

Taxol-induced neuropathy: short-term effects of local injection

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Summary

Taxol, a compound that enhances microtubule polymerization, was injected locally into the sciatic nerve of the rat and its effects examined by electron microscopy up to 21 days postinjection. The drug did not have a degenerative effect upon the P.N.S. but caused a slow accumulation of microtubules, first seen within Schwann cells and shortly thereafter, in axons. Within Schwann cells, microtubule aggregates evolved at the expense of rough endoplasmic reticulum (ER) and microtubule arrays were frequently encountered in relationship to smooth ER cisterns. Arrested mitoses were seen, there was no proliferation of Schwann cells and long stretches of axon were naked. Within axons, microtubules predominated over intermediate filaments and displayed a tendency to cluster around mitochondria. The lesion appeared to be focal and to be related to a local axonal stasis. These experiments provide yet another tool for the examination of Schwann cell–axon interactions.

Introduction

Taxol, an antitumour agent derived from the plant *Taxus brevifolia* (Wani *et al.*, 1971) promotes the assembly of microtubules and prevents the depolymerization of microtubules by cold, calcium and antimitotic drugs such as colchicine (Schiff *et al.*, 1979; Kumar, 1981; Schiff & Horwitz, 1981). The drug inhibits cell replication and fibroblast cell migration (Schiff & Horwitz, 1980). Taxol binds stoichiometrically to polymerized tubulin and its interaction with cells results in an alteration of the microtubule cytoskeleton that includes the formation of microtubule bundles (DeBrabander *et al.*, 1981; Parness & Horwitz, 1981; Brenner & Brinkley, 1982; Manfredi *et al.*, 1982). In nervous tissue, studies have been limited to the examination of organotypic

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P.N.S.-C.N.S. tissue cultures exposed to taxol where accumulations of microtubules in neurons, Schwann cells, glia and fibroblasts and unusual microtubule-endoplasmic reticulum (ER) arrays were observed (Masurovsky *et al.*, 1981, 1983).

Although microtubular networks are known to play an important role in the structural integrity and normal function of nervous tissue *in situ*, no studies have as yet appeared upon the effect of this compound on nervous tissue *in vivo*. The purpose of the present study was to examine the short-term morphologic consequences of local injections of low levels of taxol into the rat sciatic nerve and to attempt to dissect the spatio-temporal events *vis-à-vis* axonal versus Schwann cell response. The results confirm previous observations *in vitro* (Masurovsky *et al.*, 1981, 1983) and demonstrate that the greatest effect of taxol is on the Schwann cell. Although the axon contains massive accumulations of microtubules, it retains its integrity while the microtubule-laden Schwann cell retracts, fails to proliferate, and leaves long segments of axon naked. These findings might have important implications at the levels of axonal physiology and regeneration.

Materials and methods

A total of 11 adult male Sprague-Dawley rats were used. Animals were anaesthetized with sodium pentobarbital and the left sciatic nerve was exposed at the level of the thigh. During surgery, a sterile procedure was followed and care was taken not to interfere with nerve blood supply. The left sciatic nerve was injected with 0.01–0.02 ml 10 µM taxol in 0.1% dimethyl sulphoxide (DMSO) using a 30 gauge needle on a 1 ml disposable tuberculin syringe. The needle was placed beneath the perineurium of the sciatic trunk and fluid was allowed to inflate the nerve. The needle was gently withdrawn. A suture was placed in a nearby muscle to identify the site of injection. The right sciatic nerve was then exposed and, for control purposes, injected with a similar amount of 0.1% DMSO only. Again, a suture was placed close to the point of injection. After each operation the wound was sutured with No. 3 black silk and sprinkled with a topical antibiotic. Animals were sampled after 2 h and at days 1, 2, 5, 7, 14 and 21 postinjection (PI). At these time points, the animals were anaesthetized and perfused through the heart with cold 5% glutaraldehyde in phosphate buffer. The sciatic nerves were exposed and the site of the injection identified. The nerves were carefully removed and kept in 5% glutaraldehyde until dissected. Samples for morphological examination were taken from the injection site and from proximal and distal segments. Each was trimmed into pieces 2–3 mm long and the proximal end was cut at 45° while the distal end was cut square to facilitate orientation (Raine *et al.*, 1983). The tissue was post-fixed, dehydrated with a graded series of ethyl alcohol and embedded in Epon. Transversely and longitudinally orientated 1 µm sections were stained with toluidine blue for light microscopy (LM) and thin sections for electron microscopy (EM) were stained with uranyl acetate and lead citrate (Raine, 1982).

Results

CLINICAL FINDINGS

Except for some minor transient postoperative weakness, no animal displayed neurological signs over the three-week period of study.

MORPHOLOGICAL FINDINGS

DMSO-treated control samples

By light microscopy, a few fibres showed Wallerian degeneration during the first week PI. This occurred in focal areas only and, by 14 days PI, abnormalities were rarely found. In general, therefore, samples from control DMSO-treated nerves were distinctly different from nerves treated with taxol (*vide infra*) in that they showed a short period of nerve fibre response but then appeared normal by two weeks PI, while taxol-treated samples displayed a progression of changes with time, most notable at two weeks PI. By electron microscopy, focal areas of Wallerian degeneration were seen in control samples which showed swollen axons with an increased number of intermediate filaments and a loss of microtubules. The axonal damage progressed for a few days and was resolved by two weeks PI. Thereafter, no obvious pathological findings could be noted at the EM level. Axonal microtubules were in normal numbers and cytoplasmic abnormalities were not apparent.

Taxol-treated animals

Light microscopy. After 2 h, only slight oedema and some haemorrhage was seen near the injection site. During the following two days PI, pathological findings consisted mainly of early Wallerian degeneration of nerve fibres near to the site of injection. There was a gradient of pathological changes which was greatest at the injection sites. Axonal swelling was seen in both myelinated and unmyelinated nerve fibres and in some, a granular axoplasm was present (Figs. 1–2). Short segments of myelin attenuation (Fig. 2) and naked axons were common within the lesion site (Fig. 3). The endoneurial space was markedly increased (more so near the injection site) and the epineurium contained occasional inflammatory cells consisting of macrophages, polymorphonuclear leucocytes and lymphocytes. Mast cells were also seen. In samples taken later than two days PI, reactive changes in axons were more apparent but it was difficult by LM to distinguish whether these were taxol-related or trauma-related. By five days PI, focal areas of myelin degradation were observed in addition to some perivascular inflammation. Control material at this time showed only minor axonal reactions consisting of some granularity of the axoplasm. The changes increased in the experimental group and by seven days PI, focal degeneration of myelin, axonal spheroids, macrophages and mast cells were quite prominent.

By 14 and 21 days PI, changes were seen throughout most of the test nerves sampled. At earlier time points, alterations were most marked at the site of the lesion but samples taken after 14 days PI demonstrated some axonal granularity distal to the site of injection. Within the lesion proper, long stretches of naked axons were common. These were sometimes associated with macrophages and on occasion clearly originated at a node (Fig. 4). Giant axonal swellings were numerous and were often located at or near to nodal areas (Fig. 5). Mitotic figures were common in Schwann cells (Fig. 6), although cell proliferation *per se* was not marked. Across the lesion centre, large diameter, normal appearing naked axons, ballooned Schwann cell bodies and macrophage activity persisted for up to 21 days PI (Figs. 7, 8). Nerve fibre breakdown was rarely seen at these

later time points although both axons and Schwann cells were distended. Proximal to the site of injection, some oedema but no nerve fibre pathology was seen.

