HELICAL SUBSTRUCTURE OF NEUROFILAMENTS ISOLATED FROM *MYXICOLA* AND SQUID GIANT AXONS

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ABSTRACT

Neurofilaments purified from invertebrate giant axons have been analyzed with the electron microscope. The neurofilaments have a helical substructure which is most easily observed when the neurofilaments are partially denatured with 0.5 M KCl or 2 M urea. When the ropelike structure comprising the neurofilaments untwists, two strands 4–5.5nm in diameter can be resolved. Upon further denaturation these strands break up into rod-shaped segments and subsequently these segments roll up into amorphous globular structures. Stained, filled densities can be resolved within the strand segments, and these resemble similar structures observed within the intact neurofilaments. The strands appear to consist of protofilaments 2–2.5 nm in diameter. These observations suggest that the neurofilament is a ropelike, helical structure composed of two strands twisted tightly around each other, and they support the filamentous rather than the globular model of intermediate filament structure.

KEY WORDS neurofilaments · 10-nm filament · axon

10-nm-thick intermediate filaments are long, unbranched fibrous structures found commonly in a variety of cell types such as nerve cells (26), smooth muscle cells (2), fibroblasts (1, 9), and macrophages (5). Although their function is not known, these filaments are thought to have a role in axonal transport in neurons and in the structural support of the neuronal cytoskeleton. Intermediate filaments are particularly abundant in the axonal processes of neurons where they are designated neurofilaments (8, 26).

Two rather different views of neurofilament structure exist in the literature. One view which is based almost entirely on electron microscope observations of sectioned axons was developed by Wuerker (26) from a model first proposed by Schmitt and Davison (21). This model holds that the neurofilament is a tubular structure composed of a helical array of globular subunits. Four of these globular subunits ~3.5 nm in diameter make up a basic unit; and these units are stacked one upon the other to form a tubule, somewhat analogous to the current view of microtubule structure. An alternative to the globular model has been suggested by the studies of Metuzals and his associates. Using both sectioned material and negatively stained preparations in their studies, they have postulated that the basic unit of the filament is a 2- to 2.5-nm fibrous protofilament (15–17). These protofilaments are thought to be interwoven in a helical pattern resulting in a ropelike structure for the neurofilament. This model has been further elaborated by Gilbert (7).

We have studied the structure of isolated intact neurofilaments from the giant axons of the marine

polycheate *Myxicola infundibulum* and the squid *Loligo pealii*. Because relatively pure axoplasm enriched in neurofilaments can be rapidly separated from these axons, they provide a valuable source of material for the purification of intact neurofilaments. Using these preparations, we developed a rapid fractionation procedure which separates the neurofilaments from the other constituents of the axoplasm and preserves the core structure of the filament in a relatively native state. The protein composition of these fractions indicates that the neurofilaments are highly purified, and it has been possible to identify their principal subunit proteins (12, 14).

Most of our studies have utilized the giant axon of *Myxicola*, because in itself it is a highly enriched preparation of neurofilaments. The generality of our observations was assessed by studying neurofilaments prepared from the giant axon of the squid, a representative of a different phylum (Mollusca). The results of these studies described below support the fibrous model of intermediate filament structure. A preliminary report has been published elsewhere (11).

MATERIALS AND METHODS

M. infundibulum were obtained from Marine Research Associates; Lord's Cove, Deer Island, M.B. Canada, and maintained in the laboratory in aerated artificial sea water at 4°C. Pure axoplasm was obtained from the nerve cord exposed by dissection of the worm. The nerve cord was cleaned from the adhering gut tissues and washed several times with fresh *Myxicola* Ringer's solution. The axon was slit open with iridectomy scissors along its entire length. The viscous axoplasm was sucked out with a 50-μl pipette and transferred to 0.2 ml of 0.1 M KCl, 10 mM Tris-HCl (pH 7.4) containing 8 mM of 2-mercaptoethanol. It was dispersed gently in a glass-to-glass tissue homogenizer. The filaments in this suspension were examined, both before and after purification, by negative staining methods. Pure filaments were obtained by a single-step purification which involved centrifugation through a discontinuous sucrose gradient (14). The filaments were found at the 1.0 M–2.5 M sucrose interface (SW 50.1 rotor [Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.] at 40,000 rpm for 3 h). Filaments were also fixed either before or after purification and compared with similar unfixed materials. Fixation was done by mixing filament suspensions with an equal volume of 5% glutaraldehyde in 0.1 M Tris buffer (pH 7.4).

Electron Microscopy

The abundance of filaments in the preparation invariably necessitated further dilution of the sample. An optimal filament concentration was obtained by adding 25 μl of the sample to 0.2 ml of buffer. A drop of the solution was placed on a thin layer of carbon film supported on a grid by a thick holey carbon film, or on a carbon-coated Formvar film. For negative staining, unbuffered 1% uranyl acetate (pH 4.4–4.5) or phosphotungstic acid at neutral pH was employed. The grids were examined in a Siemens 101 electron microscope and a Philips 200 operated at 80 kV.

Magnification and Measurements

The magnifications of the plates were read either directly from the dials on the microscope or determined with carbon gratings. In some experiments, ferritin molecules were mixed with the filament suspension for comparison of their thickness. Measurements were made on micrographs taken at a base magnification of ~55,000–120,000 and further enlarged photographically 3–10 times.

The filament thickness was also measured in thin sections of plastic-embedded materials and compared with that of fresh filaments. The worms for this purpose were fixed, processed, and embedded in Araldite, and the thin sections were double-stained in uranyl acetate and lead citrate.

RESULTS

Thin-Sectioned Material

The *Myxicola* giant axon and its surrounding sheath cells are shown in Figs. 1 and 2. Neurofilaments represent the predominant structural feature of the axon. As Gilbert et al. have noted (8), no microtubules are visible in the giant axon. Some small tubular profiles were noted which have the same dimensions as microtubules; however, these tubular profiles appear to be attenuated regions of the agranular reticulum which is a particularly prominent structure near the surface of the axon (Fig. 2).

