maintenance of form. There is evidence that tropomyosin is present in neurons
(Fine et al., 1973) and that it may be a smaller protein than muscle tropomyosin. The
other ancillary proteins have so far not been rigorously identified.

The way in which actin and myosin work in cells other than muscle is not known.
There is little doubt that they are involved in some way with movements, and
the striking similarity they show to their muscle counterparts encourages the view
that, at the molecular level, they work in a similar way. The problem in such
cells as neurons is to understand how the basic motile elements can be organized and
associated with other cellular structures such as membranes and microtubules.

The best known kind of movement shown by nerve cells is axoplasmic transport.
The extreme asymmetry of neurons requires that special mechanisms exist to carry
material to and from the soma. Fast axoplasmic flow has been shown to be blocked by
substances, such as vinblastine, that cause the breakdown of cytoplasmic microtubules.
These could produce movement directly by assembly and disassembly, by interaction
with dynein-like proteins as in cilia and flagella, or by interaction with actomyosin-like
proteins. Another activity that might well involve fibrillar elements is the release of
transmitter from synaptic endings.

The tips of regenerating and developing neurites show an amoeboid movement.
This appears to be exploratory and to determine the direction of growth (Letourneau,
1975). Ultrastructural examination of these areas does not show regular arrangements of
fibrillar structures except in the filopodia. These distinctive features of the growth cone
are long thin structures that can form rapidly and also exhibit a kind of retraction.
They do not contain microtubules, but bundles of microfilaments in longitudinal
alignment are seen. In related structures, such as microvilli, these can be decorated with
muscle myosin. It seems probable that their extension depends on actin-like proteins,
either by sliding against myosin or by polymerization, and that these must in some way
be connected to structures on the outside of the cell that are responsible for adhesion.

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Neurofilaments

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Although neurofilaments are found in most nerve cells, their molecular structure,
protein composition and functions remain to be determined. In sectioned material,
neurofilaments appear about 10nm wide. It has been suggested that they represent
one form of a ubiquitous ‘intermediate’ filament. Other related structures may be
glial filaments (Yen et al., 1976) and smooth-muscle 10nm filaments (Rice et al., 1975).
It is now agreed that neurofilaments are structurally and biochemically distinct from the
smaller 6nm microfilaments (actin) and the larger 25nm microtubules.
Attempts to isolate neurofilaments have met with only limited success. This is reflected in the disparate estimates of the molecular weights and solubilities of the main protein components of neurofilaments (Table 1). Apart from minor discrepancies in molecular-weight estimates due to differences in technique, there are clearly major differences concerning the physical properties of neurofilament protein still to be resolved. Some of these discrepancies are more apparent than real, since molecular-weight estimates from gels containing sodium dodecyl sulphate may vary greatly (+look in different electrophoretic conditions, and also depend considerably on the standards available. For example, R. J. Lasek (personal communication), using a mol.wt. standard of myosin (200000), finds substantial agreement with the molecular weights we have reported for *Myxicola* neurofilaments.

The isolation of neurofilaments from brain involves osmotic shock of myelinated axonal segments to release the neurofilaments. These preparations may be contaminated with glial filaments, because they contain two distinct morphological types of fibrillar bundles (Shelanski *et al.*, 1971). The procedure is also lengthy, so it is possible that the neurofilament proteins are degraded by the proteinases present in brain (Guroff, 1964; Hallpike & Adams, 1969; Dahl, 1976). Isolation of neurofilaments from squid giant axons is relatively more rapid (20 min) than from brain (3 h), but the most readily accessible source is the giant nerve fibre of the marine worm *Myxicola*. Axoplasm can be extracted as a gel from the worm in about 10 s. The gel is relatively free from
non-neuronal contamination, and is also free of the axonal plasma membrane (Gilbert, 1975a).

Electron-microscopical examination of sectioned whole Myxicola axoplasm shows it to consist of a densely packed array of neurofilaments. Microtubules are absent. (Little, if any, of the protein in Myxicola axoplasm has the mobility of tubulin in gel electrophoresis in the presence of sodium dodecyl sulphate.) Negatively stained preparations of Myxicola axoplasm homogenized in buffers of low ionic strength show many smooth neurofilaments, about 7 nm wide and up to 10 μm long. However, the neurofilament protein can adopt more complex configurations when exposed to fixatives, non-electrolytes (sucrose, glycerol) or changes of ionic strength (Gilbert et al., 1975).