Electron microscopy. During the first two days PI, many myelinated axons showed evidence of Wallerian degeneration in the form of accumulations of mitochondria, dense bodies and tubular profiles (Zelena *et al.*, 1968; Singer & Steinberg, 1972; Donat & Wisniewski, 1973). In addition to Wallerian degeneration, some Schwann cells manifested perikaryal swelling and cytoplasmic lakes which contained increased numbers of microtubules (Fig. 9). In most fibres, an increased number of microtubules was seen in addition to the previously described excess of intermediate filaments and vesicular profiles (Figs. 10, 11) which are associated with Wallerian degeneration (Zelena, 1968). In a few axons, increased numbers of microtubules were noted (Fig. 11) with intermediate filaments displaying no or only slight changes. The most marked increase of microtubules was observed in axons which showed areas of myelin retraction or focal swelling (Fig. 11). These microtubules were arranged in longitudinal arrays in some areas but in others, the arrangement was haphazard. By five days PI, stretches of naked axon were more frequent and occasionally consisted of a swollen Schwann cell overlying an axon from which the myelin sheath had been retracted.

At seven days PI, dilated axons with giant swellings containing cores of microtubules arranged in an irregular manner were common. Along some axons, axoplasmic protrusions were noted (see Fig. 15). Short naked areas of axon were seen covered by enlarged Schwann cells which by this time contained an obviously abnormal complement of microtubules. Changes were more widespread than at earlier time points and were most severe near the site of injection where most Schwann cells were swollen and

Figs. 1–8 are light micrographs from 1 µm epoxy sections stained with toluidine blue.

Fig. 1. Two days PI. Note the numerous reactive, densely staining myelinated axons in the centre, the enlarged Schwann cell body (arrow) and the single naked swollen axon (a) to the upper left. × 480.

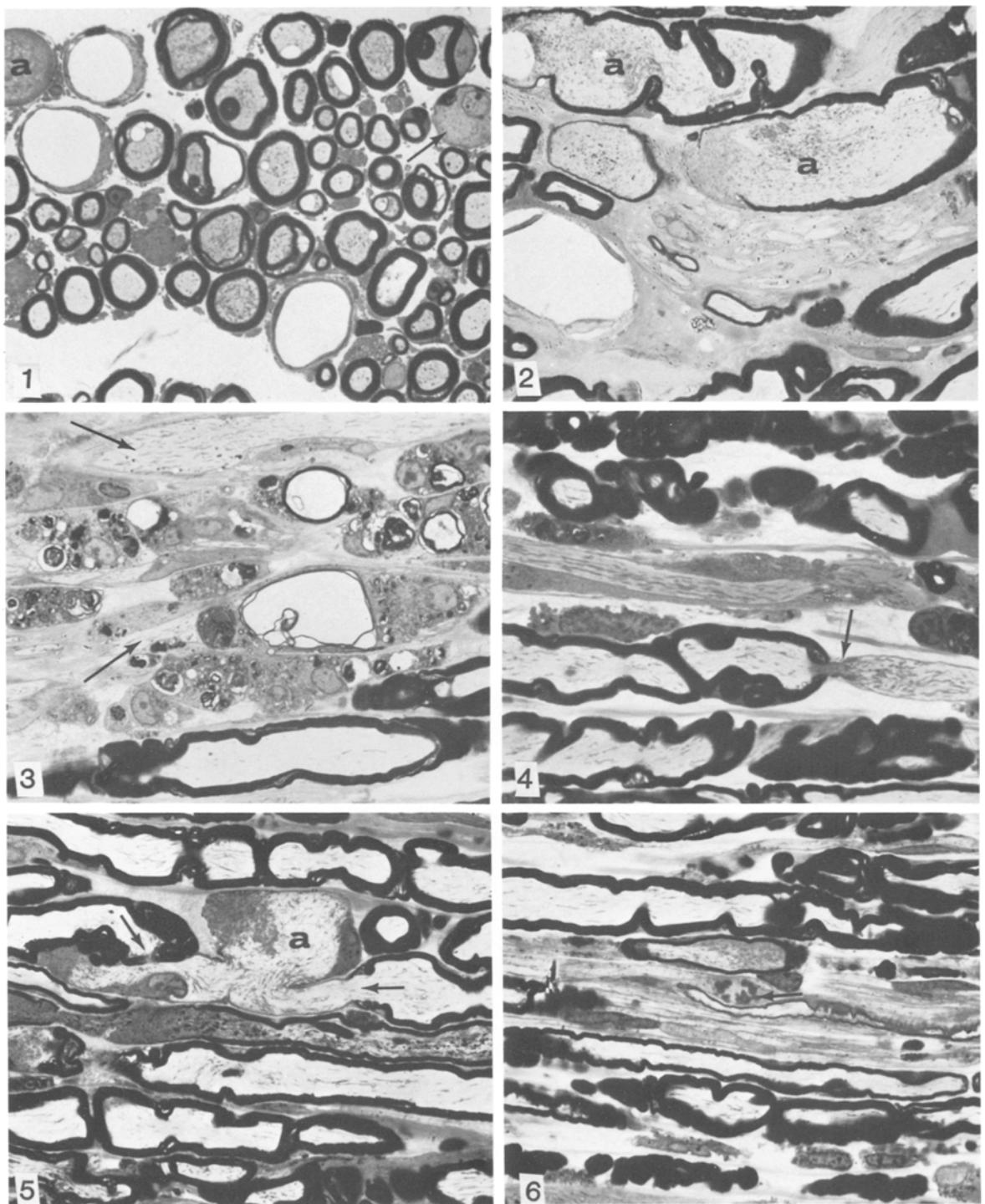
Fig. 2. Two days PI. Two dilated axons (a) display a moderate accumulation of organelles and attenuated myelin sheaths. × 750.

Fig. 3. Two days PI. Several segments of denuded axons (arrows) traverse an area of nerve which displays macrophages and Schwann cells containing myelin debris. × 480.

Fig. 4. 21 days PI. A myelinated nerve fibre displays a normal-appearing node (arrow) which continues as a naked axon containing numerous mitochondria. A second naked axon lies above. × 750.

Fig. 5. 21 days PI. The myelin sheath of a longitudinally sectioned fibre terminates at a heminode (arrow) whereafter the axon (a) enlarges into a distorted mass before it meets the opposite heminode (arrow) around which much of the myelin sheath has been retracted. Note the accumulation of organelles in the dilated axon. × 480.

Fig. 6. 21 days PI. This area of nerve shows a few naked fibres (centre) and a Schwann cell in mitosis (arrow). Some axonal pathology is also present. × 300.



contained large numbers of microtubules, an extensive Golgi apparatus and little rough ER. An increased number of microtubules was not uncommon in fibroblasts.

