THE FIBROUS STRUCTURE OF THE NERVE AXON IN RELATION TO THE LOCALIZATION OF "NEUROTUBULES"*

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PLATES 36 AND 37

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In electron microscope studies of various types of vertebrate and invertebrate nerves fragmented by sonic oscillations or by blending, De Robertis and Schmitt (1) observed characteristically dense-edged fibrils to which the descriptive term "neurotubules" was applied. While the fragmentation technique is not well suited to localization of components, reasons were given for supposing that neurotubules are axonic constituents. Particularly crucial were the facts that neurotubules were obtained from axons isolated from squid giant nerve fibers and that characteristic neurotubules were not found in nerves subjected to in vitro or Wallerian degeneration (2). Neurotubules from nerves infected with poliomyelitis virus (3) and with B virus (4) were also studied. The localization problem was particularly acute because of the similarity, under certain conditions, between neurotubules and collagen fibrils.

Subsequently data inconsistent with the view that neurotubules are of axonic origin were obtained in this laboratory. Preparations of rabbit nerves, after extensive (3 weeks) Wallerian degeneration, were found not to be devoid of neurotubules but to contain them in an abundance at least as great as that of control preparations of normal nerves (5). The divergence of these results from those previously reported (2) is presumably due to the sampling difficulty encountered with the fragmentation technique. Neurotubules were also found in aqueous dispersions of fragmented unfixed bull frog spinal roots although it had previously been supposed that neurotubules are highly unstable in unfixed preparations (this property was one of those thought to differentiate neurotubules from collagen fibrils).

Because of its significance in neurology the problem of the localization of "neurotubules" in the nerve fiber was reinvestigated. Two avenues of approach seemed capable of yielding unequivocal results: (1) a detailed investigation of axoplasm isolated from giant fibers and (2) a study of various types of nerve fibers in thin sections. A preliminary report of the fibrous structure of fresh

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and fixed axoplasm of the squid, *Loligo pealii*, and of the marine annelid, *Myxicola infundibulum*, has been given (6) and a more detailed paper is in press (7). The present paper describes observations of thin sections of squid giant fibers and of several types of vertebrate nerves. Of primary interest for present purposes is the fibrous structure of the axon and of the connective tissue; the structure of the myelin and the metatropic sheaths will be dealt with in a subsequent communication.

**Material and Methods**

The nerves studied include giant fibers of the squid, sciatic nerves of the green frog and the white rat, and interganglionic segments of human sympathetic nerve chains.

The squid nerves were fixed in 10 per cent formalin in sea water, or in 1 per cent osmic acid for 24 to 48 hours. Vertebrate nerves were fixed in 10 per cent formalin in appropriate Ringer solution, in 1 per cent osmic acid, or in formalin followed by osmic acid. Formalin fixation proved unsatisfactory for myelinated fibers because poor fixation of the lipid material of the myelin sheath produced distortions such as to leave the nerve fibers almost unrecognizable in thin sections.

After fixation and thorough washing in water, the nerves were dehydrated in alcohol and embedded in n-butyl methacrylate according to the procedure outlined by Newman, Borysko, and Swedlow (8). After polymerization of the monomer (using 1½ per cent benzoyl peroxide as a catalyst) the blocks were trimmed to about 1 mm. and sectioned with a rotary microtome adjusted to cut to 0.05 μ according to the technique of Baker and Pease (9). Best results were obtained when sectioning was done at low temperatures (2-4°C.) either by carrying out the operation in a cold room or by chilling the specimen block with CO₂. Ribbons were obtained by floating the sections on water contained in a trough attached to the knife in a manner similar to that described by Gettner and Hillier (10). Calculations from shadowed specimens indicated that the sections were somewhat thicker than the setting of the microtome indicated.

RCA type EMB and type EMU electron microscopes were used. Chromium in thicknesses of 5 to 10 Å was used for shadowing, the shadowing angle being usually between 10 and 15°.

**Electron Microscope Observations**

*Squid Giant Fibers.*—Cross or longitudinal sections of the giant fibers, 0.3 to 0.4 mm. in width, were first mapped on the grid with the light microscope. Any particular square could then be identified in the electron microscope and electron micrographs taken in various regions known to be in the sheath or in the axon.

