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(Citation)
The Kobe journal of the medical sciences,39(4):133-146

(Issue Date)
1993

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/E0034048

Kobe J. Med. Sci. 39, 133/146, August 1993

INFANTILE NEUROAXONAL DYSTROPHY

----Immunohistochemical and ultrastructural studies on the central and peripheral nervous systems in infantile neuroaxonal dystrophy----

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INDEXING WORDS

infantile neuroaxonal dystrophy; spheroid body; PAP method; electron microscopy; posterior column

SYNOPSIS

We performed pathological studies on the central and peripheral nervous systems of cases with infantile neuroaxonal dystrophy (INAD). Numerous spheroid bodies in the central and peripheral nervous systems, were seen and divided into large spheroid bodies (LSB) and small spheroid bodies (SSB) photomicroscopically. LSB had a relation to some specific neurons with weak expression of neuron specific enolase, neurofilament and chromogranin using PAP method. SSB showed a relation to the axon without immunohistochemical expression of neuron specific enolase, neurofilament, glial fibrillary acidic protein, myelin basic protein, chromogranin, S 100 protein or antitrypsin. LSB were prominent in the posterior column, gracile nucleus, cuneate nucleus, and the tegmentum of

Received for publication: August 16, 1993
Authors' names in Japanese: 伊東恭子, 根岸宏邦, 大林千穂, 林 祥剛, 埴岡啓介, 今井幸弘, 伊東 宏

the midbrain and the pons associated with neuronal loss and gliosis. SSB were observed in the thalamus, basal ganglia and the cerebral cortex. The cerebellum was sclerotic with few microtubule-like structures disposed in a dense network in association with degenerated mitochondria. Similar changes were observed in the sural nerves, autonomic nerve endings in the skin, and the nerve plexus of the digestive tract. Although INAD is a generalized neurodegenerative disease, it is suggested that the primary disorder might occur in the neurons and axons of the sensory tracts.

INTRODUCTION

Infantile neuroaxonal dystrophy (INAD) is an autosomal recessive, neurodegenerative disorder. It is characterized by the presence of numerous spheroid bodies in both the central and peripheral nervous systems. A number of morphological studies by light and electron microscopy revealed that the spheroids were present in the distal part of the axons and in the presynaptic endings. Although the exact nature of the spheroids and the pathogenesis of INAD have not been delineated, it is speculated that the abnormal axonal transport system might be a fundamental pathogenesis of INAD. We performed morphological studies on the spheroid bodies and the microtubulus in the central and peripheral nervous systems of INAD-patients to examine the nature of these spheroid bodies and the relation between microtubules and axonal transport system.

Recently Schindler disease is recognized as INAD resulting from deficient activity of lysosomal hydrase, alpha-N-acetyl-galactosaminidase, which also shows abnormal excretion of urinary oligosaccharide. We performed urine analysis by thin layer chromatography in order to identify alpha-N-acetyl-galactosaminidase deficiency.

MATERIALS AND METHODS

1. Light microscopic and immunohistochemical examinations

The central nervous system of case 1, including the cerebrum, brainstem, cerebellum and spinal cord was divided into 32 segments. Each 4-5 micron-thick sections cut in all segments were stained with hematoxylin eosin, luxol fast blue (LFB) and iron hematoxylin, and 10 micron-thick sections were stained by Bodian's method. We examined the

morphological features and distribution of the spheroid bodies. The sural nerve, skin and esophagus of case 1 and the sural nerve of case 2 were cut and stained by the same method for light microscopic examination.

Immunohistochemistry was also done on 4-5 micron-thick paraffin sections of each block by PAP method using antisera against neuron specific enolase (NSE), neurofilament (NF), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), chromogranin, S100 protein, antitrypsin, kinesin, dynein and tubulin. We studied the histochemical characteristics of the spheroid and microtubulus.

2. Electron microscopic examination

The materials from the cerebral cortex, brainstem, cerebellum,spinal cord, and those from sural nerves and peripheral nerves of the skin, from the myenteric plexus of the rectum and the esophagus, were fixed in 3% phosphate buffered glutaraldehyde and post-fixed in 2% osmium tetroxide. After dehydration in a series of ethanol and embedding in the epon mixture, silver grey coloured ultra-thin sections were made and stained with both uranyl acetate and lead citrate.

