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Light, Fluorescence, and Electron Microscopic Features of Neuronal Intranuclear Hyaline Inclusions Associated with Multisystem Atrophy

J. H. Sung

Departments of Laboratory Medicine and Pathology (Division of Neuropathology), and Neurology, University of Minnesota Medical School, Minneapolis, MN 55455, USA

Summary. Light, fluorescence, and electron microscopic features of intranuclear hyaline inclusions of neurons associated with multisystem atrophy in a 21-year-old woman are described. The neuronal inclusions resemble Marinesco bodies on light microscopy but differ from the latter in their distribution, autofluorescence, and ultrastructure. They are widespread in almost all central, peripheral, and autonomic neurons and are generally larger than Marinesco bodies. The inclusions emit yellow-green autofluorescence with ultraviolet light between 470 and 530 nm of the spectrum and are ultrastructurally composed of haphazardly arranged, uniform, fine, straight filaments (8–9 nm in diameter). The neuronal inclusions have neither the ultrastructural feature of known viral inclusions nor are associated with virus particles. Their chemical nature and pathogenesis remain to be elucidated.

Key words: Neuronal intranuclear hyaline inclusions – Multisystem atrophy – Autofluorescence and ultrastructure

Sung et al. (1979) recently reported an unusual degenerative disorder of the nervous system in a 21-year-old woman, which is characterized by widespread neuronal intranuclear hyaline inclusions associated with degeneration of multiple systems including autonomic and peripheral neurons. Although etiology of the disorder is unknown, it was asserted that the neuronal inclusions are of non-viral origin and may represent an unknown metabolic abnormality leading to involution of various types of neurons and atrophy of multiple systems. To emphasize the uniqueness of the neuronal inclusions, "neuronal intranuclear hyaline inclusion disease" was proposed to name the disorder (Sung et al. 1980). Janota (1979) also reported a case of progressive neurological disorder in a 45-year-old man, which is characterized by widespread intranuclear neuronal corpuscles associated with what was considered to be the spinal form of spinocerebellar degenerations or Friedreich's ataxia. In his case, the illness was apparently familial, the onset was delayed, and systems involved were relatively limited as compared with the case of Sung et al. (1980). As far as the character of the neuronal intranuclear inclusions and their association with chronic progressive system degeneration are concerned, both cases are remarkably similar and seem to represent the same disorder. Since no other neurological disorders are known to be associated with neuronal intranuclear inclusions of non-viral origin and the neuronal inclusions in the two cases of multisystem atrophy resemble Marinesco bodies on light microscopy, it is important to document in greater detail whether the latter neuronal inclusions represent viral inclusions or Marinesco bodies. For this reason, the neuronal intranuclear hyaline inclusions in the present case were restudied to establish the details of their morphology even though the clinical and pathological aspects of the case have recently been reported (Sung et al. 1980).

Materials and Methods

The patient was a 21-year-old woman who developed behavioral abnormality, peculiar involuntary movements, and ataxia at the age of 3–5. Subsequently, her symptoms progressed to dementia, choreoathetosis, rectal and bladder incontinence, bulbar and spinal muscle weakness, pes cavus, and kyphoscoliosis. The patient terminally developed generalized seizures and died 18 years after the onset of illness. The clinical details and autopsy findings have previously been reported (Sung et al. 1980).

For light microscopy, sections from the brain, spinal cord, dorsal spinal root ganglia, paravertebral sympathetic ganglia, and myenteric plexus of the gastric and intestinal walls were embedded in paraffin following fixation in 10% formalin for 2–5 weeks. Histological stains applied included hematoxylin and eosin (HE), periodic acid Schiff's (PAS), Luxol fast blue (LFB), methyl green-

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pyronin, Congo red, oil red O, osmic acid, cresyl violet, Mallory's trichrome, toluidine blue, and Bielschowsky's silver. Sections were also examined with polarized light.

Autofluorescence of the neuronal inclusions was studied on formalin-fixed paraffin sections by a Zeiss fluorescence photomicroscope equipped with a high pressure mercury lamp, Osram HBO 200. The exciter filters used were BG 38, BG 3, and BG 12, and the barrier filter insert contained six barrier filters (41/44/47/50/53/65).

