 74 h after crush.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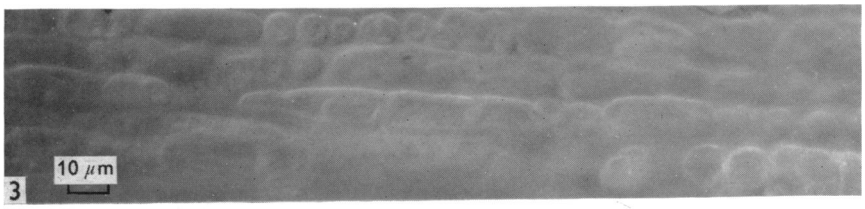
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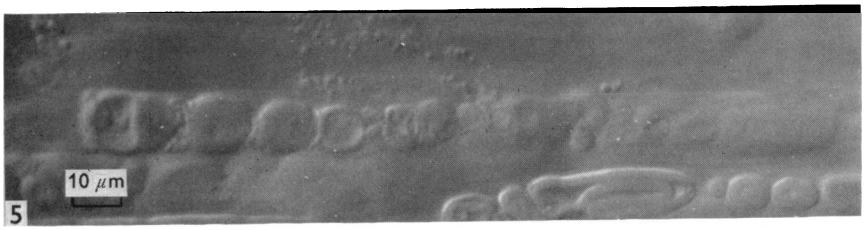
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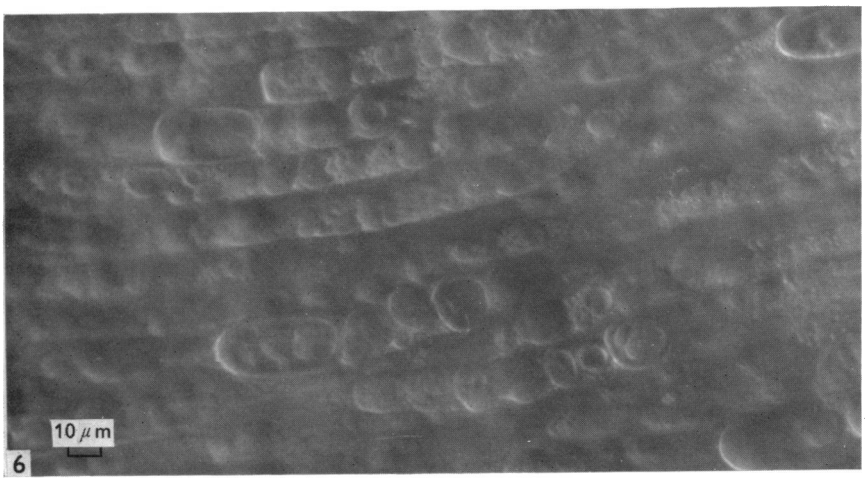
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fused (Fig. 1). In all cases the result of such changes was the progressive partitioning of the primary ellipsoid into irregular spherical and oval profiles.

By the beginning of the fifth day, the emergence of two different populations of profiles, still confined within the cylindrical territories of the degenerating fibres, was apparent: (i) numerous, homogeneous, very small circular globules 1–2 μm in diameter; (ii) relatively massive, circular and elongate profiles, up to 10 μm in diameter, which retained a rim of varying width which was optically similar to the original myelin sheath, but which often displayed internal foldings and surface irregularities. The very small globules were typically observed surrounding the large profiles, packing the spaces between them (Figs. 3–6). Continuous viewing *in vivo* demonstrated the pinching-off of the ends of ellipsoids and the margins of the larger profiles to form the small globules.

Until the fifth day the degenerating fibres, laden with the various profiles and small globules just described, were the characteristic feature, lying parallel to one another and to the longitudinal axis of the nerve, in more or less continuous columns. However, small globules were occasionally detected within flattened elongated cells which were distributed external to and between the fibres. Usually their long axes corresponded to those of the fibres, although occasionally cells were observed wrapped transversely around the fibres, particularly in those areas where the small globules were densely packed within the Schwann cell. There was a gradual increase in the number and size of the globule-containing cells within the endoneurial space between the fibres during the next 10 days. Usually the cells were approximately oval, sometimes with markedly irregular external margins, reflecting their content of very large numbers of the small globules. Their nuclei were observed as circular surface depressions devoid of globules: in some instances two or three such clear areas were present within a cell, suggesting the presence of a multilobulated nucleus.

Through-focusing at different time intervals indicated the progressive outward migration of the cells through the endoneurial space until, by the end of the first week, a few could be observed nearer the surface of the nerve than the degenerating fibres. Subsequent electron microscopy showed these cells to be immediately beneath the perineurium (*see below*). Over the period 7–30 days, the numbers of such cells increased considerably; whereas 3 days after crush none were visible, by 5 days they were observed within the endoneurial space, by 10 days they were filled with small globules, and by 20 days they constituted an almost continuous subperineurial cellular layer lying along the entire distal extent of the common peroneal and tibial nerves (Figs. 7–9).

Fig. 2. Ninety-six hours after crush. Note the persistence of a single myelinated fibre at *, with grossly dilated Schmidt-Lanterman incisures (arrows).

