Acta Neuropathol. (Berl.) 44, 211–216 (1978)

Acta Neuropathologica

A Sporadic Juvenile Case of the Amyotrophic Lateral Sclerosis with Neuronal Intracytoplasmic Inclusions

M. Oda[1, 2], N. Akagawa[1], Y. Tabuchi[3], and H. Tanabe[3]

[1] Division of Pathology and [3] Clinic of Neurology of Toranomon Hospital and Okinaka Memorial Institute for Medical Research, 2, Akasaka-aoi-cho, Minato-ku, Tokyo 107, Japan
[2] Metropolitan Medical Center of the Severely Handicapped (Fuchu-Ryoiku-Center), Fuchu, 2-9-2, Musashidai, Fuchu-city, Tokyo 183, Japan

Summary. In an autopsy case of the sporadic juvenile ALS (a 17-year-old girl) intracytoplasmic inclusions are found in the upper and lower motor neurons and in nerve cells of the dentate nucleus, pontine nucleus, brain stem reticular formation, substantia nigra, thalamus, globus pallidus and others. Histochemically they contain RNA-Protein compounds. Electron microscopically, they consist of randomly interwoven tubules with granular endoplasmic reticulums and free ribosomes in the margin. Each tubule measures 90–150 Å in diameter and shows no distinct periodic constiction. Amorphous substances as well as ribosome granules are scattered and associated with those tubules. The inclusion-bearing cells are usually swollen and chromatolytic and have a large hydropic nucleus, suggesting a close relation between the development of the inclusion and chromatolysis. Clinically, a rapid progress of the symptoms (total duration: about 12 months) and conspicuous disturbances of the autonomic nerve, such as sinus tachycardia and bladder sphincter dysfunction, should be noticed.

Key words: Sporadic juvenile ALS – Neuronal intracytoplasmic inclusion – RNA-protein compounds – Straight tubule.

In this report we would like to present some supplementary findings of the sporadic juvenile amyotrophic lateral sclerosis (ALS). A particular interest will be attached to neuronal cytoplasmic bodies which have been described in some cases of this rare disease.

Offprint requests to: M. Oda, M.D. (address see above)

Case Report

A high school girl aged 16 showed a rapidly progressing muscle weakness and atrophy 6 months before admission, beginning on the left arm and expanding to the right arm and both legs. She was hospitalized on July 22, 1974. The family and past histories were unremarkable. In addition to the severe motor disturbance, a dysarthria, hoarseness, respiratory distress, muscle fasciculation, and pyramidal tract signs were observed. In October, a difficulty of urination and many irritabilities of the autonomic nerve such as sinus tachycardia, hyperhidrosis, hypersalivation and mydriasis occurred. The cardiac arrest occurred three times and led the patient into a terminal coma over a month. She died on January 3, 1975. The autopsy was done 4 h p.m. (Autopsy No. 2770, Toranomon Hospital, Tokyo).

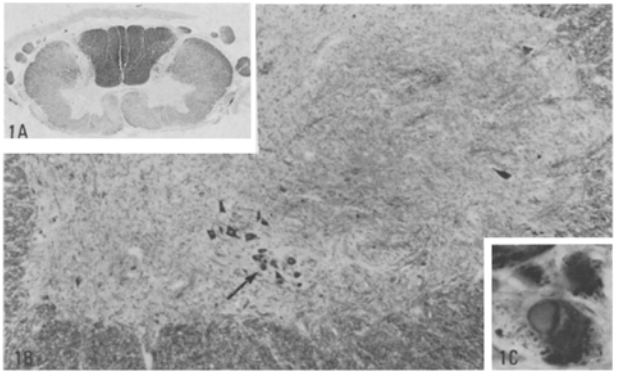

Fig. 1. A The 7th cervical cord. Diffuse pallor of myelin in the lateral and ventral funiculi; atrophic and demyelinated ventral roots. The spinocerebellar tracts are relatively well preserved. ×2.7. **B** The second sacral anterior horn. The ventral cell group is well preserved, while other motor neurons disappear. ×29. **C** Higher magnification of the area indicated by arrow in Fig. 1B. A cytoplasmic inclusion with deeply basophilic rim is visible. ×288. (**A–C**: Luxol fast blue with cresyl violet)