For electron microscopy, tissue blocks were sampled from the hippocampus and an inferior cervical (stellate) ganglion after being fixed in 10% formalin for 2 weeks. The minced tissue fragments were rinsed in phosphate-buffer soluton (pH 7.4) overnight and post-fixed in 4% phosphate-buffered glutaraldehyde (pH 7.4) for 3 h. Thereafter, the tissue was again fixed in 2% phosphate-buffered osmium tetraoxide solution for 3 h. The tissue blocks were then dehydrated in graded concentrations of ethanol and embedded in Epon. The thin sections were stained with uranyl acetate and lead citrate solution.

Results

Light Microscopic Findings

The intranuclear inclusions were discrete, round, eosinophilic, homogeneous or hyaline, and often surrounded by a halo (Fig. 1a). They were widespread in virtually all types of central, peripheral, and autonomic neurons which were otherwise normal. On rare occasions, neurons harboring the intranuclear inclusions were in the process of disintegration. The inclusions varied considerably in size ranging from a few to 15 μm in diameter. They were generally larger than the nucleolus of the neurons in which they occurred and tended to be larger in large than in small neurons. The inclusions were often multiple and more than two inclusions were occasionally encountered. Rare large inclusions contained a denser central core. The inclusions were unstained by osmic acid, oil red O and methyl green-pyronin. They were PAS-negative, non-metachromatic and pale blue in toluidine blue stain (Fig. 1b). They were stained pale brick red by Congo red but were not birefringent with polarized light before or after Congo red staining. The inclusions were stained a copper color with Bielschowsky's silver rather than black and were gray or light green in Mallory's trichrome stain.

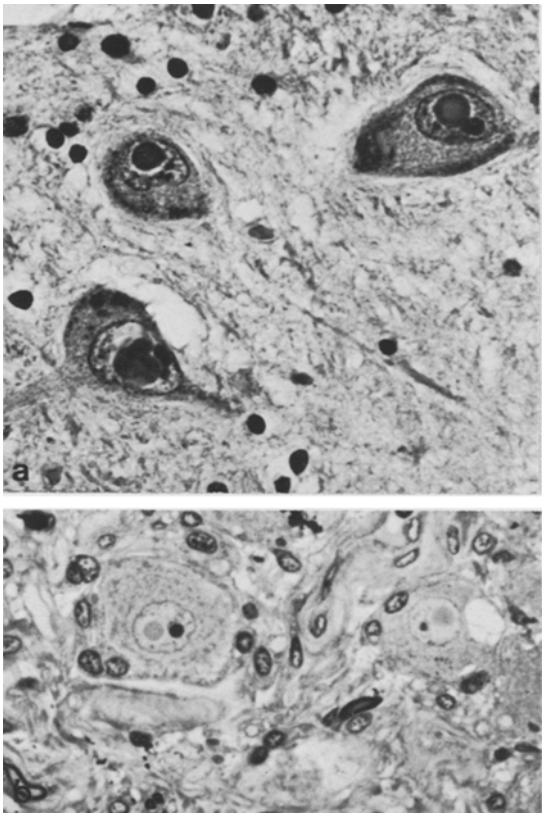

Fig. 1. Large neurons of sublenticular region (**a**) and stellate ganglion cells (**b**) show intranuclear inclusions surrounded by halo. Nucleoli are darker and are clearly seen. HE stain (**a**); Epon section and toluidin blue stain (**b**). ×480

Fluorescence Microscopic Findings

The inclusions emitted yellow-green autofluorescence with ultraviolet light and the emission band extended from 470–530 nm of the spectrum (Fig. 2d and e). In contrast, Marinesco bodies of pigmented neurons of the substantia nigra from a 67-year-old man (Fig. 2a and b) did not exhibit autofluorescence.

