

IMMUNOFLUORESCENCE STUDIES OF NEUROFILAMENTS IN THE RAT AND HUMAN PERIPHERAL AND CENTRAL NERVOUS SYSTEM

W. W. SCHLAEPFER and R. G. LYNCH

From the Department of Pathology, Washington University School of Medicine, St. Louis, Missouri
63110

ABSTRACT

Localization of antisera to neurofilament antigens derived from rat peripheral nerve was carried out in tissues of rat and human peripheral and central nervous systems by indirect immunofluorescence. Unfixed and chloroform-methanol-fixed frozen sections of tissues were incubated in purified IgG of the experimental rabbit antisera and subsequently exposed to goat anti-rabbit IgG conjugated with fluorescein isothiocyanate. Control studies were conducted on identical tissue preparations incubated in the same concentrations of nonspecific rabbit IgG or in experimental rabbit IgG absorbed with extracts of rat peripheral nerve containing neurofilament antigen. Extensive immunofluorescence was observed in rat and human peripheral and central nervous systems. The distribution and configuration of immunofluorescence corresponded to neurofilament-rich structural components of these tissues. Prominent immunofluorescence occurred along axons of peripheral nerve fibers. Immunofluorescence was also noted in neuronal cell bodies of spinal sensory ganglia, especially in perikarya of the large neuronal type. Immunofluorescence of the central nervous system was located predominantly in myelinated axons of the white matter in cerebrum, cerebellum, brain stem, and spinal cord. Less intense immunofluorescence was also seen in neuronal perikarya and in short thin linear processes of grey matter.

Neurofilaments are a very conspicuous but poorly understood neuronal organelle. They can be recognized in neuronal perikarya but occur in great abundance within large neuritic processes (44). In fact, neurofilaments comprise the major structural component of many large axons (16, 17, 40) and some large dendrites (31, 39, 44, 45). Slow axonal transport has been considered to be the mechanism of their replenishment (13, 22, 33). Their function, however, remains obscure. It has been suggested that they play some role in axonal transport (4, 22, 36) or that they may furnish structural support for long neuritic processes (18, 39).

Our lack of knowledge about neurofilaments may be due in part to methodological limitations regarding the visualization and detection of these organelles within tissues. Identification of neurofilaments is presently limited to transmission electron microscopy. Silver impregnation preparations are capricious and lack rigorous organelle specificity (31).

A technique that has recently been developed in this laboratory enables the isolation of intact neurofilaments and neurofilamentous polypeptide components from rat peripheral nerve (34). Antisera which have been raised to this polypeptide

fraction react specifically with intact neurofilaments isolated from peripheral nerve (34). The present study has utilized these antisera along with standard indirect immunofluorescence methodology to localize neurofilament-containing structures in the peripheral and central nervous systems of rat and human tissues. Some of these findings have appeared in abstract (35).

MATERIALS AND METHODS

Brain, spinal cord, and peripheral nerves were obtained from 250–300 g rats in which the tissue vasculature had been cleared by cardiac perfusion of isotonic saline under pressure. Human tissues were obtained from autopsies performed within an 8-h postmortem interval. Peripheral nerves were desheathed of perineurium. All tissues were immersed and frozen in phosphate-buffered saline (PBS), and 8–10 μm frozen sections were cut from tissues within this medium and placed on dry, clean cover slips. Multiple frozen sections of different tissues were air-dried (15–60 min) on the same cover slip. Some tissues were subsequently fixed for 30 min by immersion in chloroform-methanol (2:1) at 4°C and then washed in PBS.

Both fresh and fixed tissues were incubated for 4 h at 4°C on a rocker platform in 1:10 dilutions of experimental and control IgG in PBS. Experimental IgG was isolated from rabbit antisera raised against extracts of rat peripheral nerve (34). Both experimental and control rabbit IgG were prepared by DEAE-column chromatography (15) from a 40% saturated $(\text{NH}_4)_2\text{SO}_4$ precipitate of sera. Stock solutions of experimental and control IgG contained 8–9 mg/ml protein, as determined by the Lowry procedure (27), using bovine serum albumin (BSA) as a standard reference.

Tissues were washed overnight at 4°C in several changes of PBS after the primary incubations. All subsequent treatments of tissues were identical, including secondary incubations for 2 h at 4°C in 1:40 dilutions of fluorescein-conjugated goat anti-rabbit IgG in a PBS media. Goat anti-rabbit IgG was obtained from Gateway Immunoserum, Cahokia, Ill. The 40% saturated $(\text{NH}_4)_2\text{SO}_4$ precipitable fraction was fluorescein con-

jugated as described (8). The conjugate had a fluorescein:protein ratio of 0.44 determined by absorbance at 490 λ and 278 λ . After incubation with conjugated anti-globulin, all tissues were subjected to a 4–6 h wash in several changes of PBS. Cover slips were mounted in glycerin-PBS, and samples were immediately examined and photographed with a Leitz Orthoplan microscope with Ploem illumination.

Experimental and control absorption studies were carried out in parallel with the primary incubations of tissues. Fresh and fixed tissues were incubated in 1:10 dilutions of experimental IgG in PBS containing either rat peripheral nerve extract (0.2 mg/ml) or BSA (0.2 mg/ml). The primary incubations with absorbants were conducted for 4 h at 4°C. All subsequent treatments were identical to those of other experimental and control incubations.