Figs. 3, 4. Five days after crush. The primary ellipsoids have been replaced by smaller, circular profiles and larger myelin whorls. The debris is still confined within longitudinal parallel chains, and no other cell types are present in the endoneurial space.

Fig. 5. Six days after crush. Small globules are present within the Schwann cell tubes, around the borders of larger myelin profiles, and scattered within the endoneurial space.

Fig. 6. Seven days after crush. Many small globules are present around the myelin whorls and within the endoneurial space. At several points, through-focusing revealed a continuity between the globules external to the fibres and those still within.


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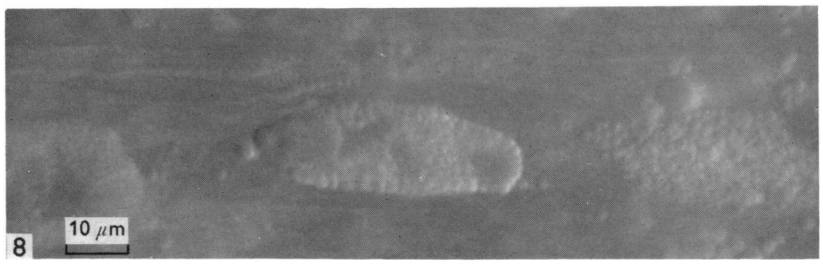
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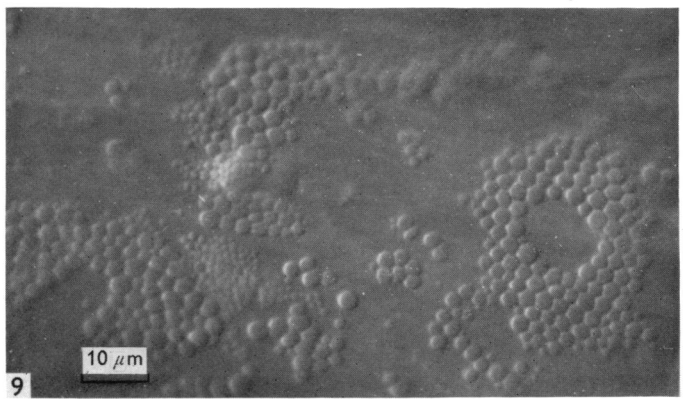
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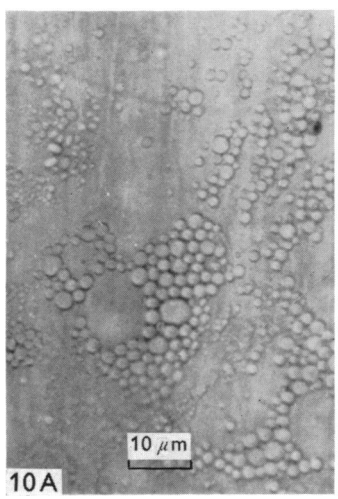
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During the remainder of the 60-day period there was a decrease in the population of globule-laden cells lying between and around the fibres, so that by the end of the second month only occasional profile-containing cells and mast cells remained between the fine remyelinating fibres which had now appeared.

Over a short distance (0·5 mm) proximal to the crush a few isolated small globules were seen between the otherwise normal nerve fibres, and also in a subperineurial position, but there was never any close packing between neighbouring globules, such as occurred distally. Further proximally, such globules were never seen, neither were any present in normal uncrushed fibres.

In the four preparations of the perineurium removed 20 days after the crush and examined *in vitro*, the only cellular features were flattened perineurial cells and adherent large epineurial fat cells. Examination of the remaining denuded nerve bundles revealed that the globule-laden cells remained in the bundle and, after teasing in isotonic saline, birefringence of the small globules was demonstrated by polarized light (Fig. 10A, B).

It is interesting that occasional fibres were markedly slower in reacting to the crush than their neighbours. For example, Fig. 2 shows a fibre in which dilatation of the incisures had only recently commenced, whilst in the surrounding fibres breakdown of the primary ellipsoids was already well advanced.

Electron microscope findings

During the first 4 days after the crush, there was a progressive disruption of the compact myelin sheath, the initial changes involving the incisures and nodes. At the nodes there was a retraction and peeling-back of the outer myelin lamellae, and separation of the paranodal terminal loops of Schwann cell cytoplasm, with the development of large electron-lucent spaces between individual loops and between the loops and the outer aspect of the axolemma. At the Schmidt–Lanterman incisures, dilatation followed the widening of the incisural intraperiod line gap, splitting of the intraperiod line in the peri-incisural region, and subsequent confluence of the spaces so formed (Hall & Williams, 1970). At both the node and incisure, strands of material derived from the peri-incisural and paranodal myelin, and exhibiting a 4 nm repeat distance of alternating dark and light bands, collapsed into these spaces and gradually came into contact with the axon, which became constricted at these points. Finally, fusion of the 4 nm repeat material across the fibre resulted in the formation of islands of axoplasm totally enclosed within myelin-bounded ellipsoids, the rounded ends of which were thus frequently composed of 4 nm repeat material. Simultaneously, wide spaces developed between the axoplasm and the degenerating wall of the ellipsoid, within which various lamellar and membranous profiles were seen.

