Isolation and Partial Characterization of Neurofibrillary Tangles and Amyloid Plaque Core in Alzheimer’s Disease: Immunohistological Studies

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Abstract. Fractions enriched in neurofibrillary tangles (NFT) and amyloid fibrils were isolated from the cerebral cortex of three cases of senile dementia of the Alzheimer type. Distilled water suspensions of these fractions were excluded from all pore size gels and resisted digestion with various proteolytic enzymes. Formic acid/chloroform treatment of each fraction resulted in the appearance of 4,000-6,000, 15,000-17,000 and 24,000 molecular weight proteins, with concomitant diminution in the amount of excluded material at the top of each gel. The 4,000-6,000 dalton band was best seen in fractions containing randomly arranged amyloid fibrils, and its amino acid composition resembled that of the recently reported “beta” protein. A polyclonal antiserum to purified NFT reacted with tangles in neurons and in dystrophic neurites around plaques by immunoperoxidase staining. No reaction was obtained with cerebrovascular or plaque core amyloid immunohistologically, or with the 4-6 kD protein on immunoblots. Cross-reactivity with the neurofibrillary lesions occurring in Pick’s disease, progressive supranuclear palsy, postencephalitic Parkinsonism and dementia pugilistica was also seen. Specific binding of this antiserum to the double filamentous structure was confirmed by immunoelectron microscopy. Although the presence of “beta” protein in both NFT and amyloid-containing fractions suggests that it may be an important constituent of both, cross-contamination cannot be excluded.

Key Words: Aging; Alzheimer’s disease; Amyloid; Dementia, senile; Neurofibrillary tangles; Paired helical filaments; Senile plaque.

INTRODUCTION

Neuropathologic features of senile dementia of the Alzheimer’s type (SDAT) include neurofibrillary tangles (NFT), cerebral amyloidosis, and granulovacuolar degeneration (1–3). The electron micrographic features of NFT have been defined in situ (1, 2, 4, 5), as well as in partially purified material (6–9), and been shown to consist of single and double helically wound fibrils, or paired helical filaments (PHF). Similar structures have also been identified in the aging brain, postencephalitic Parkinsonism, progressive supranuclear palsy, Guam-Parkinson dementia complex, apparently normal Guamanians, dementia pugilistica, Down’s syndrome, and subacute sclerosing panencephalitis (10–14). Some of these conditions differ, however,
in the amount of straight fibrils described in addition to PHF present (4, 14), the lack of associated amyloid, and the anatomic distribution of NFT (11).

Recent biochemical studies have provided some information as to the composition of the amyloid subunit protein comprising the vascular deposits and amyloid core in SDAT and Down's syndrome with senile dementia. A novel "beta" protein, with an apparent molecular weight of 4-6 kD and no amino acid sequence homology to other known proteins, has been characterized by two groups using different isolation techniques (15–17). Largely because of similarities in amino acid composition (18), it has been suggested that the NFT, amyloid plaque, and cerebrovascular amyloid may in fact all be different forms of the same protein (19).

We obtained enriched NFT- and amyloid-containing fractions from three cases of Alzheimer's disease (AD) by modifications of previous methods and we report the characterization of a monospecific polyclonal antiserum to the former by immunogold and peroxidase techniques.