RESULTS

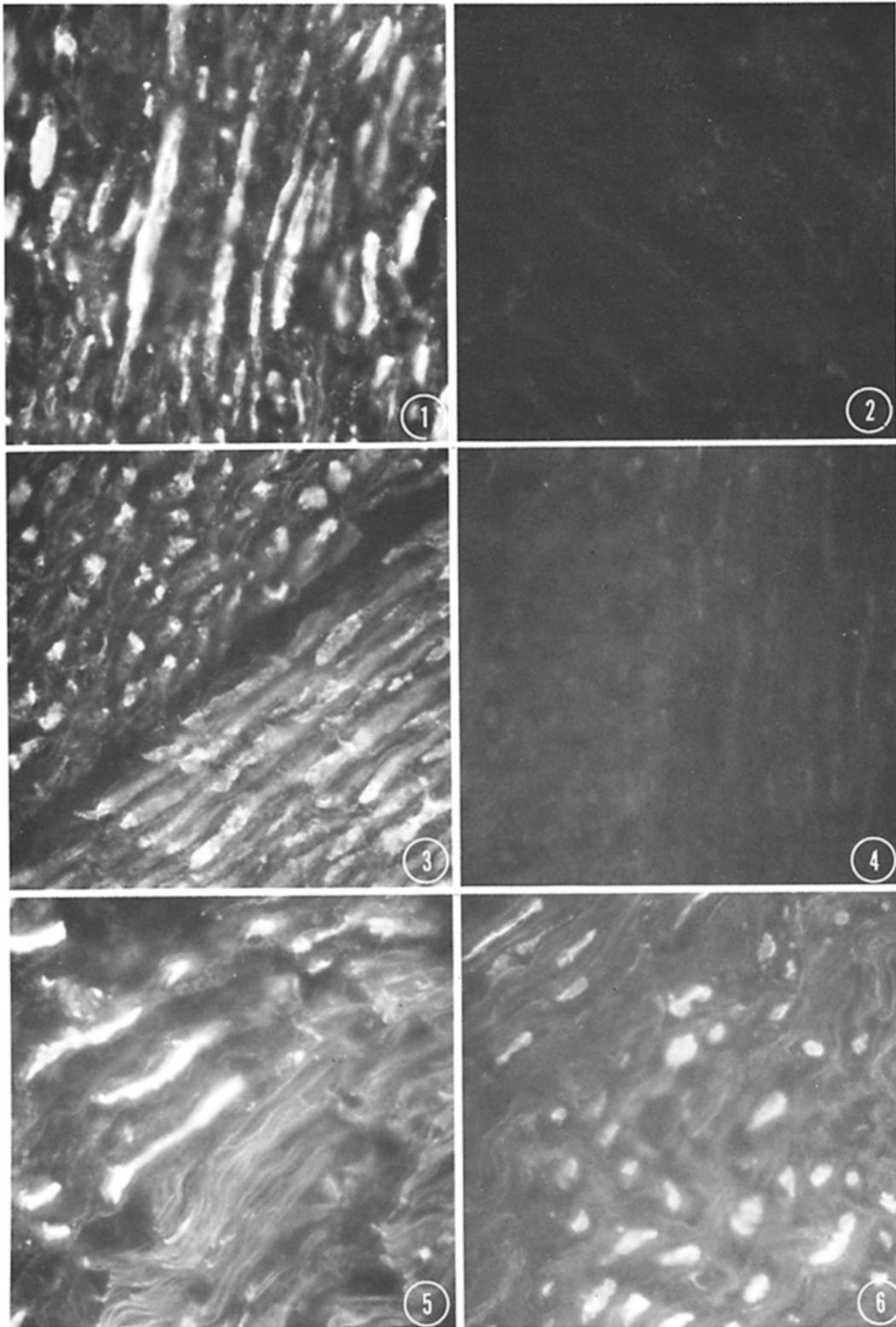
Peripheral Nerve

Bright-green immunofluorescence was readily observed in rat and human peripheral nerves which had been incubated in experimental IgG (Figs. 1–6). The dimensions, configurations, and overall distribution of immunofluorescent stain coincided with the location of axons within these tissues. The cylindrical-shaped axons appeared as linear or punctate profiles of immunofluorescence when sectioned in a longitudinal or transverse orientation, respectively (Fig. 3). Large myelinated axons were the most intensely fluorescent components of nerve fascicles. Unstained myelin sheaths could often be recognized surrounding the fluorescent axonal cores. Unmyelinated axons were infrequently encountered, appearing as clusters of parallel thin linear arrays which coursed along the longitudinal axis of the nerve (Fig. 5). Individual linear structures with faint fluorescence and more irregular course were also noted. Their anatomic identities were uncertain but some of them could represent Schwann cell processes.

FIGURES 1–2 Identical unfixed frozen sections of rat sciatic nerve incubated in experimental and nonspecific rabbit IgG, respectively. Axonal immunofluorescence is seen as partially truncated, linear profiles due to the sectioning of cylindrical axonal structures in a longitudinal-oblique orientation. $\times 250$.