By 14 days PI, nerve fibre changes were abundant. Phagocytosis of myelin was seen, leaving many segments of naked axons (Fig. 12). The phagocytic process consisted of the peeling of myelin lamellae from the axon (Figs. 13, 14). Between the folds of the macrophage membrane, formations resembling coated pits were occasionally seen, apparently involved in the removal of myelin, a process akin to receptor-mediated phagocytosis of myelin (Epstein *et al.*, 1983). Some dilated axons had axoplasmic protrusions full of haphazardly arranged microtubules (Fig. 15). Large groups of unmyelinated axons occurred which contained increased numbers of microtubules (Figs. 16, 17). Schwann cell cytoplasm associated with these axons was rich in microtubules but contained more intermediate filaments than did axons (Fig. 17). That these collections of unmyelinated axons were indicative of sprouting was likely but could not be definitely discerned. Large diameter axons were often completely naked for long stretches and lacked Schwann cell investment (Figs. 18, 19), the cell body being located elsewhere along the fibre. The denuded axons were covered by basal lamina and an increased amount of collagen deposition was evident in the endoneurium. Intermediate filaments, large numbers of microtubules and smooth-membrane cisterns were the major axoplasmic constituents. By this time, the microtubule defect had become the major feature of the tissue, particularly near the injection site although some changes were seen distally. Axons displaying thick myelin sheaths (as opposed to naked segments which had, at earlier time points, tended to reveal the greatest amount of change), showed a preponderance of microtubules (Fig. 20). These microtubules had a tendency to be aligned along mitochondria (Fig. 21). Similar associations were also encountered in unmyelinated axons where 2–3 concentric rows of microtubules sometimes encircled a mitochondrion (Fig. 22), each row being separated by an electron-dense leaflet.

Schwann cells continued to display a cytoplasm with increasing numbers of microtubules, sometimes almost to the exclusion of other organelles (Figs. 23, 24).

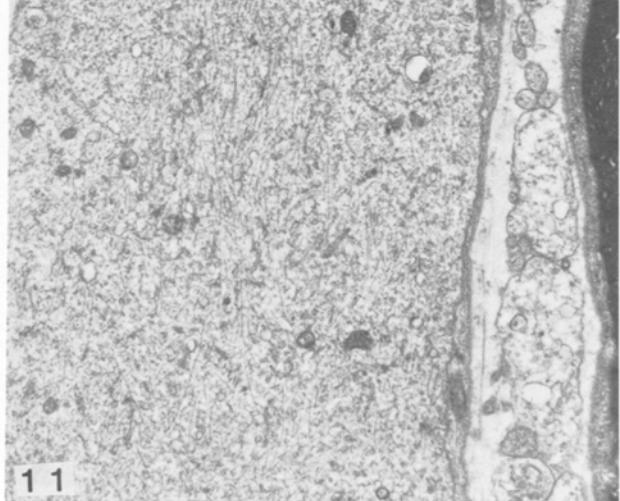
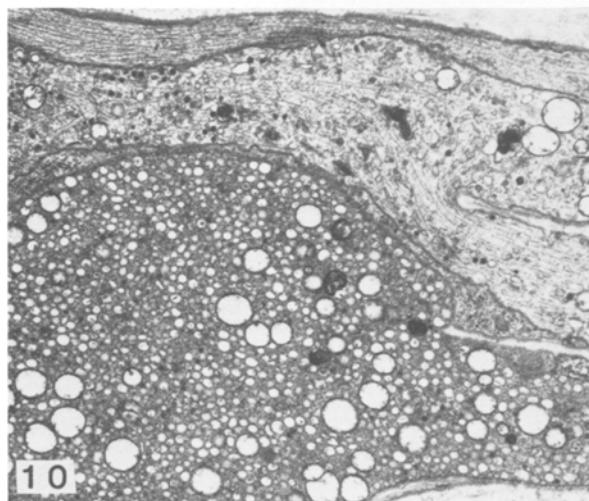
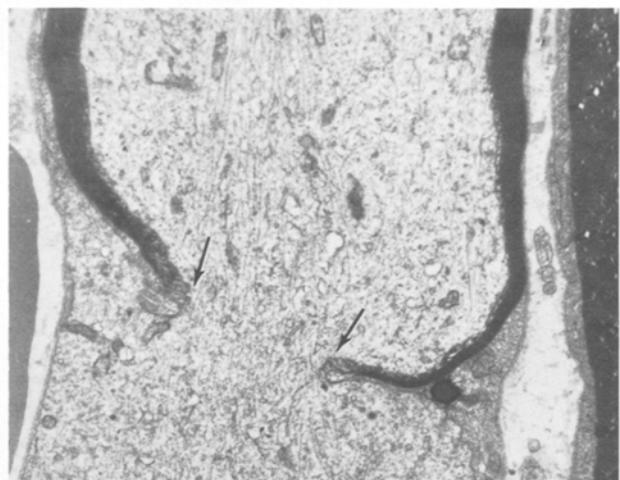
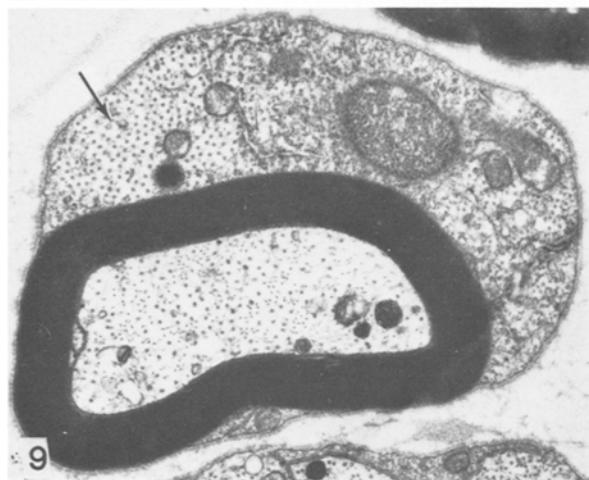
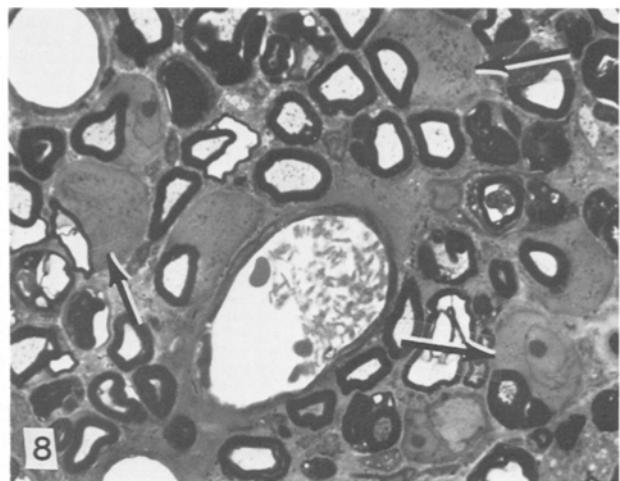
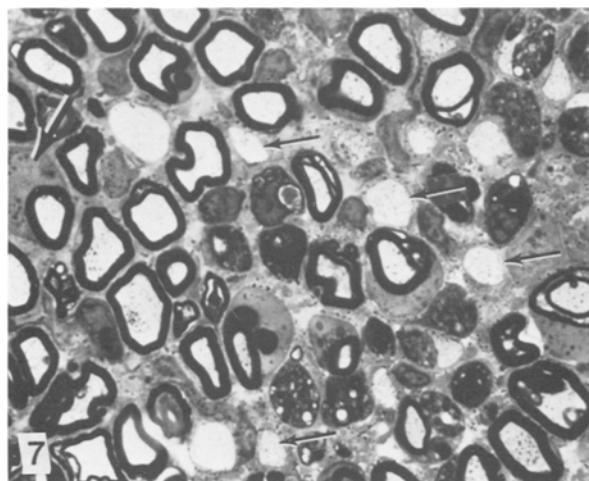
Fig. 7. 21 days PI. Several transversely sectioned large diameter nerve fibres lack myelin (small arrows). An enlarged Schwann cell (large arrow) and many myelin macrophages are also present. $\times 300$.