The glia cells which invest the giant axon (Fig. 2) also contain filaments which may be similar to the glial filaments seen in the astrocytes of vertebrates. Comparisons of the neurofilaments and the glial filaments in *Myxicola* indicate that they differ from one another. For one thing, whereas the glial filaments are packed tightly together in bundles, the neurofilaments are generally spaced farther apart. The glial filaments are considerably smaller than neurofilaments. Measurements of their diameters in cross sections revealed values of 7–8 nm for glial filaments and 10–11 nm for neurofilaments. Wuerker (26) has reported similar values for the width of glial filaments and neurofilaments in mammals. Thus, the differences which have

been found between glial filaments and neurofilaments of mammals appear to be generalizable to animals of phyla other than vertebrates.

The morphology of the squid giant axon viewed in sectioned material has been described in the literature (16), and our observations are consistent with these descriptions. The neurofilaments in the squid giant axon have an appearance similar to those in *Myxicola*. However, unlike the *Myxicola* giant axon, the squid giant axon contains a large complement of microtubules and therefore more closely resembles the typical axons of vertebrates.

Morphology of Neurofilaments Prepared From Axoplasm

Resolution of the fine structure of neurofilaments is much greater in preparations which are spread on a grid and negatively stained than in thin-sectioned material. Therefore, most of our observations have been made on negatively stained material. Because we did not observe any differences between squid and *Myxicola* filaments, our description will focus primarily on *Myxicola*, and references to the squid will be limited to cases where we believe that it is important to document a particular example.

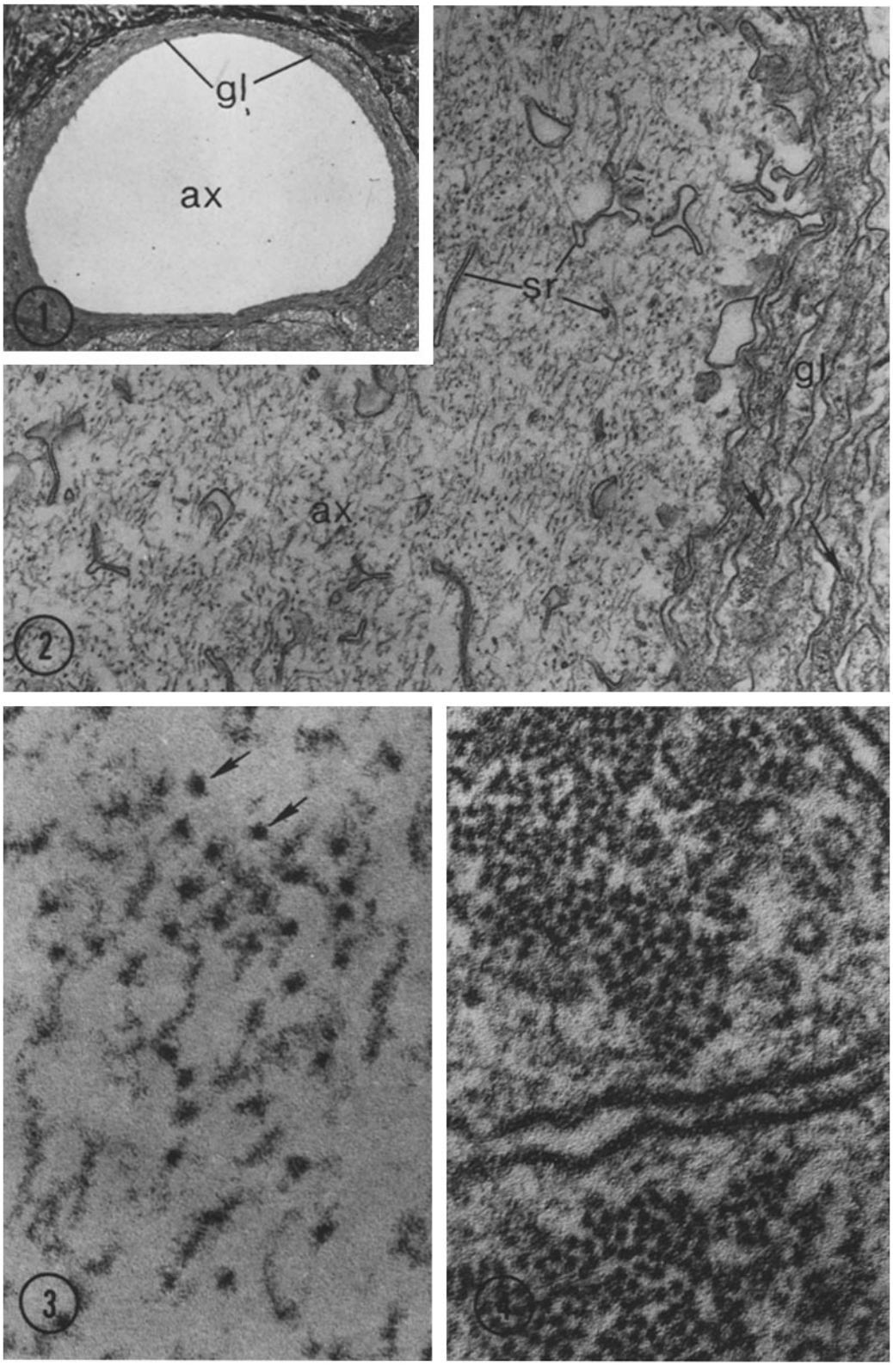
Neurofilaments prepared from whole axoplasm provided a reference point for our studies on purified filaments. The fine structure of the filaments was indistinguishable in preparations of freshly dispersed axoplasm whether the axoplasm was dispersed in fixatives or in buffer. Our principal reason for using purified filaments from *Myxicola* was to remove the soluble proteins and other more slowly sedimenting material which we thought might interfere with a high-resolution analysis of the core structure of the filaments. In the case of the squid giant axon, an added goal was to remove the microtubules. To maintain the native form of the neurofilament, both for these studies and for biochemical analyses, we designed a purification scheme which was relatively rapid and had as few steps as possible. Highly purified filament preparations were obtained by subjecting axoplasmic homogenates to sedimentation through a discontinuous sucrose gradient (see the companion paper for the details of this procedure). The longest neurofilaments sedimented through the gradient, leaving the soluble proteins and membranes behind on the gradient.