0001-6322/78/0044/0211/$1.20

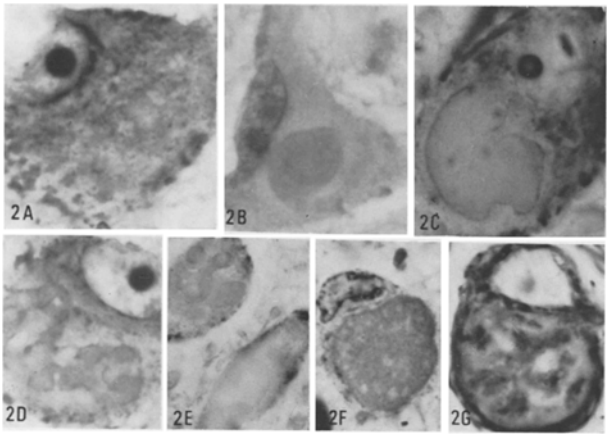

Fig. 2A–G. Various forms of neuronal intracytoplasmic inclusions. **A** A small and faintly basophilic body in the center of the slightly chromatolytic cytoplasm of an enlarged nerve cell in the occulomotor nerve nucleus. The nucleus is pushed aside to the cell periphery and enlarged and bright, and has an enlarged nucleolus. × 750. **B** A round basophilic granular body in the center of an ischemic-degenerated Betz cell. × 750. **C** A faintly basophilic and homogeneous body with distinct rim in an enlarged occulomotor nerve cell. × 750. **D** Foliated or fragmented inclusions in a markedly chromatolytic occulomotor nerve cell with enlarged hydropic nucleus. × 750. **E** Two highly chromatolytic nerve cells with fragmented or almost vanishing cytoplasmic inclusions. × 300. **F** Nerve cell in the dentate nucleus. A round cytoplasmic inclusion shows a brown silver-impregnation and fine-vesicular structure. × 750. **G** Nerve cell in the occulomotor nerve nucleus. Black or brown silver-impregnated fibrils or skeins are scattered in the lucent background of the cytoplasmic inclusion. × 750. (**A**, **C**–**E**: Luxol fast blue with cresyl violet, **B**: H.-E., **F** and **G**: Bodian)