MATERIALS AND METHODS
Isolation of Fractions

Brain tissue from three cases of known AD (Dr. G. G. Glenner furnished two of them from the National Alzheimer's Disease Brain Bank) were frozen at −70°C; the presence of NFT and amyloid plaques in all cortical lobes was confirmed histologically in formalin-fixed material by silver staining. Temporal, parietal and frontal lobes and hippocampus were thawed and extracted following the procedure of Selkoe et al (20) with some modifications. Cortex was carefully dissected free of white matter, finely minced, and incubated with gentle sporadic shaking for three hours (h) at 1:1 (wt/vol) ratio in 50 mM Tris, pH 7.6, containing 0.1 M 2-mercaptoethanol and 2% sodium dodecyl sulfate (SDS) (wt/vol). This material was diluted two-fold in the same buffer, homogenized at very low speed for ten minutes (min) at room temperature in a VirTis homogenizer, and then heated to 100°C for ten min. The homogenate was sieved under reduced pressure through a 74 μm nylon mesh and centrifuged at 100,000 × g, 4°C, one h (Beckman L5-65). The pellet was then resuspended in the same buffer but now including 1% SDS (wt/vol). It was centrifuged in conical tubes at 200 × g, 4°C for ten min and the resulting supernatant resuspended at 100,000 × g, 4°C for one h. The pellet obtained was composed of beige fluffy upper and brown lower hard layers; the former could be separated from the latter by gentle pipetting with a two-fold excess of fresh buffer. Each fraction was applied separately to discontinuous sucrose gradients (1.0–2.1–1.4–2.0 M) in 50 mM Tris, pH 7.5, 1% SDS (wt/vol) and centrifuged at 220,000 × g, 20°C for 20 h (Beckman SW41 Rotor). Interfaces and layers were easily visualized and aspirated with Pasteur pipettes. The 1.4 M layer and the 1.4–2.0 M interface obtained from the brown lower pellet were designated S3 and S4 respectively and the 1.4–2.0 M interface from the beige upper layer was designated S6 (Fig. 1). Individual fractions were resuspended in distilled water, water bath sonicated, and centrifuged at 100,000 × g, 4°C for 60 min (Beckman TI80 Rotor). Pellets were washed twice in distilled water and finally resuspended in a small volume of distilled water, vortexed, and sonicated in a water bath. Samples were checked for apple-green birefringence by polarizing microscopy following prolonged staining in alkaline Congo red, as well as by electron microscopy. They were also run on various (3–15%) SDS-polyacrylamide gels, applied with and without urea sample buffer, under both reducing and non-reducing conditions (21). Following fixation, gels were stained by both Coomassie brilliant blue and silver stains (22).

Electron Microscopy

Dilute solutions of sonicated pellets in distilled water were applied to carbon-coated grids, freshly glow-discharged, and negatively stained with 1% aqueous uranyl acetate, pH 6.0. Samples were viewed with a Philips 301 electron microscope. Scanning transmission electron microscopy
Fig. 1. Discontinuous sucrose gradient of SDS-insoluble AD cerebral cortex pellet prepared as described in Methods, showing the appearance and location of the S3, S4 and S6 fractions.

microscopy (STEM) was performed at the Brookhaven Biotechnology Resource Facility, Upton, New York, under the direction of Dr. Joseph S. Wall.

Enzymatic Digestions

Aliquots of extensively sonicated suspensions of individual fractions were digested with trypsin (Worthington), chymotrypsin (Worthington) or subtilisin (type VIII, Sigma), both separately and in combination. Additional aliquots were treated with pancreatic elastase (kindly provided by Dr. A. Janoff, SUNY, Stony Brook), pronase (Sigma), urea-pancreatin (hog stomach mucosa, Sigma), collagenase type III (Advance Biofactures Corp.), hyaluronidase type IV (Sigma), lipase (Nutritional Biochemicals), phospholipase A₂ (kindly provided by Dr. Peter Elsbach, N.Y.U. Medical Center) and phospholipase C (Sigma). All digestions were carried out at 1:25 to 1:100 w/w enzyme; protein ratios, at 37°C and at 60°C, for times ranging from one to 16 h, after which reactions were terminated by dilution in distilled water and freeze-drying.

Reduction and Alkylation

Reduction with dithiothreitol (DTT) and alkylation with iodoacetic acid (IAA) was performed in distilled water or denaturing buffers (urea, guanidine) made 100 mM in DTT and brought to pH 11.5 with NH₄OH.
Amino Acid Analysis and Sequence Studies

Performic acid oxidized or unoxidized samples were hydrolyzed under vacuum with 200 μl 6 N HCl, 40 μl 0.1% phenol for 24 h at 110°C and analyzed on a Durrum D-500 automated amino acid analyzer. Automated amino acid sequence analysis was carried out on a Beckman 890C sequencer with a 0.1 M Quadol program (pH = 8.9) as previously described (23).