Electron Microscopic Findings

In neurons of the hippocampus, subcellular organelles of the cytoplasm were poorly preserved because of postmortem autolysis, but mitochoria and lipofuscin granules were still well recognized. The nuclei were generally better preserved than the cytoplasm and the nucleoli were usually identified. The chromatin often occurred in coarse clumps which were randomly dispersed in most neuronal nuclei (Fig. 3). In contrast to postmortem alterations of hippocampal neurons, cytoplasmic organelles of the stellate ganglion cells were relatively better preserved. The nuclei of the ganglion cells (Fig. 6) had generally less chromatin than the nuclei of the hippocampal neurons. In both hippocampal neurons and sympathetic ganglion cells, the nuclei frequently contained one or two sharply defined, round inclusion bodies without limiting membrane (Figs. 3 and 6). The inclusions were strikingly less electron-dense than the nucleolus and chromatin. They were usually single in the hippocampal neurons while they were often double in the sympathetic ganglion cells (Fig. 6) and the double inclusions were occasionally coalescent. The inclusions consisted of uniform, fine, straight filaments which were indefinite in length and 8–9 nm in diameter. The filaments were haphazardly arranged (Fig. 4a), but occasionally, they showed ra-

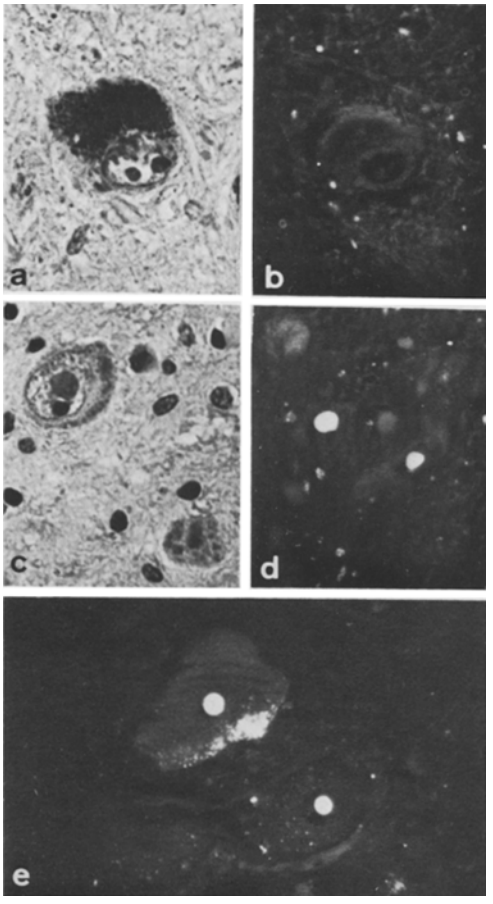

Fig. 2. Marinesco body of pigmented substantia nigra neuron (**a**) of an elderly man shows no autofluorescence (**b**) as compared with neuronal intranuclear inclusions of substantia nigra neuron (**c, d**) and dorsal spinal ganglion cell (**e**) exhibiting strong autofluorescence with UV light. HE stain; ×480 (**a, c**). Paraffin section; ×450 (**b, d, e**)

dial or concentrical arrangement at the periphery (Fig. 4b). Rare large inclusions contained a central core which was extremely electron-dense and amorphous (Fig. 5). In a few stellate ganglion cells, the "rodlets of Roncoroni" were encountered side by side with the inclusions but they were unrelated to the latter (Fig. 6). The rodlets were also observed in a few ganglion cells harboring no inclusions. No counterpart of the halo around the inclusion observed on light microscopy was noted on electron microscopy and the halo was probably a fixation artefact.

Discussion

Neurological diseases associated with neuronal and/or glial intranuclear inclusions are generally regarded as viral infections regardless of virological confirmation. The neuronal intranuclear hyaline inclusions (neuronal inclusions), described herein have neither the ultrastructural feature of known viral inclusions nor are they associated with virus particles. They are composed of uniform, fine, straight filaments of indefinite length and 8–9 nm in diameter, which are haphazardly arranged. Their unique ultrastructure when taken together with the progressive course of the illness for 18 years seems to speak against a viral etiology. The neuronal inclusions also differ ultrastructurally from the widespread intranuclear inclusions described by Lindenberg et al. (1968) in a mentally retarded 28-year-old man with progressive spasticity and ataxia since childhood. The latter inclusions were widespread not only in the brain but also in every visceral organ while the former inclusions were limited to neurons.

Certain neurological degenerative disorders are well known to be associated with characteristic neuronal cytoplasmic inclusions, such as Parkinson's disease (Lewy body), myoclonic epilepsy (Lafora body), amyotrophic lateral sclerosis (Bunina body and others), and Pick's disease (Pick body). The intranuclear inclusions in question, however, differ clearly from these intracytoplasmic inclusions not only in their location but also in their ultrastructure.