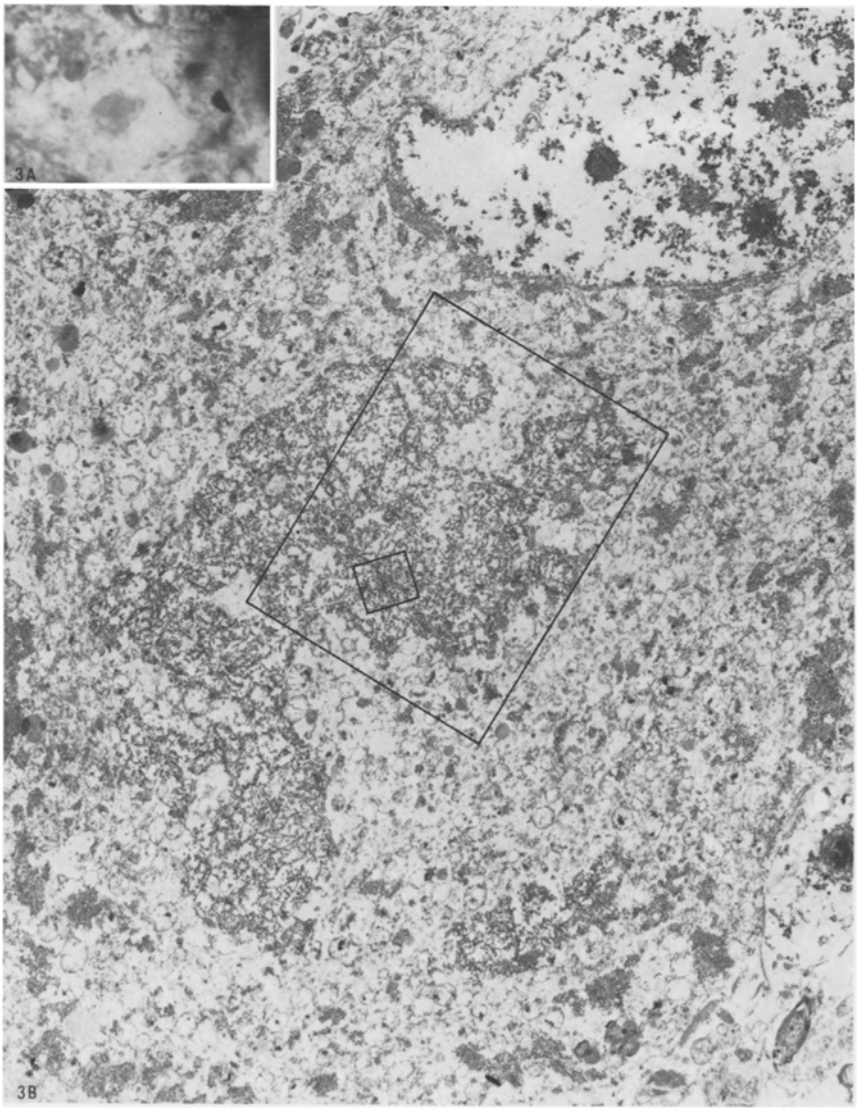

Fig. 3A and B
A nerve cell of the cervical anterior horn
A 1-μ thick section for light microscopy. An intracytoplasmic body in the swollen cytoplasm with central chromatolysis. × 643
B Electron microscopic view of the same cell. An irregularly shaped body consisting of interwoven filamentous and granular materials is seen in the cytoplasm with numerous mitochondria, a few dense bodies and lipid granules and juxtanuclearly and peripherally displaced small groups of ERg. No limiting membrane is visible around the inclusion. × 4,020. (**A** Toluidine blue, **B** Uranil acetate and lead citrate)

Neuropathological Examination

This examination revealed the following changes which were distinguishable from co-existing anoxic damages at the endstage. In the corticospinal tract throughout the spinal cord, both the crossed and uncrossed, and the medulla oblongata, a moderate diminution of myelinated fibers was observed; in addition, the lateral and ventral funiculi were diffusely pale in the myelin preparations (Fig. 1 A). A glial proliferation was minimal. In the upper level of the brain stem, the degeneration of the pyramidal tract was less prominent. However, in the middle one-third of the posterior limb of the internal capsule, a narrow band of myelin loss was evident. With Sudan-III stain, a circumscribed area of fat mobilization was observed in the white matter of the central hemispheric region, presumably showing a degeneration of the descending fibers from the motor cortex. The nerve cell loss was obvious in the anterior horn of every level of the spinal cord and in the 7th and 12th cranial nerve nuclei. The dorsal nucleus of Clarke as well as spinocerebellar tracts were well preserved. The intermediolateral nucleus of the thoracic cord and the ventral cell group of the 2nd sacral anterior horn (Group X of Onufrowicz) were also well preserved, showing several intraneuronal inclusions (Fig. 1 B and C). A severe loss of nerve fibers was seen in the atropic ventral roots, and a neurogenic degeneration was evident in all examined muscles of the extremities and trunk.

Most impressive in this case were a number of neuronal intracytoplasmic inclusions distributed mainly in the motor system of the CNS: in Betz cells, pyramidal cells in the precentral and neighboring frontal cortex, nerve cells in the 3rd (including Edinger-Westphal nucleus), 4th, 6th, 7th and 12th cranial nerve nuclei, and in nerve cells of the motor nucleus of the trigeminal nerve, ambiguus nucleus, dorsal motor nucleus of the vagus and of the anterior horn. The neuronal inclusions were found also in the dentate nucleus, pontine nucleus and in large cells of the brain stem reticular formation. A small number of them were detected in the medial and lateral thalamic nuclei, globus pallidus, red nucleus, in pigmented cells of the substantia nigra, in the spinal lateral horns and rarely in the vestibular nuclei, superior colliculus and posterior horns.

The nucleus of the inclusion-bearing cells was usually enlarged, bright, and pushed aside to the apical side of the cytoplasm and had a large distinct nucleolus (Fig. 2 A, C and D). The center of the swollen chromatolytic cytoplasm was occupied by a basophilic, large or small, round, oval, kidney- or horseshoe-shaped body with deeply stained rim and fine granular, vesicular, or faint homogeneous central zone (Fig. 2). With Bodian stain, some of round inclusions were compactly brown-impregnated (Fig. 2 F), but in the majority only black or brown fibrils or skeins were visible on the lucent background (Fig. 2 G). Occasionally, the inclusion consisted of feebly stained multiple leaf-like segments in the lower motor neurons, especially in the occulomotor nerve nucleus (Fig. 2 D). There were several highly chromatolytic nerve cells, occasionally with vanishing inclusion (Fig. 2 E). The inclusion was stained very faintly blue or red-violet with Masson's trichrom and Azan stain, respectively, slightly black with gallocyanin-chromalum, pink with pyronin, positive to coupled tetrazonium and Millon's reaction, but negative to Feulgen, PAS, alcian blue, congo red and various lipid reactions such as Sudan-III, Sudan black B, and luxol fast blue.

