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Reappraisal of the Fine Structure of Alzheimer's Neurofibrillary Tangles

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Summary. Alzheimer's neurofibrillary tangles were studied by electron microscopy. The study includes four cases of Alzheimer's disease, two cases of atypical senile dementia, and one case of progressive supranuclear palsy. In Alzheimer's disease the tangles were composed of either straight filaments or paired helical filaments. In progressive supranuclear palsy the tangles were composed of 15 nm straight filaments or helical filaments. A few straight filaments were mixed with paired helical filaments. In atypical senile dementia, both straight and paired helical filaments comprised the tangles and one type of filaments appeared to intermingle with the other in the same neurons.

Key words: Alzheimer's disease – Neurofibrillary Tangles – Senile dementia – Supranuclear palsy – Ultrastructure

It has been generally considered that Alzheimer's neurofibrillary tangles are composed of paired helical filaments and their presence is the ultrastructural hallmark of Alzheimer's type of dementia (Wisniewski et al. 1976). However, Oyanagi (1979) and Hirano et al. (1968) have observed that 15 nm straight filaments occasionally comprise the tangles and are usually seen in the dendrites. On the other hand, most tangles in progressive supranuclear palsy are composed of 15 nm straight filaments (SF) (Tellez-Nagel et al. 1973; Bugiani et al. 1979) and in a few cases of the tangles were composed of paired helical filaments (PHF) (Ishii and Itoh 1979) or of both types of neurofibers (Tomonaga 1977). Recently, Shibayama and Kitoh (1978) reported the co-existence of PHF and SF in a case of atypical senile dementia. Furthermore, Yagishita et al. (1979) illustrated the co-existence of these two types of neurofibers in the same neurons. A transition of the neurofilaments to PHF has been described by Oyanagi (1974). However, the fine structure of neurofibrillary tangles has not been fully elucidated up to date and their morphogenesis is still a matter of dispute.

The purpose of the present study is to reappraise the fine structure of neurofibrillary tangles and comment on the nature and origin of these abnormal neurofibers.

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Subjects and Methods

Seven autopsy brains with many Alzheimer's neurofibrillary tangles were usued for this study: four cases of Alzheimer's disease including two familial cases; two cases of atypical senile dementia; and one case of progressive supranuclear palsy. their age, sex, and clinical diagnosis are listed in Table 1.

Besides the conventional examination of the central nervous system (CNS), electron-microscopic research was conducted. The specimens were taken from formalin-fixed material of the frontal cortex, Ammon's horn, and on occasion subcortical nuclei. The tissues were placed in 2.5 % glutaraldehyde solution, post-fixed in 1 % osmic acid, dehydrated in graded alcohol, and embedded in epon mixture. Ultrathin sections were contrasted with uranyl acetate and lead citrate and observed in a Hitachi HU-12 electron microscope at 75 KV.

Results

The neuropathologic findings of all cases are shown in Table 2.

Table 1

Case 1 44 yr	female	familial Alzheimer's disease
Case 2 37 yr	male	familial Alzheimer's disease
Case 3 53 yr	male	sporadic Alzheimer's disease
Case 4 72 yr	female	sporadic Alzheimer's disease
Case 5 69 yr	male	progressive supranuclear palsy
Case 6 59 yr	female	atypical senile dementia
Case 7 74 yr	female	atypical senile dementia

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Table 2

Case 1	Neuronal loss and gliosis in cerebral cortex.
Case 2	Marked Alzheimer neurofibrillary tangles,
Case 3	granulo-vacuolar degeneration, and senile plaques
Case 4	
Case 5	Marked neurofibrillary tangles in subcortical nuclei, especially in mid-brain and brain stem. No senile plaques
Case 6	Marked neuronal loss and gliosis in cerebral cortex.
Case 7	Countless neurofibrillary tangles, Hirano bodies and senile plaques. In addition, the presence of widespread Lewy type inclusions in the cerebral cortex as well as substantia nigra is a peculiar finding in these two cases

Electron-microscopic Findings

Although the tissues were considerably damaged by long-term preservation in commercial formalin, the detailed structure of neurofibrillary tangles could be easily ascertained.