While the convex terminations of the primary ellipsoids were predominantly com-

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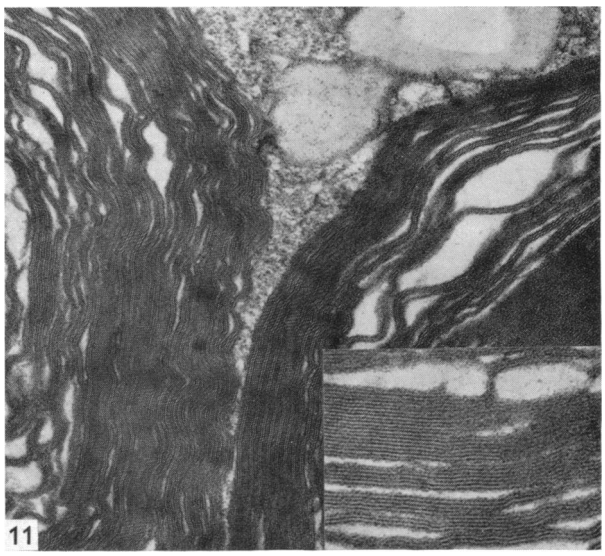
Figs. 7–9. Large globule-containing cells, observed at a level of focus higher than that employed in examining the degenerating fibres, i.e. the cells are occupying a subperineurial position. (Figs. 7, 8: 10 days after crush; Fig. 9: 15 days after crush.)

Fig. 10. Similar globule-laden cells, observed in an excised, slightly teased nerve bundle, the perineurium having been carefully removed. Twenty days after crush. A, Köhler illumination; B, plane polarized light.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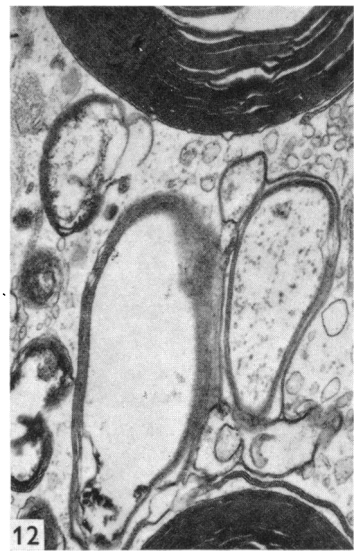
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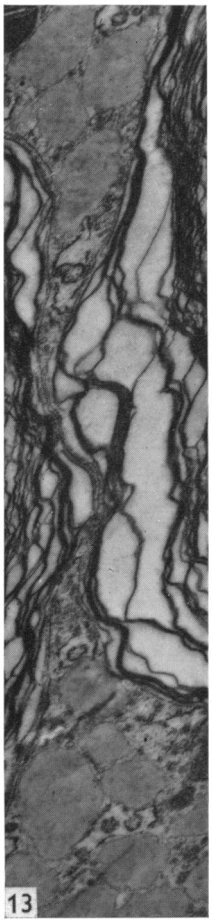
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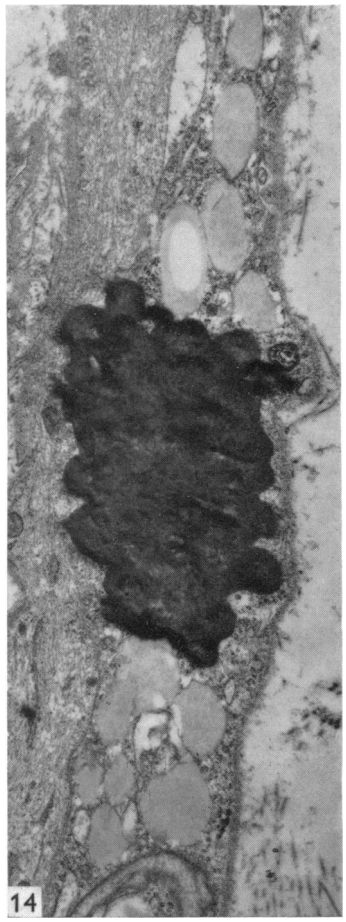
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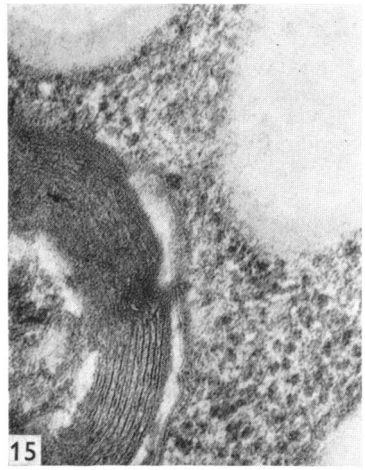
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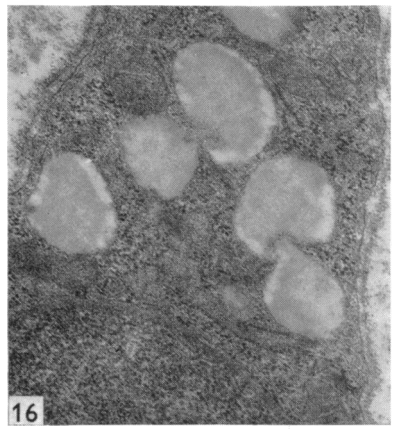
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posed of 4 nm repeat material, the walls of the larger profiles exhibited both 10 nm repeat lamellar myelin (Fig. 15) and 6–8 nm repeat material. Presumably these represented the ends of ellipsoids or profiles which had been formed by secondary infolding of the walls of the longer primary ellipsoids. The Schwann cell cytoplasm contained lamellar structures with varying repeat distances and density characteristics, ranging in shape from small hollow spheres enclosing granular material to longer ovoids, the latter often containing material of more than one lamellar type. By the fourth day a new type of degradation product appeared in the vicinity of the ends of the ellipsoids; that is, round or oval moderately electron-dense small bodies (Fig. 11). Some were membrane-bound, either completely or for only part of their circumference, while others possessed no limiting membrane and merged with the surrounding cytoplasm. Occasionally fragments of 4 nm repeat material were observed within these bodies, which corresponded to the small globules observed *in vivo* around the ellipsoid. Gradually the spaces between the ends of two apposed ellipsoids became filled with these globules (Fig. 13), which also extended into the neighbouring Schwann cell cytoplasm (Figs. 14–16). Finely granular material was often associated with the margins of the globules, particularly in situations where the globules appeared to be interlocking (Fig. 16).