Fig. 8. 21 days PI. In this region, taken from the centre of a lesion, note the presence of extracellular exudate and many swollen Schwann cells (arrows). $\times 750$.

Fig. 9. One day PI. This electron micrograph shows a small myelinated nerve fibre, the Schwann cell cytoplasm of which possesses an abnormal collection of microtubules (arrow). $\times 16\,000$.

Fig. 10. Two days PI. A group of myelinated fibres is shown, one of which is dilated and contains swollen mitochondria and clear vesicular profiles, another below has many swollen mitochondria and dense-cored vesicles. $\times 7500$.

Fig. 11. Two days PI. A longitudinally sectioned myelinated axon shows attenuation and retraction of paranodal myelin (arrows) and a long stretch of denuded swollen axon at the node. The axoplasm contains increased numbers of microtubules. $\times 7000$.



Rough ER was diminished extensively but Golgi apparatus remained prominent. The globular body of the Schwann cell appeared retracted and was often seen in sections devoid of an axon (Fig. 23) and for the first time, reticulated areas of cytoplasm became apparent. At higher magnification, these reticulations consisted of linear arrangements of smooth vesicles or ER cisterns among the bundles of microtubules (Fig. 24), an appearance superficially similar to sarcoplasmic reticulum of muscle. Schwann cells and

Fig. 12. 14 days PI. A nerve fibre demonstrates myelin breakdown with the sheath being degraded and stripped (arrow) by macrophages beneath the basal lamina, one of which (below asterisk) is leaving the fibre. The axon contains increased numbers of microtubules, dense bodies and mitochondria. $\times 6800$.

Fig. 13. Detail from near the asterisk in Fig. 12. Note the gap in the basal lamina of the fibre (arrows) through which the macrophage extrudes. The axon (above) contains a predominance of microtubules. $\times 20\,000$.

Fig. 14. Detail from area at arrow in Fig. 12. Note the pincer-like processes from a macrophage which flank the detached lamellae of myelin. $\times 35\,000$.

Fig. 15. The node of Ranvier shown in Fig. 5 is shown. Note the attenuated myelin sheath (lower left) and the axoplasmic extrusion at the node. $\times 6000$. The insert illustrates the predominance of microtubules within the axon. $\times 50\,000$.

Fig. 16. 14 days PI. Near to the site of injection, mosaics of nonmyelinated axons are seen to be invested in part by darker staining Schwann cell cytoplasm lying within a background matrix of increased collagen deposition and proliferated basal lamina material. $\times 4500$.

Fig. 17. Detail of two axons (a) and an area of Schwann cell cytoplasm(s) from Fig. 16. Note that the axoplasm contains only a few neurofilaments amongst the increased number of microtubules while the Schwann cell cytoplasm has more filaments. $\times 80\,000$.

Fig. 18. Two segments of large diameter, naked axons (a) are shown invested only by basal lamina. The endoneurial space contains excessive amounts of collagen. $\times 8400$.

Fig. 19. Detail from Fig. 18. Note the many microtubules, fewer neurofilaments and scattered smooth-walled cisterns within the axon. $\times 50\,000$.

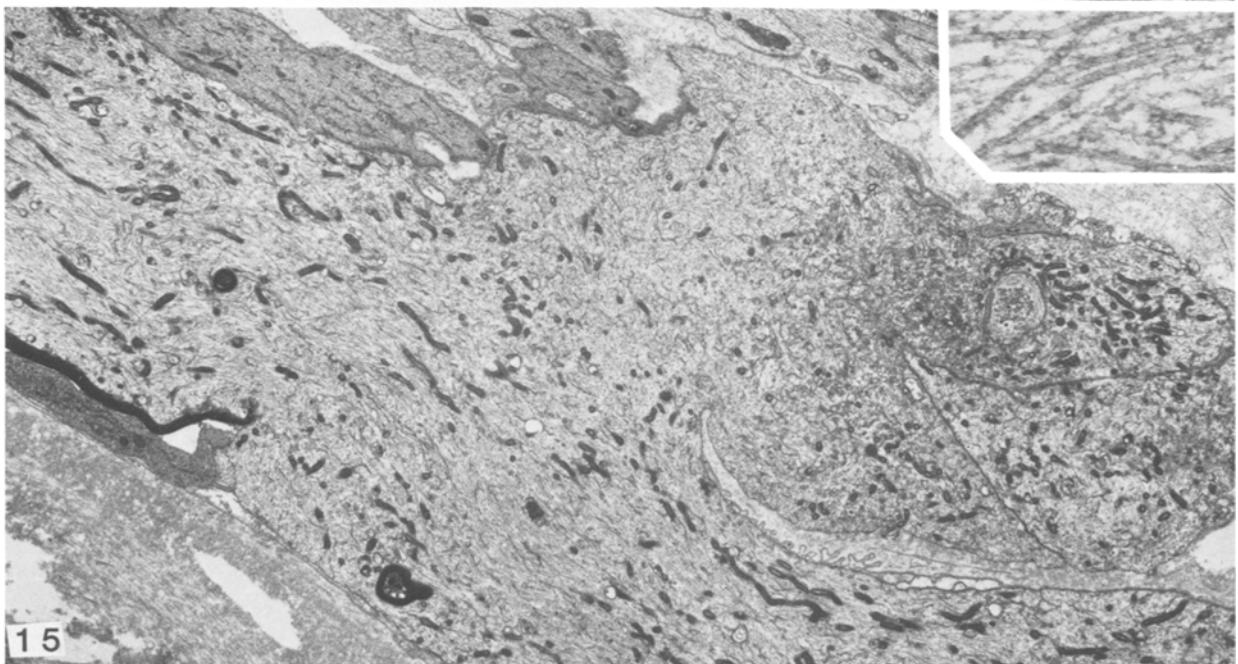
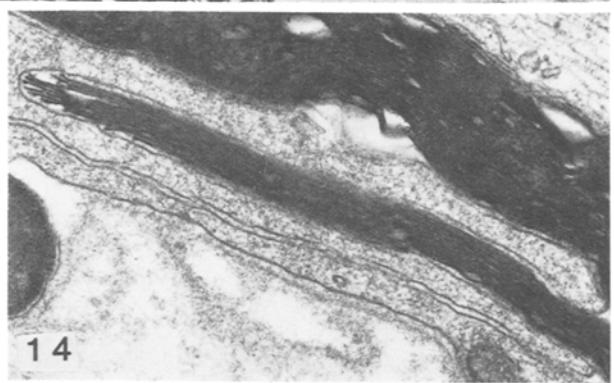
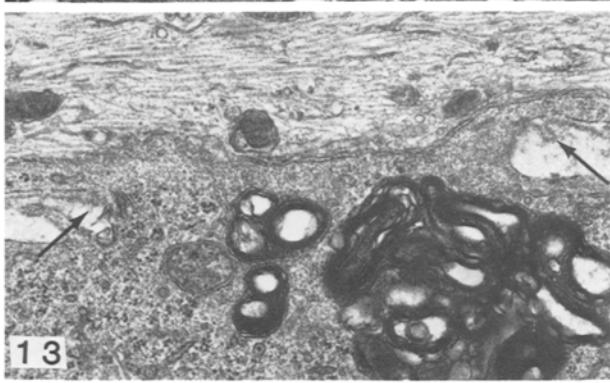
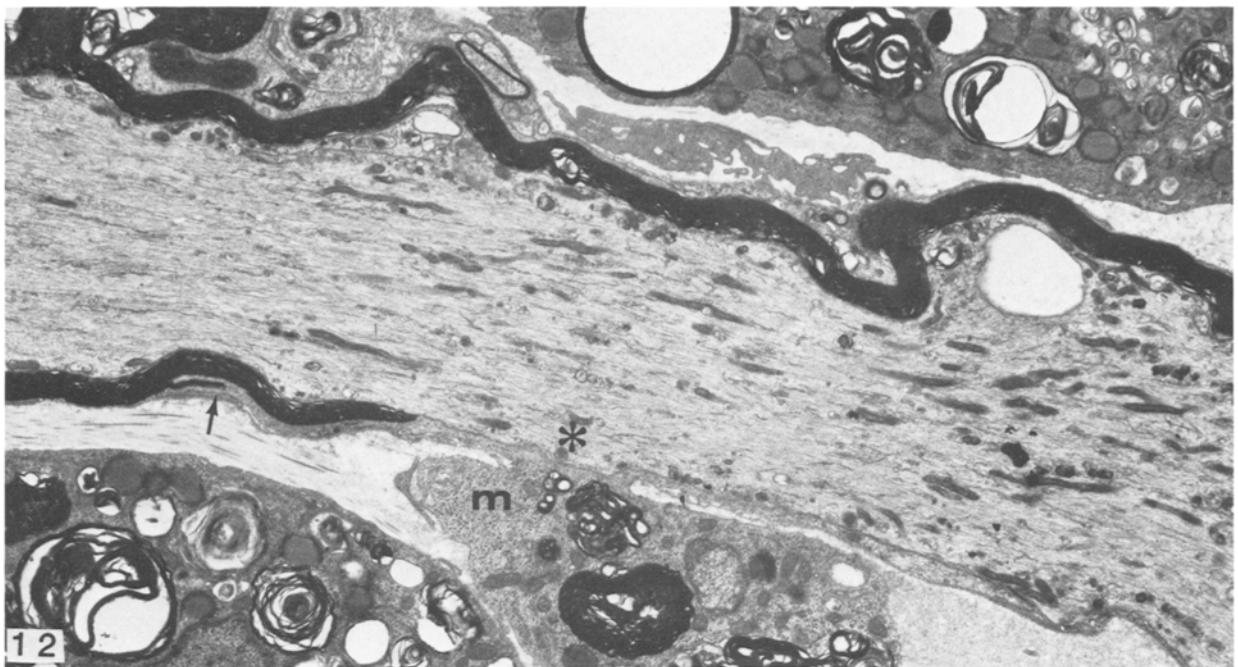
Fig. 20. 14 days PI. A myelinated axon is filled with microtubules and mitochondria, some of which (arrows) are closely associated. The Schwann cell(s) cytoplasm above is extraordinarily rich in microtubules. The area at the asterisk is shown in Fig. 27. $\times 13\,500$.