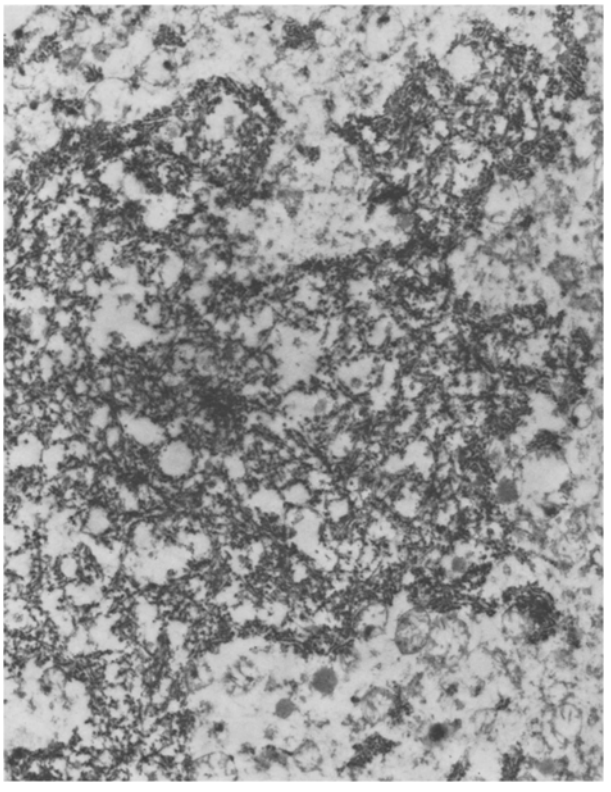

Fig. 4. Magnified view of the area of the inclusion body encircled by a large square in Fig. 3 B in another preparation. Aggregated ERg and ribosomes margin and demarcate it from the matrix. Ribosome-like granules are interspersed among the filamentous structures. × 8,520. (Uranil acetate and lead citrate)

Electron Microscopic Findings

The material had preserved in 15% formol solution for about a year. Several small blocks from the cervical anterior horn, occulomotor nerve nucleus, precentral cortex and dentate nucleus were post-fixed with 1% osmic acid in phosphate buffer, embedded in epon and cut with LKB-microtome. Nerve cells laden with basophilic cytoplasmic bodies were found in each toluidine blue-stained 1-μ section (Fig. 3 A). Some of them were prepared for electron microscopic examination. The ultrathin sections were doubly stained with 2% uranil acetate and 0.2% lead citrate, and studied with Hitachi Hu-12 electron microscope.

In the cervical anterior horn, the inclusion-bearing cells were swollen and a small number of granular endoplasmic reticulums (ERg) were displaced to the peripheral and juxtanuclear zones of the cytoplasm; numerous mitochondria, some neurofilaments, a few round dense bodies and lipid granules were present (Figs. 3 B and 4). Corresponding to the cytoplasmic inclusion in the toluidine blue preparation for light microscopy, an irregularly shaped cluster of loosely packed granular and filamentous materials was visible rimmed by aggregated ERg and free ribosomes (Figs. 3 and 4). The inclusion had no limiting membrane. Its high power view disclosed loose networks of interwoven filaments, each of which showed a tubular profile with outer diameter of 90 – 150 Å; an electron lucent central space was visible also in cross sections; they were straight or gently curved, with no periodic constrictions nor side branches (Fig. 5 A and B). The length of those tubules could not be measured because of their random arrangement. Fuzzy and less electron dense materials as well as free ribosome granules were interspersed or associated with them (Fig. 5). A few mitochondria and neurofilaments were enclosed in some of the inclusions. In nerve cells of the precentral cortex and dentate nucleus, which had round and deeply stained inclusions light microscopically (Fig. 2 B and F), the inclusions seemed to contain more mitochondria, compared to those in the