FIGURES 3–4 Identical chloroform-methanol-fixed frozen sections of rat sciatic nerve incubated in experimental rabbit IgG absorbed with BSA and peripheral nerve extract, respectively. Nonspecific BSA absorbant fails to abolish axonal immunofluorescence in transversely (upper left) and longitudinally (lower right) sectioned nerve. $\times 250$.

FIGURES 5–6 Unfixed and chloroform-methanol-fixed frozen sections of human femoral nerve incubated in experimental rabbit IgG, respectively. Immunofluorescence can be seen both in large myelinated axons and in bundles of small unmyelinated axons, especially in the fresh frozen section. $\times 250$.



The same pattern of immunofluorescence was noted in rat (Figs. 1 and 3) and human (Figs. 5 and 6) nerves as well as in unfixated tissues (Figs. 1 and 5) and in frozen sections of nerve which had been immersed in chloroform-methanol (Figs. 3 and 6). The immunofluorescence was less intense but more uniformly distributed in chloroform-methanol fixed nerve. Fixation in formalin, paraformaldehyde, or glutaraldehyde was less successful in preserving immunofluorescence of the tissues.

Immunofluorescence of nerve was not seen in control incubations run in parallel on fresh (Fig. 2) or fixed (Fig. 4) tissues. It was not observed when nerve tissues were incubated with nonspecific rabbit IgG followed by incubation with fluorescein-conjugated goat anti-rabbit IgG (Fig. 2). The addition of peripheral nerve protein extract to the primary incubation completely inhibited the specific antibody and prevented the immunofluorescence of nerve tissue (Fig. 4), whereas an equal concentration of BSA did not inhibit (Fig. 3).

Spinal Cord

Immunofluorescence of rat and human spinal cord occurred predominantly in the parallel myelinated tracts which comprise the white matter of the cord (Figs. 7-9). In transverse section, immunofluorescent stain outlined multiple punctate profiles within these longitudinal nerve fiber tracts (Figs. 7 and 9). Occasional oblong configurations arose from focal oblique transections. The distribution and configuration of stain corresponded to the location of axons in the respective nerve tracts. Axons of the anterior spinal nerve root tracts were also outlined with immunofluorescence, appearing as aggregates of parallel linear profiles extending from the margin of the anterior horn to the edge of the spinal cord at the root entry zone and separating the longitudinally coursing long fiber tracts of the anterolateral funiculus (Fig. 7).

Less intense immunofluorescence was manifest in the grey matter of the spinal cord where staining was largely localized to fine short linear processes with irregular course (Fig. 7). Large neuronal perikarya were outlined by a diffuse immunofluorescence of moderate intensity. Limiting glial membranes along the pial surface or in perivascular locations did not stain.

Immunofluorescence of spinal cord of rat (Fig. 7) and human (Fig. 9) showed similar patterns but was localized with greater precision and consist-

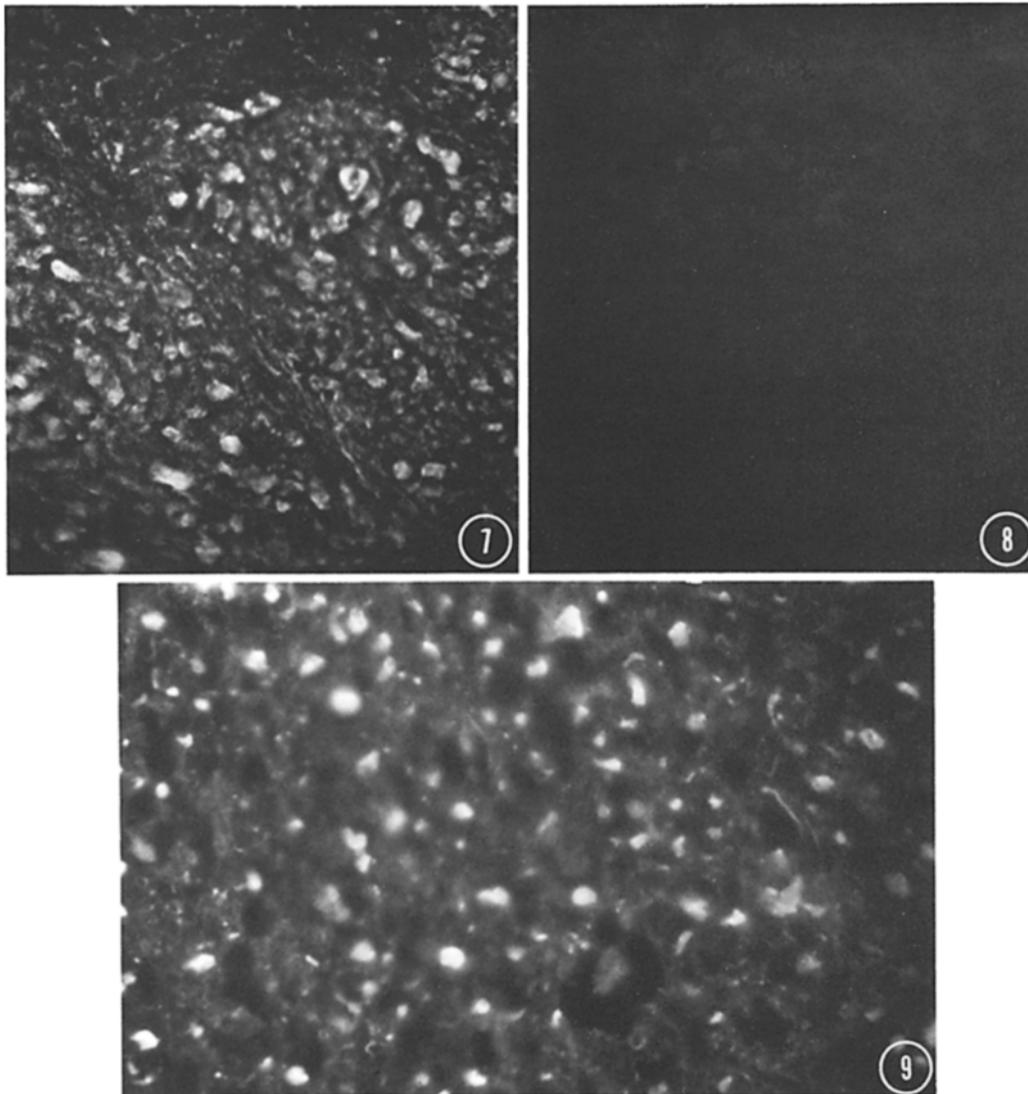
ency in fixed (Fig. 7) rather than fresh (Fig. 9) frozen sections. Control incubations of spinal cord were negative, including complete inhibition of immunofluorescence by the addition of purified peripheral nerve extracts to the first antibody incubation (Fig. 8).