Towards the end of the first week, the endoneurial space contained three main cellular populations: (i) the original Schwann cells, characterized by the presence of long ellipsoids containing patches of 4 nm repeat material, profiles containing 6–8 nm lamellar repeat material, and masses of small electron-dense globules, together with axoplasmic remnants; (ii) elongated narrow cells identified as newly differentiating Schwann cells, and characterized by a patchy basement membrane, pseudopodial processes, numerous free RNP granules, mitochondria and a few small globules; (iii) globule-containing macrophages, identified by the relative density of their cytoplasm, the absence of a basement membrane, and the presence of numerous pseudopodial processes and lysosomes. Occasionally, large mononuclear cells devoid of debris, eosinophils, degranulating mast cells and endoneurial fibroblasts were observed, the last two cell types particularly in the later stages of the response to crush.

Macrophages were observed after 3 days near the small blood vessels within the endoneurial space, and by the fifth day they were present throughout the distal extent of the nerve, lying adjacent to the outer aspects of the Schwann cell membranes of

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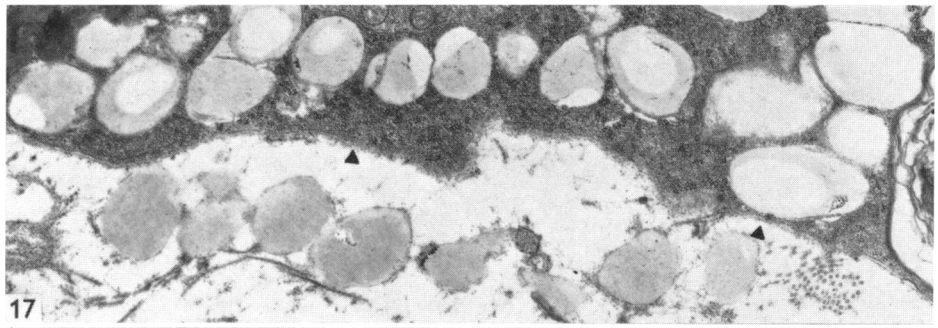
Fig. 11. Electron micrograph of an adult mouse sciatic nerve, 6 days after crush. Note the rounded ends of two ellipsoids containing 4 nm repeat lamellar material (see inset). Two lipid globules are present in the Schwann cytoplasm between the ellipsoids. Some extraction of lipid has occurred in the upper globule. × 56000; inset, × 96000.

Fig. 12. Six days after crush. Various forms of lamellar debris between the ends of ellipsoids (not shown) with the persistence of some apparently normal 10 nm repeat myelin. × 34200.

Fig. 13. Twelve days after crush. Numerous small lipid globules are packed in the spaces between the two ellipsoids; compare with Figs. 5, 6. × 11000.

Fig. 14. Seven days after crush, adult mouse sciatic nerve. Lipid globules, various lamellar forms and a dense irregular mass of degrading myelin are contained within a Schwann cell. × 16200.

Figs. 15, 16. The adult mouse common peroneal nerve 8 days after crushing the sciatic in the upper thigh. Note the varying lamellar repeat distances in the myelin form shown in Fig. 15, and the apparent interlocking of the small lipid globules in Fig. 16. Fig. 15, × 93000; Fig. 16, × 30000.