3. Urine analysis

A screening test for abnormal urinary oligosaccharide was performed by thin layer chromatography method. Fresh urine samples obtained from case 2 and control individual were analysed.

CASE REPORT

CASE 1.

[redacted] was the first boy of consanguinous healthy parents, who were first cousins. He was born after forty-one-week gestation, weighing 3450g. Initial development was normal until the age of 12 months, when he began to walk with support. He was uttering repeated syllables. At the age of 14 months he lost interest in his surroundings and was no longer able to stand or sit alone. Deterioration progressed rapidly, and he was markedly hypotonic and lying in the frog position by 24 months old. By 18th of age he lost visual following and showed pale optic disc. Examination at the age of 7 years showed an emaciated child with rigid extremities. He was blind and developed horizontal nystagmus. His optic discs appeared pale and atrophic, and corneal reflex was absent. Responses of the lower extremities to pinprick disappeared. The CSF was normal. Numerous laboratory

studies, including determination of lysosomal enzymes in leucocytes and amino-acids analysis, gave normal results except for slight high levels of GOT(46 IU/L) and LDH(604 IU/L) in serum and CSF. He became unable to swallow and required tube feeding by the age of 8 years and died at 10-year-old because of respiratory failure.

CASE 2.

was a younger brother of case 1. He was born at thirty-four-week gestation, weighing 2180g after an uncomplicated pregnancy. His early development was within normal limits. He held a rattle at 4-month old, rolled over at 6-month old. Then he progressed somewhat more slowly, standing and walking with support at 18-month old. He could never speak. After 18 months remarkable hypotonia appeared in his lower extremities and there was gradual regression in mortor and mental states. By the age of 24 months, he was no longer able to sit alone and showed decreasing interest in his surroundings. At the age of 24 months, neurological examination showed profound hypotonia of the trunk and lower extremities and poor voluntary movements. Deep tendon reflexes of the lower extremities were exaggerated and Babinski reflex was induced. A left convergent strabismus was present. Corneal reflex was sluggish and tapping jaw jerk was induced. Horizontal nystagmus and bilateral optic atrophy were found at 2 and 6-month-old. By 3-year-old, seizure of neck myoclonus developed. Numerous laboratory studies including determination of lysosomal enzymes in leucocytes and amino-acid analysis gave normal results but slightly high levels of GOT (88 IU/L) and LDH (1065 IU/L) in serum and CSF. A biopsy of the right sural nerve was taken at 3-year-old.

RESULTS

1. Light microscopic and immunohistochemical examinations

The spheroid bodies, diffusely distributed mainly in axons of neuronal cells in the central and peripheral nervous systems, were divided into 2 groups on light microscopic examination. The first group was large spheroid bodies (LSB) that were rather large and distributed in the pons, medulla oblongata and posterior column of spinal cord (Fig.1). LSB were seen often in axons and included fragments resembling nucleus or nucleolus. The second group was small spheroid bodies (SSB) which were smaller in size

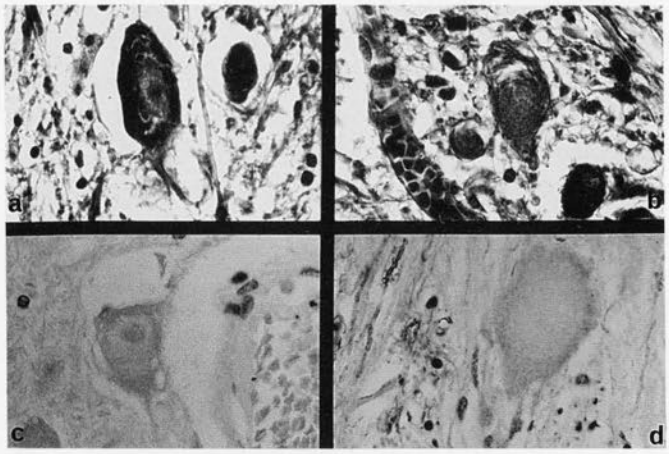

Fig. 1
Neuronal Spheroid Body (NSB) a: LFBx400, b: Bodian x400,
c: Chromogranin x400, d: Neurofilament x400