Brain

Localization of immunofluorescence to specific structures in brain was limited by the cytological complexity of the tissues as well as by artifacts incurred by the use of fresh frozen sections. Nevertheless, general patterns of reproducible immunofluorescence could be recognized. Most of the immunofluorescence in rat cerebellum (Figs. 10 and 11), cerebrum (Fig. 12), and brain stem was located in areas of white matter and outlined discrete linear structures which had been cut in transverse, oblique, or longitudinal orientation. This characteristic immunofluorescence of white matter was exemplified by the staining pattern seen in the myelinated core of cerebellar folia (Fig. 11). These stained profiles correspond to the general configuration and distribution of myelinated axons which are known to comprise the major constituency of white matter substructure.

The immunofluorescence of neuronal cell bodies was less intense than that of the linear structures in white matter. Purkinje cells of the cerebellar cortex revealed a diffuse cytoplasmic stain which was partially obscured due to the encirclement of Purkinje cells by multiple converging processes with intense immunofluorescence (Fig. 10). Occasionally, smaller neuronal perikarya in the overlying molecular layer were also outlined by the convergence of fine immunofluorescent processes (Fig. 10). Some groups of neurons in the rat cerebral cortex showed a localization of perikaryal immunofluorescence in crescentic or ring-shaped configurations which often appear to surround the nucleus (Fig. 12). A similar cytoplasmic distribution of neurofilamentous immunofluorescence was observed in neuroblastoma cells (25).

Not all of the morphological constituencies of immunofluorescence could be identified within brain tissues. Nevertheless, the glial limiting membranes along the pial surfaces and around perivascular spaces were not the sites in which immunofluorescence could be detected. Furthermore, control studies with nonspecific IgG and immunoabsorbants were indicative of specificity of brain immunofluorescence.



FIGURES 7-8 Identical chloroform-methanol-fixed frozen sections of rat spinal cord incubated in experimental rabbit IgG absorbed with BSA and peripheral nerve extract, respectively. Axonal immunofluorescence occurs predominantly in long myelinated tracts in the white matter which have been sectioned in a transverse and focally oblique manner. A finer linear axonal immunofluorescence is present in grey matter of the anterior horn (upper left) and in anterior rootlets coursing laterally between the white matter tracts. Immunofluorescence is completely abolished by absorption with peripheral nerve extract. $\times 250$.

FIGURE 9 Intense axonal immunofluorescence in unfixed transverse frozen section through lateral funiculus of human spinal cord incubated in experimental rabbit IgG. $\times 250$.

Spinal Sensory Ganglia

Immunofluorescence occurred in neuronal cell bodies of rat spinal sensory ganglia (Fig. 13). Prominent immunofluorescent staining was ob-

served in perikarya of large ganglion cell type. These neurons revealed a lattice-like pattern of staining throughout their cytoplasm, the central nucleus remaining unstained. Immunofluorescence of the small ganglion cell type was consider-