Nucleic Acid Content

The presence of small amounts of DNA or RNA was assessed by a modification of the ethidium bromide method (24). Five microliters of S3, S6 and a series of DNA standards (0.5-20 μg/ml) were spotted onto a plastic wrap stretched over a UV transilluminator. An equal volume of distilled water containing 2 μg/ml ethidium bromide was added to each sample and mixed by pipetting. A short wavelength source was used to produce the emitted fluorescence.

Antiserum

A New Zealand white (NZW) rabbit was injected weekly subcutaneously in the foot pads and the back with approximately 150 μg (dry weight) of NFT-enriched fractions emulsified in complete Freund’s adjuvant. The animal received three weekly injections prior to the collection of high titer antiserum and was subsequently boosted monthly. The antiserum was absorbed 1:4 (w/w) with collagen and purified neurofilament triplet proteins (kindly provided by Dr. Ron Liem, N.Y.U. Medical Center) prior to use.

Immunohistological Studies

Paraffin-embedded blocks of tissue from cases of aging brain with senile lesions (five cases), familial (one case) and nonfamilial (five cases) AD, adult Down’s syndrome with dementia (one case), demenita pugilistica (two cases), progressive supranuclear palsy (one case), Parkinson’s disease (two cases postencephalitic, two idiopathic), Pick’s disease (one case) and sporadic (three cases) and hereditary (one case, from a Dutch kindred) (25) congophilic angiopathy were selected for study. Neurofibrillary change was determined by hematoxylin and eosin (H&E) and Bodian stains, and amyloid by birefringence in Congo red stains by polarizing microscopy. Regions selected for study included the hippocampus and adjacent temporal cortex (AD, Down’s syndrome), rostral pons, midbrain and hippocampus (dementia pugilistica), rostral pons, midbrain, hippocampus and basal ganglia (progressive supranuclear palsy) and midbrain (Parkinson’s disease). Controls included matched blocks from individuals dying traumatically (two cases) or age-matched with cerebral arteriosclerosis (two cases) but without senile changes.

Six micrometer (μm) sections were mounted on albumin-subbed slides and serially developed with primary antiserum, biotinylated antirabbit immunoglobulin, avidin peroxidase–antiperoxidase complex (Vector ABC) and diaminobenzidine as colorimetric reagent (26). Primary antiserat tested included anti-NFT (1:100–1:1,000 dilutions), anti-gamma trace protein (kindly provided by Dr. A. Grubb, 1:500 dilution), antiser to neurofilament triplet proteins (1:500 dilution), glial fibrillary acidic protein (1:500), tubulin (1:200, all provided by Drs. Ron Liem and Michael Shelanski, N.Y.U. Medical Center), antymyelin basic protein (1:500; Dr. David Colman, N.Y.U. Medical Center) and prealbumin (transhyretin) monomer (1:100 and 1:500 dilutions) (27). Controls for each run included (a) normal rabbit serum and (b) phosphate-buffered saline, pH 7.2 substituted for the primary antiserum. Absorptions to prove specificity were done by incubating antiserum with purified antigen, 1–10 mg/ml, overnight at room temperature.

Immunoelectron microscopy was carried out on purified NFT prepared as described above, studied by the immunogold technique (28). Preparations were incubated with primary antisera (1:100 dilution) and then a preparation of 5 nm staphylococcal protein A colloidal gold (kindly provided by Dr. C. De Lemos, Department of Cell Biology, N.Y.U. Medical Center). Controls included antineurofilament antibody and normal rabbit serum.
Western Blots

Neurofilament triplet proteins and S3, S4, and S6 were run onto 10% SDS-polyacrylamide gels, and blotted onto aminobenzoxymethyl (ABM) paper as previously described (27). Blots were developed with primary antisera and 125-iodine labeled staphylococcal protein A (New England Nuclear), erased with 10 M urea 0.1 M beta-mercaptoethanol, 60°C, 30 min, and washed with Tris-gelatin-NP40 buffer, before exposure to a second antibody (27).