In all four cases of Alzheimer's disease, the neurofibrillary tangles were composed of both 15–20 nm SF and PHF. Most tangles in the neuronal perikarya were composed of PHF and the remaining consisted of SF, while most tangles in the neuronal processes were composed of both SF and PHF. However, both types of filaments were observed separately in each neuron and did not co-exist in the same neuron.

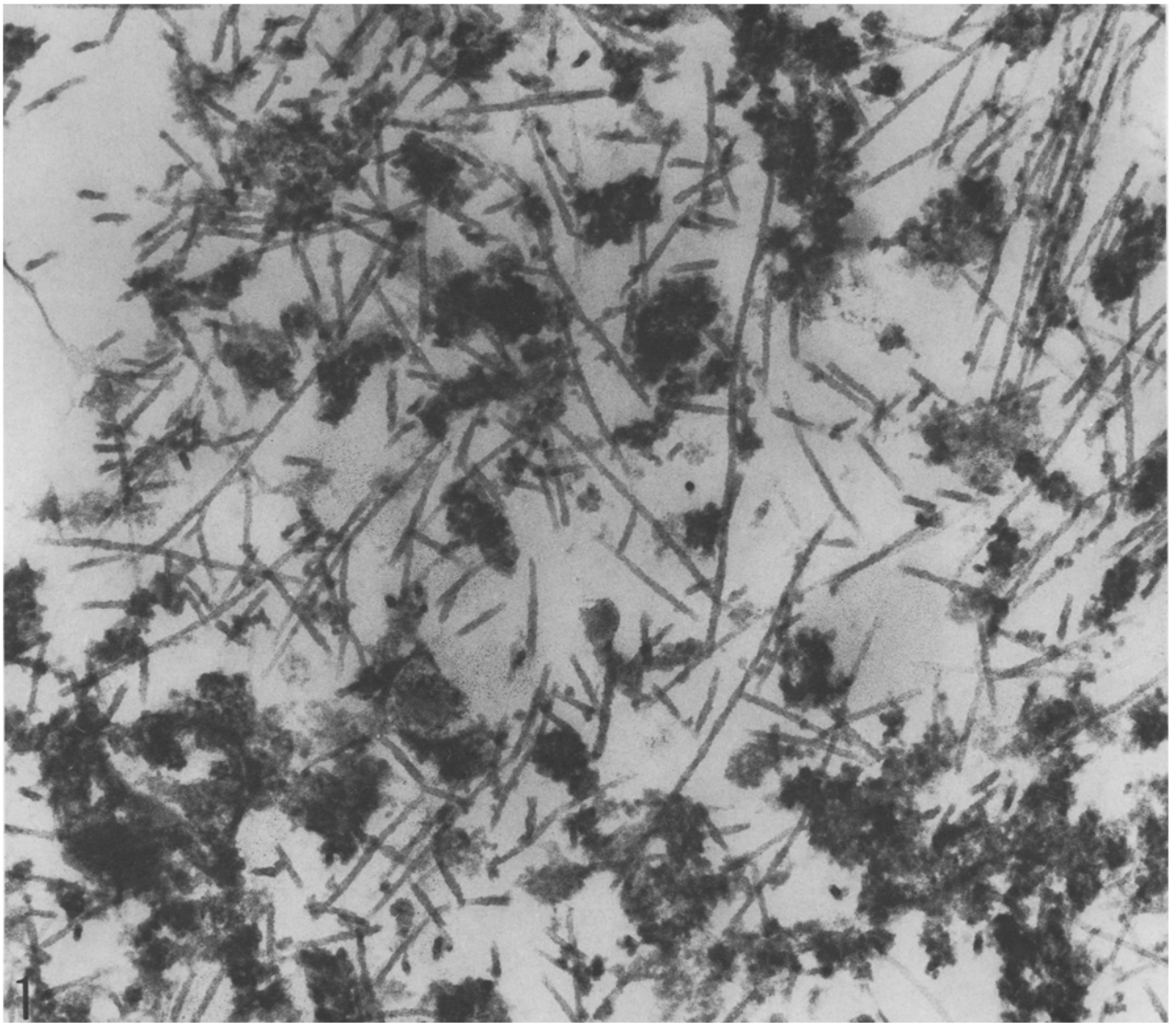

Fig. 1. Part of a tangle. Straight filaments are seen running in an overall random distribution. Some filaments seem to have slightly narrow points. × 54,480, Case 7