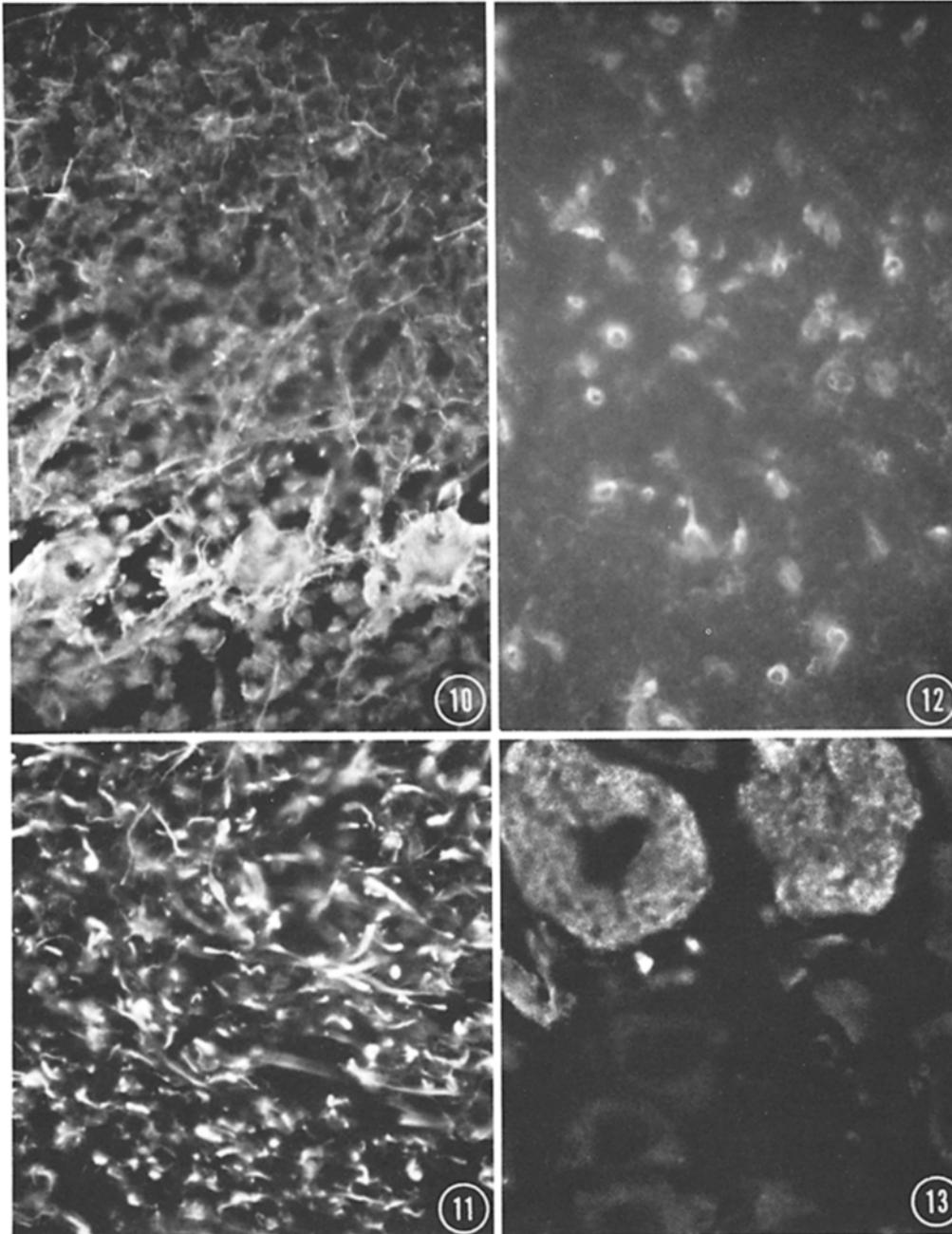


FIGURE 10 Chloroform-methanol-fixed frozen section of rat cerebellar cortex incubated in experimental rabbit IgG. Diffuse cytoplasmic immunofluorescence of three adjacent Purkinje cells is partially obscured by their encirclement by multiple converging immunofluorescent axonal processes. Immunofluorescent staining can also be seen in fine linear axonal processes in overlying molecular cortex. $\times 250$.

FIGURE 11 Chloroform-methanol-fixed frozen sections of white matter in rat cerebellar folium after incubation in experimental rabbit IgG. Intensely fluorescent linear profiles occur in irregular orientation, corresponding to the configuration and distribution of myelinated axons. $\times 250$.

FIGURE 12 Chloroform-methanol-fixed frozen section of rat cerebral cortex incubated in experimental rabbit IgG. Immunofluorescence can be seen in crescent- and ring-shaped configuration within neurons. Immunofluorescence of surrounding neuropil is faint but occurs in short, thin linear structures. $\times 250$.

FIGURE 13 Chloroform-methanol-fixed section of rat spinal ganglion incubated in experimental rabbit IgG. Immunofluorescence of two large ganglion cells (above) outlines central nucleus (top left) as well as mottled areas of cytoplasm, contrasting with the very faint diffuse immunofluorescence of small ganglion cells (below). Brightly fluorescent punctate profiles around large ganglion cell (top left) correspond to the location of emerging axon. $\times 500$.

ably less than that of the large ganglion cell type and tended to be diffusely distributed throughout the cytoplasm. Occasional punctate or short linear profiles of intense immunofluorescence could be seen immediately surrounding the large ganglion cells, corresponding to the axons emerging from these cells which are known to take a circuitous course before dividing into their bipolar processes (32).

DISCUSSION

The present study has demonstrated the feasibility of visualization by immunofluorescence of neurofilaments by the use of antisera which has been raised to neurofilament protein extracted from rat peripheral nerve. The specific reactivity of these antisera with isolated and intact neurofilaments from rat nerve has been demonstrated by immune electron microscopy (34). Wider application of these antisera is now evidenced by the *in situ* demonstration of neurofilaments among a variety of neural tissues by indirect immunofluorescence.