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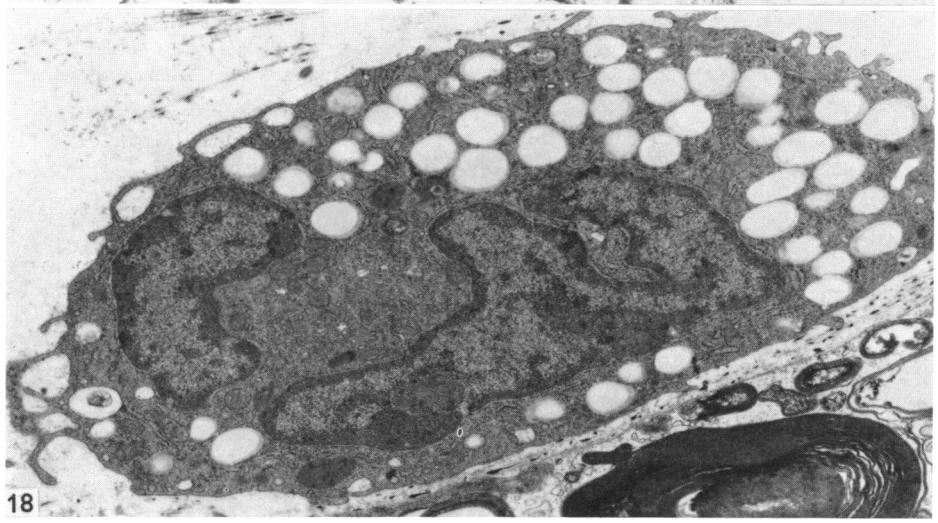

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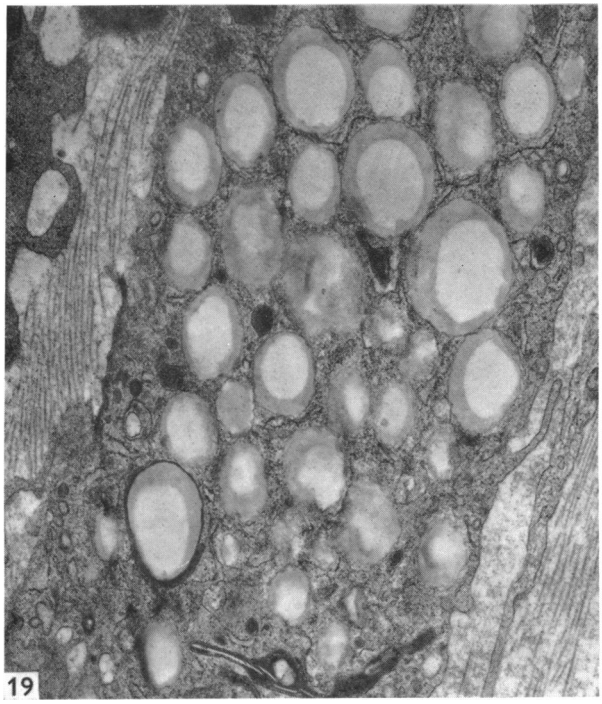

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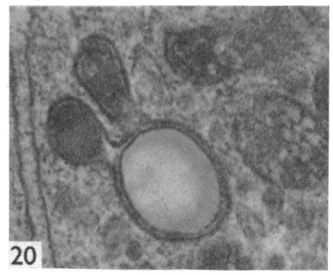

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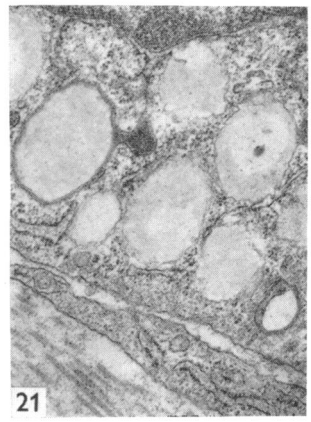

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the degenerating fibres. Very occasionally, long electron-dense macrophage processes were observed within the basement membrane of Schwann cells surrounding both myelinated and non-myelinated fibres (Fig. 22). However, there was little evidence to suggest the involvement of macrophages in the initiation of myelin breakdown, as has been described in experimental allergic encephalomyelitis (Wiśniewski, Prineas & Raine, 1969).

As degeneration continued, many globules passed from the Schwann cells into the endoneurial space, apparently extruded via discontinuities in the Schwann cell plasma membrane and basement membrane (Fig. 17). Usually the globules were membrane-bound, frequently with fragments of granular material adherent to them.

From the fifth day the macrophages became filled with small globules and, far less frequently, with more complex profiles. In most cases processing had extracted varying amounts of the material (presumably lipid) from the granules, which consequently only contained material in their periphery (Figs. 18, 19). Many of the globules, still membrane-bound, were associated with small dense bodies of the primary lysosome type (Figs. 20, 21), apparently following a fusion of their respective limiting membranes. The larger masses of debris, i.e. the lamellar profiles, remained within the original Schwann cells for a considerable time, and were still present when remyelination began. Little of this relatively gross debris was seen within the macrophages, presumably because it was not acceptable for phagocytosis by them, or because degradation followed a specific pattern within the Schwann cells prior to release of the small globules.