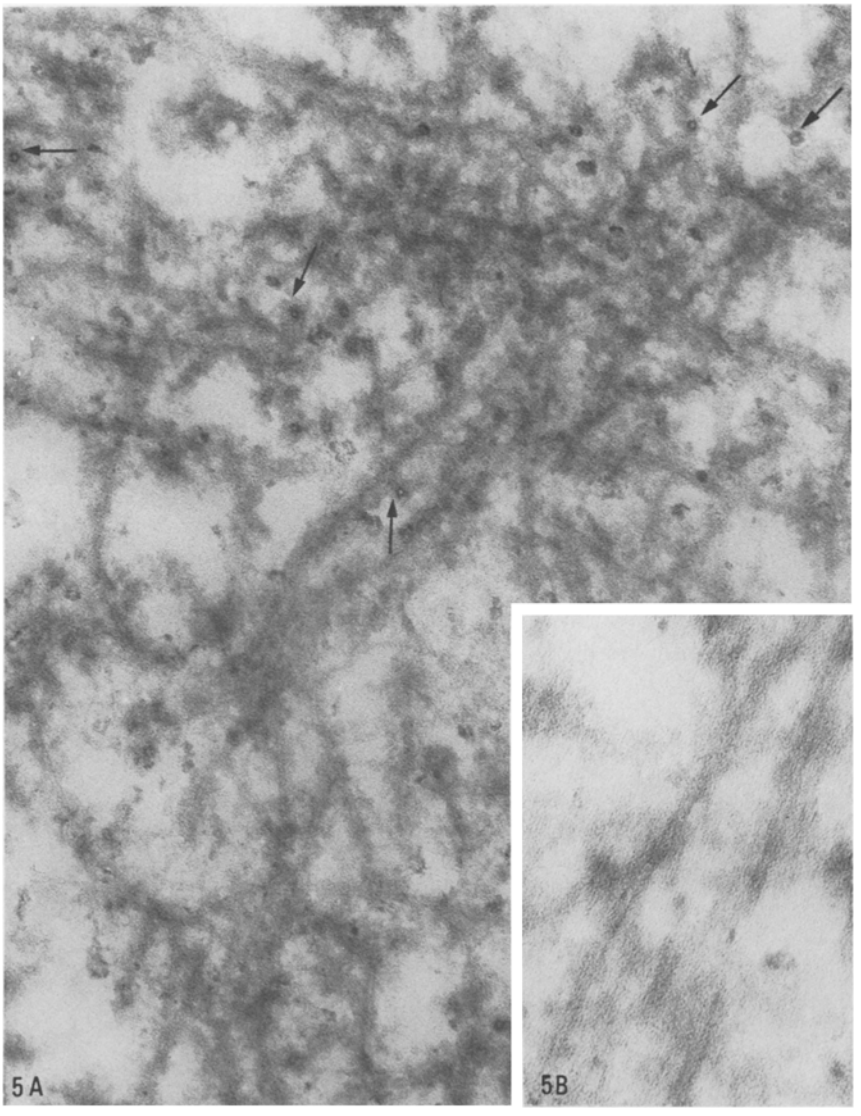

Fig. 5A and B
Part of the cytoplasmic inclusion in cervical anterior horn cells
A Magnified area indicated by a small square in Fig. 3B. The inclusion consists of interwoven tubular elements of 120–140 Å in diameter. Circular profiles of transverse sections are also visible (*arrows*). Fuzzy substance is associated with the tubules. ×90,000
B Higher magnification of two tubules. No periodic constriction is visible. ×150,000. (Uranil acetate and lead citrate)

anterior horn (Fig. 6). In contrast, in feebly stained inclusions of the occulomotor nerve nucleus (Fig. 2C–E and G), tubular elements were more loosely packed, compared to those in the inclusions of the anterior horn, and granular elements were less conspicuous (Fig. 7).