The purified filaments appeared as long, unbranched fibrous structures which measured many microns in length. The surface of the filaments was relatively smooth (Fig. 5), and there was no clear evidence of the side arms which are commonly observed projecting from the filaments in thin sections. The side arms were not observed even in preparations of whole axoplasm which were rapidly dispersed into buffer A and immediately fixed by the addition of glutaraldehyde. Metuzals and Mushynski (17) have observed side arms which appear to interconnect adjacent neurofilaments in unfixed spreads of cytoplasm from rabbit Deiters' neurons. These observations indicate that the projecting side arms are relatively labile and either are detached from the filaments during isolation or collapse onto the filament so that they are not observed as projections.

The thickness of the spread filaments in fixed and unfixed preparations was similar. In Fig. 9, a ferritin particle is included with neurofilaments for size comparison. Filaments purified by sucrose density gradient centrifugation range in width between 7 and 9 nm. This width is somewhat larger than that of 7.2 nm found by Gilbert et al. (8) for *Myxicola*. This difference probably reflects the difficulty in deciding where the edge of the filament is in negatively stained preparations and a difference in choice in our studies and those of Gilbert et al. (8).

Fine Structure of the Neurofilament

A number of investigators have indicated that no clear substructure can be observed in negatively stained preparations of neurofilaments. However, we noted that the stain penetrated the structure of the purified filaments, apparently filling spaces in the filament and producing an appearance of repeating links of a chain (Figs. 5–7). The chainlike configuration was a constant feature of purified filaments from both squid and *Myxicola*. In favorable regions of the filaments the links appeared to have a periodicity of ~5 nm from the center of one stained-filled density to another (Fig. 6). Strings of 6–8 of the repeating densities could be followed along the filament, and these linear arrays tended to be aligned at an angle relative to the main axis of the filaments (Figs. 7). Fine filamentous structures measuring 2.0–2.5 nm in width appeared to make up the links of the chain (Fig. 6) and resembled the 2-nm helically intercoiled protofilaments which have been observed in positively stained neurofilaments (17). These appear to be similar to the "unit filaments" which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Metuzals and Izzard (16) have observed in negatively stained preparations of squid filaments.

Dissection of the Filament Structure with Denaturants

The suggestions of a helical substructure in the filaments seen in the intact filaments can be clearly demonstrated when the neurofilaments are teased apart either with high concentrations of salt (0.5 M KCl) or urea. The filaments begin to fall apart when they are treated with 0.5 M KCl or 2 M urea for a few minutes before staining and are completely disrupted by 4 M urea. This susceptibility has been noted by others for *Myxicola*, squid, and mammalian neurofilaments (3, 8, 17, 20). In 2 M urea the chainlike strands begin to separate from one another, and the neurofilaments have the appearance of an untwisted rope made up of two strands. Various stages of this unraveling can be seen in Figs. 8–11. In some instances the ends of neurofilaments appeared frayed, as others have noted. The strands measured 4–5.5 nm in width and were twisted around each other in a right-handed helix. The pitch length of the helix measured in 35 cases in which the filaments were partially unraveled ranged between 100 and 130 nm (Fig. 11).

Another notable feature of the denatured neurofilament preparations was the appearance of globular structures which were attached to the neurofilaments. These globular structures could be produced either by shearing the filaments or by treating them with urea. Fig. 12 shows an example of a neurofilament in a preparation of purified filaments in Buffer A which were subjected to a vigorous homogenization in a glass-glass homogenizer. A number of globular swellings can be seen along the filament which is attenuated and in regions appears to be reduced to a single 4- to 5.5-nm strand. The appearance of globular elements in 2 M urea is illustrated in Fig. 13. All of the filaments have globular regions which are apparently strung together by a structure resembling one of the strands which comprise the filament. The complete conversion of the filaments into globular structures of varying sizes by 4 M urea is illustrated in Fig. 14. The majority of these globular structures range in size between 120 and 180 nm. The globular material disappeared and was completely converted into short filaments when this preparation was dialyzed exhaustively against 10 mM Tris-HCl, pH 7.4 at 4°C (Fig. 15). These observations indicate that the globular structures associated with neurofilaments represent one of the denatured states of the neurofilament core protein.

