TUBULAR ARRAYS DERIVED FROM MYELIN BREAKDOWN DURING WALLERIAN DEGENERATION OF PERIPHERAL NERVE

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When a myelinated nerve fiber is interrupted, its distal portion undergoes Wallerian degeneration, which involves the disruption and removal of the axon and myelin. Although a number of electron microscope studies on Wallerian degeneration have been published (1–6), insufficient information is so far available for a systematic description of the process of myelin breakdown and removal to be made. Light microscope studies have demonstrated that the myelin breaks up into "ovoids" and smaller droplets (7, 8). This brief communication describes a type of breakdown of the myelin ovoids that has not been reported previously.

The observations were made on the nerve to the medial head of the gastrocnemius muscle of 8 adult male albino rabbits. The animals were anesthetized with pentobarbital sodium and ether, the nerve exposed under aseptic conditions, and cut with scissors 3 cm above the muscle. The central stump was separated from the tibial trunk as far proximally as possible and avulsed. Biopsy specimens were taken 2 cm below the point of section after survival periods of 7, 10, 14, and 21 days, again during pentobarbital sodium and ether anesthesia. The specimens were fixed *in situ* for 5 minutes in 1 per cent potassium permanganate in mammalian Ringer solution buffered with veronal-acetate to pH 7.4 and then immersed in this solution for 3 hours at 4°C. They were subsequently dehydrated in graded concentrations of ethanol, embedded in Araldite, sectioned with a Porter-Blum microtome, and examined with an RCA EMU 3E or Siemens Elmiskop 1b. Some sections were stained with lead hydroxide (9).

In the material obtained at 7 days after nerve section, all the myelinated nerve fibers had undergone Wallerian degeneration. Myelin ovoids surrounded by Schwann cells and their processes were present within the confines of the basement membranes that had surrounded the Schwann cells of the intact nerve. Such ovoids were numerous in the material obtained at 7, 10, and 14 days, but at 21 days were less common, myelin remains being visible within phagocytic cells. At all stages, some of the myelin ovoids displayed circumferential splits. At the surface of the ovoids and within the splits, a proportion of the ovoids showed aggregates of circular or oval profiles (Fig. 1). In addition, aggregations of such profiles were seen within the central spaces enclosed by the myelin ovoids and which also contained material derived from the degenerating axons. Other collections were present between the processes of the proliferating Schwann cells (Fig. 2). Such appearances were commonest in the material obtained at 14 and 21 days after nerve section, but were seen to a lesser extent in the material obtained 7 and 10 days after operation.

The profiles were sometimes of very uniform diameter (~600 A), and were grouped in closely packed arrays with a regular hexagonal arrangement (Fig. 2). The examination of longitudinal and oblique sections revealed that they were tubular structures with their long axes usually parallel to the long axis of the nerve trunk. At higher magnification (Fig. 3), they were shown to consist of a clear central area bounded by a ~75 A membrane. This had a three-layered structure with an intermediate light zone bounded by two opaque layers, each ~25 A in thickness. The composite structure thus corresponded to the "unit membrane" of Robertson (10). Where the membranes of adjacent tubules were opposed, a five-layered arrangement was often present, no separation being visible between the two outer opaque layers.

In preliminary observations on material fixed in osmium tetroxide, although similar changes were seen to be present, the appearances were not so uniform as those observed in the permanganate-fixed nerves, and regular hexagonally packed arrays of tubules were not commonly encountered. This raises the question as to the influence of fixation and subsequent treatment of the tissue on the appearances obtained. Rosenbluth (11) has reported differences in membrane arrangement between osmium-fixed and permanganate-fixed toad spinal ganglia and suggested that, with both fixatives, membrane stabilization was not sufficient to prevent alterations occurring during dehydra-

tion and embedding. The differences between the appearances found in permanganate-fixed and osmium-fixed nerve undergoing Wallerian degeneration are being further investigated.

Lampert, Blumberg, and Pentschew (12) have described ~600-A tubular structures arrayed in closely packed bundles present within dystrophic axons in the gracile and cuneate nuclei of vitamin E–deficient rats. There was no associated disintegration of myelin sheaths. However, in view of their close association with myelin, the tubules observed in the present investigation were considered to be derived from myelin breakdown. Since they were found lying between Schwann cells, it can be assumed that they were surrounded by extracellular tissue fluid, but within the basement membranes of the original Schwann cells. The precise manner in which the lamellar structure of the myelin breaks down to give rise to this arrangement is being studied. Most *in vitro* observations of lipid-water systems examined by electron microscopy or x-ray diffraction methods have revealed lamellar structures. It is of considerable interest, in relation to the present observations, that Luzzati and Husson (13), who studied lipid-water systems under various conditions by x-ray diffraction techniques, were able to detect hexagonal arrays of cylinders of uniform diameter as well as lamellar structures. The suggested arrangement for one such system was a hexagonal array of lipid cylinders with the hydrocarbon chains of the lipid molecules filling the interior of the cylinders, the hydrophilic polar groups at their surfaces and water between the cylinders. Of greater interest in the present context was the suggested arrangement for another system in which the lipids formed a cylindrical shell composed of a double layer of lipid molecules, with water filling the central cavities and the spaces between the cylinders. Stoeckenius (14) has obtained electron micrographs of hexagonal arrays of a phospholipid-water system that was also studied by Luzzati and Husson. It is possible that, in the breakdown of myelin during Wallerian degeneration, or during fixation, there is a change in the physical condition of the Schwann cell membrane which results in the formation of hexagonal arrays of tubules similar to those observed *in vitro*. The tubules may be comparable to the "vesicles or tubules" bounded by ~75-A membranes observed by Robertson (15) in myelinated nerve fibers immersed in distilled water before fixation. A further instance of the disruption of a lamellar system into tubules is provided by the changes occurring in the outer segment of retinal rods during vitamin A deficiency (16). The ordered array of flattened disks breaks down into irregular tubules and vesicles.