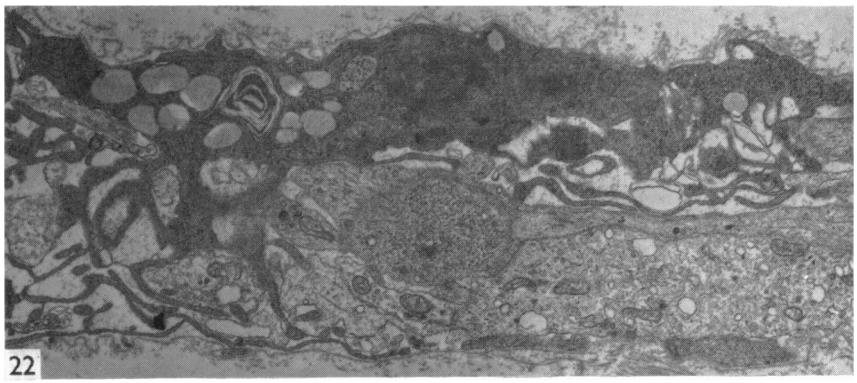
By the second week many globule-laden macrophages were present immediately beneath the innermost layer of the perineurium (Fig. 23); these corresponded in shape and position to the cells observed *in vivo* (*see above*). There was a gradual increase in the overall size of the globules with time, probably due to their progressive fusion, and eventually some macrophages showed large areas (6–8 μm wide) containing the same homogeneous material.

By the 25th day after the crush the cells of the innermost layer of the perineurium contained small globules (Fig. 24), and isolated globules were also present between the individual layers of the perineurium. The cells in question were unmistakably identified as perineurial since they were narrow, flattened, and possessed a continuous basement membrane, numerous pinocytotic vesicles and only a few mitochondria; they differed markedly from the globule-laden macrophages which occupied a subperineurial position. No macrophages were observed crossing the perineurium during the 60-day period, and yet most of the endoneurial macrophages disappeared,

Fig. 17. Seven days after crush, adult mouse sciatic nerve. Note the Schwann cell cytoplasm in the upper part of the photograph, laden with small lipid globules, and the small globules which have been extruded into the endoneurial space; the latter have some associated fragments of cytoplasm. Note the absence of a Schwann cell basement membrane (between arrows). × 16200.

Fig. 18. Adult mouse sciatic nerve, 6 days after crush. A large macrophage filled with small lipid globules lies adjacent to a degenerating fibre. No other form of myelin debris is present in the macrophage. × 11000.

Figs. 19–21. Further views of small lipid globules within the cytoplasm of macrophages which are occupying the endoneurial space. Figs. 19 and 21 are 21 days after crush, Fig. 20 is 12 days after crush. Note the association between dense membrane-bound primary lysosomes and individual lipid globules. Fig. 19, × 16200; Fig. 20, × 60400; Fig. 21, × 23900.



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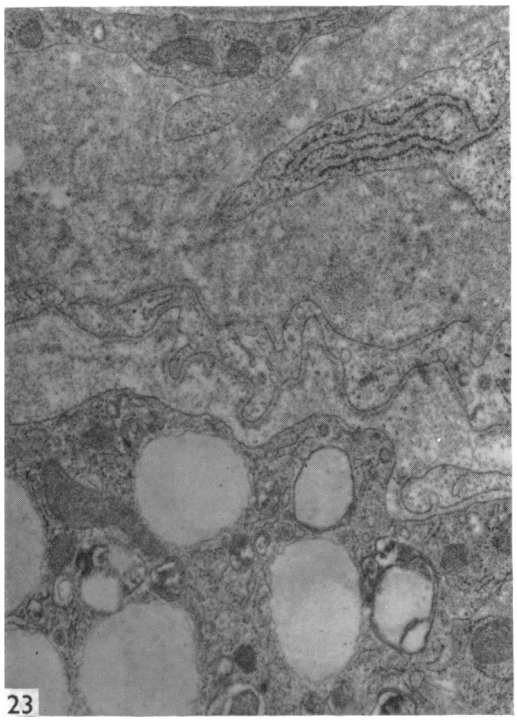

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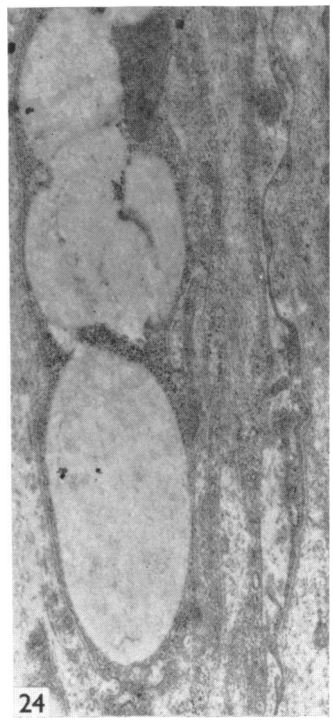