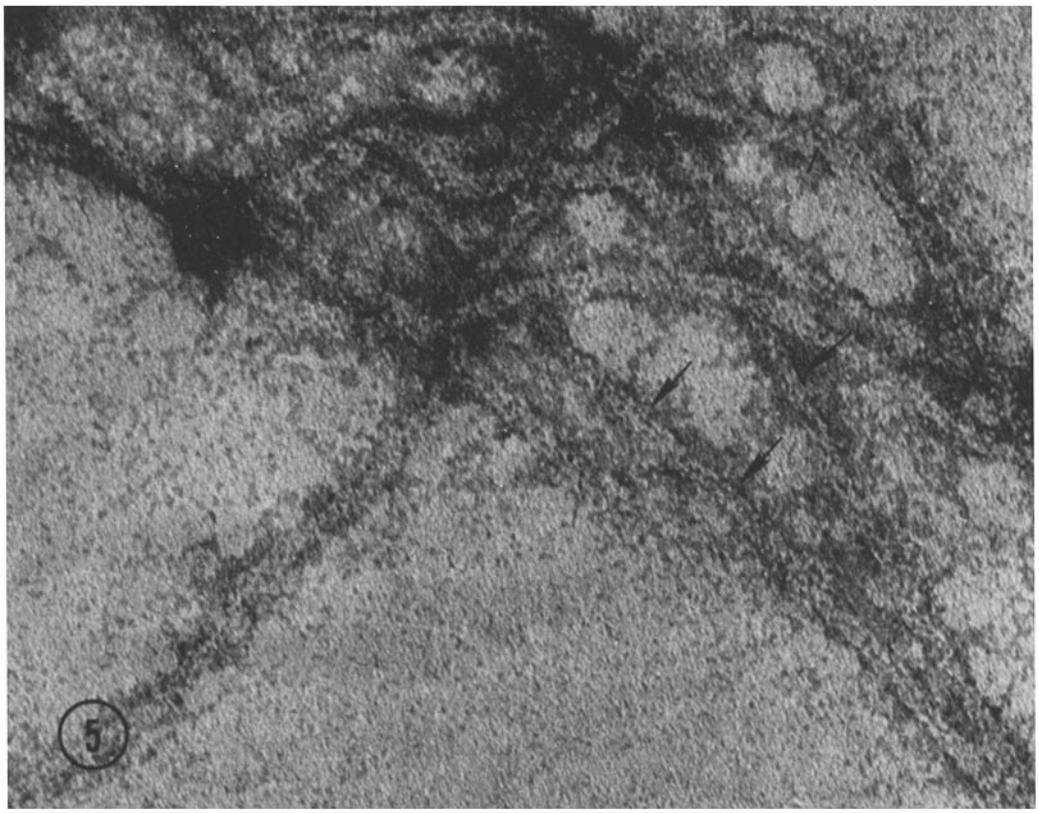
The intermediate stages in the conversion of the filament proteins into globular structures were useful for further examining the substructure of the strands which comprise the filaments. Before rolling up into the amorphous globular structures, many of the disrupted strands appear to remain rod-shaped. In a few favorable cases when neurofilaments were completely disrupted by 4 M urea but had not changed into the globular structures, individual strands could be resolved measuring 4–5.5 nm in width (Fig. 16). The chainlike

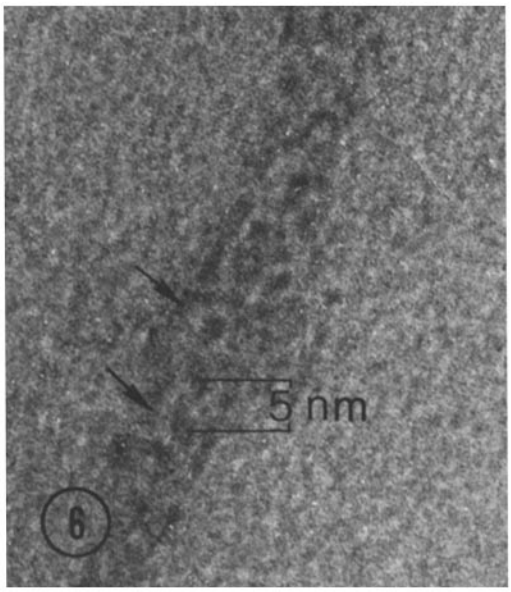
FIGURE 1 A light micrograph of a transversely sectioned *Myxicola* giant fiber stained with toludine blue. A thick glial sheath (*gl*) invests the giant axon (*ax*). × 150.

FIGURE 2 This electron micrograph of a thin section of *Myxicola* giant fiber shows a region of adaxonal glial sheath (*gl*) and a representative portion of the axoplasm (*ax*). Neurofilaments are the most abundant structural elements in the axoplasm. The small tubular profiles (*sr*) in the axoplasm are transversely sectioned smooth endoplasmic reticulum. The glial cells and their processes commonly contain bundles of filaments (arrow). These are shown at higher magnification in Fig. 5. Stained in uranyl acetate and lead citrate. × 46,000.

FIGURE 3 This micrograph of a thin section through neurofilaments in a *Myxicola* giant axon illustrates their typical appearance in materials embedded in plastic. In cross sections, the neurofilaments (arrows) show a heavily stained central region surrounded by a less electron-dense coat. Stained in uranyl acetate and lead citrate. × 233,000.

FIGURE 4 The glial filaments seen in this electron micrograph of a thin section through a *Myxicola* giant fiber can be compared with the neurofilaments shown in Fig. 3. The glial filaments which are smaller in diameter are also packed more tightly together than the neurofilaments. Stained in uranyl acetate and lead citrate. × 162,000.