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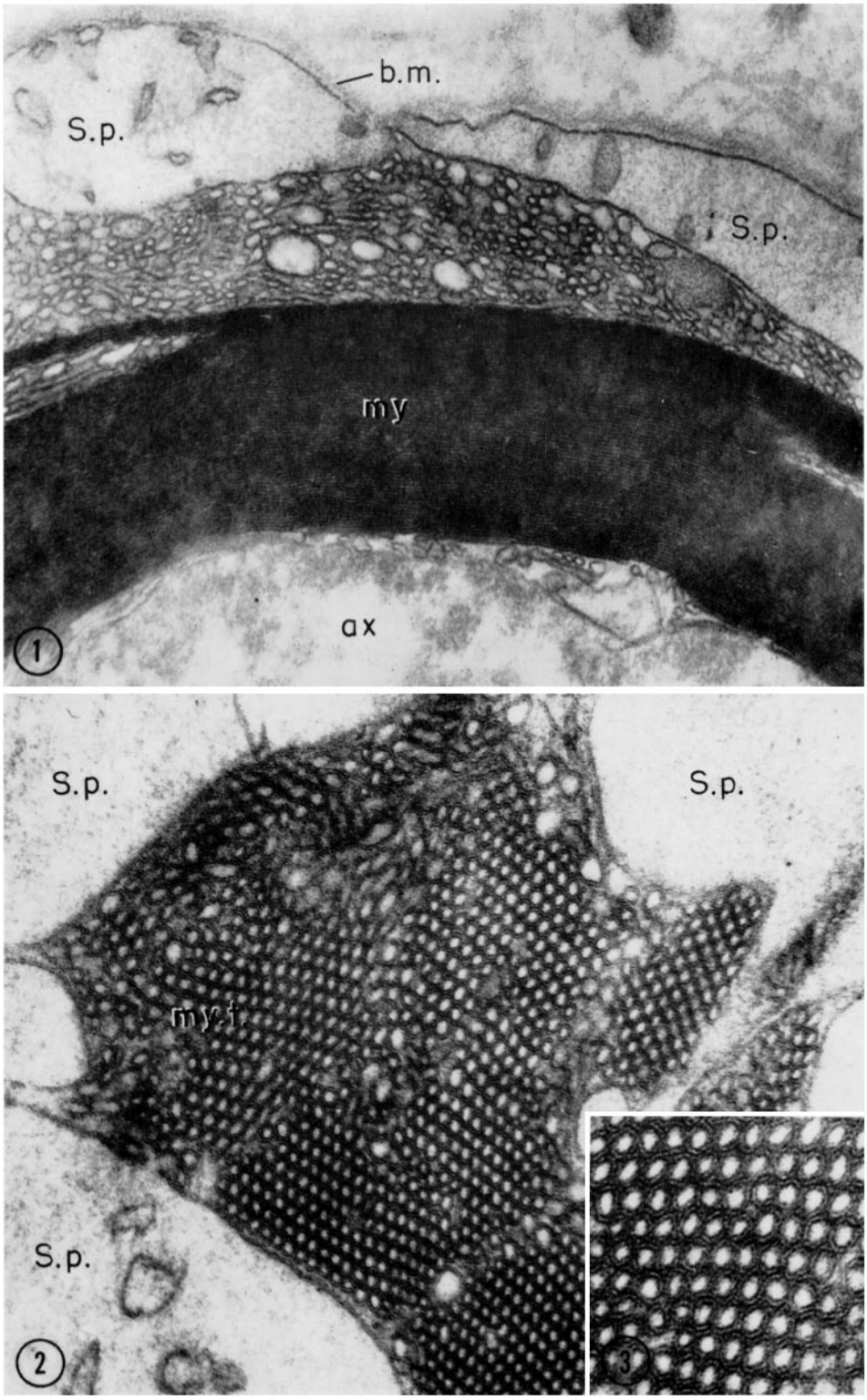
FIGURE 1 Transverse section through portion of a myelin ovoid (*my*) 21 days after nerve section. Numerous circular and oval profiles are seen lying between the degenerating axon (*ax*) and the internal surface of the ovoid, within splits in the myelin and between the outer surface of the myelin and Schwann cell processes (*S.p.*). The basement membrane (*b.m.*) that surrounded the Schwann cell of the intact fiber is seen external to the Schwann cell processes. × 21,000.

FIGURE 2 Transverse section through nerve 14 days after operation. A closely packed hexagonal array of tubules of uniform diameter derived from myelin breakdown (*my. t.*) is seen lying between Schwann cell processes (*S.p.*) × 75,000.

FIGURE 3 (inset) Portion of hexagonally packed aggregate of myelin tubules 14 days after operation to show ~ 75 A triple-layered membrane walls. Where walls of adjacent tubules are in contact, a single five-layered membrane is present. × 150,000.


b.m.
S.p.
S.p.
my
ax
1
S.p.
S.p.
my.f.
S.p.
2
3

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