Amyotrophic Lateral Sclerosis

Inclusion bodies in a case of the classic sporadic form*

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ABSTRACT

Postmortem light and electron microscopic studies of a 52 year old black male who died 17 months after the onset of upper and lower motor neuron signs showed: (1) degeneration of cortico-spinal tracts, (2) loss of spinal neurons and gliosis and (3) cellular inclusions with neurotubules, neurofilaments and granular material. Although these cellular inclusions resemble Lafora bodies, they differ in that, to the authors' knowledge, the latter were not reported to have microtubules. Review of the literature revealed no previous report of these inclusions in cases of amyotrophic lateral sclerosis.

Tissue cultures of cord, hindbrain and cerebrum did not show cytopathic effect during a three-week observation period.

Introduction

Cellular inclusions observed in patients afflicted with amyotrophic lateral sclerosis (ALS) rekindle the hope for elucidation of the etiopathogenesis of this disease. To date, the following inclusions have been described: Hirano bodies, Bunina bodies, and Lewy-like bodies.2,3,13 Others have noted neurofibrillary tangles, granulovacuolar bodies, and amorphous inclusions in anterior horn cells.4,5,11 Studies of cellular inclusions are presented which, to our knowledge, have never before been observed. Additionally, evidence is offered denying the role of conventional viruses in the etiology of ALS.

Case History

A 52 year old Negro male was admitted to the V.A. Hospital in September, 1971 with a chief complaint of progressive weakness and spasms in all extremities. He had served in New Guinea, Western Solomons and the Philippines between 1944 and 1946 and had been unable to work for six months prior to the time of admission because of weakness which was first noted in the right upper and lower extremities. During the four months prior to admission he had lost 15.5 kilos. At the time of admission, he was only capable of ambulation with the aid of a walker. The general physical examination upon admission was normal.
The neurological examination revealed weakness, present bilaterally, which was more severe in the right upper and lower extremities. There was marked atrophy of the intrinsic muscles of the hands. Fasciculations were observed in both upper extremities. Cranial nerves and intellect were normal. No nystagmus or visual defects were noted. The reflexes were hyperactive in all limbs, especially in the legs and particularly on the right side. Hoffman’s sign was present bilaterally but extensor plantar responses were not elicited.

The coordination tests were performed adequately, although slowly. Superficial abdominal reflexes were present and symmetrical. The sensory examination was normal except for some vibratory loss in the lower extremities. The brain scan, EEG and myelogram were normal. Serum VDRL was nonreactive. The CSF contained 84 mg per 100 ml of protein but was otherwise normal. Urine arsenic level was reported to be 112 micrograms per liter (normal = less than 100 micrograms per liter).

Suspecting arsenic intoxication, a course of BAL therapy was administered. However, the arsenic level in a 24-hour urine specimen collected following the said treatment was normal, thus making the diagnosis of arsenic poisoning untenable. The patient had a course characterized by slow, progressive deterioration over the ensuing months. He developed Myerson’s sign, snout reflex and bilateral patellar clonus. Babinski reflexes were not fully present until one month following the first admission.

Electromyographic tests revealed fibrillations in all the muscles of the extremities including respiratory muscles of the chest. Nerve conduction velocity of the left median, ulnar and peroneal nerves were normal. The muscular weakness was notably increased; the patient coughed and choked when attempting to swallow foods because of paralysis of pharyngeal muscles. On readmission, eleven months later, the patient was in a terminal state. He appeared emaciated and cachectic owing to the impairment for deglutition of liquid or solid foods and expired 48 hours afterward.

Materials and Methods

Authorization for partial autopsy (brain only) allowed for the fixation and sampling of brain and cervical spinal cord within one hour after death. Unfortunately, samples of skeletal muscle were not obtained although portions of temporalis muscles could have been removed despite the imposed limitations.

For light microscopic studies tissues were fixed in 10 percent buffered formalin and embedded in Paraplast Plus. Sections were stained with Bodian’s protargol for neurofibrils, Weil and Luxol-Fast Blue for myelin, as well as by conventional hematoxylin and eosin.

Electron microscopic studies were performed on samples from cervical spinal cord, medulla oblongata, pons, cerebellum, cerebral cortex, thalamus and white matter of frontal and occipital lobes. These tissues were minced into small blocks in 4 percent glutaraldehyde in 0.1 M phosphate buffer pH 7.3 post-fixed in 1 percent OsO₄ for one hour, dehydrated in graded ethanol, passed through propylene oxide and embedded in Epon 812. Sections were stained with uranyl acetate and lead citrate.

Representative portions of the thalamus, pons, medulla oblongata and spinal cord were obtained for tissue culture (TC). These portions of tissue were minced, washed in Hank’s balanced salt solution (HBSS) and subsequently placed in sterile, 25 cm² plastic TC flasks. One half of a cm² of modified Eagle’s minimum essential culture medium prepared in HBSS supplemented with pyruvate, non-essential aminoacids, 5 percent calf serum, 100 u of penicillin and 50 micrograms of streptomycin per ml were added to each flask.

The total amount of culture medium was such that the portions of tissue were simultaneously in contact with the surface of the flasks and bathed in the culture medium. TC was incubated in a stationary position for about four to five days. During this interval some cells became attached to the surface of the flasks. Finally, four to five ml of fresh culture medium were added for additional cultivation every three or four days. Microphotographs were obtained 15 days after cultures were initiated.

Results

Neuropathological Observations

The brain weighed 1,250 grams. There was minimal atherosclerosis in the circle of Willis. Moderate atrophy of the frontal
and parietal gyri producing widening of the sulci of these lobes. The ventricles were symmetrical and moderately dilated. Sections through the cervical portion of spinal cord revealed white-gray discoloration of the dorsal one-half of the lateral columns.