Extensive immunofluorescence of neurofilaments from the central and peripheral nervous systems of rat and human tissues is indicative of a common antigenicity of neurofilaments from diverse origins, suggesting a similarity in the chemical composition of neurofilaments from cerebral and peripheral nervous systems. Yet, neurofilament preparations isolated from mammalian brain are dominated by a 54,000-dalton polypeptide (11, 12, 24, 38, 46), a moiety which is not apparent in electrophoretic analyses of neurofilament-rich peripheral nerve (22, 26) or pure axoplasmic samples (19, 29) obtained from the giant axons of invertebrate species. Over 70% of the protein composition of peripheral nerve extracts containing neurofilamentous antigen migrated as a single electrophoretic band which corresponded to an apparent mol wt of 68,000 daltons (34). The presence of this 68,000-dalton polypeptide within the axial core of peripheral nerve neurofilaments was evidenced by the successful absorption of experimental antisera with fractions of this polypeptide eluted from polyacrylamide gels (34). The likelihood that this 68,000-dalton polypeptide is an antigenic component of neurofilaments in general is now indicated by the cross immunofluorescence reactivity between peripheral and central neural tissues, as well as by the widespread absorptive capacity of the same peripheral nerve extracts in successfully preventing immunofluorescence of neurofilament-rich structures in central and pe-

riphery nervous systems. This does not exclude the possible existence of other antigenic reactive components within peripheral nerve extracts or the presence of the same antigenic site(s) in larger or smaller polypeptide moieties.

Antisera to neurofilament preparations from brain tissue have been noted to cross-react with a glial fibrillary acidic protein (14, 23, 46) with a mol wt of about 54,000 daltons (10). Immunofluorescence localization of these antisera in mammalian brain revealed a distributional pattern which was indistinguishable from that noted with antisera to glial fibrillary acidic protein (14, 47). The cross-reactivity could be indicative of chemical similarities between glial filaments and neurofilaments (23, 46), or it could also result from copurification of polypeptides of similar molecular weights (2, 14). The latter possibility is supported by morphological (12, 38, 46) and immunological (2, 3, 12) evidence of admixtures of glial and neural filaments in brain fractions. Nevertheless, the cross-reactivity with glial filaments would limit the general utility of antisera to brain neurofilaments as an immunofluorescent probe of neurofilaments, even though it has been successfully used to demonstrate these organelles in neuroblastoma tissue culture cells (25).

Localization of immunofluorescence to neurofilament-rich components could be determined with greater precision in peripheral nerve (Figs. 1, 3, 5, and 6). Intense immunofluorescent staining was observed in large myelinated axons which are known to contain the largest number and highest density of neurofilaments (17, 40). Clusters of small unmyelinated axons were occasionally seen but were much less conspicuous than larger axons. Some short, thin linear arrays of faint immunofluorescence may also have represented Schwann cell processes. Schwann cells are known to contain intermediate-sized filaments (6; 30) which share many features of neurofilaments (see reference 34). Immunological cross-reactivity between neurofilaments and intermediate-sized filaments of Schwann cells could not be excluded from the present study.

Accurate localization of immunofluorescence to the cytological distribution of neurofilaments was also evidence in the spinal ganglia (Fig. 13). Large ganglion cells are distinguished cytoarchitecturally by the clustering of rough endoplasmic reticulum, separated by sheaths of neurofilaments and microtubules (1, 7, 41), endowing these cells with a lattice-like network of neurofilaments similar to

that described for large neurons in Deiter's nucleus (28). Small ganglion cell populations possess a diffusely distributed endoplasmic reticulum and few neurofilaments (1, 7, 41). The differential densities and patterns of neurofilaments between large and small ganglion cells were reflected in their respective immunofluorescence.

Precise cytological localization of immunofluorescence was obscured by the structural complexity of the brain and, perhaps, by the high lipid content of this tissue. Nevertheless, the pattern of staining in the central nervous system corresponded to the distribution and configuration of neurofilament-rich structures. Neurofilaments are particularly concentrated in large myelinated axons (30, 31, 44) and, to a lesser extent, in some large dendrites (30, 39, 45), but are generally less conspicuous in neuronal perikarya (30, 44). Accordingly, the prominent immunofluorescence of white matter is due to the predominant axonal constituency of this tissue. It is noteworthy that immunofluorescence staining was not observed along the glial limiting membranes which underlie the pial surfaces and surround the perivascular spaces of the brain. These locations are rich in glial fibers and are characteristically stained with fluoresceinated anti-glial sera, as exemplified in immunohistochemical studies of glial acidic fibrillary protein (5, 9).

Some distortions of structural components undoubtedly arose through the use of fresh frozen sections and chloroform-methanol fixation. The latter treatment presumably removed large amounts of lipid-soluble components from the brain, thereby enhancing the penetration of aqueous solutes into the tissues. Similar fixation has been successful in immunofluorescence studies of other brain antigens (20, 21). Some loss or alteration of antigenicity of the fixed tissues may have been countered by the enhanced exposure of tissue components. Both formalin and glutaraldehyde fixations were far less successful in maintaining immunofluorescence in brain and nerve tissues. Unfortunately, the inadequate preservation of either structural integrity or tissue antigenicity seems to be an inherent limitation for immunohistological studies on brain tissue.