RESULTS

Following homogenization in 2% SDS, preparations enriched in NFT and amyloid fibrils could be isolated from SDS-insoluble pellets by discontinuous sucrose gradient ultracentrifugation. The 1.4 M sucrose layer contained in its midportion a macroscopically fibrillar zone with brownish yellow color (S3), while the 1.4–2.0 M sucrose interface was white (S6) (Fig. 1). The S3 fraction was rich in paired helical filaments (PHF) by electron microscopy, though it was still contaminated with small amounts (less than 10%) of lipofuscin, collagen and straight, single fibrils. The S4 fraction contained 90% Congo red-birefringent material, some of which was present in the configuration of spherical plaque cores, seen as melasene crossies by polarizing microscopy. The S6 fraction consisted of a very fine amorphous Congo red-birefringent material that contained straight amyloid fibrils (Fig. 2). Paired helical filaments were also present ultrastructurally in both S4 and S6, comprising up to 20% of the former, but less than 5% of the latter; contamination with lipofuscin and granular material was minimal in the S6 fraction. Each fraction was resuspended, sonicated and re-centrifuged in distilled water, resulting in further purification from contaminants, though with some loss of material from the original preparations.

The S3, S4 and S6 fractions all contained less than 0.5 μg/ml nucleic acid by the ethidium bromide reaction. All were unaffected by enzymatic digestion with hyaluronidase, lipase, phospholipase A2, and phospholipase C. All three fractions resisted complete dissolution in 1% or 2% SDS, various salts (NaCl, Tris, phosphates, Ca++) reducing agents (DTT, 2-mercaptoethanol), chaotropic agents (6 M urea, 5 M guanidinc), chelating agents (EDTA, citrate) and organic solvents (ethanol, methanol, chloroform : ether, propylene oxide, dimethylsulfoxide). All fractions were excluded from various concentration SDS-polyacrylamide gels when applied from the untreated water suspension, when both were run under reducing (DTT) and denaturing (urea) conditions, or run without reducing or dissociating agents present. No lower molecular weight material entering the gels could be seen by either Coomassie or silver stains (Fig. 3A). Furthermore, material removed from the top of the gel by aspiration with a Pasteur pipette, followed by sonication and centrifugation, retained the ultrastructural characteristics (PHF or amyloid fibrils) of the starting material by electron microscopy. Trypsin, chymotrypsin, subtilisin, pepsin, urea-pepsin, elastase and collagenase, did not result in additional bands entering gels when digestions were analyzed by SDS-PAGE and fractionations of high-speed supernatants by high pressure liquid chromatography were blank (not shown). These findings confirm and extend previous observations of resistance of purified NFT to proteinase K, papain, pepsin, cathepsins and elastase (29, 30).

When S3, S4 and S6 fractions were brought to a concentration greater than 60% in formic acid, extensively shaken for one h at 37°C with an equal volume of chloroform, dried down and resuspended in 6 M urea sample buffer, 100 mm DTT, each fraction penetrated 3% stacking gels to a significant extent, yielding three major lower molecular weight bands at 24, 15–17 and 4–6 kD, respectively (Fig. 3B). Additional bands were seen in S3 at 14,500 and 39,000 molecular weight and at
Fig. 2. Negatively stained preparation of amyloid fibrils seen in S6. ×74,500; insets at higher magnification. Top right, ×160,000; bottom left, ×300,000.

21,000 molecular weight in S4. Although the three major bands were common between S3, S4 and S6, their relative contribution varied between fractions. Amino acid analyses of S3, S4 and S6 are shown in Table 1. Each fraction was digested with collagenase, washed and repelleted prior to hydrolysis. The S3 fraction contained 12% glycine, 9% glutamic acid (glutamine), 10% alanine, 6% aspartic acid (asparagine), 1% cysteine, 2% methionine, 11% basic residues and 25% hydrophobic residues (9% leucine). The amino acid composition of the S4 and S6 fractions resembled that reported for plaque core protein isolated from AD and Down’s syndrome (17, 18).
Fig. 3. A. 10% SDS-PAGE run under reducing conditions, showing exclusion of untreated water suspensions of S3 and S6 fractions from the gel; B. 12.5% SDS-PAGE with 3% stacking gel, showing low molecular weight bands at 4-6, 15-17, and 24 kD of the S3, S4 and S6 fractions following formic acid treatment; Coomassie stain. Four times as much material (dry weight) was loaded per lane in B as in A.