Discussion

At least 7 pathologically verified cases of the sporadic juvenile ALS have been reported till now (van Bogaert, 1925; Cognazzo et al., 1970; Nishigaki et al., 1971; Wohlfart et al., 1941; Berry et al., 1965; Nelson et al., 1972; Tsujihata et al., 1978). In the last four cases peculiar intracytoplasmic bodies similar to those of the present case were observed in anterior horn cells or, as in the case of Nelson et al., more widespreadly with almost the same distribution as that of ours. Berry et al. described "dense aggregates of parallel filaments with similar characteristics to those in cortical neurons in Alzheimer's disease" in a short abstract of their electron microscopic study. However, the still unsatisfactory information about those inclusions necessitates further studies especially of their fine structures, not only to search for their significance for cell metabolism, but also to contribute to the pathology of various fibrillar bodies in neurons. It should indeed be judged carefully whether our findings about the inclusion in autopsy material preserved in formalin reproduce precisely a phenomenon occurring intra vitam. However, this unusual neuronal change, which can never be found in other control autopsy cases, could represent a significant abnormality of neurons in this case (or disease), because of its selective localization in the motor systems and of appearance of the abnormal tubules in its fine structure.

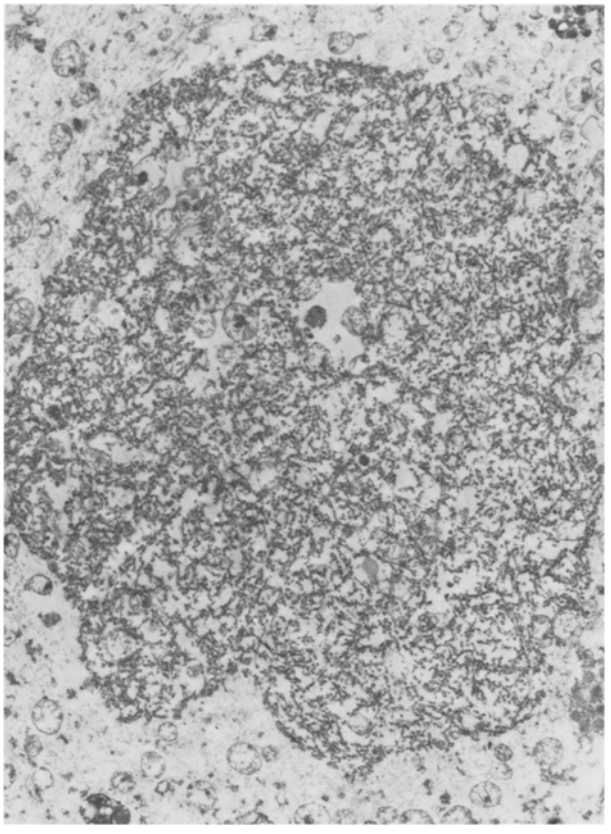

Fig. 6. Nerve cell of the dentate nucleus. An intracytoplasmic inclusion with the same structures as in the anterior horn. Several mitochondria are enclosed. × 6,390. (Uranil acetate and lead citrate)