The neuronal inclusions do, however, resemble Marinesco bodies on light microscopy as has been pointed out (Janota, 1979; Sung et al. 1980) but differ from the latter in several other respects. Marinesco bodies rarely occur in children and young adults but increase in frequency with advancing age (Yuen and Baxter 1963). They are limited to pigmented neurons of the brain stem and have never been observed in association with any particular disease (Yuen and Baxter 1963). In contrast, the neuronal inclusions are widely distributed in almost all central, peripheral, and autonomic neurons and are associated with chronic progressive multisystem atrophy. According to Leestma and Andrews (1969), Marinesco bodies consist of ovoid aggregates of fine granular material (20 nm in diameter) without limiting membrane, which is often associated with "a lattice-like filamentous (10–12 nm in diameter) array". The neuronal inclusions are, therefore, different from Marinesco bodies in their filamentous nature. Moreover, the neuronal inclusions are never associated with the "lattice-like filamentous array". A "lattice-like filamentous array" was, however, observed in a nucleus having the inclusions but was unrelated to the latter. The "lattice-like filamentous arrays" together with the "nuclear bodies" and "rodlets of Roncoroni" have been observed by other investigators in various neurons unrelated to any particular diseases in man as well as in animals; the subject has been extensively reviewed by Schochet (1972). The "rodlets of Roncorni" were also observed in a few nuclei with or without the inclusions in the present case but were unrelated to the latter. The

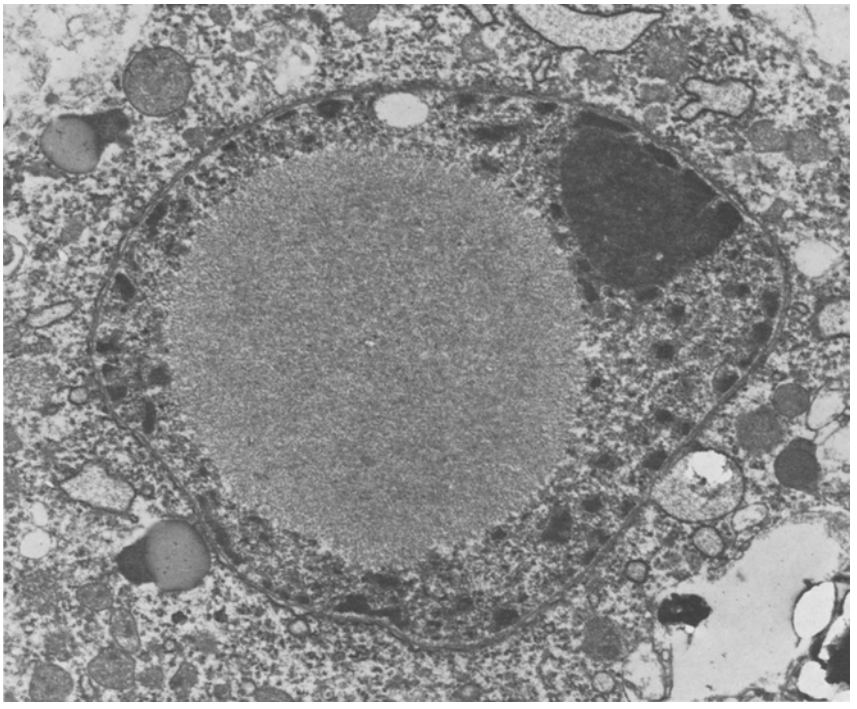

Fig. 3. Hippocampal neuron with intranuclear inclusion, nucleolus and clumped chromatin; no halo around inclusion. × 12,100