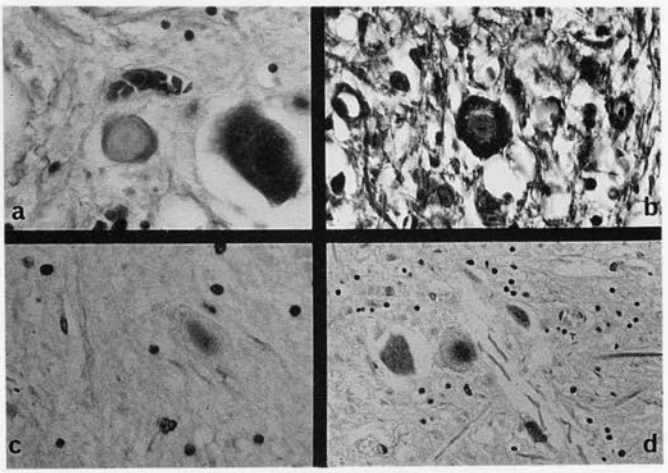

Fig. 2
Axonal Spheroid Body (ASB) a: LFB x400, b: Bodian x400,
c: Chromogranin x400, d: Neurofilament x400

than that of LSB, and found in the cerebral cortex, thalamus, basal ganglia (Fig. 2). In case 1, the cerebral cortex, composed of granular and pyramidal cells, was destroyed and widespread gliosis was evident. Numerous SSB, small sized and lightly eosinophilic stained, were diffusely

dispersed in the cerebral cortex (Fig. 3). Spheroid bodies were infrequnet in the white matter. Loss of Purkinje cells and granular cells from the cerebellar cortex was almost complete. Although widespread gliosis and loss of myelin were evident, spheroid bodies were rare in the cerebellum (Fig. 4). The thalamus showed a widespread neuronal loss and spongy degeneration as well as diffuse gliosis and demyelination. SSB were sparsely distributed. In tne basal ganglia, the neurons of caudate nucleus, putamen and globus pallidus were degenerated and markedly reduced. SSB and mineralization by calcium and iron substances were diffusely dispersed. In the midbrain, the inferior colliculi showed LSB and remarkable gliosis. The nerve fibers were reduced in number associated with SSB in the superior cerebellar peduncule. Melanin granules were slightly decreased in amount in neurons of the substantia nigra. The principal trigeminal sensory and mortor nuclei showed normal large pyramidal neurons with frequent deposit of LSB and SSB. Neuronal atrophy and loss were evident with marked distribution of SSB and corresponding gliosis in the principal trigeminal nuclei. In the medulla oblongata, the dorsal motor nucleus of vagus and solitary nucleus showed diffuse neuronal loss and marked distribution of LSB and SSB same as seen in the principal trigeminal nuclei. The degeneration and loss of neurons associated with dispersed LSB were remarkable in the posterior funicular nuclei. These phenomena as well as dispersion of LSB were especially striking in the gracile nucleus. In the olivary nucleus, neurons lost their dendrites and degenerated to round shape. There were numerous LSB and SSB, in addition to neuronal loss and gliosis in the posterior column of the whole length of spinal cord. These changes were also observed in the dorsal horn of spinal cord. A few neurons remained in the upper thoracic and cervical cords. Ventral horn cells were enlarged with small and fine vesicles in their cytoplam in the sacral cord. The nuclei were degenerated and lost in the thoracolumbar cord. In the cervical cord these changes were less. Some SSB showed myelin sheath-like structure with surrounding spiculate features and small vesicles.

Immunohistochemically some of LSB were stained weakly with NSE, NF and chromogranin, however, SSB showed no positive staining.

2. Electron microscopic examination

On electron microscopic examination, the most striking feature was the

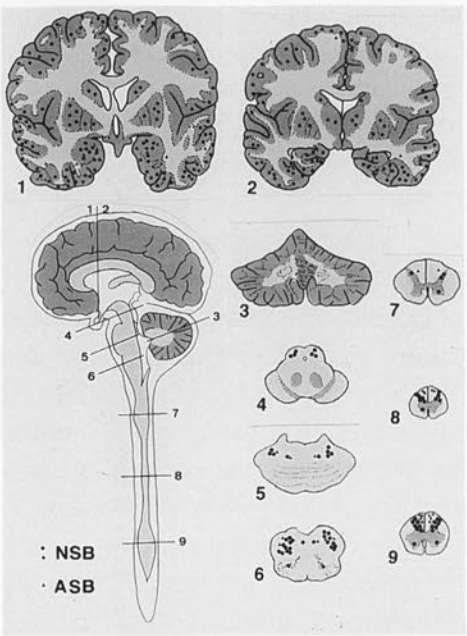

Fig. 3
Spheroid distribution in CNS (case 1)