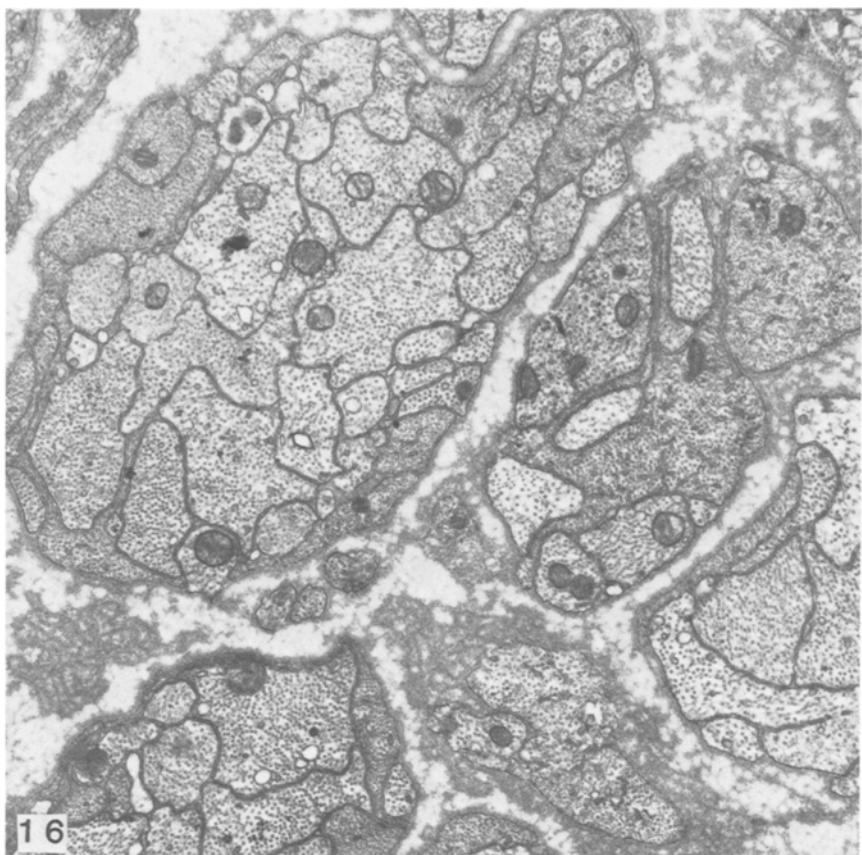
Fig. 21. Detail from Fig. 20. Note how groups of microtubules in longitudinal section display a preference to associate with mitochondria. $\times 58\,500$.

Fig. 22. 14 days PI. A variation of the phenomenon in Fig. 21 is the occurrence within axons of concentric rows of microtubules around a mitochondrion, as shown here. Between each row, an electron-dense lamina is apparent. $\times 50\,000$.

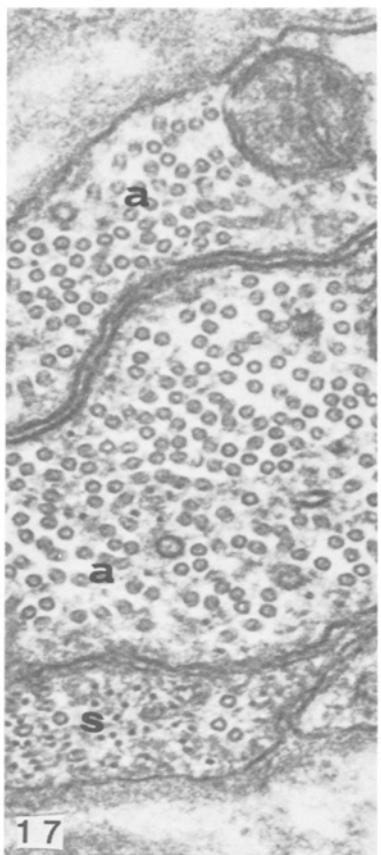
Fig. 23. 14 days PI. The body of a Schwann cell, devoid at this level of an associated axon, is rich in Golgi apparatus and most other organelles except rough ER. Schwann cell fingers (arrow) protrude from the cell surface beneath the basal lamina. The area of cytoplasm around the asterisk possesses reticular formations. $\times 5000$.

