Amyotrophic Lateral Sclerosis: Effect of Serum on Anterior Horn Cells in Tissue Culture

Abstract. *A high proportion of diluted serums of patients with amyotrophic lateral sclerosis were toxic to the anterior horn cells of the mouse in tissue culture. This is not a general cytotoxicity, since apparently only the neurons were killed. Serums from other degenerative neurological diseases were inactive.*

Amyotrophic lateral sclerosis (ALS) is an inexorable and fatal motor neuron disease of man. Its onset is often in middle life, and it is characterized by weakness, fasciculations, muscular atrophy, and paralysis. There is degeneration of the Betz cells of the motor cortex, the motor nuclei of the brainstem, and the anterior horn cells of the spinal cord, although in an individual case all of these areas may not be involved. The etiology of ALS is unknown, but a circulating toxin with a predilection for motor neurons has been considered (*1*). To determine if there is anything in the serums of patients with ALS that is deleterious to motor neurons we have exposed cultures of anterior horn cells of mice to diluted ALS serums. These in vitro studies made it possible to circumvent the blood-brain barrier and allowed direct contact of the serums with the anterior horn cells and their processes.

Three-day-old inbred Swiss-Webster mice were anesthetized by hypothermia and decapitated. The lower thoracic and lumbar segments of the spinal cord were removed with the use of a dissecting microscope and with sterile technique. The meninges were removed, and transverse slices were made about 1 mm thick. Each anterior horn was dissected free and explanted onto a sterile cover slip coated with gelled rat tail collagen. The explant was covered with two drops of a medium consisting of 40 percent fetal calf serum, 35 percent Simm's balanced salt solution, and 25 percent Eagle's medium with glutamine. Glucose was added to a final concentration of 600 mg/100 ml. The cultures were maintained in Maximow double cover slip assemblies at 37°C and the medium was changed three times a week.

The cultures were examined at weekly intervals by phase microscopy, and in 3 weeks they had usually flattened to monolayers. Only monolayer cultures with a dense outgrowth of nerve fibers were used for testing the serums. At this time the cultures had the appearance of a sheet of fibroblasts on top of which was a network of nerve fibers (Fig. 1A). These fibers spread from the center of the explant to the edge of the layer of fibroblasts. The anterior horn cells were visible in the center of the flattened explants (Fig. 1B). The central canal of the spinal cord was often present, as were a few oligodendrocytes and macrophages.

The serums to be tested were diluted with the tissue culture medium to a final concentration of 30 percent human serum. The cultures were fed with the diluted serums three times in the course of a week, and they were examined by phase microscopy before each feeding. At the end of the test period they were fixed in phosphate-buffered formalin and stained by the Holmes silver method (*2*). This procedure stained the nerve fibers in their entirety and the nuclei of all of the cells (Fig. 1C). The staining of the anterior horn cells was erratic and uncontrollable, and, in contrast to the complete staining of the fibers, the neuronal somas were usually unstained.

Thirty-seven cases of ALS were tested and 26 of these (70 percent) selectively destroyed the anterior horn cells and their processes. The loss of these cells and their nerve fibers could be seen in the living cultures. The total loss of nerve fibers was confirmed by examining the stained cultures (Fig. 1D). There was no obvious correlation between the age and sex of the patients or their type of ALS (upper or lower motor neuron disease) and the toxicity of the serums to the anterior horn cells in the cultures.

The ALS serums did not have a general cytotoxic effect, since the cells in the cultures, other than the neurons, remained intact. The factor in the ALS