Efforts at obtaining an amino terminal sequence of undigested S3, S4 or S6, both under reducing and nonreducing conditions, were limited by the insolubility of the material applied to the cup. Nevertheless the S6 fraction yielded a heterogeneous amino terminus for two to three steps that included aspartic acid, phenylalanine, serine and glycine, i.e. amino terminal residues reported for APC "beta" protein by Masters et al (17). This material is currently under study.

Electron microscopically, S3 fractions were predominantly PHF, the double filamentous structure of which was apparent by routine (Fig. 4A) and scanning transmission (Fig. 5A) electron microscopy. Paired helical filaments were composed of both single and double filaments (Fig. 4B). Single filaments in some fields appeared to turn and intertwine with themselves to form the configuration of double filaments. Single filaments may participate in the formation of several double filaments, terminating involvement in each as a tight hairpin loop or truncated, as recently described (8) (Fig. 4C). Dimensions of these structures were corroborated on unstained specimens by mass density measurements in the STEM studies. Double filaments have apparent periodic crosses at an average of 66.5 nm, with maximum width of 17-18 nm and minimum width at crossing points of 9-9.5 nm (Fig. 5A). When observed alone, single filaments twist over themselves to give the appearance of a flat ribbon (Fig. 5B). Average diameters ranged from 6.5 nm at the narrowest points to 9.3 nm at the widest points.

A polyclonal antiserum to S3 reacted specifically by immunoperoxidase staining with intraneuronal tangles in the hippocampus of cases of sporadic and familial

SDAT (Fig. 6A). Reactivity was also seen in dystrophic neurites around amyloid plaque cores, though this was somewhat variable from case to case. No reaction was seen with the amyloid itself (Fig. 6B). Similarly, no staining of vascular amyloid deposits associated with SDAT or with cases of sporadic or hereditary hemorrhage due to congophilic angiopathy was seen. Positive staining was specifically removed by preabsorption of the antiserum with purified NFT (S3), but not with normal brain homogenates or purified neurofilament triplet protein. Western blots of formic acid-treated fractions were positive for the material excluded from the gels and showed no reactivity with homogenates of normal brain processed similarly to the cases of SDAT, purified neurofilament proteins, or the 4-6 kDa and other bands present in formic-acid treated preparations noted above (Fig. 7).

Polyclonal anti-NFT antibody reacted similarly with neurofibrillary tangles present in aging brains, as well as in cases of postencephalitic Parkinsonism (Fig. 8A), progressive supranuclear palsy (Fig. 8B), dementia pugilistica (Fig. 8C), one case of Pick's disease studied, and Down's syndrome with senile dementia. In each instance, there was adequate clinical and pathologic documentation of the diagnosis, and reactivity was retained despite differences in anatomic distribution. For example, there was a striking concentration of NFT in tegmental midline nuclear groups of the pons and midbrain in dementia pugilistica. No reactivity was seen with sections of control brains. Similarly, there was no reactivity of plaques, tangles or congophilic angiopathy with anti-neurofilament, antitubulin, anti-gamma trace protein, or anti-prealbumin.

The specificity of the reaction of our antiserum with PHF was confirmed at the ultrastructural level using the immunogold technique. Gold particles decorated predominantly the double filaments in purified NFT preparations (Fig. 9).

### Table 1

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<th>S3</th>
<th>S4</th>
<th>S6</th>
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<tr>
<td>Cysteine†</td>
<td>1.54</td>
<td>ND</td>
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<tr>
<td>Aspartic acid</td>
<td>5.88</td>
<td>5.35</td>
<td>7.10</td>
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<td>Threonine</td>
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<td>Glutamic acid</td>
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<td>7.73</td>
<td>10.07</td>
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<td>Glycine</td>
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<td>Alanine</td>
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* Mean of three determinations.
† Cysteine was determined as cysteic acid after performic acid oxidation.
Fig. 4. A and B. Electron micrograph of the S3 fraction showing a grouping of double (arrowheads) and single (open arrows) filaments. x177,500. C. Single filaments participating in double filamentous structure. A and B: x177,500 and C: x250,000.