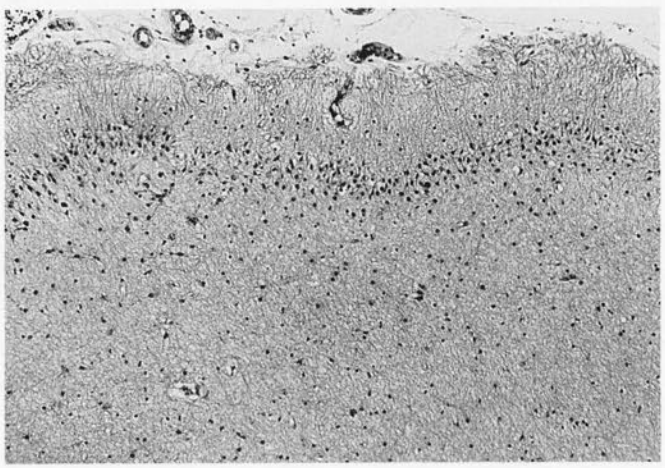

Fig. 4
Loss of Purkinje cells and granullar cells in the cerebellar cortex. cerebellum (case 1) HE x100

presence of numerous smooth membranes, tubulovesicular structures randomly disposed in the myelinated and unmyelinated axons (Fig. 6a). Amorphous structures including synaptic vesicles, fine filaments and numerous small vesicles were noticed (Fig. 5a). Compact aggregates of enlarged and degenerated mitochondria were seen around the tubulo-membranous accumulations (Fig. 5b). The distribution of membranes, small vesicles, microtubules, neurofilaments was irregular and discontinuous in longitudinal sections. There were finger print-like structures produced by accumulation of parallel or crossed membranes in the cerebral cortex. Bundles of parallel membranes, showing myelin-like features, were frequently noted (Fig. 6b). Glycogen-like granules, electron dense bodies and multilamellar bodies were found too. The myelin sheaths of the swollen axons were thinner than those of normal control. In the plexus of the digestive tract, unmyleinated nerve fibers were proliferated in the esophagus. The ganglion cells were reduced in number in the esophagus, stomach and intestine. Some nerve fibers and axonal terminals were enlarged by accumulations of numerous granules, fine tubular structures and small vacuoles. In the skin, a few SSB were dispersed in the

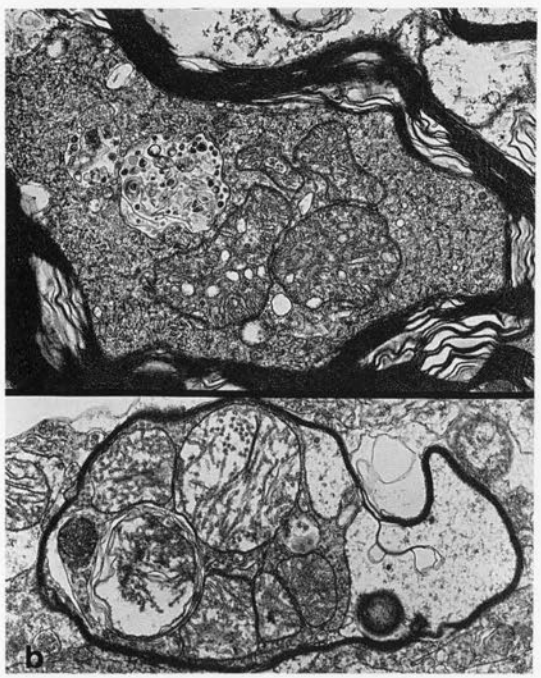

Fig. 5
a: Enlarged mitochondria and amorphous bodies containing cored vesicles and fine filaments in a myelinated axon. spinal cord (case 1) x30000
b: Accumulation of abnormal mitochondria in association with small vesicle and microtubules.
midbrain (case 1) x30000.