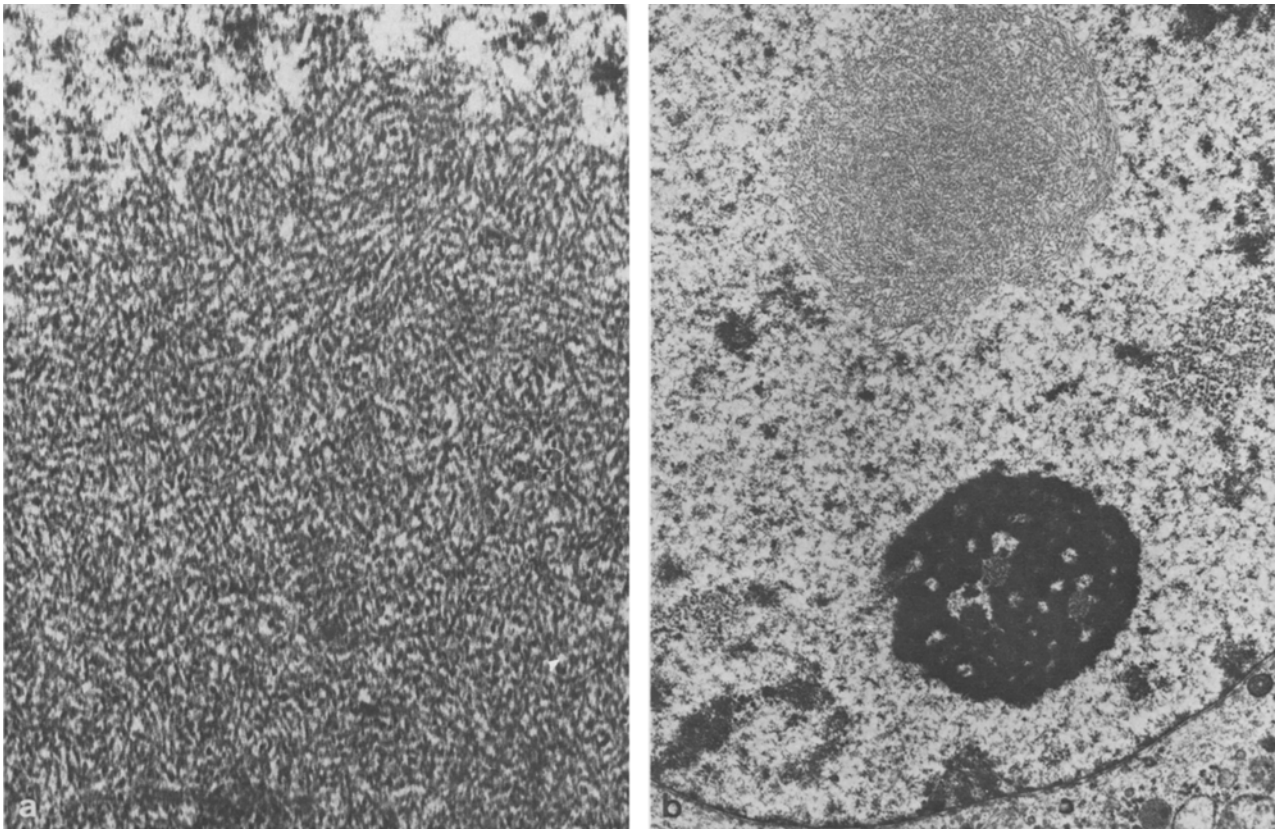

Fig. 4a and **b.** Intranuclear inclusions of stellate ganglion cells. Filaments are uniform, straight, and disorderedly arranged (**a**), but they tend to be concentrically arranged at the periphery (**b**). × 48,300 (**a**); × 13,900 (**b**)