At both extremes of ionic strength the neurofilaments are either partially or whole disassembled. Negatively stained preparations of neurofilaments dispersed in distilled water show them frayed into narrower fibres, some as small as 2 nm wide. In low concentrations of neutral buffer (ionic strength below 0.2; usually 20 mM-histidine, pH 7.0) neurofilaments are readily sedimented in the ultracentrifuge (2 h at 150000 gav), and by repeated sedimentation (three to six times) preparation of ‘washed neurofilaments’ are obtained. As the ionic strength is raised beyond 0.3 with KCl, fewer neurofilaments sediment. Analytical ultracentrifugation of washed neurofilaments in 0.75 M-NaCl (with 20 mM-cacodylate, pH 7.0) shows a single peak, somewhat hypersharp, with a leading foot, and sedimenting very much as myosin does at high ionic strength. The sedimentation coefficient has been measured over a fivefold range of protein concentration and an s20, w value of about 6.0 S obtained by extrapolation to zero concentration. Since the sedimentation properties are similar to those of myosin, we interpret these data to mean that at high ionic strength the neurofilaments are dissociated to give a single highly asymmetric subunit. This interpretation is consistent with both electron-microscope observations and with X-ray-diffraction studies (Day & Gilbert, 1972).

The latter have shown that neurofilaments are made up of coiled β-helical coils, placing them in the KMEF class of fibrous proteins. The intact filament must therefore consist of polymerized fibrous subunits of high β-helical content.

The disassembly of neurofilaments by solutions of high ionic strength appears to be reversible, since dialysis to low salt concentrations results in the reassembly of fibrous structures that are morphologically indistinguishable from the native neurofilaments.

Analysis of whole Myxicola axoplasm by gel electrophoresis in the presence of sodium dodecyl sulphate shows the predominant polypeptides to have mol.wts. of 152000 (comprising about 40% of the total protein) and 160000 (about 10%; Gilbert et al., 1975). These major polypeptides, plus a number of minor components, are also present in three types of purified preparations. These are: washed neurofilaments, neurofilaments reassembled from solutions of high ionic strength, and filaments reassembled after exposure to 6 M-urea. We conclude that these two major polypeptide components comprise Myxicola neurofilaments, and that several of the minor components are also likely to partake in the structure.

It has long been known that Ca2+ ions rapidly disperse the gel structure of squid axoplasm (Hodgkin & Katz, 1949). The same is also true for Myxicola axoplasm. Furthermore, incubation of whole Myxicola axoplasm with Ca2+ (CaCl2) at concentrations greater than 2 mM (in 20 mM-histidine, pH 7.0) results in the degradation of the major neurofilament polypeptides to products with mol.wts. in the 50000–80000 range. With 20 mM-CaCl2 the major polypeptides are completely degraded within a few minutes. The pattern of digestion on gels suggests that there are only a few sites susceptible to proteolysis, and that these sites lie quite distant from the ends of the chains. The digestion is prevented by preincubation with 1 mM-p-chloromercuribenzoate, 1 mM-1-chloro-4-phenyl-3-tosylamidobutan-2-one (TPCK) or 1 mM-7-amino-1-chloro-3-tosylamidoheptan-2-one hydrochloride (TLCK) but not by 1 mM-phenylmethanesulphonyl fluoride. These observations are consistent with the presence of an endogenous Ca2+-activated thiol proteinase in the extracted axoplasm.

This proteinase can be separated from the neurofilaments simply by sedimenting the latter out of solution. If the whole axoplasm gel is treated with 1 mM-p-chloromercuri-
benzoate, then subsequent addition of Ca\(^{2+}\) fails to produce dispersal of the gel (Gilbert, 1975b).