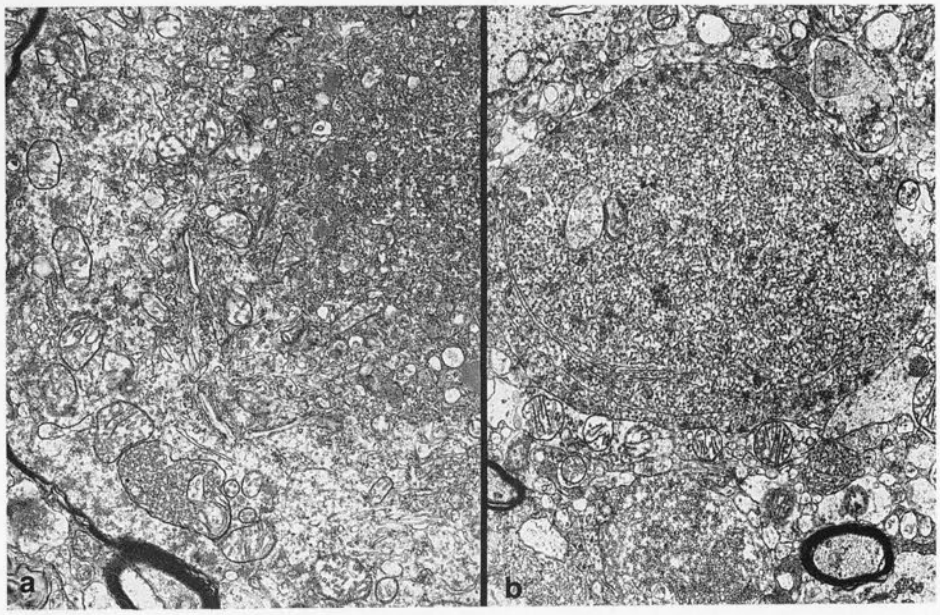

Fig. 6
a: Membranous, tubulo-vesicular profiles and small vacuoles associated with accumulation of mitochondria in a myelinated axon. cerebellum(case 1) x25000. b: Aggregation of microtubules-like profiles in unmielinated fiber. cerebellum(case 1) x20000.

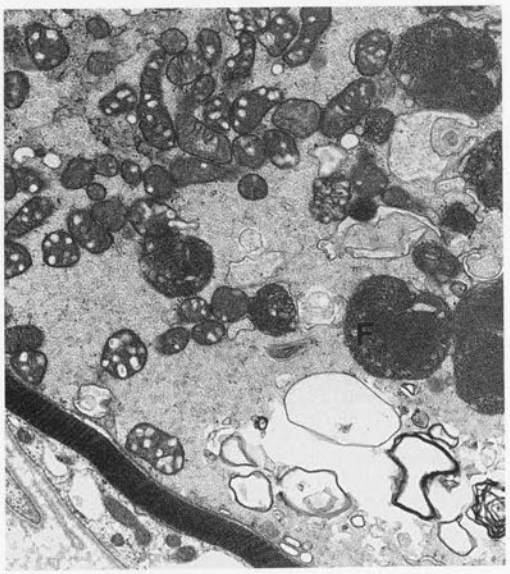

Fig. 7
Microtubules, small vacuoles, finger print-like profiles(F) and accumulation of mitochondria. sural nerve(case 2) x10000.

cutaneous nerve bundles around the sweat glands. Each spheroid body was filled with membranes, microtubules and small vacuoles. The sural nerve of case 1 contained SSB surrounded by a narrow lamellated structure. They consisted of numerous microtubules, small electron dense bodies, round vacuoles and various inclusions. The sural nerve of case 2 had SSB deposits but they were less than those of case 1, whereas some axons were swollen in case 1 and were observed irregularly. There were aggregates of low electron dense microtubules, smooth membranes, small sized vacuoles and finger print-like figures associated with accumulation of mitochondria in myelinated and nonmyelinated axons (Fig. 7). Furthermore, some large sized round vacuoles, surrounded by membranous materials, were seen in the sural nerve in case 2.

3. Urine analysis

Thin layer chromatography showed no abnormal urinary oligosaccharide in case 2.

DISCUSSION

Infantile neuroaxonal dystrophy (INAD) is a rare, autosomal recessive, neurodegenerative disease characterized by the presence of numerous dystrophic axons in the central and peripheral nervous systems. Since Seitelberger described the first case in 1952, more than 100 cases have been reported in the world literature (1-10, 12-15, 17). Spheroid bodies were considered to be divided into 2 groups; large spheroid bodies (LSB) and small spheroid bodies (SSB). Its connection to dendrite or cell body of neuron, immunopositivity for NSE and NF suggested LSB being derived from the neuron. On the other hand, SSB bore some relationships to the axon. These various morphological figures are considered to result from the stage of neuroaxonal degeneration; spheroid bodies showed many features not only by the primary accumulation of various materials and micro-organelles in the neuron and axon but also by the secondary degeneration and absorption of normal structures.