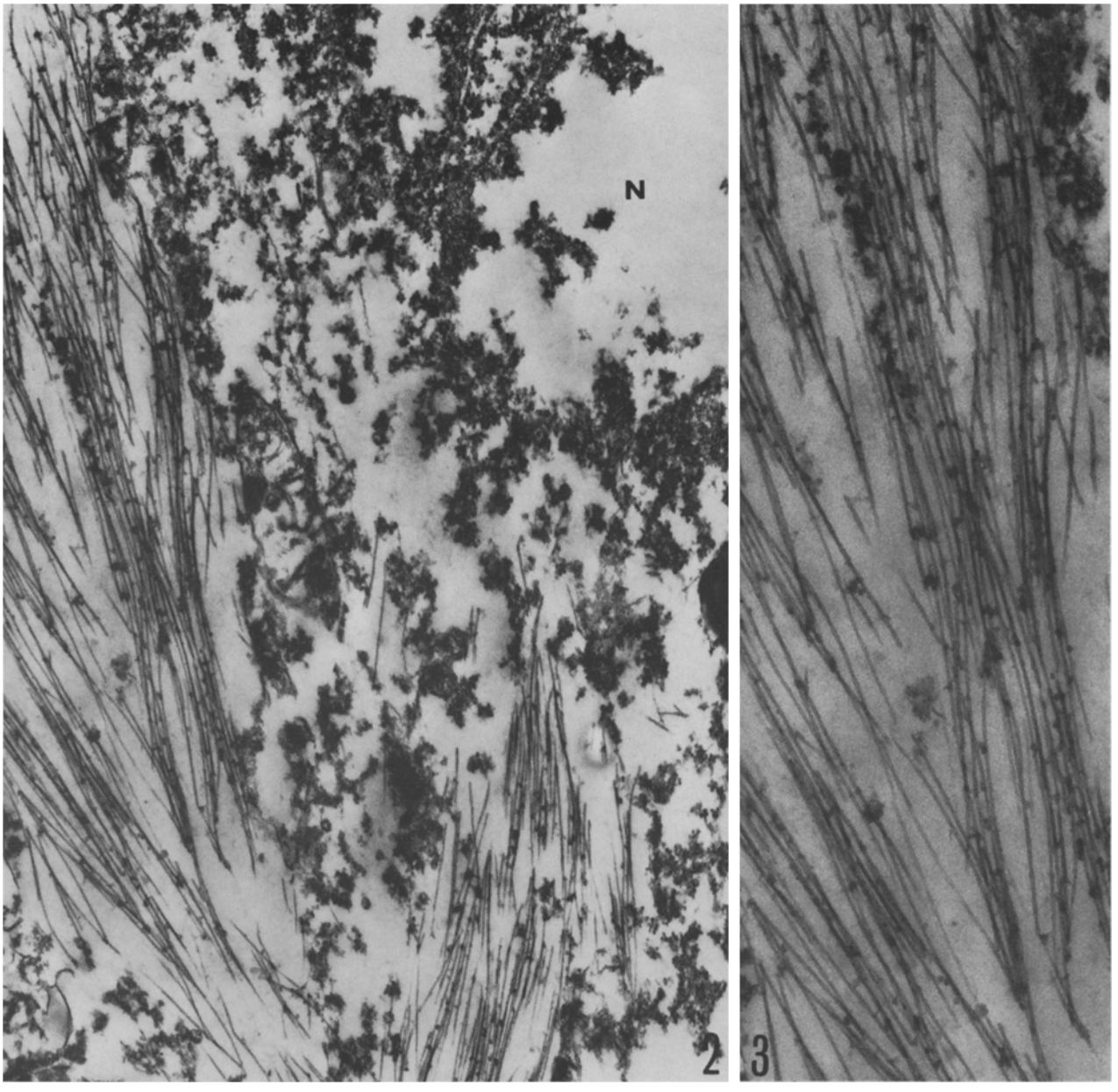

Fig. 2. Two bundle of straight filaments in neuronal perikarya. *N* nucleus. × 24,270, Case 6

Fig. 3. Higher magnification of Fig. 2. × 39,200

In progressive supranuclear palsy, the majority of subcortical tangles was composed of the aggregates of interlacing bundles of 12 – 17 nm SF, and a few tangles were composed of PHF. The frequency of these tangles in the cerebral cortex was reversed, most tangles being composed of PHF and the minority of SF. A few SF were mixed with PHF in the cortical tangles, and the reverse was not seen (for detailed information see Yagishita et al. 1979).

In two cases of atypical senile dementia, the tangles were composed of two types of filaments; one was the straight type of 15 – 20 nm filaments and the filaments were most often arranged in either loose or compact accumulation in overall random distribution (Fig. 1). Skeins of SF often comprised the tangles in the neuronal perikarya in contrast to those of Alzheimer's disease (Figs. 2, 3). The other type of filaments was twisted, showing characteristic periodic constrictions.