One curious feature of the proteinase is its lability in the presence of its activator, Ca\(^{2+}\). If axoplasm is pretreated with 0.5mM-CaCl\(_2\) for 20h at 0°C (subthreshold for detectable neurofilament digestion) and then more Ca\(^{2+}\) is added to give a final concentration in excess of 5mM-CaCl\(_2\), no further digestion results. Likewise, if the time-course of digestion at 0°C in the presence of 10mM-CaCl\(_2\) is followed, one sees an initial degradation in the first 2 min of incubation, but no further change, even after several days. One simple explanation for these observations is that the proteinase, once activated, rapidly degrades itself. In the absence of Ca\(^{2+}\) the proteinase is quite stable for up to 90h at 0°C, since on subsequent addition of 5mM-CaCl\(_2\) digestion is observed. Mg\(^{2+}\) does not activate the proteinase.

Our findings with this unusual proteolytic activity indicate that caution must be exercised in assigning molecular weights to neurofilament polypeptides. Since this particular proteinase is rapidly self-inactivated, it might be impossible to detect it once degradation of neurofilaments has taken place. Ca\(^{2+}\)-activated neutral proteinases have also been reported in brain (Guroff, 1964).

One further noteworthy observation concerning the Ca\(^{2+}\)-induced degradation of neurofilaments is its effect on X-ray-diffraction patterns. After partial digestion of the major Myxicola neurofilament polypeptides with 5mM-CaCl\(_2\) (in 20mM-histidine, pH 7.0), the X-ray diagram shows greatly increased detail that is, as yet, difficult to interpret, although the basic \(\alpha\)-pattern remains. Fractionation of the digestion products is likely to be important in interpreting these diagrams and may lead to a greater understanding of the neurofilament structure, much as studies of the proteolytic fragments of myosin have aided the elucidation of myosin structure.

We have recently discovered that the addition of cytochrome \(c\) (a protein of opposite charge to neurofilaments) to homogenates of either Myxicola or squid axoplasm causes the precipitation of neurofilaments. In the electron microscope aggregated neurofilaments are seen, many in the form of rings (Gilbert et al., 1975), similar in size and shape to those found in nerve endings (Boycott et al., 1961). When the protein composition of these aggregates from squid axoplasm is examined by gel electrophoresis in the presence of sodium dodecyl sulphate, two main components are found (in addition to the cytochrome \(c\)) having mol.wts. 65000 and 180000. The proportion of protein in the 65000-mol.wt. band is slightly greater than that in the 180000-mol.wt. band. This procedure appears to give remarkable enrichment of neurofilaments, and may be of general applicability.

In conclusion, we suggest that the main Myxicola neurofilament polypeptides have mol.wts. near 152000 and 160000 and that those from squid have mol.wts. of about 65000 and 180000. The rapid and self-destructive behaviour of proteolytic enzymes in neurofilament preparations, however, suggests that these and other molecular-weight data be viewed with caution. Nevertheless, our findings for squid neurofilaments are in fairly close agreement with those obtained by Rice et al. (1975) and Lasek et al. (1975), even though our isolation procedure is entirely novel.

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Biochemical Properties of Microtubules

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The biochemical properties and subcellular distribution of microtubular subunit protein (tubulin) in nervous tissue have been investigated, with special reference to enzymes and/or substrates involved in phosphoprotein and phospholipid metabolism that co-purify with brain microtubules during their reassembly in vitro (for recent references, see Lagnado et al., 1975; Lagnado & Kirazov, 1975). Some of the interactions between various antimitotic (see Wilson et al., 1975) and neuroleptic drugs and microtubular components need to be considered in relation to their observed pharmacological effects on processes associated with synaptic function.


Microtubules and Synaptic Structure

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Microtubules and Vesicle Migration in Neurons

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Many of the difficulties inherent in analysing the movement of intracellular components can be minimized by studying their manifestations in neurons. Within the long processes of neurons (axons and dendrites) materials move to and from the cell body; the importance of this bidirectional traffic for all regions of the cell is reflected in the death of axons separated from their cell bodies and in the changes that occur in neuronal cell bodies whose axons have been sectioned. The nature of the materials moving and their rates of progress can be determined by constricting axons and examining the progressive accumulation of organelles and substances on both sides of the blockage. Isotopically labelled materials incorporated by the cell body can also be followed as they enter and pass along the axon. Finally, drugs can be applied selectively to either cell bodies or axons in attempts to separate transport phenomena from the biosynthesis of the transported materials (Banks & Mayor, 1972).