Ultrastructurally, spheroid bodies consisted of numerous membranes, vesiculo-tubular structures, electron dense bodies and synaptic vesicles associated with the accumulation of abnormal mitochondria (1-10, 14, 15, 17). It is suggested that large spheroid bodies, mainly located in the

tegmentum of the brain stem, posterior column of spinal cord and peripheral nerves, were dystrophic axons of polymorphic synapses or terminal axons, and small spheroid bodies (pale eosinophilic bodies) observed in the cerebral cortex, basal ganglia, thalamus, hypothalamus, midbrain, anterior column of spinal cord, sural nerve, were those of the small synaptic endings (6, 8, 9, 15). Kimura et al. investigated ultrastructurally the dystrophic axons and suggested that the primary and fundamental structures were tubulo-membranous structures in axon terminals, and accumulation of mitochondria, neurofilament and other micro-organelles was the sequela to axonal flow disturbance in larger axons (8). It was also suggested that the aggregates of microtubules and small vacuoles were the primary changes and the accumulation of enlarged mitochondria, electron dense bodies and finger-print figures were secondary. Because the primary changes were always associated with those secondary changes and they were located in the larger myelinated axons. The sural nerve of case 2 showed variable and gradual changes similar to those observed in the central and peripheral nervous systems of case 1, which suggested that neuroaxonal degeneration in INAD was progressive from infancy to terminal.

Nothing has been known about the pathogenesis of INAD. Kimura et al. speculated that the transmission abnormality of chemical substance between terminal axons and their receptors or anomalous formations of presynaptic vesicles in synaptic junctions were the fundamental pathogenesis of INAD. From the result that LSB and SSB were the accumulation of microtubule-like structures and variable micro-organellae, the disorder of neuroaxonal transport system was speculated for the main pathogenesis of INAD. Because posterior funicular nuclei, especially in the gracile nucleus, and posterior column showed the most severe degenerations; the primary changes could occur in these areas. Since the lower cord was more severely affected in the posterior column, and neuronal loss and spheroid deposits were most frequent in the gracile nucleus, these changes might be caused the downward degeneration of the long axon of spinal ganglia. Because the neuroaxonal degeneration was most profound in the posterior column in the lumbosacral cord compatible with the early sensory loss of lower extremities, posterior sensory neurons could be upwardly degenerated. In the mortor tracts, neuronal loss and spheroid bodies were frequent in the cortex of pre- and post-central gyri

and neuron enlargements and axonal swellings were remarkable in the anterior column of lower spinal cord. It is suggested that these changes were owing to the disorder of neuron metabolism or the disturbance of efferent axonal transport. Although the loss of cortical neurons was secondary to the respirator brain as 6 months' vegetal state preceded in case 1, the possibility exists that the degeneration of mortor neurons was caused by the primary loss of stimulation from the sensory tracts. The pathological changes in the posterior column are compatible with dying-back neuropathy of the ganglion cells of dorsal roots, those end in the posterior funicular nuclei (10). However variable figures and distribution of spheroid bodies have remained unexplained only by neuropathy.

Although the cerebellum showed remarkable sclerosis associated with severe loss of Purkinje cells and granular cells, spheroid bodies were rare, which are the same result as found in the previous investigations (2, 6, 7, 9). It remains obscure whether once spheroid bodies had been formed in the active stage and disappeared in the terminal stage or a degenerative mechanism, different from that in posterior funicular nuclei, acted in cerebellum. On the other hand, neuronal INAD without remarkable cerebellar atrophy or INAD associated with little spinal degeneration have been reported (4, 12, 16). Although these pathological differences are considered to be due to the wide variation or to the difference in stage of INAD, more studies are necessary to distinguish INAD from similar diseases such as Hallervorden-Spatz disease. Recently Schindler disease is a recognized INAD resulting from deficient activity of the lysosomal hydrase, alpha-N-acetylgalactosaminidase (11, 18). Elevation of GOT and LDH levels in serum and CSF is considered as the result of nonspecific neuronal degeneration, however, there might be a deficiency of unknown enzymes which act in the axonal transport system. Further studies on the axonal transport system and biochemical aspects even in molecular lesion are necessary to discriminate the pathogenesis of INAD .