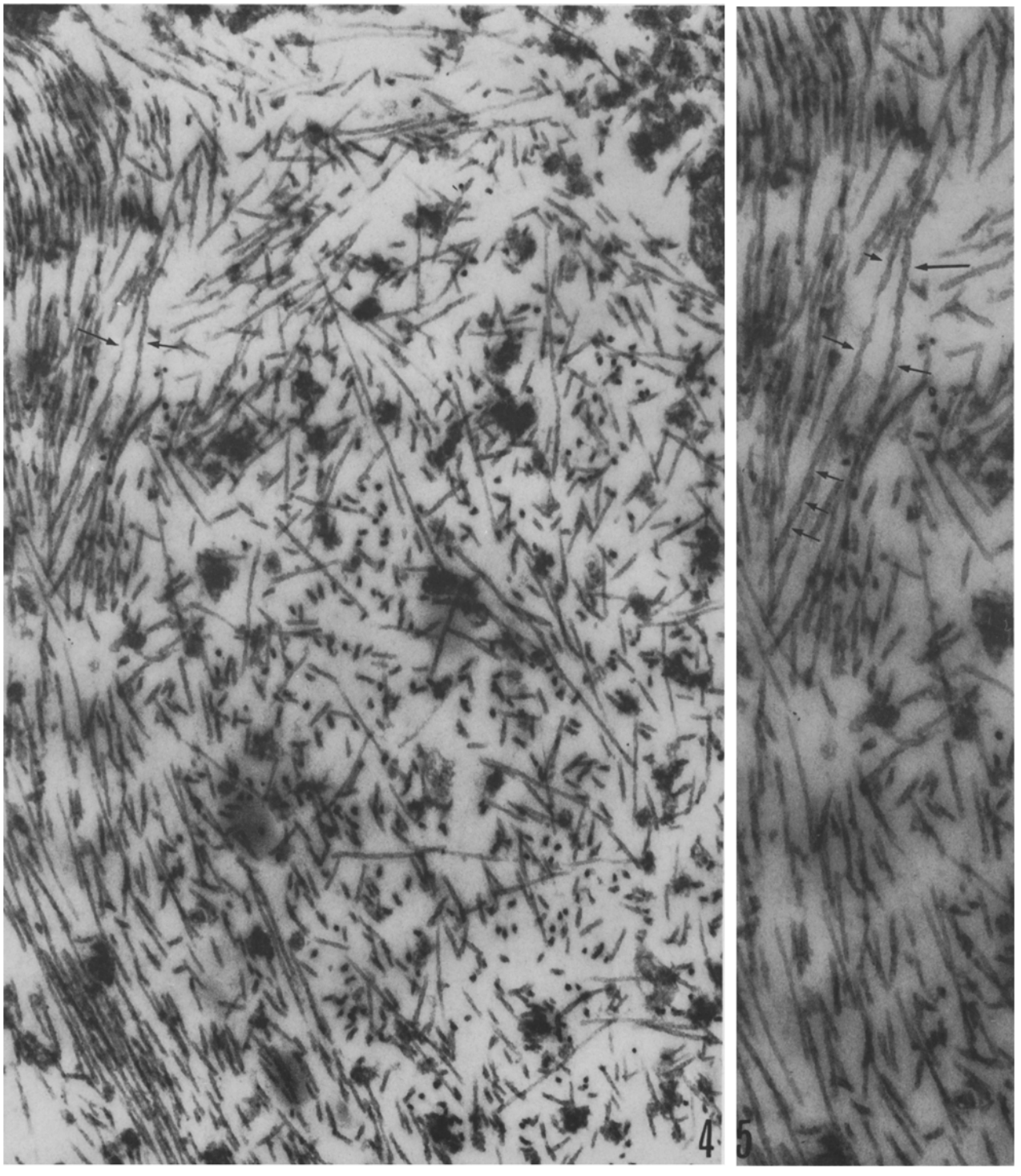

Fig. 4. Part of a tangle. Two paired helical filaments are seen among the randomly distributed straight filaments. Arrows show paired helical filaments. ×46,670, Case 7

Fig. 5. Higher magnification of Fig. 4. Arrows show paired helical filaments. ×54,135

These two types of filaments usually appeared separately in each neuron; however, some PHF seemed to intermingle occasionally with the SF in the same neuron (Figs. 4, 5) and on the contrary, some of SF seemed to be mixed among the PHF (Fig. 6). However, nearly equal numbers of both types of filaments were not admixed together. The majority of the filaments in the neuronal processes seemed to be regularly arranged