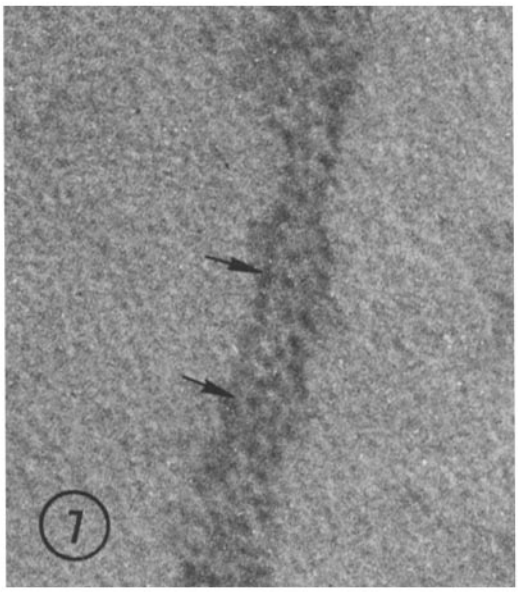
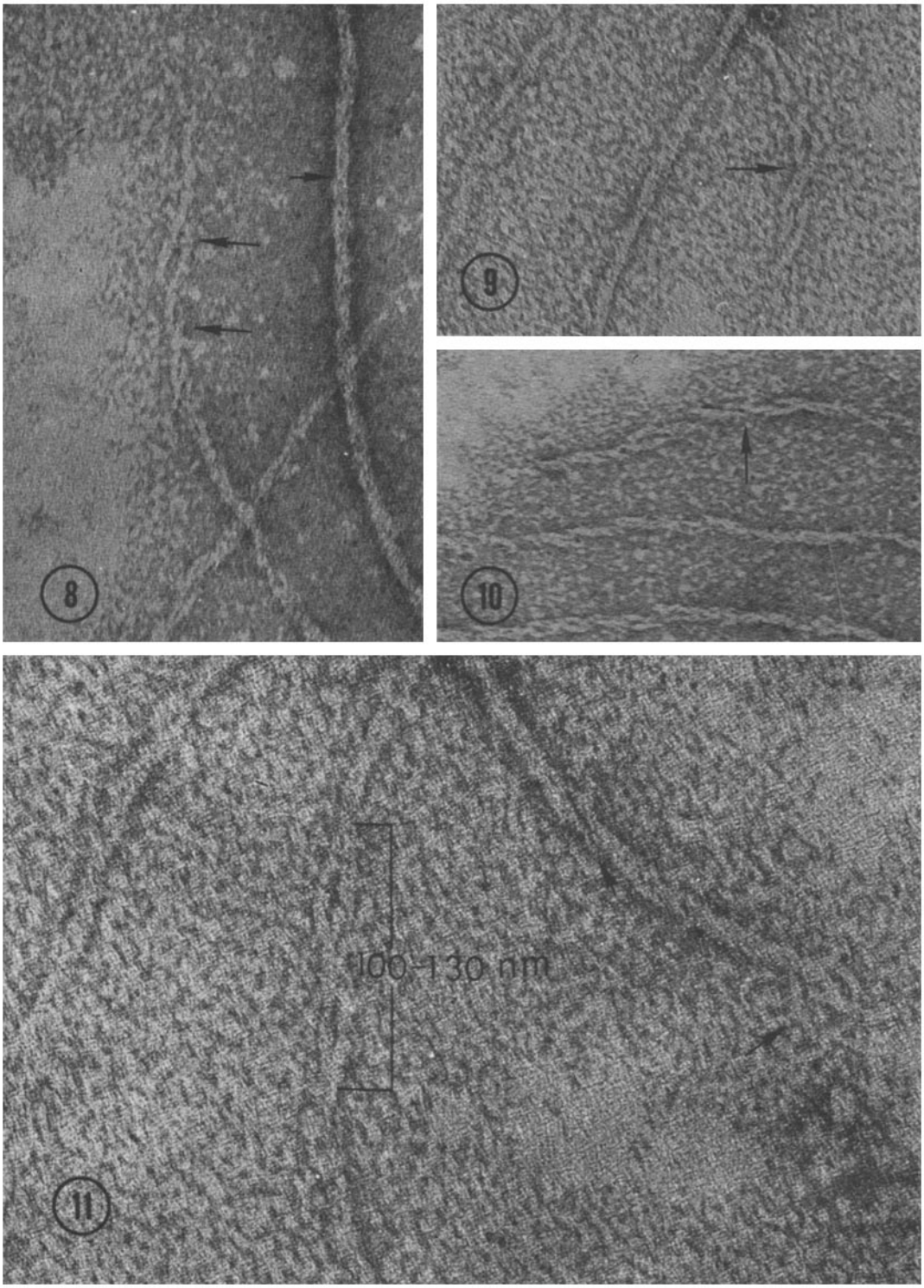
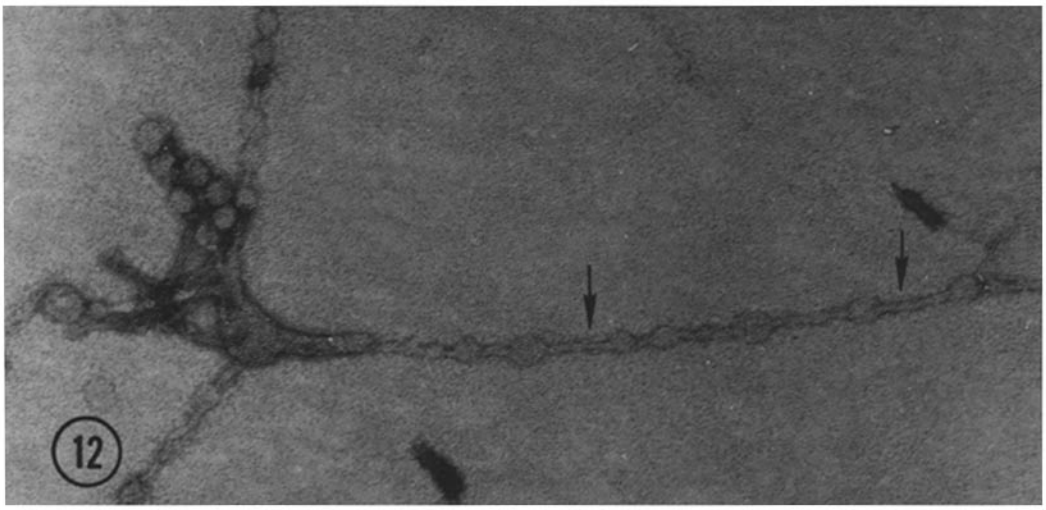


FIGURE 5 A high resolution micrograph of negatively stained *Myxicola* neurofilaments showing certain substructural characteristics. It illustrates the stain-filled depressions forming a series of dots along the length of the neurofilaments which are the most prominent feature of negatively stained intact filaments. This appearance is shown more clearly at higher magnification in Figs. 6 and 7. Negatively stained with 1% aqueous uranyl acetate. × 400,000.

FIGURES 6 and 7 These micrographs of negatively stained *Myxicola* neurofilaments illustrate the stain-filled depressions located along the length of the neurofilaments. These densities give the neurofilament a chainlike appearance (arrow). The densities appeared to be periodic measuring ~5 nm from center to center. The densities are bounded by a 2.0- to 2.5-nm filamentous structure which is seen most clearly in Fig. 6 (arrows). Negatively stained with 1% aqueous uranyl acetate. Fig. 6 × 1,254,000; Fig. 7 × 860,000.