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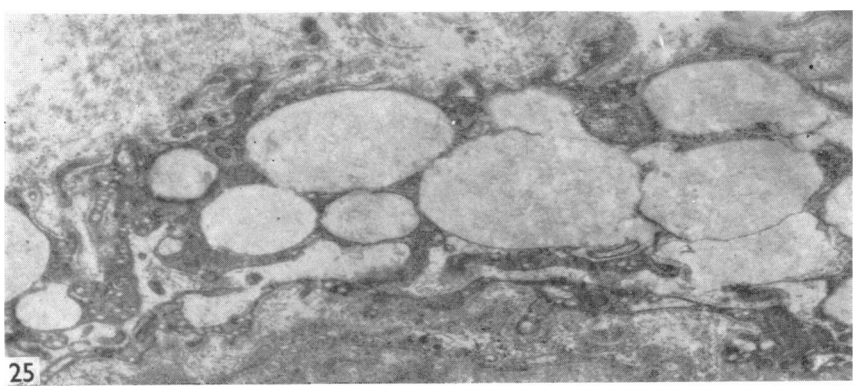

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presumably returning to the endoneurial blood vessels. Globules were observed within cells of all the layers of the perineurium (Fig. 25) and also in epineurial cells.

Little mention has been made of regenerating and remyelinating fibres during this survey. Many of the observations were made 2–4 cm distal to the lesion, and it was therefore possible, for much of the period, to analyse the cellular responses unobscured by the presence of regenerating fibres. The sequence of events observed *in vivo* during regeneration will be the subject of a further communication.

DISCUSSION

Continuous viewing *in vivo* during Wallerian degeneration has revealed not only the rapidity of the initial fibre response, but also the sequential morphological changes occurring within the next 60 days along the distal extent of the sciatic nerve and its main branches. Of particular interest has been the demonstration of the involvement of the Schmidt-Lanterman incisures in the early breakdown of the fibre into primary ellipsoids, the rounded ends of which subsequently disintegrated to produce large, dense, whorled masses surrounded by numerous small globules. Somewhat later, globule-laden macrophages migrated radially outwards to assume a subperineurial position, and finally globules appeared within perineurial and epineurial cells.

Ultrastructurally the changes were correlated with a progressive loss of myelin-sheath architecture, expressed initially by the production of 4 nm repeat material associated with irreversible dilatation of the incisures and comparable changes in the paranodal myelin. The appearance of this material at the ends of many of the primary ellipsoids correlates well with the correspondence of these ellipsoids to enclosed cylindrico-conical and initial segments of the internode. Further breakdown of the remaining compact sheath, and of the 4 nm repeat material, resulted in the formation of irregular masses of collapsed lamellar material and homogeneous globules of lipid lying within the Schwann cell cytoplasm. The possible role of Schmidt-Lanterman incisures in the initiation of myelin breakdown has already been discussed (Williams & Hall, 1971).

The manner in which the simple, optically birefringent small globules are extruded from the Schwann cells is reminiscent of some forms of apocrine secretion; it bears a close ultrastructural resemblance to recent descriptions of the extrusion of membrane-bound lipid globules during milk secretion (Wooding, Peaker & Linzell, 1970).

The source of the phagocytic cells responsible for the removal of the globules from the immediate vicinity of the fibres has been disputed. According to Cajal (1928),

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Fig. 22. Adult mouse sciatic nerve 7 days after crush. The long electron-dense processes of an invading macrophage, the cytoplasm of which contains a few lipid globules, are present within the confines of the basement membrane of a Schwann cell that previously enveloped several non-myelinated axons. The degenerating axons may be observed in the lower right-hand segment of the photograph. ×11000.

Fig. 23. Adult mouse common peroneal nerve, 20 days after crush. Portion of a lipid-laden macrophage lies immediately beneath the innermost cellular layer of the perineurium. ×23900.

Figs. 24, 25. Adult mouse common peroneal nerve, 25 days after crush. The flattened perineurial cells contain accumulations of lipid and numerous pinocytotic vesicles. Fig. 24, ×9625; Fig. 25, ×10000.

most of the early investigators believed the cells to be 'migrated large leucocytes', which he described as actively invading the degenerating Schwann tubes. Others (Causey & Barton, 1961; Ohmi, 1961; Wechsler & Hager, 1962; Satinsky, Pepe & Liu, 1964) considered the Schwann cell to be solely responsible for phagocytosis, particularly after it was demonstrated that in tissue culture Schwann cells become phagocytic (Weiss & Wang, 1945). However, the autoradiographic studies of Konigsmark & Sidman (1963) demonstrated that less than 50% of the macrophages appearing in the first 2 days after a cerebral stab wound, and less than 30% arriving thereafter, arose endogenously. Further autoradiographic evidence has supported the haematogenous origin of the majority of the macrophages both centrally (Huntington & Terry, 1966) and peripherally (Olsson & Sjöstrand, 1969).

The work described in this paper has demonstrated *in vivo* the appearance, throughout the distal extent of the fibre, of cells which actively phagocytose myelin globules within the endoneurial space; these cells have been identified ultrastructurally as macrophages. These findings differ from those of Cravioto (1969), who stated that 'the histiocytes, phagocytes and other mesenchymal cells found in every degenerating nerve... are seen only in the area of injury...'; and from those of O'Daly & Imaeda (1967) in that no evidence of bulk movement of macrophages outwards through the perineurium was obtained. *In vivo* cinematographic studies now in progress should enable a further clarification of the origin and ultimate fate of the macrophages during Wallerian degeneration.