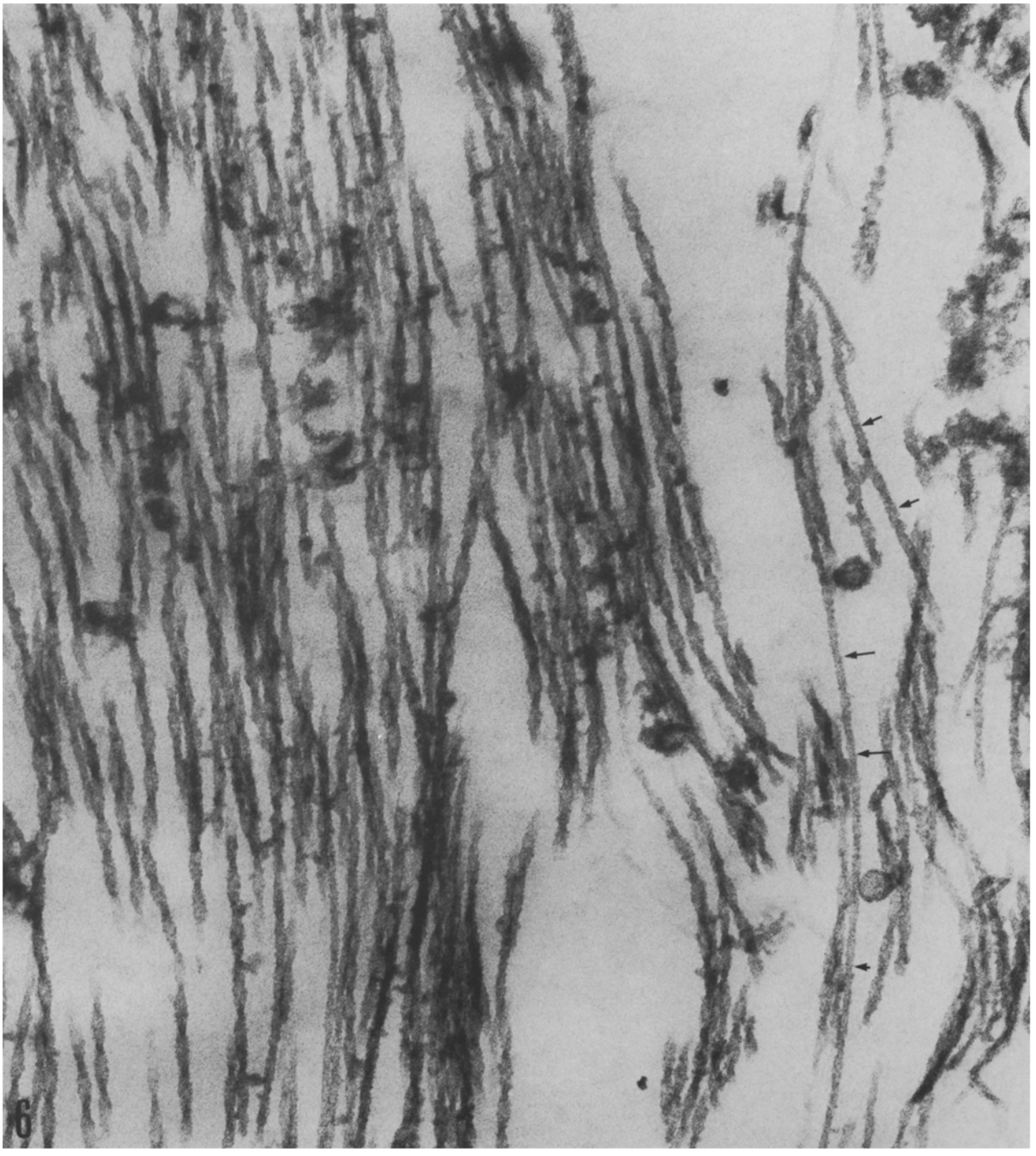

Fig. 6. Part of a tangle. Straight filaments seem to intermingle with paired helical filaments. Arrows show straight filaments. × 102,480, Case 6

on the long axis, while most filaments in the neuronal perikarya were randomly distributed or running in curved fashion. On very rare occasions, a few twisted filaments appeared alone or in small groups among the pre-existing organelles (Fig. 7). Some tangles appeared to be solely composed of abnormal neurofibers while other proper organelles were scanty or absent, indicating the end stage of tangle formation (Fig. 8).

Discussion

In the present study the neurofibrillary tangles in all cases examined were found to be composed of two types