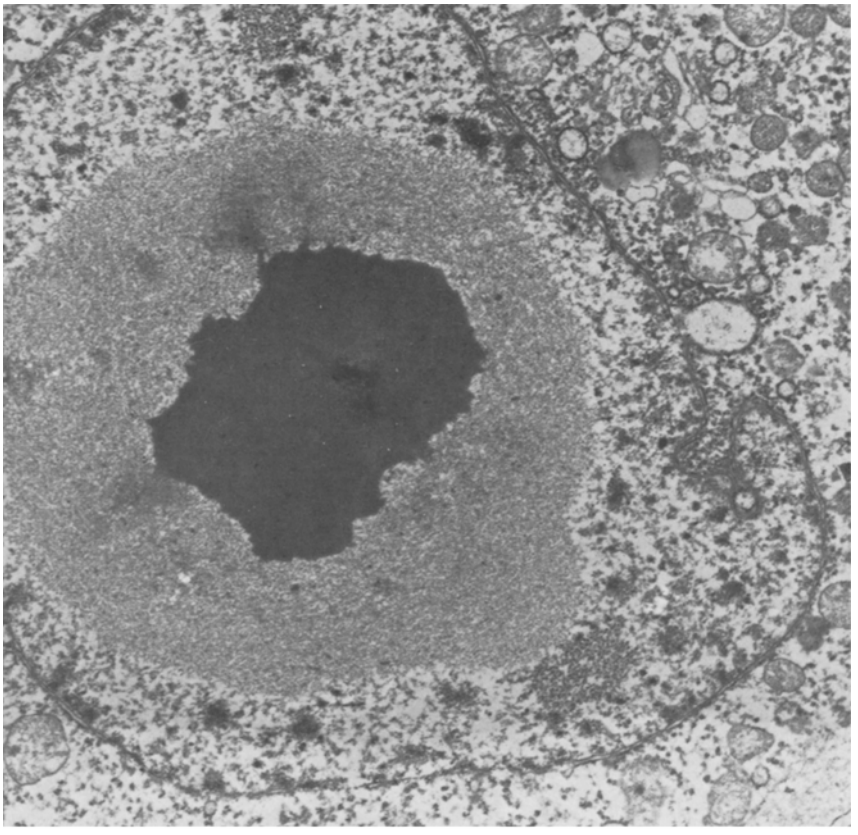

Fig. 5. Hippocampal neuron having large intranuclear inclusion with electron-dense amorphous core. ×12,000

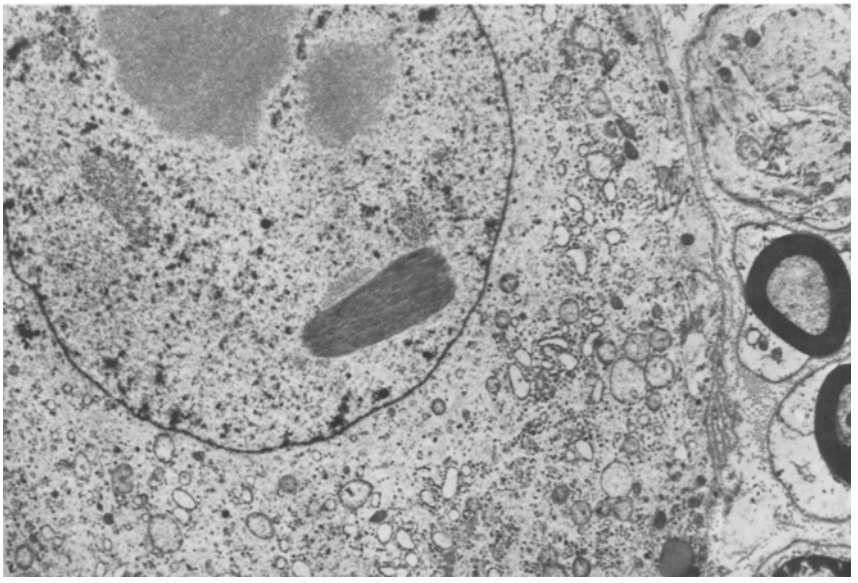

Fig. 6. Stellate ganglion cell harboring two intranuclear inclusions and a "rodlet of Roncoroni" (parallel arrays of fine filaments). ×5,000

neuronal inclusions also differ from Marinesco bodies in another respect. The inclusions with ultraviolet light emit strong yellow-green autofluorescence with the emission band extending from 470–530 nm of the spectrum while Marinesco bodies do not emit autofluorescence. By virtue of their characteristic ultrastructure and autofluorescence, it is reasonable to conclude that the neuronal inclusions are distinct from Marinesco bodies despite their light microscopic resemblance to the latter.

No attempt was made in this study to investigate histochemical differences between the neuronal inclusions and Marinesco bodies. However, the neuronal inclusions exhibited negative response to various lipid (Osmic acid, oil red O), carbohydrate (PAS), amyloid (birefringence after Congo red), and nucleic acid (methyl green-pyronin) stans applied herein. This suggests that the inclusions may represent protein, but their chemical nature remains to be elucidated.

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