The axon contains a dispersion of nodose filaments, fine granules, and relatively amorphous material. In transverse sections, as illustrated in Figs. 1 and 2, the filaments appear as relatively short segments, due probably to

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the fact that the axon filaments pursue a course predominantly parallel with
the axis. In longitudinal sections, as in fragmentation preparations (6), the
filaments are of indefinite length. The axon filaments, in formalin-fixed as well
as in osmic acid-fixed preparations, have a characteristically beaded or nodose
appearance. Filament widths range from somewhat less than 100 Å to about
200 Å. The nodes occur more or less regularly along the filaments, giving rise
to a pseudoperiodic appearance. In some cases the filaments appear devoid of
nodes, presenting a smooth contour. Granules apparently independent of fila-
ments were also observed. Conceivably the filaments are a complex of fibrous
and globular components. The resolution so far obtained with sections does
not permit more detailed description of the structure of the filaments.

The axon filaments as described above are obviously distinctly different
from the dense-edged neurotubules previously supposed (1) to characterize
the fibrous component of the squid axon.

The sheath of the giant fiber is composed of a packing of fibrils of variable
widths. The inner portion of the sheath bounding the axon, the metatropic
sheath, has special significance for it contains radially oriented lipid molecules
and is presumably homologous to the myelin sheath (11). The structure of
this component will be examined in more detail in subsequent experiments.
For present purposes interest centers on the outer, connective tissue com-
ponents of the sheath. These fibrils are cross-striated and are presumably col-
lagenous in nature, though a study of the detailed structure (intraperiod
structure, etc.) has not yet been made. In one preparation the fibrils in the
external region of the connective tissue were found to have dense edges, re-
sembling neurotubules (Fig. 3). The width of these connective tissue fibrils
(230 to 350 Å) agrees well with that reported for the neurotubules of squid
giant fibers (1). Apparently the demonstration of dense-edged fibrils in thin
sections of squid giant fibers requires very particular conditions. In the few
cases when such structures were seen in these experiments they were found to
occur in the sheath rather than in the axon. The characteristic appearance of
the connective tissue in these sections is that of cross-striated fibrils with a
period in the range of that characteristic of collagen. That neurotubules are
not axonic constituents is confirmed by the fact that characteristically dense-
edged fibrils have been observed in fragmented squid fibers in which axon struc-
ture had degenerated, as indicated by complete loss of positive birefringence,
after standing in cold sea water for 3 days.

Frog and Rat Sciatic Nerves.—Transverse and longitudinal sections have
been studied. In osmic acid or formalin-osmic acid-fixed preparations, mye-
linated fibers are readily recognized by virtue of the extremely dense myelin
sheath.

Fig. 4 shows a longitudinal section of a 5 μ medullated fiber of rat sciatic
nerve. The axon contains a fine network of smooth or nodose filaments which
are shown in more detail in Fig. 5. The beaded structure is not as evident as in shadowed preparations. The widths of the filaments range between 130 and 180 Å. In regions where the nodes were more or less regularly spaced the internodal distance averaged between 200 and 350 Å. A similar range of values was manifested by axon filaments of frog medullated fibers.

Closely adhering to the periphery of the myelin sheath and forming a fibrous matrix between nerve fibers are bundles of fibrils presumably collagenous in nature. In occasional cases (NT in Fig. 4) the fibrils have dense edges, resembling neurotubules.

**Human Sympathetic Nerve Fibers.**—In the sections studied the unmyelinated fibers were readily recognized by the characteristic network of nodose filaments in the axon and by the cross-striated collagen fibrils of the connective tissue. The axon is bounded by a thin sheath whose detailed structure has not yet been resolved.

The axon filaments have a structure similar to that described in sciatic nerves. Filament widths average about 200 Å. In several cases the axon presented a granular appearance, probably due to poor fixation.

In some cases the compact fibrils (presumably connective tissue) separating axons had dense edges reminiscent of neurotubules (Fig. 6).

**DISCUSSION**

The present evidence clearly demonstrates that neurotubules are not axonic constituents. The fibrous material of the axon occurs as filaments considerably thinner than neurotubules, manifesting only a rather irregular axial periodicity due to the arrangement of bead-like nodes along the filaments. The average distance between axial discontinuities ranges between 200 and 350 Å in mammalian nerves. Although further investigation may reveal greater regularity in this pseudoperiod and establish its value more accurately, the axial pattern differs unmistakably from the collagen-like pattern of neurotubules. The axon filaments have no dense edges in formalin- or in osmic acid-fixed material.