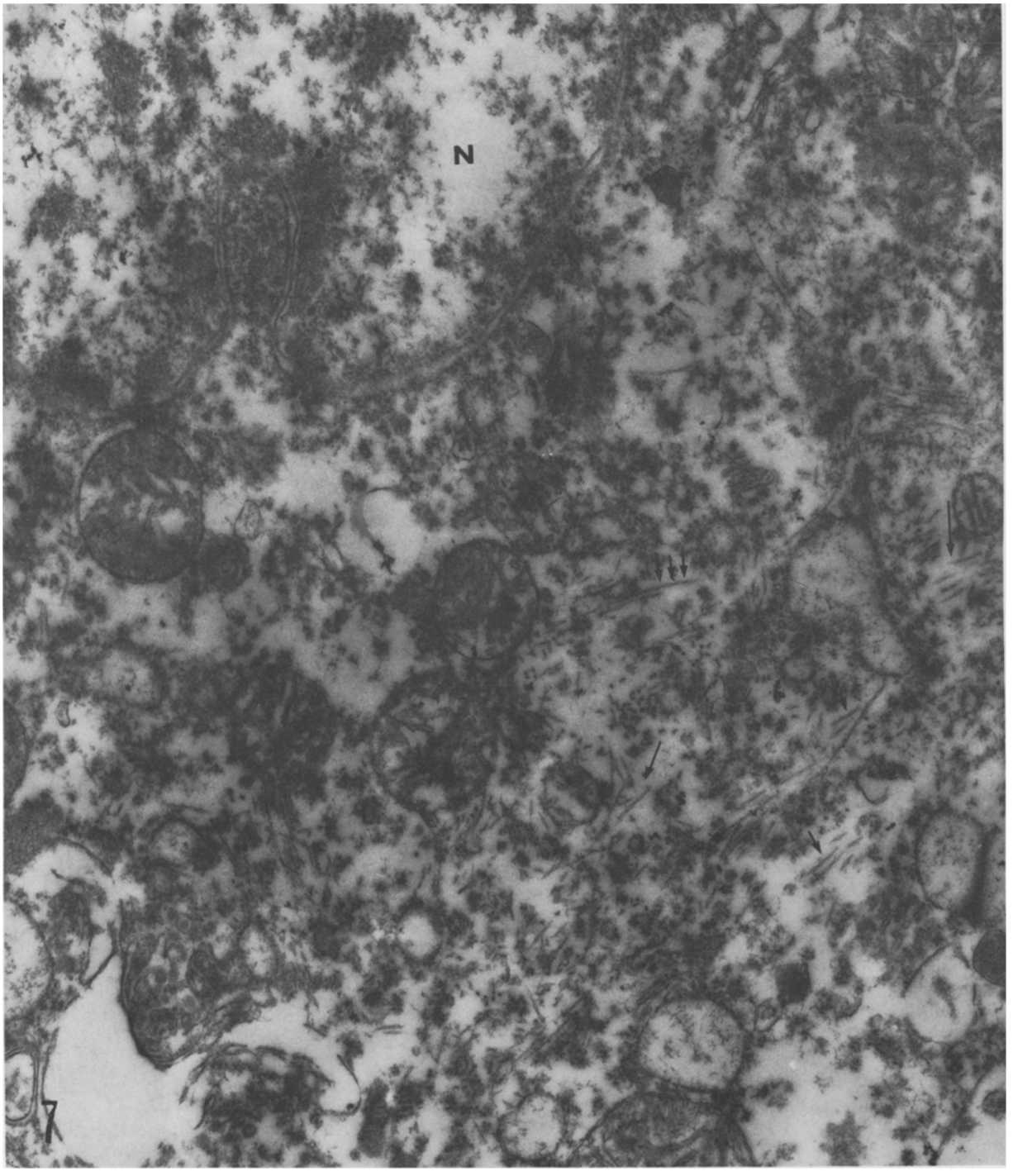

Fig. 7. Many paired helical filaments are seen among abundant cytoplasmic organelles. They are distributed singly or in small groups. No straight filaments are identified. ×27,000, Case 6

of filaments, viz., SF and PHF. First of all, many filaments showed clear periodic constrictions, while some filaments were apparently uniform in diameter, and there were some morphological variations in different cases. In Alzheimer's disease both types of filaments were seen separately in each neuron. In progressive supranuclear palsy some SF seemed to intermingle with PHF. In atypical senile dementia both types of filaments appeared occasionally in the sme neuron. However, one type of filaments exceeded the rest and did not intermingle with each other in nearly equal number.