Fig. 24. Detail of the area at the asterisk in Fig. 23. Note how the trabeculae are made up of cisterns of smooth ER (arrow) and that the background is extraordinarily rich in microtubules. A few free ribosomes are seen. $\times 50\,000$.

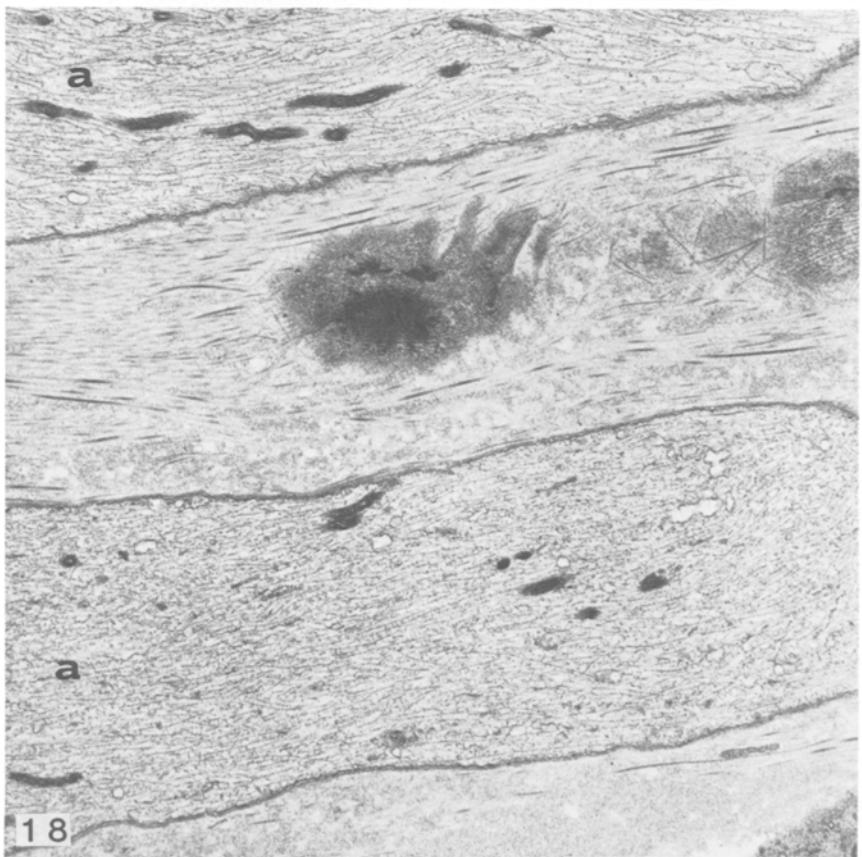




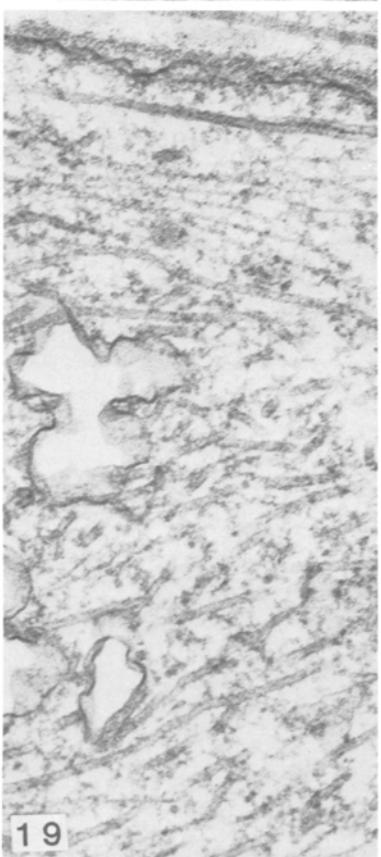
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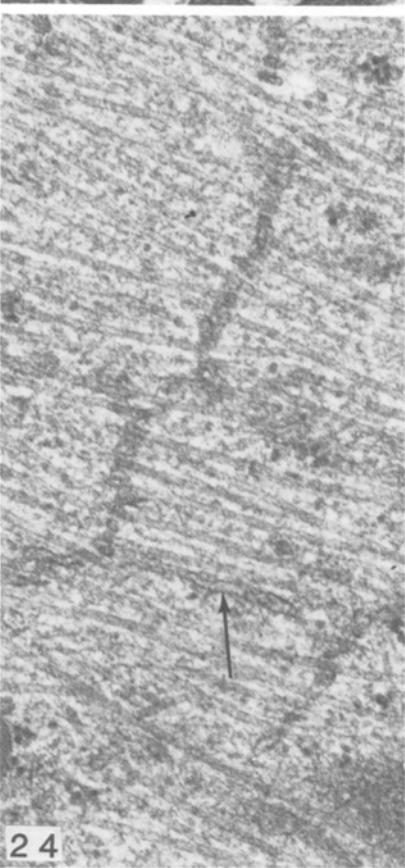
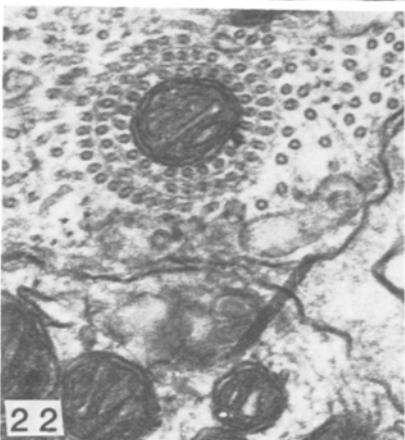
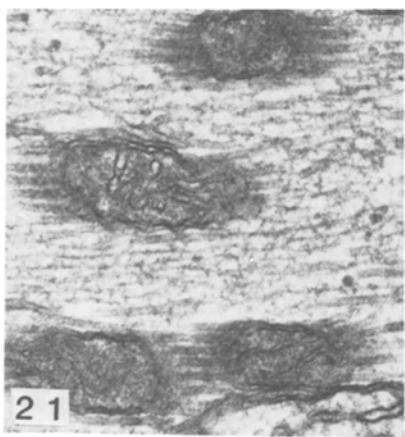
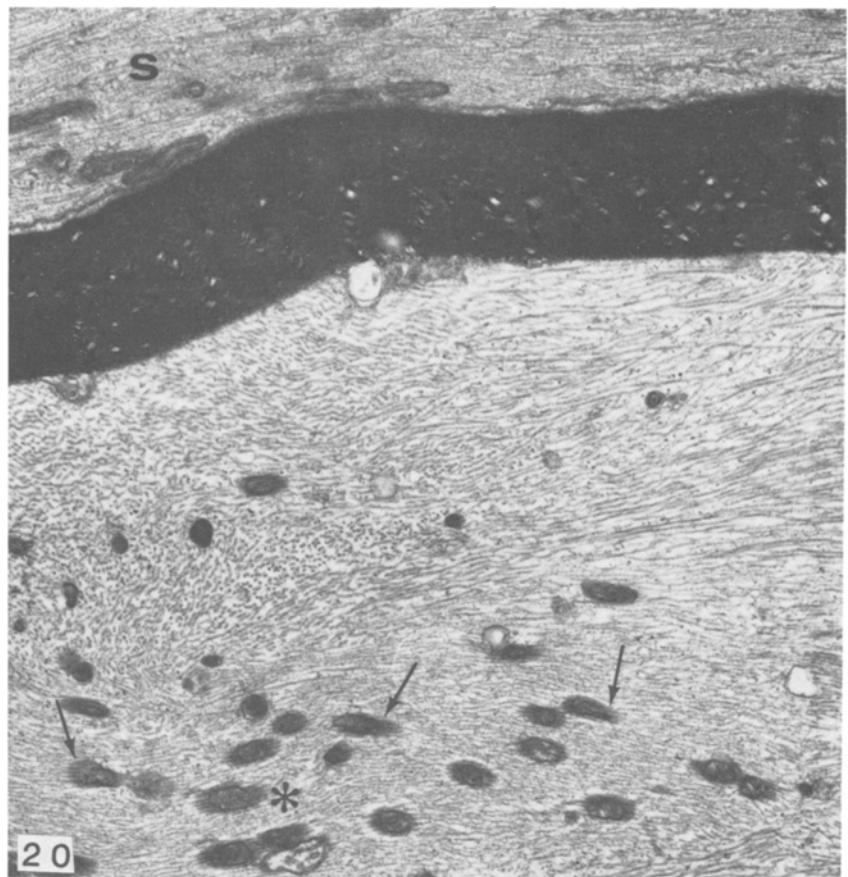
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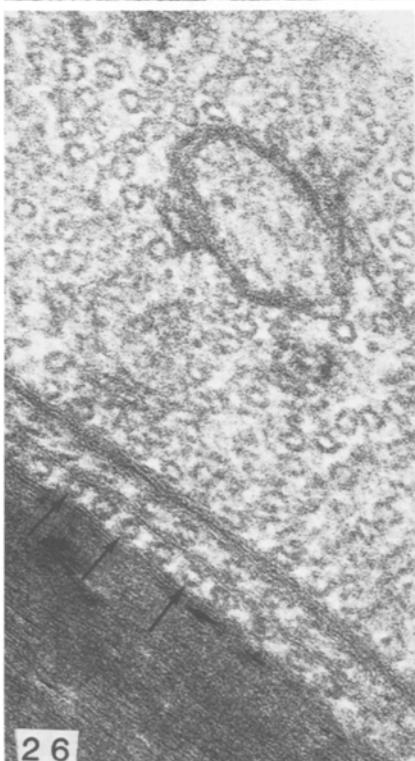
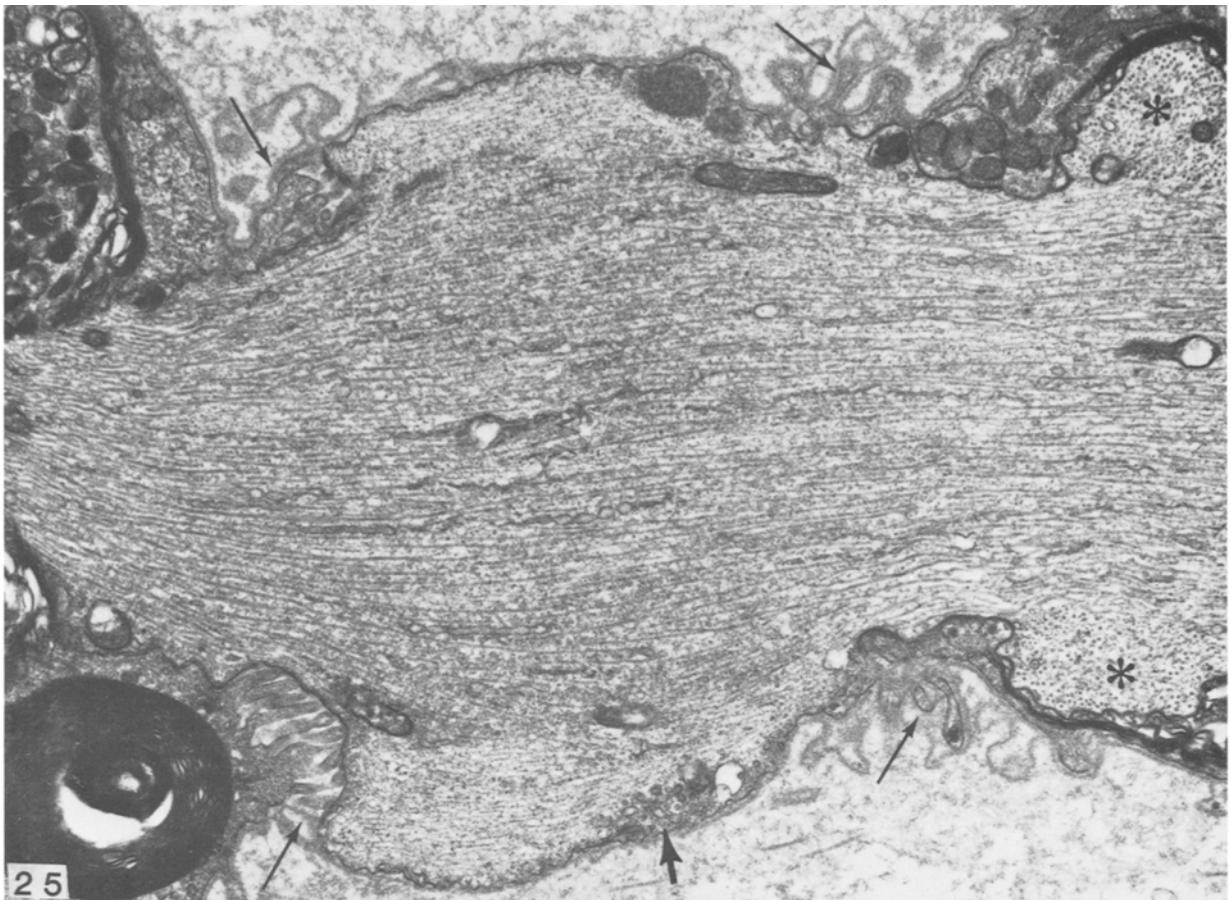


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fibroblasts were encountered in mitosis. It appeared as if the mitotic process was arrested at this stage (i.e. metaphase). Endothelial cells displayed no remarkable changes.

By 21 days PI, large diameter naked axons were very common. Within the denuded axons microtubules still predominated and on occasion, were associated with mitochondria. Intermediate filaments were present but rare. Nodes of Ranvier displayed axonal swellings which often extended paranodally and which were covered laterally by retracted Schwann cell cytoplasm and Schwann cell fingers, leaving much of the nodal surface covered only by basal lamina (Fig. 25). Sometimes, coated, invaginated vesicles were seen beneath the nodal axolemma, perhaps indicative of pinocytosis (Fig. 25). Paranodally, an increased number of microtubules was occasionally encountered in a collar-like formation orientated at right angles (i.e. circumferentially) to the main aggregates of axoplasmic microtubules (Fig. 25). In addition to previously described features, Schwann cells were more retracted and displayed microvillous-like Schwann cell fingers (Raine, 1982) from the cell surface (see Fig. 23), away from the node. These were located below the basal lamina and protruded into the endoneurial space. Also, Schwann cell microtubules were observed in cytoplasmic layers between the outer myelin lamellae (Fig. 26) and within some Schwann cell perikarya, vacuoles were traversed by cytoplasmic bridges filled with rows of microtubules. In places, these bridges fused to form myelin lamellae (Fig. 27). Elsewhere, flat sheets of smooth ER were flanked by microtubules (Fig. 28) while rough ER was not prominent. Evidence of Schwann cell replacement and proliferation (Raine, 1977) was not apparent. Finally, Schwann cell nuclei occasionally showed grotesque changes with the formation of multiple nuclear lobules and in some cells, the possibility of multiple nuclei could not be excluded. These nuclear changes were also seen in fibroblasts in which an increased number of microtubules was always observed.