When formalin-fixed vertebrate nerves are fragmented as described by De Robertis and Schmitt (1), it is relatively easy to demonstrate characteristically dense-edged fibrils in abundance. However, in sections of formalin- or osmic acid-fixed nerves such structures are, in our experience, seen very rarely and then only in the connective tissue components (Figs. 3, 4, and 6).

The question arises whether the dense-edged fibrils are morphological entities in the sheath or are artifacts produced by the particular procedure used by De Robertis and Schmitt (1). If the material of the dense edges is lipid in nature it is possible that the material, though present in untreated nerves, is removed in the sectioning procedure, involving treatment with organic solvents. However, it seems more likely that the dense edges result from the fragmentation procedure. Fibrils resembling neurotubules were obtained in this labora-
tory by fragmenting washed collagen fibrils (from skin) in water containing dispersed nerve lipid. Possibly other electron-dense materials, if adsorbed to collagen fibrils, may similarly produce dense edges. It would be pertinent to determine the nature of the material constituting the dense edges of the fibrils prepared by the method of De Robertis and Schmitt (1). However, since the dense-edged fibrils are not constituents of the axon or of the neuron proper but derive from the connective tissue sheath, such an analysis seems of secondary importance. Unless evidence to the contrary can be adduced, it seems best to assume that “neurotubules” are not morphological entities in normal, intact nerve. This makes interpretation of observations on nerves infected with neurotropic viruses (3, 4) uncertain since it must now be assumed that the characteristic particles were associated with fibrils from connective tissue rather than from the axon. Possibly a study of infected nerves in thin sections may prove rewarding. Experiments along these lines are being planned.

The present observations obviously constitute but an introduction to the problem of axon structure. The nodose filaments are probably characteristic of fixed nerve axons generally for they have been found in two invertebrate phyla and in a variety of vertebrate forms. The role of the fixative may be quite important in producing the characteristic nodose appearance since the filaments observed in unfixed axoplasm are usually smooth-contoured rather than nodose (7). Obviously much improvement in technique will be required to resolve the detailed structure of the filaments.

SUMMARY

In squid, frog, rat, and human nerves examined in thin sections with the electron microscope the axon contains, in addition to certain other particulates, characteristic filaments. These filaments have diameters ranging from about 100 to 200 Å and have indefinite length. They frequently have a nodose appearance due to the presence of discontinuities sometimes fairly regularly spaced along the filaments. This structure differs unmistakably from that of the dense-edged fibrils called “neurotubules” and it is clear that the latter are not axonic constituents. Though dense-edged fibrils can readily be demonstrated in fragmented formalin-fixed nerve preparations, they are seldom observed in thin sections. When such structures were seen in these experiments they were located in the connective tissue sheath. The present evidence offers no support for the view that “neurotubules” are structural entities of normal intact nerves.

BIBLIOGRAPHY


**EXPLANATION OF PLATES**

**PLATE 36**

Electron micrographs of thin transverse sections of three squid giant fibers. In all figures the scale indicates 1 μ.

Fig. 1. Squid giant fiber fixed in formalin. After removal of n-butyl methacrylate embedding medium, sections were shadowed with chromium. Only a small portion of the sheath (S) and the axon (A) is shown. × 4,400.

Fig. 2. Region of axon from a section prepared as in Fig. 1. Note segments of axon filaments showing beaded structure. × 35,000.

Fig. 3. Fibrils of outer layers of connective tissue sheath of squid giant fiber. From thin transverse section of a fiber fixed in osmic acid. Note dense edges characteristic of "neurotubules." × 49,000.
(Schmitt and Geren: Fibrous structure of nerve axon)
Thin longitudinal sections of mammalian nerve fibers.

Fig. 4. Myelinated fiber of rat sciatic nerve fixed in formalin followed by osmic acid. Myelin sheath forms the dense border of the fiber. Note filamentous structure of the axon and connective tissue fibrils adherent to the periphery of the fiber. (In some regions (NT) these fibrils may appear dense-edged, characteristic of "neurotubules") X 11,000.

Fig. 5. Enlargement of region of axon indicated in rectangle in Fig. 4. Note axon filaments showing beaded structure in regions. X 37,000.

Fig. 6. Human sympathetic fiber fixed in osmic acid. Axon has characteristic filamentous structure. Connective tissue fibrils bordering the fiber show relatively dense edges. X 18,000.
(Schmitt and Geren: Fibrous structure of nerve axon)