Immunofluorescence of the neurofilamentous constituency within tissues provides a methodology which has considerable utility for elucidating many puzzling aspects of this widespread but still obscure organelle. Immunohistochemical studies may help elucidate the ontogeny, phylogeny, and

universality of neurofilaments, their relationship to other filamentous or contractile proteins, their synthesis, assembly, and movements within the complex confines of the neuron as well as their possible altered distribution and character in certain pathological states. Filamentous accumulations characterize the major pathological change of numerous experimental conditions and human disease states (43). The twisted tubules which aggregate in cortical neurons of some neurological diseases (37, 42) may also represent a manifestation of neurofilament alteration. Studies in which an immunological marker is used as a probe of tissue neurofilaments employ inherent chemical specificity which may enable us to narrow the gap between the biochemistry and the morphology of these organelles.

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REFERENCES

1. ANDRES, K. H. 1961. Untersuchungen über den Feinbau von Spinalganglien. *Z. Zellforsch. Mikrosk. Anat.* **55**:1-48.
2. BENITZ, W. E., D. DAHL, K. WILLIAMS, and A. BIGNAMI. 1976. CNS fractions enriched in nerve and glial fibers. Immunological and biochemical studies. *J. Neuropath. Exp. Neurol.* **35**:345.
3. BENITZ, W. E., D. DAHL, K. W. WILLIAMS, and A. BIGNAMI. 1976. The protein composition of glial and nerve fibers. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **66**:285-289.
4. BERTOLINI, B., G. MONACO, and A. ROSSI. 1970. Ultrastructure of a regular arrangement of microtubules and neurofilaments. *J. Ultrastruct. Res.* **33**:173-186.
5. BIGNAMI, A., and D. DAHL. 1973. Differentiation of astrocytes in the cerebellar cortex and the pyramidal tracts of the newborn rat. An immunofluorescence study with antibodies to a protein specific to astrocytes. *Brain Res.* **49**:393-402.
6. BLÜMCKE, S., and H. R. NIEDORF. 1966. Electron microscope studies of Schwann cells during the Wallerian degeneration with special reference to the cytoplasmic filaments. *Acta Neuropathol.* **6**:46-60.
7. BUNGE, M. B., R. P. BUNGE, E. R. PETERSON, and M. R. MURRAY. 1967. Light and electron microscope study of long-term organized cultures of rat dorsal root ganglia. *J. Cell Biol.* **32**:439-466.
8. CLARK, H. F., and C. C. SHEPARD. 1963. A dialysis technique for preparing fluorescent antibody. *Virology.* **20**:642-644.