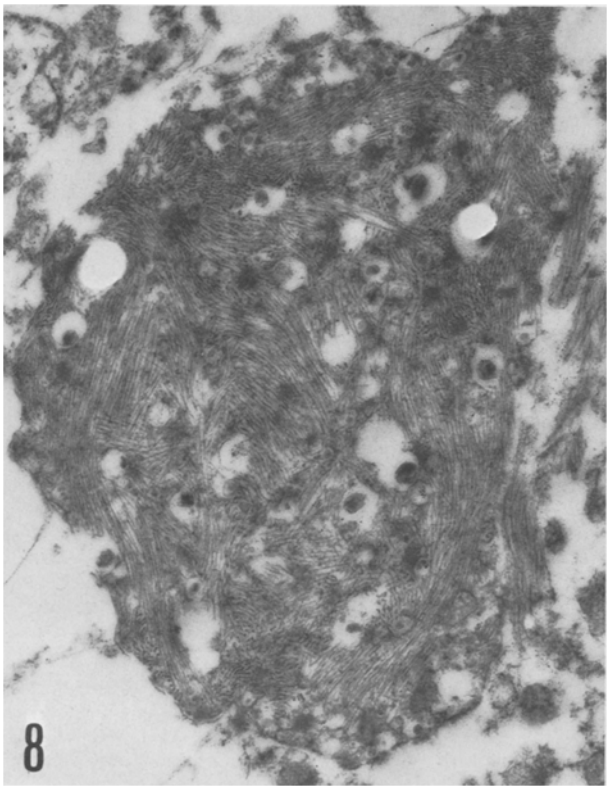

Fig. 8. A tangle is solely composed of paired helical filaments. The proper organelles are scarce in number. ×15,250, Case 2

The majority of neurofibrillary tangles in the human brain are composed of twisted tubules or paired helical filaments (Lampert 1971; Wisniewski 1976). The present study clearly shows that the tangles are composed of two types of filaments: SF and PHF.

The 20–24 nm neurotubules are unstable and when the tissues are fixed in formalin, the neurotubules disappear easily after a relatively short time. However, the SF described here, being both 12–17 nm and 15–20 nm in diameter, respectively, were stable to formalin and autolysis. It is tenable, therefore, to interpret them as abnormal and pathologic neurofibers different from normal neurotubules.

Co-existence of both types of filaments has been rarely reported (Hirano et al. 1968; Niklowitz et al. 1975; Tomonaga 1977; Shibayama and Kitoh 1978). Only Shibayama and Kitoh (1978) described that rare helical filaments were intermingled among SF, but they did not offer any photographic demonstrations. Recently, Yagishita et al. (1979) described co-existence of both types of filaments in the same neuron.

Hirano et al. (1968) and Tomonaga (1977) considered that the uniform tubules were twisted into a spiral configuration and SF was in a pre-stage of the formation of PHF. Oyanagi (1974) reported that neurofilaments seemed to transform into paired helical filaments. However, all these authors have considered that neurofilaments and SF do transform into paired helical filaments. We could not observe that the one filament did transform into the other filaments in the present study, and as shown in Fig. 7, a few PHF are scattered in the abundant proper organelles and ST could not be discerned anywhere, suggestive of an early stage of tangle formation, although we could not completely eliminate the possibility that the sections might cut just through the edge of the tangle.

The paired helical filaments protein subunit is closely related chemically to the normal neurofilament protein subunit and the beta-tubulin (Iqbal et al. 1978; Grundke-Iqbal et al. 1979). Iqbal et al. (1974) reported unique 50,000 dalton protein in a fraction band enriched in PHF. These data may suggest that unique 50,000 dalton protein is a key protein for the assembly of PHF. We would venture to consider, from the present study and these chemical data, that tangle formation may be a result of reactive response of neuronal cells misprogrammed to induce synthesis of abnormal neurofibers by assembly of lower molecular subunit protein and not by transformation of the pre-existing neurofibers.

In addition to the aforementioned abnormal neurofibers, skins of several other types of neurofibers comprise neurofibrillary tangles; neurofilaments (Higgins et al. 1977; Crapper et al. 1976; Schochet et al. 1968), 12 nm straight wavy filaments (Kuroda et al. 1979), paired helical filaments with twist every 30 nm (Volk 1980) and paired helical filaments with 50 nm spaced constrictions (Wisniewski et al. 1973). The nature of these fibrous proteins has not yet been elucidated. Further chemical and ultrastructural analyses of these neurofibers will throw a light on the implication and origin of these normal and abnormal neurofibers.

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