DISCUSSION

Fractions enriched in neurofibrillary tangles (NFT), amyloid cores or amorphous amyloid fibrils can be obtained by discontinuous sucrose gradient centrifugation, utilizing the insolubility of these structures in SDS and most reducing and dissociating agents (20, 29). Although methods that were originally developed for the isolation of low molecular weight amyloid subunit proteins in immunoglobulin light chain, amyloid angiopathy and prealbumin (transthyretin)-related amyloidosis appear to be of some value in the purification of leptomeningeal amyloid in brain (15, 16), they have not proven useful for the isolation of plaque core amyloid (unpublished observations). With short centrifugations, using a modification of the method de-
Fig. 5. A: Ribbon-like configuration of paired and, B: single filaments from an unstained sample of S3 visualized directly by scanning transmission electron microscopy. Rod-shaped structure in B is tobacco mosaic virus, included as a size control. Bar 18 nm.
Fig. 6. A: Low and B: higher power photomicrographs of the hippocampus of a case of familial Alzheimer's disease following immunoperoxidase staining with polyclonal antiserum raised to S3 at 1:250 dilution. Reaction product specifically decorates neurons with neurofibrillary pathology, as well as plaque neurites, but no staining is seen of the amyloid plaque core. A: ×100. B: ×200.
scribed by Selkoe et al (20), two fractions (S3 and S4) are obtained, the first being predominantly NFT and the second being greatly enriched in plaque cores. With longer centrifugations, greater separation one from the other is achieved. Nevertheless, cross-contamination remains a significant variable and must be considered in the evaluation of any reports claiming the isolation of subunits from one or the other. A third fraction (S6), which appears to be more than 90% amyloid fibrils not in the configuration of the core is obtained from the upper part of the initial SDS-insoluble pellet. The S3, S4 and S6 fractions share the distinctive insolubility of NFT in most dissociating agents, resistance to protease digestion and exclusion from all
pore-size SDS-polyacrylamide gels. These biochemical properties differ strikingly from fibrils occurring in the systemic amyloidoses and suggest unique features inherent to, or resulting from, the milieu of the cerebral cortex.

Detailed study of extensively purified NFT from different cases of AD revealed it to be predominantly protein, yielding a consistent amino acid composition on acid hydrolysis (Table 1). Carbohydrate, nucleic acid or lipid do not appear to be significant constituents of S3, S4 or S6. The high and equal content of acid, basic and hydrophobic residues is consistent with that of an amphiphilic protein capable of adopting a beta-pleated sheet and fibrillar configuration (31), a prediction that has recently been directly confirmed by X-ray diffraction studies (32). This same amphiphilic structure might also contribute to insolubility following SDS treatment, and the poor digestibility of the purified NFT with a wide variety of proteases.

Similarity of the amino acid composition obtained for purified S4 and S6 (Table 1) and that reported by Glenner and Wong (15) for the “beta” protein makes it likely that this protein is a major constituent of S4. Low yield following Edman degradation of S6 may reflect insolubility of the material in buffers routinely used for automatic amino acid sequencing, heterogeneity, or steric conditions that interfere with amino terminal reactivity. The heterogeneous sequence obtained by Masters et al (17) for core protein may thus be due to the lack of SDS in their extraction protocol or, alternatively, the use of a gas/liquid phase sequencer rather than Edman degradation as a means of N-terminal analysis.