REFERENCES

1. Aicardi, J. and Castelein, P. : Brain 1979. 102. 727/748. Infantile neuroaxonal dystrophy.
2. Berard-Badier, M., Gambarelli, D., Pinsard, N., Hassoun, J. and Toga, M.: Acta. Neuropathol. (Berl). 1971. Suppl.V.30/39. Infantile

neuroaxonal dystrophy or Seitelberger's disease: II. Peripheral nerve involvement: electron microscopic study in one case.

3. Cowen, D. and Olmstead, E.V. : J. Neuropathol. Exp. Neurol. 1963. 22. 175/236. Infantile neuroaxonal dystrophy.
4. Haberland, C., Brunngraber, E.G. and Witting ,L.A.: Acta . Neurol . 1972. 26. 391/402. Infantile neuroaxonal dystrophy: neuropathological and biochemical study of a case.
5. Hedley-Whyte, E.T., Gilles, F.H . and Uzman , B.G.: Neurology 1968. 18. 891/906. Infantile neuroaxonal dystrophy: a disease characterized by altered terminal axons and synaptic endings.
6. Hunter, A.G.W., Jimenez, C.L., Carpenter, B.F. and MacDonald, I.: Am. J. Med . Genet. 1987. 28. 171/180. Neuroaxonal dystrophy presenting with neonatal dysmorphic features, early onset of peripheral gangrene, and a rapidly lethal course.
7. Huttenlocher, P.R. and Gilles, F.H.: Neurology 1967. 17. 1174/1184. Infantile neuroaxonal dystrophy : clinical, pathologic, and histochemical findings in a family with 3 affected siblings.
8. Jellinger, K.: In:Progress in Neuropathology (Ed.). H.M. Zimmerman. New York and London: Grune and Stratton 1973. 2. 129/180. Neuro-axonal dystrophy : Its natural history and related disorders.
9. Kamoshita, S., Neustein, H.B. and Landing , B.H. : J. Neuropathol. Exp. Neurol. 1968. 27. 300/323. Infantile neuroaxonal dystrophy with neonatal onset: Neuropathologic and electron microscopic observations.
10. Kimura, S.: Yokohama Med. Bull. 1988. 39. 21/32. Ultrastructual investigations on dystrophic axons in infantile neuroaxonal dystrophy.
11. Sandbank, U., Lerman, P. and Geifman, M.: Acta. Neuropathol. (Berl) . 1970. 16. 342/352. Infantile neuroaxonal dystrophy: cortical axonic and presynaptic changes.
12. Schindler, D., Bishop, D.F., and Wolfe, D.E., et al. : N. Engl . J. Med . 1989. 320. 1735/1740. Neuroaxonal dystrophy due to lysosomal alphaN-acetylgalactosaminidase deficiency.
13. Seitelberger, F.: In: Proceedings of the First International Congress of Neuropathology (Rome, Sept. 8-13, 1952) vol.3 Turin: Rosenberg & Sellier: 323/333. Eine unbekannte Form von infantiler lipoidspeicher Krankheit des Gehirns.
14. Seitelberger, F. : Acta. Neuropathol . (Berl). 1971. Suppl .V . 17/29.

Neuropathological conditions related to neuroaxonal dystrophy.

15. Shimono, M., Ohta, M., Asada, M. and Kuroiwa, Y.: Acta. Neuropathol. (Berl). 1976. 36. 71/79. Infantile neuroaxonal dystrophy: ultrastructual study of peripheral nerve.
16. Tachibana, H., Hayashi, T., Kajii, T., Takashima, S. and Sasaki, K.: Brain . Dev . (Tokyo). 1986. 6. 605/609. Neuroaxonal dystrophy of neonatal onset with unusual clinicopathological findings.
17. Wang, A.M., Schindler, D. and Desnick, R.J.: J. Clin. Invest. 1990. 86. 1752/1756. Schindler disease: the molecular lesion in the alphaN-acetylgalactosaminidase gene that causes an infantile neuroaxonal dystrophy.
18. Yagishita, S. and Kimura, S.: Acta. Neuropathol. (Berl). 1974. 29. 115/126. Infantile neuroaxonal dystrophy (Seitelberger's disease): histological and electron microscopic study of two cases.