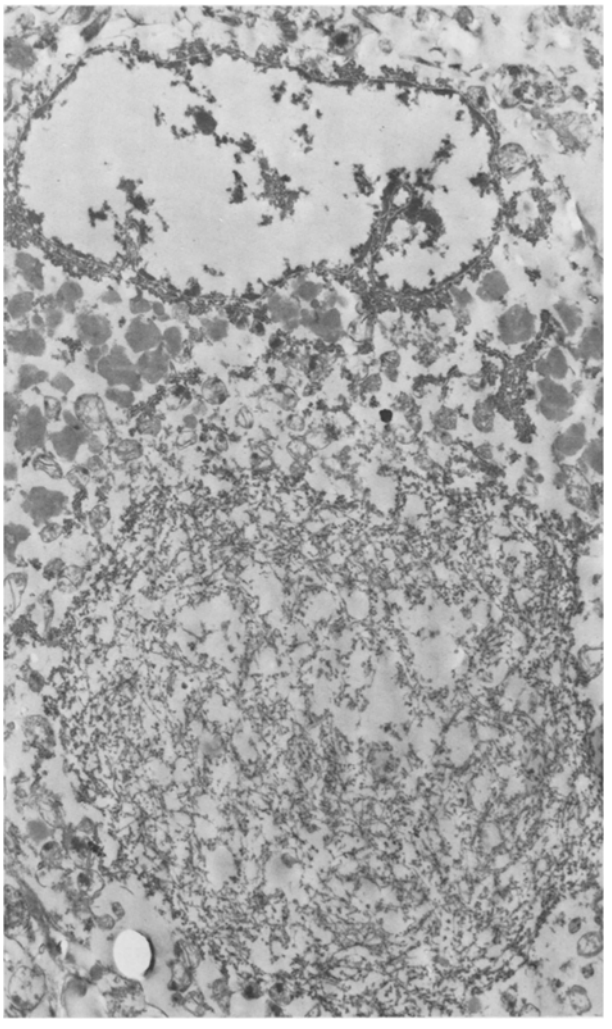

Fig. 7. Nerve cell of the occulomotor nerve nucleus. Same kind of cytoplasmic inclusion is observed, but granular and filamentous elements are more loosely packed. × 7,110. (Uranil acetate and lead citrate)

In various diseases and experimental conditions, intraneuronal fibrillar bodies have been described, such as Alzheimer's and experimentally induced neurofibrillary changes, Pick bodies, Lewy bodies and Lafora bodies. In the motor neuron diseases, Lewy-like bodies in familial cases of ALS (Hirano et al., 1967; Takahashi et al., 1972) and Lafora-like bodies (Orthner et al., 1973; Sun et al., 1975), conglomerates of interwoven skeins of neurofilaments (Schochet et al., 1969) and Bunina-like bodies (Tomonaga et al., 1977) in sporadic and familial cases have been delineated. Furthermore, Norman (1974), Mendell et al. (1971), Roessmann et al. (1971) and others described hyaline cytoplasmic inclusions in motor neurons in cases with or without nervous disease. The inclusions as well as their fibrillar fine structures of our case are, however, different at least partially from any of those above mentioned and also from amyloid fibrils, normal neurofilaments and microtubules in randomly interwoven arrangement of probably straight tubules with 90–150 Å diameter, in clear margin of ERg and ribosomes and in routine and histochemical stainabilities.

The cytoplasmic inclusions in the present example contain RNA-protein compounds with similar, but lesser stainability to that of Nissl bodies or ribosomes. They showed various forms presumably corresponding to developing stages, such as round and compact bodies in early stage, which seemed to contain electron microscopically more abundant mitochondria and ribosomes (Figs. 2A, B, F and 6), and faint and fragmented ones with relatively loose fibrillar fine structure in advanced stage (Figs. 2C–E, G and 7). No definite neuronal deterioration was observed in the inclusion-bearing neurons. But they showed swelling and central chromatolysis and a large hydropic nucleus, suggesting a certain stimulated state of neuronal metabolism in the pyramidal and other motor control systems, in relation to the development of the inclusion.

Lastly, a few words are given about the tubular elements, probably the most essential component of the inclusion. In spite of limited reproducibility of our material, they seem to appear in the neuronal cytoplasm in contact with ERg and ribosomes. In addition, the following points would be noteworthy. First, Alzheimer's neurofibrillary changes have been described together with such neuronal bodies in 2 cases of the sporadic juvenile ALS (Berry et al., Nelson et al.). Second, Kuroda et al. (1976) reported a case of the pyramidal tract degeneration with neurofibrillary tangles in the upper and lower motor neurons. Third, many neurofibrillary tangles appear also in the progressive supranuclear palsy (PSP), and individual fibrils with straight tubular profile of the PSP-tangles (Tellez-Nagel et al., 1973) show a remarkable resemblance of the structure to those of the present case. An interesting question, whether the intraneuronal fibrillar structures of this case and neurofibrillary tangles have something in common in their etiology and pathogenesis, remains to be solved.

The eight referred cases of the sporadic juvenile ALS including our case, five males and three females, are characterized clinically by teenaged onset of the disease and rapid progress of 12–18 months of duration. In our case, the lateral and ventral funiculi were affected diffusely and equally at every level of the spinal cord. Furthermore, a possible dysfunction of the inclusion-bearing neurons in the 3rd and 10th cranial nerve nuclei, intermediolateral nuclei of the thoracic cord and in the ventral cell group of the sacral anterior horn (Onufrowicz) could have contributed to the disturbance of the autonomic nerve including mydriasis, sinus tachycardia and bladder sphincter dysfunction, although no distinct nerve cell loss was observed in these regions, as said in usual cases of ALS (Mannen et al., 1975).