After formic acid and chloroform treatment, all three fractions showed similar intermediate-to-low molecular weight bands on gels. It is not yet clear whether the three major bands obtained are due to in vitro aggregation of a 4–6 kD subunit, as has been suggested by Masters et al (17, 33), or in fact are different proteins. Nevertheless, a correlation can be seen (Fig. 3B) between the structural appearance of PHF (S3) and amyloid (S4, S6) and the relative contribution of the bands. The S3 fractions appear to contain proportionately more of the 25,000 molecular weight band, whereas S4 and S6 fractions contain more of the 4,000–6,000 molecular weight protein. Although these findings are compatible with the existence of a common subunit structure, this issue must remain unsettled until completely purified preparations are available or until the “beta” protein can be shown to assume the configuration of PHF in vitro.

Purified NFT fractions have been utilized by several groups to raise polyclonal and monoclonal antisera that have proven useful for defining NFT in tissue sections (34–38). In agreement with the findings of Ishara et al (39), our antiserum does not appear to react with normal brain or purified neurofilament triplet proteins, either by immunoblot analysis or immunohistology (34). It also does not react on immunoblots with purified neurofilament triplet or the 4–6 kD band released from S3 and S6 by treatment with formic acid and chloroform (Fig. 7). The latter finding is consistent with lack of reactivity with plaque core or congophilic angiopathy in tissue sections and strongly suggests that such antisera may be directed to conformational antigenic determinants on PHF or perhaps to other components of tangles as yet undefined. Reactivity with a conformational determinant unique to the tangle is also suggested in turn by the immunogold studies and similar specificity for ultrastructural antigenic determinants has recently been reported (36, 38). By contrast two recent reports (33, 40) presented apparently conflicting results regarding reactivity of monospecific antisera prepared to intact beta protein or synthetic peptides corresponding to its published sequence (15–17) with NFT in tissue sections. Further studies will be needed to confirm the presence of beta protein as an integral component of PHF.

Fig. 8. Immunoreactive neurofilamentous aggregates in brainstem neurons: A. Substantia nigra in a case of postencephalitic Parkinson's disease; B. red nucleus in a case of progressive supranuclear palsy; C. dorsal raphe nucleus in a case of dementia pugilistica. Paraffin-embedded sections stained with anti-NFT antibody, 1:250 dilution. × 200.
Specificity of the staining obtained with anti-NFT was confirmed by immunocytochemistry using monospecific antisera to myelin basic protein (MBP), glial fibrillary acidic protein (GFAP), tubulin, neurofilament triplet protein, gamma-trace protein and prealbumin (transthyretin). Positive controls (not shown) included the specific staining of myelinated nerve fibers in white matter (MBP) (41) astrocytes (GFAP) occasionally encircling senile plaques in AD and Down's syndrome with dementia (42), neuronal cell processes (tubulin, NF) (43), pituitary cells (gamma-trace) (44) and choroid plexus (45), pancreas (46) (prealbumin).

Anti-NFT also recognized determinants in NFT present in normal aged individ-

Fig. 9. Immunoelectron microscopy of S3, showing specific labeling with gold particles. ×112,500.
uals, Down's syndrome, *dementia pugilistica*, postencephalitic Parkinsonism and progressive supranuclear palsy, as well as Pick bodies in the one case of Pick's disease studied. No reactivity was seen with Lewy bodies in two cases of idiopathic Parkinsonism without apparent NFT. Cross reactivity with Pick bodies (47, 48) as well as with the straight filaments of progressive supranuclear palsy (38, 49) has been reported using both polyclonal and monoclonal antibodies to NFT (47, 48). These studies demonstrate a commonality of antigenic specificity shared by NFT occurring in a wide variety of pathologic conditions, many distinct from cerebral amyloidosis.

The recent identification of major subunit proteins comprising amyloid core and vascular amyloid deposits in SDAT, Down's syndrome (15–17) and in the scrapie-associated fibril (50) make it likely that the structural basis for the neurofibrillary changes in cerebral amyloidosis and various other pathological states of man will soon be clarified. Although the functional significance of these proteins is still obscure, they provide tools for defining the relationship between neurofibrillary changes and amyloidosis in Alzheimer's disease.

*Note added in proof:* More recent studies have shown reactivity of our anti-NFT with Tau protein, but not with a synthetic peptide corresponding to the reported (15–17, 33) sequence of the "beta" protein (unpublished data).

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