FIGURES 8–11 *Myxicola* neurofilaments treated with 2 M urea before negative staining. The filaments are shown in a variety of stages of disruption, varying from intact to partially disrupted filaments. Note that two strands can be resolved in the disrupted filaments and that these strands apparently twist around each other in a helix (arrows), and that the helix is right handed (Figs. 8 and 10). In Fig. 11 one of the filaments has a frayed appearance at its end (arrow). Negatively stained with 1% aqueous uranyl acetate. Fig. 8 × 283,000; Fig. 9 × 277,000; Fig. 10 × 200,000; Fig. 11 × 419,000.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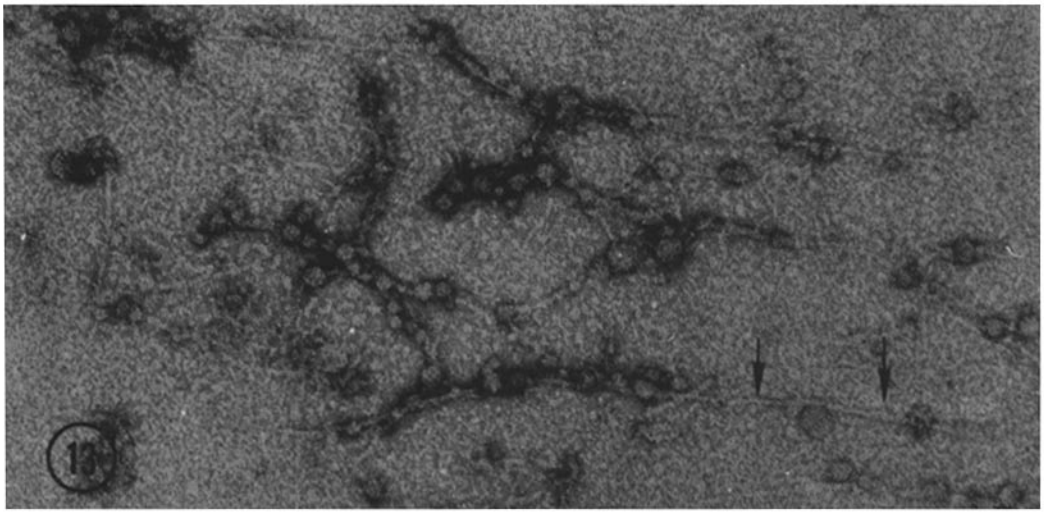
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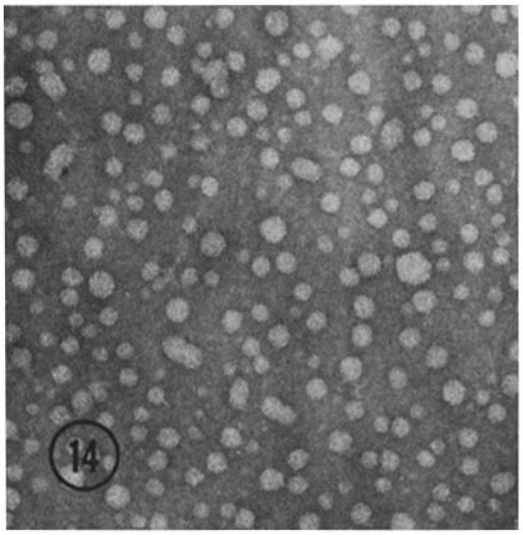
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configuration which was observed in the native neurofilament could be resolved in a number of these rod-shaped strands (Figs. 17, 18). The similarity between these structures and those seen in the native neurofilament suggests that the 4- to 5.5-nm strands observed in 4 M urea are equivalent to the chainlike strands observed in intact filaments. The rod-shaped structures varied in length from 20 to >40 nm. As in the case of the native neurofilaments, a 2- to 2.5-nm fibrous structure made up the loops surrounding the stained densities in the strand. In Fig. 18, the 2- to 2.5-nm fibrous structure has the appearance of a continuous protofilament which is twisted upon itself. These observations indicate that at least part of the chainlike repeat seen in the intact neurofilaments reflects the intrinsic structure of the strands which comprise the neurofilaments.

DISCUSSION

Our observations of purified neurofilaments from animals of two phyla, Annelida and Mollusca, indicate that the neurofilament is a rope constructed from protofilaments rather than a tubule composed of globular subunits. Thus, these studies confirm and broaden the fibrous model for neurofilament structure (7, 15–17); our observations are inconsistent with the globular model (26). We have found that two 4- to 5.5-nm fibrous strands which are ~½ the diameter of the 10-nm filament are twisted together to form a two-stranded rope, and the rope has the form of a right-handed helix with a pitch of 100–130 nm. Small and Sobieszek (22) have come to a similar conclusion from their studies of the intermediate filaments in smooth muscle; optical diffraction patterns of long intact filaments led them to suggest that the filament is a two-stranded helix with a pitch of 124 nm. Schlaepfer (19) has also suggested that mammalian neurofilaments have a helical substructure, and he has occasionally observed the presence of two strands in filaments treated with urea.