**Light Microscopy**

These studies revealed loss of neurons and gliosis in the anterior horns of the cervical segments of the spinal cord, hypoglossal nuclei and nucleus ambiguous. The extent of axonal degeneration, demyelination, and gliosis with corpora amylacea of the corticospinal tracts decreased from the spinal cord towards the hindbrain (figure 1). The internal capsule, centrum ovale and motor cortex appeared normal.

**Electron Microscopy**

The one hour delay preceeding the fixation of tissues resulted in some autolytic fragmentation of the various cellular membranes. The principle positive findings were the cellular inclusions noted in the cerebellum and cervical spinal cord. These inclusions were sparse, measured from 7 to 8 \( \mu \text{m} \) in diameter and exhibited a core of intertwined microtubules (figure 2). The cores measured about 0.2 \( \mu \text{m} \) in diameter. Intermingled with the microtubular matrix, dense granules ranging between 60 and 120 nm were seen (figure 3). The peripheral area of the inclusion bodies was composed of radiating microtubules which diameters ranged from 7 to 10 nm. The tubules measured up to 0.4 \( \mu \text{m} \) in length (figure 4).

**Tissue Culture**

The samples of tissue from the cervical cord, the cerebellum, and the motor cortex produced viable fibroblasts. They appeared to migrate from tissue fragments within a few days and seemed normal on routine observation. After the first week in which cellular multiplication seemed to take place, the cultures became self limited. Cells remained viable for several days but no additional cellular division was evident. The gradual degeneration of all cultures did not appear to be related to specific viral cytopathic changes.
Discussion

Certain clinical and morphological peculiarities are readily apparent in this case. The relatively short course of the illness and the fact that the patient served in the Western Pacific indicate that the patient may have suffered from the Guamanian form of ALS. There were, however, no intellectual deficits or symptoms of Parkinsonian as observed in the Chamorros and patients from the continental United States afflicted with Guamanian ALS. The morphologic anomalies do not give support to a diagnosis of Guamanian ALS or hereditary ALS. The inclusion bodies noted in the case are compared, in table I, with
those structures which were considered in the differential diagnosis. Histochemical comparison was impossible because the bodies were not found in the portions of nervous tissue examined by light microscopy.

Figure 3. Dense granules (arrow) intermingled with microtubules (MT) are seen in the central core of the cellular inclusion. × 42,500.

Figure 4. High magnification of twisted microtubules are shown in the peripheral area of the inclusion. × 240,000.
AMYOTROPHIC LATERAL SCLEROSIS

TABLE I

Ultrastructural Features of Some Cellular Inclusions of the Central Nervous System

<table>
<thead>
<tr>
<th>Name and Location</th>
<th>Morphology</th>
<th>Diseases</th>
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<tbody>
<tr>
<td><strong>Corpora Amalycea</strong> (astrocytic)⁸,¹²</td>
<td>Spheroidal, central core with fibrils (100 to 125A) amyloid bodies (?), glycogen and dense granules (500A)</td>
<td>Epilepsy, ubiquitous in CNS</td>
</tr>
<tr>
<td>**Hirano bodies (axonal, and extracellular?)**¹³</td>
<td>Rod-like, made of filaments (60 to 100A diameter) interspersed with a sheet-like material about 100 to 150A in thickness</td>
<td>Guamanian ALS, Pick's disease, normal aged brains, Kuru, Kuru-infected chimpanzees</td>
</tr>
<tr>
<td><strong>Lafora and Lafora-like bodies (neuronal, hepatocytes, striated muscle)⁶,¹⁰,¹⁴</strong></td>
<td>Spheroidal; stellate core of granular material surrounded by branching fibrils (70 to 100A diameter)</td>
<td>Myoclonus epilepsy type IV, glycogenosis, presenile dementia, olivopontocerebellar atrophy</td>
</tr>
<tr>
<td><strong>Lew and Lewy-like bodies (neuronal)⁹,¹¹</strong></td>
<td>Spheroidal; dense core with fibrils and granules; outer zone of radically arranged fibrils (100A diameter) and scattered granules and circular profiles (500 to 800A)</td>
<td>Parkinson's disease, hereditary ALS</td>
</tr>
<tr>
<td><strong>Present case (neuronal?)</strong></td>
<td>Spheroidal; intertwined microtubules (70A diameter) in the core with dense granules (600A); periphery of radiating microtubules</td>
<td>Sporadic ALS</td>
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</table>

In retrospect, the authors think that the inclusion bodies of this case represent a cellular manifestation of metabolic anomalies which were not suspected clinically. Supporting this hypothesis, there is a study of pancreatic functions of 16 patients with ALS in which: (1) five had abnormal and four had borderline glucose tolerance tests, (b) 10 had abnormal response to tolbutamide injection and (3) nine had abnormal uptake of I.131 triolein.⁷

The same study alluded to clinical improvements of some patients with ALS who received pancreatic enzymes for treatment of pancreatic dysfunction. Another supporting fact is that the inclusion bodies ultrastructurally resembling those of our case have been observed in glycogenosis type IV.¹⁰

These findings from our case and the experience of others suggest that the inclusion bodies may be nonspecific or that various illnesses could have a common derangement of cellular metabolism which result in formation of inclusions. The negative findings of virological studies confirm the experience of others.¹

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**References**

6. PETTIO, C. K., HART, M. N., PORRO, R. S., AND EARLE, K. M.: Ultrastructural studies of