9. DAHL, D., and A. BIGNAMI. 1973. Immunochemical and immunofluorescence studies of the glial fibrillary acidic protein in vertebrates. *Brain Res.* **61**:279-293.
10. DAHL, D., and A. BIGNAMI. 1975. Glial fibrillary acidic protein from normal and gliosed human brain. Demonstration of multiple related polypeptides. *Biochim. Biophys. Acta.* **386**:41-51.
11. DAVISON, P. F., and B. WINSLOW. 1974. The protein subunit of calf brain neurofilament. *J. Neurobiol.* **5**:119-133.
12. DeVRIES, G. H., L. F. ENG, D. L. LEWIS, and M. G. HADFIELD. 1976. The protein composition of bovine myelin-free axons. *Biochim. Biophys. Acta.* **439**:133-145.
13. DROZ, B., H. L. KOENIG, and L. DIGIAMBERARDINO. 1973. Axonal migration of protein and glycoprotein to nerve endings. I. Radioautographic analysis of the renewal of protein in nerve endings of chicken ciliary ganglion after intracerebral injection of [³H]lysine. *Brain Res.* **60**:93-127.
14. ENG, L. F., G. H. DeVRIES, D. L. LEWIS, and J. BIGBEE. 1976. Specific antibody to the major 47,000 MW fraction of bovine myelin-free axons. *Fed. Proc.* **35**:1766.
15. FAHEY, J. L. 1967. Chromatographic separation of immunoglobulins. *Methods Immunol. Immunochem.* **1**:321-332.
16. FRIEDE, R. L., T. MIYAGISHI, and K. H. HU. 1971. Axon calibre, neurofilaments, microtubules, sheath thickness and cholesterol in cat optic nerve fibres. *J. Anat.* **108**:365-373.
17. FRIEDE, R. L., and SAMORAJSKI. 1970. Axon caliber related to neurofilaments and microtubules in sciatic nerve fibers of rats and mice. *Anat. Rec.* **167**:379-388.
18. GILBERT, D. S. 1975. Axoplasm architecture and physical properties as seen in the *Myxicola* giant axon. *J. Physiol. (Lond.)* **253**:257-301.
19. GILBERT, D. S., B. J. NEWBY, and B. H. ANDERTON. 1975. Neurofilament disguise, destruction and discipline. *Nature (Lond.)* **256**:586-589.
20. HARTMAN, B. K. 1973. Immunofluorescence of dopamine- β -hydroxylase. Application of improved methodology to the localization of the peripheral and central noradrenergic nervous system. *J. Histochem. Cytochem.* **21**:312-332.
21. HARTMAN, B. K., and F. L. MARGOLIS. 1975. Immunofluorescence localization of the olfactory marker protein. *Brain Res.* **96**:176-180.
22. HOFFMAN, P. N., and R. J. LASEK. 1975. The slow component of axonal transport. Identification of major structural polypeptides of the axon and their generality among mammalian neurons. *J. Cell Biol.* **66**:351-366.
23. HONG, B. S., and P. F. DAVISON. 1976. Characterization of mammalian neurofilament protein. *Fed. Proc.* **35**:1766.
24. IQBAL, K., H. M. WISNIEWSKI, I. GRUNDKE-IQBAL, J. K. KORTHALS, and R. D. TERRY. 1975. Chemical pathology of neurofibrils. Neurofibrillary tangles of Alzheimer's presenile-senile dementia. *J. Histochem. Cytochem.* **23**:563-569.
25. JORGENSEN, A. O., L. SUBRAHMANYAN, C. TURNBULL, and V. I. KALNINS. 1976. Localization of the neurofilament protein in neuroblastoma cells by immunofluorescent staining. *Proc. Natl. Acad. Sci., U. S. A.* **73**:3192-3196.
26. KELLY, P. T., and M. W. LUTTGES. 1975. Electrophoretic separation of nervous system proteins on exponential gradient polyacrylamide gels. *J. Neurochem.* **23**:1077-1079.
27. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
28. METUZALS, J., and W. E. MUSHYNSKI. 1974. Electron microscope and experimental investigations of the neurofilamentous network in Deiter's neurons. Relationship with the cell surface and nuclear pores. *J. Cell Biol.* **61**:701-722.
29. METUZALS, J., and W. E. MUSHYNSKI. 1974. Filamentous network of the axoplasm, as revealed by freeze-etching of the squid giant nerve fiber, in relation to actin, tubulin and myosin components. *Biol. Bull. (Woods Hole)* **147**:491.
30. PETERS, A., S. L. PALAY, and H. DEF. WEBSTER. 1976. *The Fine Structure of the Nervous System: The Neurons and Supporting Cells.* W. B. Saunders Company, Philadelphia.
31. POTTER, H. D. 1971. The distribution of neurofibrils co-extensive with microtubules and neurofilaments in dendrites and axons of the tectum, cerebellum, and pallium of the frog. *J. Comp. Neurol.* **143**:385-410.
32. RAMON Y CAJAL, S. 1906. Die Struktur der sensiblen Ganglien des Menschen und der Tiere. *Ergeb. Anat. Entwicklungsgesch.* **16**:177-215.
33. SCHLAEFFER, W. W. 1971. Vincristine-induced axonal alterations in rat peripheral nerve. *J. Neuro-path. Exp. Neurol.* **30**:488-505.
34. SCHLAEFFER, W. W. 1977. Immunological and ultrastructural studies of neurofilaments isolated from rat peripheral nerve. *J. Cell Biol.* **74**:226-240.
35. SCHLAEFFER, W. W., and R. G. LYNCH. 1976. Immunofluorescent studies of neurofilaments in the peripheral and central nervous system of rats and humans. *J. Neuropathol. Exp. Neurol.* **35**:345.
36. SCHMITT, F. O. 1968. Fibrous proteins-neuronal organelles. *Proc. Natl. Acad. Sci., U. S. A.* **60**:1092-1101.
37. SCHOCHET, S. S., P. W. LAMPERT, and R. LINDENBERG. 1968. Fine structure of the Pick and Hirano bodies in a case of Pick's disease. *Acta Neuropathol.* **11**:330.
38. SHELANSKI, M. L., S. ALBERT, G. H. DeVRIES,

- and W. T. NORTON. 1971. Isolation of filaments from brain. *Science (Wash. D.C.)*. **174**:1242-1245.
39. SMITH, D. E. 1973. The location of neurofilaments and microtubules during the postnatal development of Clarke's nucleus in the kitten. *Brain Res.* **55**:41-53.
40. SMITH, R. S. 1973. Microtubule and neurofilament densities in amphibian spinal root nerve fibers: Relationship to axoplasmic transport. *Can. J. Physiol. Pharmacol.* **51**:798-806.
41. TENNYSON, V. M. 1965. Electron microscopic study of the developing neuroblast of the dorsal root ganglion of the rabbit embryo. *J. Comp. Neurol.* **124**:267-318.
42. TERRY, R. D. 1963. The fine structure of neurofibrillary tangles in Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* **22**:629-642.
43. WISNIEWSKI, H., R. D. TERRY, and A. HIRANO. 1970. Neurofibrillary pathology. *J. Neuropathol. Exp. Neurol.* **29**:163-176.
44. WUERKER, R. B., and J. B. KIRKPATRICK. 1972. Neuronal microtubules, neurofilaments and microfilaments. *Int. Rev. Cytol.* **33**:45-75.
45. WUERKER, R. B., and S. L. PALAY. 1969. Neurofilaments and microtubules in the anterior horn cells of the rat. *Tissue Cell* **1**:387-402.
46. YEN, S. H., D. DAHL, M. SCHACHNER, and M. L. SHELANSKI. 1976. Biochemistry of the filaments of brain. *Proc. Natl. Acad. Sci. U. S. A.* **73**:529-533.
47. YEN, S. H., C. VAN HORN, and M. L. SHELANSKI. 1976. Immunohistological localization of the neurofilament protein in the mouse. *J. Neuropathol. Exp. Neurol.* **35**:346.