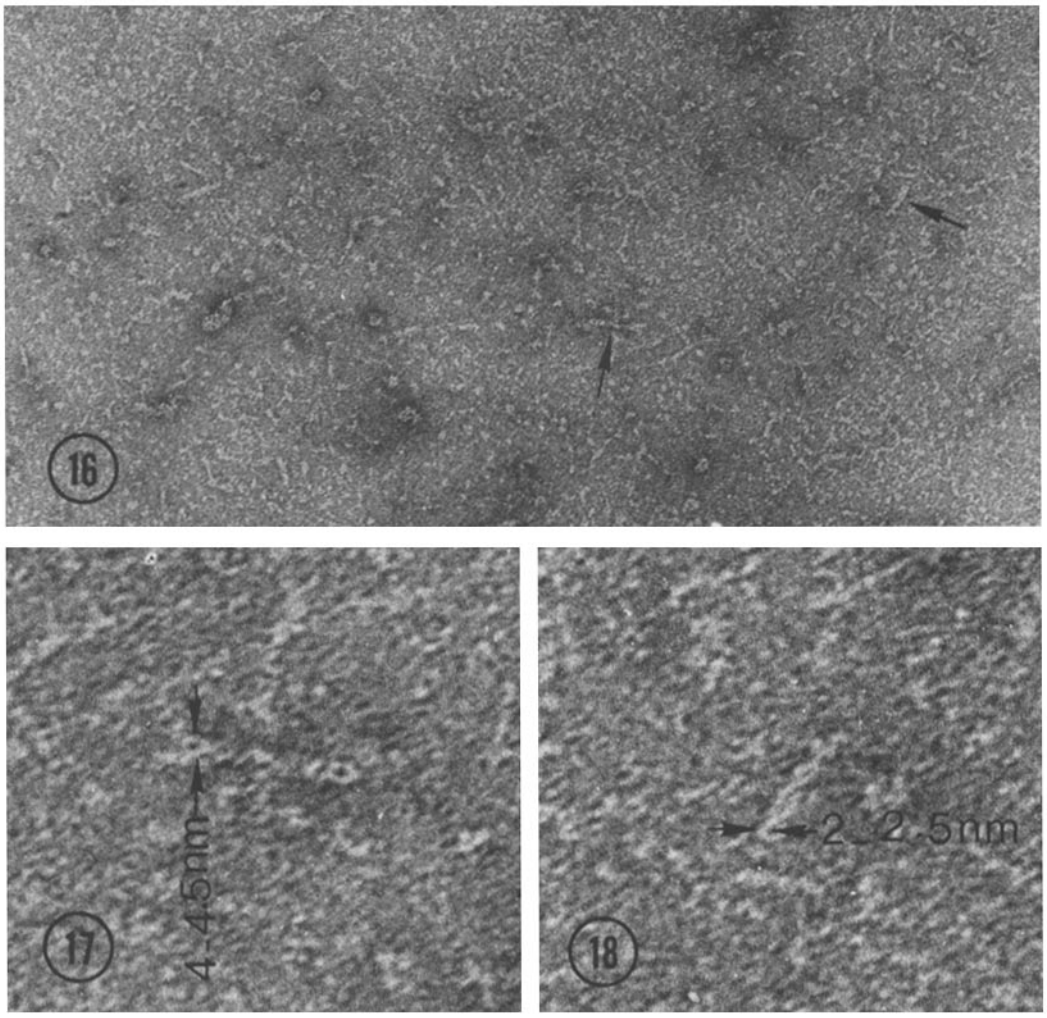
The primary electron microscope evidence for the presence of strands in intact filaments are the chainlike structures which result from the deposition of stain in periodic depressions in the filament. One fraction of the periodic depressions is apparently intrinsic to the strand itself because it can be seen when the structure of the neurofilament is disrupted so that individual strands are resolved. Another fraction of the periodic densities may reflect the presence of a groove between the two strands.

The filament strands appear to be flexible structures, reflecting the inherent flexibility of the entire neurofilament which can be bent at rather acute angles without any evidence of breakage. When one of the strands is stripped away from its complementary partner, the remaining strand can exist as an intact elongated fibrous structure with lengths in excess of 700 nm (Fig. 13). However, the strands also break into shorter segments (Figs. 16–18). The minimum length of the strands is an interesting dimension because it is probably directly related to the length of the protofilament; however, we were unable to accurately determine the minimum strand length.

FIGURES 12 and 13 *Myxicola* neurofilaments were found to undergo a change in structure which resulted in globular elements. This transition could be produced either by vigorous shearing in a glass-glass homogenizer in buffer A (Fig. 12) or by 2 M urea (Fig. 13). In the preparation which was sheared in low ionic strength buffer (Fig. 12), a single 4- to 5.5-nm strand (arrow) is decorated with amorphous globular structures. In Fig. 13 the globular structures have a relatively constant size. In one case (arrows), a long single 4- to 4.5-nm strand has been left intact even though its partner appears to have been almost completely separated from it except for a few remaining segments which are present as decorating globules. Negatively stained with 1% aqueous uranyl acetate. Fig. 12 × 121,000; Fig. 13 × 132,000.

FIGURE 14 In this preparation of *Myxicola* neurofilament treated with 4 M urea, the neurofilaments have undergone a complete transition to amorphous globular structures. Negatively stained with 1% aqueous uranyl acetate. × 184,000.

FIGURE 15 A preparation in which *Myxicola* neurofilaments were renatured after disruption into globular structures by 4 M urea. Renaturation was produced by prolonged dialysis against 10 nM Tris-HCl buffer, pH 7.4 at 4°C. The renatured products are shorter than intact neurofilaments and have a branching appearance. Negatively stained with 1% aqueous uranyl acetate. × 179,000.



FIGURES 16–18 In a few fortuitous cases, treatment with 4 M urea resulted in complete disruption of the *Myxicola* neurofilaments without a complete transition to the globular form. This field is filled with rod-shaped structures (strand segments) measuring 4- to 5.5-nm in diameter. Two of the strand segments (arrows) are shown at higher magnification in Figs. 17 and 18. Note that the periodic densities observed in the intact neurofilaments illustrated in Figs. 9–11 also appear to be present in the strand segment and that these stain-filled areas are surrounded by a 2- to 2.5-nm protofilament. Negatively stained with 1% aqueous uranyl acetate. Fig. 16 × 140,000; Fig. 17 × 519,000; Fig. 18 × 510,000.

Evidence for a Rod-Shaped Protofilament Subunit

Although it has been possible to resolve the structure of the neurofilament into strands, the intrinsic structure of the strand itself remains a puzzle. Our observations support those of Metuzals (15) who has proposed that the smallest resolvable element in the filament is a 2- to 2.5-nm protofilament, and we have observed comparable protofilaments in intact filaments and in the individual strands. Markham rotation of cross sections of intermediate filaments from heart Purkinje fibers indicates that the intermediate filament is composed of four protofilaments (4). If the neurofilament also contains four protofilaments, then each of the strands probably contains two protofilaments. Our observations of the fully disrupted neurofilaments are consistent with this possibility (Fig. 18). Furthermore, Metuzals and Mushynski

(17) have suggested that the protofilaments are arranged in an intercoiling helix, and such an arrangement could explain the chainlike structures that we have observed in the strands.