It is interesting to compare the relative roles of the Schwann cell and the macrophage in Wallerian degeneration with their suggested roles in some experimental demyelinating conditions such as diphtheritic polyneuritis, experimental allergic encephalitis (EAE) and experimental allergic neuritis (EAN). In Wallerian degeneration and diphtheritic polyneuritis the enzyme-mediated degradation of the sheath is initiated by the Schwann cell (Holtzman & Novikoff, 1965; Weller & Mellick, 1966; Hallpike & Adams, 1969), and overt myelin damage is present long before macrophages enter the bundle. In the inflammatory conditions of EAE and EAN, however, the Schwann cell appears to be of secondary importance, and monocyte (Lampert, 1967) or macrophage (Wiśniewski *et al.* 1969) infiltration of the sheath is the apparent *sine que non* for myelin breakdown. As in Wallerian degeneration, the source of the macrophages is uncertain: it has been suggested that they may derive from transformed lymphocytes (Howard, Boak & Christie, 1966), from histiocytes (Trowell, 1958), from Schwann cells (Holtzman & Novikoff, 1965) or circulating monocytes (Volkman & Gowans, 1965). Whereas in Wallerian degeneration the macrophage activity is assumed to be entirely non-specific, in the delayed-hypersensitivity type reactions of EAE and EAN, the cellular attack on the myelin has been described as a form of immune phagocytosis (Wiśniewski *et al.* 1969). It is possible that the lipid-laden macrophages of Wallerian degeneration are also in some way associated with the sensitization of immuno-competent cells to peripheral myelin antigens; circulating lymphocytes sensitized to peripheral nerve antigen have recently been demonstrated in the Guillain-Barré syndrome (Currie & Knowles, 1971). It is known that a proportion of fibres degenerate during the remodelling associated with growth during normal ontogeny (Hughes, Egar & Turner, 1969) and that a far smaller proportion are constantly degenerating and regenerating within

normal nerve bundles (Weddell & Glees, 1941; Hall, unpublished *in vivo* observations). In these situations it is reasonable to assume that the potential auto-antigenic peripheral myelin debris is taken up by macrophages as in experimental Wallerian degeneration. The number of subsequently sensitized cells would perhaps be far too small, in the absence of further precipitating stimuli, to perpetuate any demyelinative response *in vivo*.

There were no morphological differences between the degeneration displayed by fibres in the zone immediately distal to the crush lesion (Williams & Hall, 1971) and by those further distal: the only demonstrable difference was a 36 h time lag. Since no gradual continuous proximo-distal gradient of degenerative change was observed beyond the initial zone, it is unlikely that the latter acted as a mechanical or chemical 'trigger' for the rest of the bundle. The basic difference between degenerative and demyelinative lesions, irrespective of the introduction of exogenous cellular populations, is the axonal degeneration associated with the former. In this context it is interesting that successive doses of lysophosphatidyl choline (LPC) to fibres already undergoing demyelination as a result of LPC injection (Hall & Gregson, 1971) produced axonal damage and subsequent Wallerian degeneration distal to the injection site (Hall, unpublished observations). Again, Asbury, Arnason & Adams (1969) conclude that focal necrosis of axons in severe EAN is responsible for isolated patches of Wallerian degeneration in experimental material. The loss of axonal permeability characteristics along the distal extent of the fibre, together with alterations in the normal axon–Schwann cell relationship, might be expected to precipitate degenerative changes.

The results described in this paper have emphasized the relatively rapid breakdown of myelin to simpler lipids, in the form of small birefringent globules (possibly of cholesterol ester), within the first week of the response to crush. According to present chemical views (e.g. Adams, 1962) such degradation occurs only after physical destruction of the sheath and does not begin until 8–10 days after crushing. However, the use of whole nerve extracts in chemical analyses may mask the recognition of small lipid globules such as those described *in vivo* in the present communication.

SUMMARY

Continuous *in vivo* observation of Wallerian degeneration during a 60 day post-operative period has demonstrated: (*a*) the activity of the Schmidt-Lanterman incisures in the initiation of the morphological changes and the production of primary ellipsoids; (*b*) the appearance of small lipid globules at the ends of the ellipsoids, coincident with the progressive degeneration of the latter; (*c*) the later appearance of cells between the fibres which became stuffed with the small lipid globules, and then gradually moved out through the bundle to assume a subperineurial position.

Ultrastructurally, it was found that the small lipid globules were derived predominantly from 4 nm repeat material which constituted the ends of these ellipsoids. The globules were extruded from the Schwann cells, and taken up in great numbers by macrophages which entered the endoneurial space from the fifth day. Subsequently, the lipid-laden macrophages were observed in a subperineurial position; at a much

later stage, the lipid was present within the perineurial cells and in epineurial macrophages, although no evidence was obtained to suggest a transperineurial passage of macrophages.

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