Fig. 25. 21 days PI. A node of Ranvier is flanked by aberrant, retracted Schwann cell fingers (small arrows) and contains an excess of axoplasmic microtubules. To the right, at the asterisks, a belt of microtubules at right angles to the longitudinal axis, runs beneath the axolemma in the paranodal area where the myelin sheath is attenuated. A cluster of subaxolemmal vesicles is present at the large arrow. $\times 15\,000$.

Fig. 26. 21 days PI. Within the Schwann cell surrounding a nerve fibre, abundant microtubules are present within the cytoplasm and between the outer layers of the myelin sheath. $\times 100\,000$.

Fig. 27. 21 days PI. Within a Schwann cell, bridges of cytoplasm contain rows of microtubules. Some cytoplasmic bridges fuse to form myelin-like lamellae (below). $\times 60\,000$.

Fig. 28. 21 days PI. Flattened cisterns of smooth ER are flanked by microtubules within the body of a Schwann cell. Scattered intermediate filaments occur throughout the cell and rough ER is rare. $\times 75\,000$.

Discussion

The present study represents the first demonstration of the effects of taxol upon P.N.S. tissue *in situ*. In general, the effects were maximal at the site of injection and decreased with distance from this point so that a dose concentration gradient was observed. Taxol affected the nerve in focal areas up to two weeks PI after which the entire lesion area and some distal segments were involved. While changes observed at earlier time points were sometimes difficult to distinguish from events associated with Wallerian degeneration, at later times they were related to the accumulation of microtubules within axons and Schwann cells. Microtubule accumulation was seen in Schwann cells as early as one day PI and increased dramatically over the 21 day period of study. By 14 days, the cell body was dilated, packed with microtubules which had formed at the expense of most other organelles and which possessed unusual relationships with reticular networks of smooth ER-like material, and the cytoplasm had an appearance reminiscent of sarcoplasm. Analyses of Schwann cell and axonal events merit deeper study and will be the subjects of a separate study.

Microtubule proliferation in axons and axonal enlargement began after 2–5 days PI and were rarely associated with axonal disintegration. As a consequence of axonal enlargement and Schwann cell retraction, significant stretches of naked axons developed, some of which displayed unusual axon–Schwann cell relationships. Finally, the frequent observation of mitotic figures in Schwann cells and fibroblasts suggests that taxol arrested cell division at metaphase and in doing so, prevented Schwann cell migration and remyelination of naked axons. Indeed there was no apparent increase in the overall Schwann cell population, an unusual observation in that Schwann cell proliferation is a common, nonspecific sequela of most insults to the P.N.S.

In general, the synopsis described above is in close agreement with the observation of previous works on foetal P.N.S. tissue developing *in vitro* (Masurovsky *et al.*, 1981a, 1983) in which microtubule proliferation was seen within neurons and supporting cells exposed to taxol. Interestingly, frank degeneration of immature cultured dorsal root ganglion neurons (DRG) by a direct toxic effect was prevented by the addition of nerve growth factor (Peterson & Crain, 1982). In contrast to the progressive restoration of normal cytoplasmic structure in the DRG neurons following removal of taxol, replicating Schwann and satellite cells showed long-lasting nuclear and cytoplasmic abnormalities, excess microtubules and sustained impairment of function during test periods up to 6 weeks (Masurovsky *et al.*, 1981b). At the LM level, taxol intoxication could resemble giant axonal neuropathy of varied etiology (Davenport *et al.*, 1976; Prineas *et al.*, 1976; Spencer & Schaumburg, 1976; Griffin & Price, 1981) which includes giant axons and myelin retraction as major features. However, ultrastructural examination after taxol treatment shows clear differences, the main one being that instead of the commonly described accumulations of intermediate filaments, an overabundance of microtubules in the axoplasm occurs. To our knowledge, this is the

first demonstration of this phenomenon *in vivo*. Furthermore, that this microtubule lesion leads to loss of myelin with no apparent subsequent remyelinative response, is unusual and might reflect an essential role for microtubules in the behaviour of the Schwann cell, its remyelinative capacity and its interaction with the axon. These responses are different from those that follow disturbances in neurofilament synthesis after which remyelination is seen (Griffin & Price, 1981). Thus, when phagocytosis of myelin has been completed after taxol intoxication, precipitated mainly by axonal swelling, Schwann cells are apparently unable to divide and migrate to invest the naked segments. Inhibition of cell migration after taxol treatment has been described as well as the effects of the drug upon mitosis (DeBrabander *et al.*, 1981; Schiff & Horwitz, 1981).

Since taxol was injected directly into the sciatic nerve in the present study, some Wallerian degeneration was expected. In control samples, this type of degeneration was seen rarely after the first week PI. Thus, at least some of the early axonal reactions in taxol-treated nerves were attributable to Wallerian degeneration but the later, progressive changes were unequivocally due to taxol intoxication. Whether or not some of the groups of unmyelinated axons were the result of axonal sprouting could not be determined since it was not always possible to determine origin and orientation. It is possible that the prolongation of axonal reactions was due to polymerization of axonal microtubules which resulted in axonal stasis.

It seems reasonable to suggest that the unusual stability of microtubules in the presence of taxol is responsible for the accumulation of the unique structures seen after treatment with the drug. Interestingly, some of these have specific forms which exclude them as being incidental findings. In this respect, the described microtubule arrays (previously seen by Masurovsky *et al.*, 1981, 1983), the sarcoplasmic appearance of affected Schwann cells and the association of microtubules with myelin loops may be significant functionally. In the microtubule arrays surrounded by electron-dense material shown previously by Masurovsky *et al.* (1983), there was no association with mitochondria, as seen in the present study. Associations between microtubules and mitochondria and flattened cisterns of axoplasmic smooth ER have been reported previously in normal and pathological situations (Raine *et al.*, 1971; Raine, 1977; Heath *et al.*, 1982), but to what extent these associations observed in the presence of taxol represent exaggeration of normal relationships must remain speculative. Thus, the possible effects of taxol upon axoplasmic flow provide substance for future investigations.

Of the observed myelin pathology, most of the changes were almost certainly related to changes in axonal diameter. Recently, such changes were also suggested to be a primary cause of toxin-induced demyelination in peripheral nerves (Griffin & Price, 1981) where chronic B,B'-iminodipropionitrile intoxication led to repetitive demyelination and onion-bulb formation. In this regard, continuing studies with taxol will investigate the long-term effects of the compound upon remyelination and axonal regeneration for further insights into the role of microtubules in nerve integrity.

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