In the Schmitt and Davison (21) model which was further elaborated by Wuerker (26), the subunit of the neurofilament is viewed as a globular molecule 3.5 nm in diameter. If the protofilament actually consisted of a string of 3.5-nm globular subunits, then such substructure should be resolvable in negatively stained spreads of neurofilaments. However, such globular substructure was not seen in our preparations; instead, the protofilaments have the appearance of a relatively uniform fibrous structure. In addition, the relatively large size of the principal subunit of *Myxicola* neurofilaments which has a mol wt of 150,000 when it is reduced with 2-mercaptoethanol in the presence of SDS is not easily reconciled with a globular model for the neurofilament subunit. If the subunit protein were roughly spherical in shape, then it would have a diameter in excess of 10 nm, which is the width of the entire filament itself.

Although the evidence for a rod-shaped neurofilament subunit remains indirect, far more thorough analyses of a similar intermediate filament, bovine epidermal 7- to 8-nm filaments (tonofilaments), have provided a relatively advanced picture of the structure of its subunit (23). The epidermal filament is apparently composed of repeating three-chain units containing segments of triple-chain coiled-coil alpha helix (23, 24). The three chain units seem to contain two discrete coiled-coil alpha helical regions interspersed with regions of nonhelix. At present, it is not clear whether the three-chain model applies to neurofilaments, and a two-chain model has been proposed for the *Myxicola* neurofilament subunit (7).

Denaturation and Renaturation of the Protofilament

Denaturation of purified neurofilaments leads eventually to a complete loss of recognizable filament structure and to the appearance of amorphous globular structures with an average diameter between 120 and 180 nm. Such amorphous globular structures in association with neurofilaments have been noted previously (8, 17, 18). It has been suggested that these structures might represent accessory proteins, possibly the side arms which are attached to the neurofilaments (8). However, our observations and those of Schlaepfer (18) on mammalian neurofilaments indicate that these globular structures are composed principally of the neurofilament core protein in an altered state. For example, we have found that the globular structures appear in highly purified preparations in which neurofilament subunit proteins constitute almost all of the protein, and we have also observed the transitional stages in the transformation of the neurofilaments into these globular structures. The discovery that the neurofilament proteins can take on this globular form is of some practical importance because these structures appear spontaneously in neurofilament preparations as they age, and the presence of these structures in neurofilament protein preparations may be an index of denaturation. For those investigators who are principally interested in isolating neurofilament protein, the presence of globular structures such as those seen in our preparations does not necessarily indicate contaminating proteins.

Gilbert (7) has convincingly argued that the subunit of *Myxicola* neurofilaments contains a significant amount of alpha helix, apparently in the coiled-coil form, and he has suggested that at least part of the filament subunit is an elongated fibrous protein. In this regard, it is interesting that the neurofilament proteins assume a globular shape when they are denatured but recover the elongated form when the denaturant is removed by dialysis. This effect may in part represent a conversion of the alpha-helical component of the protein into a random coil by denaturation and a subsequent recovery of the elongate coiled-coil alpha helix when the denaturant is removed. Neurofilaments from squid and mammals have been reconstituted after disruption with 6 M guanidine hydrochloride and subsequent dialysis against dilute buffers (3, 10); the reconstitution of neurofilaments after such harsh treatment indicates that the determinants which are required for reassembly must be recoverable after denaturation. The stability of these determinants in the neurofilament protein is consistent with the view that they represent a large number of interactions between the surfaces of two or more elongate molecules.

Our observations suggest the following sequence of events during invertebrate neurofilament denaturation and renaturation: The lateral interactions between the strands of the neurofilament appear to break first, resulting in the separation of the strands of the filament. The strands then begin to break up into segments of varying lengths.

Finally, the segments lose their rod-shaped structure and roll up into amorphous globular structures. Although we have not observed the steps involved in the renaturation process, we expect that they represent a reversal of the progression during denaturation. However, the renatured products are not exact replicas of the original filaments but are shorter and may be branched; these differences may reflect mismatching of the strands or incomplete renaturation of the subunit.

The Helical Structure of Neurofilaments and the Helical Structure of Axons

On the basis of our observations and those in the literature, we propose the following model for neurofilament structure: The basic unit of intermediate filaments is a protofilament 2–2.5 nm in width. The protofilament consists of an unknown number of elongate protein subunits with a substantial content of alpha helix (7). Two protofilaments are associated with one another to form a strand segment 4–5.5 nm in width. Strand segments are bonded together at their ends to form a polymeric strand, and two polymeric strands interact laterally with one another to form a double-stranded rope.

The ropelike character of the neurofilament may be an important contribution to the helical properties of axoplasm. Helical structure has been observed at a number of levels in the axoplasm of the squid and the *Myxicola* giant axons (6, 13, 15). Gilbert and his colleagues have made the most careful analysis of the helical structure of axoplasm in their studies of *Myxicola*. They have concluded that this helical structure is derived from the properties of the neurofilaments because the neurofilaments represent the major formed element in the *Myxicola* giant axon. The tendency of neurofilament proteins to polymerize into helical structures has also been observed in vitro (13); and Gilbert (7) has postulated that the helical twist which is present in the axoplasm could be explained if the neurofilaments behave like twisted elastic rods.

Our observation that the neurofilament consists of two strands which are twisted tightly around each other in a ropelike configuration adds further support to the idea that the neurofilament is a twisted elastic rod; such a rod would contain an inherent torsional force that could readily explain the tendency of neurofilaments to become organized into helical bundles of continually increasing size. This torsional force might also be involved in the formation of the twisted filaments in nerve cells of patients with Alzheimer's disease. The pathology of this disease involves the accumulation of neurofibrillary tangles within nerve cells bodies, and these tangles (which also occur in the brains of most aged humans) appear to be made up of pairs of neurofilaments twisted tightly around one another (25). The twisted Alzheimer filaments have a periodic constriction with an average length of 65 nm (25), suggesting that the helix formed by the paired neurofilaments has a pitch of 130 nm. It is interesting that the helical pitch of the paired neurofilaments is similar to the helical pitch of the paired strands that comprise the individual neurofilaments. Thus, both the helical properties of axoplasm and the character of the neurofibrillary tangles of aged neurons may be derived from the intrinsic helicity within neurofilament structure.

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