Acknowledgements. The authors wish to thank Dr. M. Hara, Chief of Division of Pathology of Toranomon Hospital, for courtesy to offer the material to this study and for many supports, and Miss Koide and Mr. Ito for their technical efforts. We are also indebted to Prof. Dr. H. Shiraki, Dr. K. Fujisawa, and Dr. M. Okawa, Tokyo, for many useful suggestions.

References

Berry, R. G., Chambers, R. A., Duckett, S.: Clinicopathological study of juvenile ALS. Neurology (Minneap.) **19**, 312 (1969)

Bogaert, L. van: La sclérose latérale amyotrophique et la paralysie bulbaire progressive chez l'enfant. Rev. Neurol. **1**, 180–192 (1925)

Cognazzo, A., Martin, L.: A sporadic case of juvenile ALS. Semiquantitative and histoenzymatical study of the denervated muscles. Eur. Neurol. **3**, 211–230 (1970)

Hirano, A., Kurland, L. T., Sayre, G. P.: Familial ALS: a subgroup characterized by posterior and spinocerebellar tract involvement and hyaline inclusions in the anterior horn cells. Arch. Neurol. **16**, 232–243 (1967)

Kuroda, S., Hayahara, T., Tateishi, J.: An autopsy case of spastic tetraparesis, showing Alzheimer's neurofibrillary changes with peculiar localization. 17. Congress of Jap. Neuropathol. Society, Niigata 1976

Mannen, T., Iwata, M., Toyokura, Y., Nagashima, K.: Pathology of anterior horn of the sacral cord in cases of ALS and its clinical significance. Neurol. Med. (Shinkeinaika) **3**, 169–175 (1975)

Mendell, J. R., Markesbery, W. R.: Neuronal intracytoplasmic hyaline inclusions. J. Neuropathol. Exp. Neurol. **30**, 233–239 (1971)

Nelson, J. S., Prensky, A. L.: Sporadic juvenile ALS. A clinicopathological study of a case with neuronal cytoplasmic inclusions containing RNA. Arch. Neurol. **27**, 300–306 (1972)

Nishigaki, S., Takahashi, A., Matsuoka, Y.: Ein Obduktionsfall der sporadischen juvenilen ALS. Clin. Neurol. (Tokyo) **11**, 500–506 (1971)

Norman, M. G.: Hyaline (colloid) cytoplasmic inclusions in motoneurones in association with familial micrencephaly, retardation and seizures. J. Neurol. Sci. **23**, 63–70 (1974)

Orthner, H., Becker, P. E., Müller, D.: Recessiv erbliche ALS mit "Lafora-körperchen". Arch. Psychiatr. Nervenkr. **217**, 387–412 (1973)

Roessmann, U., McFarland, D. E.: Hyaline cytoplasmic inclusions in motor neurons. J. Neuropathol. Exp. Neurol. **30**, 551–556 (1971)

Schochet, S. S., Hardman, J. M., Ladewig, P. P., Earle, K. M.: Intraneuronal conglomerates in sporadic motor neuron disease. A light and electron microscopic study. Arch. Neurol. **20**, 548–553 (1969)

Sun, C. N., Araoz, C., Lucas, G., Morgan, P. N., White, H. J.: ALS. Inclusion bodies in a case of the classic sporadic form. Ann. Clin. Lab. Sci. **5**, 38–44 (1975)

Takahashi, K., Nakamura, H., Okada, E.: Hereditary ALS. Arch. Neurol. **27**, 292–299 (1972)

Tellez-Nagel, I., Wiśniewski, H. W.: Ultrastructure of neurofibrillary tangles in Steele-Richardson-Olszewski syndrome. Arch. Neurol. **29**, 324–327 (1973)

Tomonaga, M., Saito, M., Yoshimura, M., Shimada, H., Mochizuki, H., Yamanouchi, H., Togi, H.: Neuronal inclusions (Bunina body) in a case of ALS. 18. Congress of Jap. Neuropathol. Soc., Matsumoto 1977

Tsujihata, M., Taguchi, H., Oku, Y., Takamori, M., Terao, H.: A sporadic case of juvenile ALS. Clin. Neurol. (Tokyo) **18**, 82–87 (1978)

Wohlfart, G., Swank, R. L.: Pathology of ALS. Fiber analysis of the ventral roots and pyramidal tracts of the spinal cord. Arch. Neurol. Psychiat. (Chicago) **46**, 783–799 (1941)

Received July 29, 1977/Accepted July 3, 1978