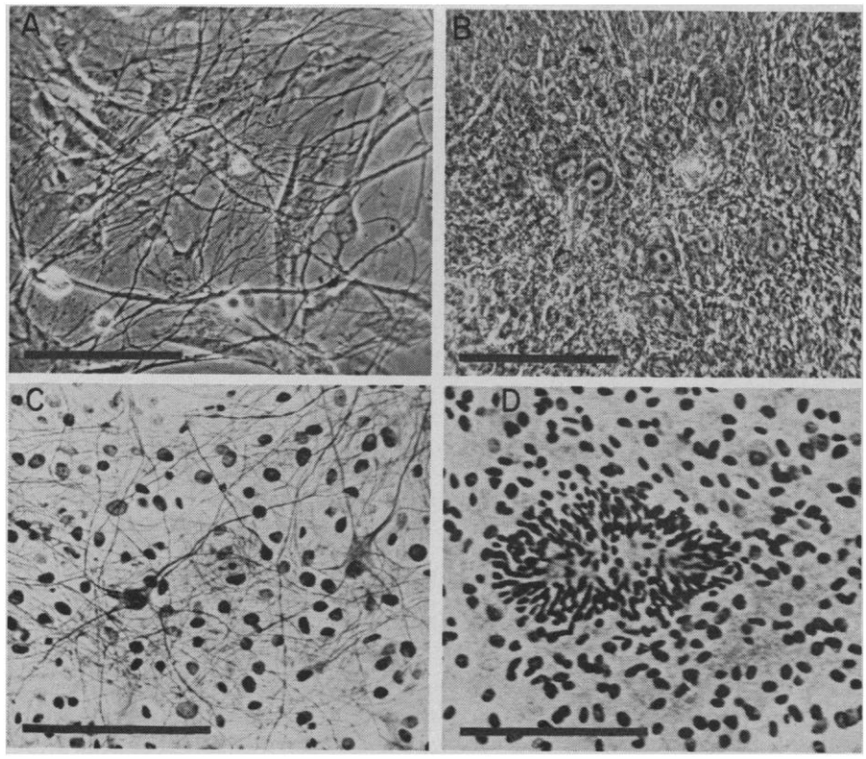

Fig. 1. Selected areas of cultures of the anterior horn of the spinal cord of the newborn mouse. The outgrowth of nerve fibers over a sheet of fibroblasts as seen in a living culture by phase optics is shown in (A) (× 215). A group of living anterior horn cells in the center of a monolayer culture is seen in (B). These cells are distinguished by their location in the explant, their size, the large pale nucleus and prominent nucleolus of each cell, and by their granular cytoplasm (× 215). An area of a stained culture that was exposed to a control serum is shown in (C). The somas of two anterior horn cells and a tangle of nerve fibers are on top of a sheet of fibroblasts whose nuclei are stained (× 255). An area of a stained culture that was exposed to serum from an ALS case is shown in (D). The ring of cells is the ependyma of the central canal of the spinal cord. Note the total absence of nerve fibers (× 255). The bars represent 0.1 mm.

serum that destroys the neurons and nerve fibers is nondialyzable and therefore is presumably a protein. We have inferred that its site of action is at the somas of the anterior horn cells because when 5-day in vitro cultures were used the ALS serums usually had no effect. In these cases, there was a good outgrowth of nerve fibers which were directly exposed to the ALS serum in the culture medium. However, the center of the explants had not yet flattened out and the anterior horn cell somas were covered by other cells. It was only when the cultures were monolayers and the somas of anterior horn cells were exposed to the culture medium that they were destroyed by the ALS serum.

Fifty-four control serums from 14 different degenerative neurological diseases were tested, and they had no effect on the cultures (Table 1). Of particular interest was the lack of toxicity to anterior horn cells in the serums of the cases of Werdnig-Hoffman disease, which is an inherited degenerative disease of the anterior horn cells of infants and children. From this it would seem that the toxicity of the ALS serums is not due to some general phenomenon secondary to the degeneration of the anterior horn cells in the ALS patients. Antibodies that demyelinate cultures of cerebellum are present in a high proportion of the serums of patients with multiple sclerosis (*3*). Approximately 60 percent of ALS serums also demyelinate these cultures (*3*, *4*), and this is inexplicable, since ALS is not a demyelinating disease. The serums of our cases of multiple sclerosis had no effect on the anterior horn cells in the cultures, indicating that the toxic factor for these cells in ALS serum is not the same as the demyelinating antibodies that have been reported in multiple sclerosis and ALS serums. To our knowledge this is the first demonstration of toxicity to living neurons by serum from a neuronal degenerative disease of man. The significance of our observation in terms of the pathogenesis of ALS can be determined only after the toxic factor has been identified.

Table 1. Neurological diseases used as controls.

Disease	Cases (No.)
Multiple sclerosis	15
Werdnig-Hoffman	6
Guillain-Barré	5
Presenile dementia	5
Huntington's chorea	4
Parkinson's disease with dementia	4
Myasthenia gravis	3
Spinocerebellar degenerations	4
Charcot-Marie-Tooth disease	3
Parkinson's disease	2
Jacob-Creutzfeld disease	1
Carcinomatous neuropathy	1
Polio	1
Total	54

FREDERICK WOLFGRAM
LAWRENCE MYERS

Department of Neurology, Reed Neurological Research Center, University of California, School of Medicine, Los Angeles 90024

References and Notes

1. Lord Brain, *Diseases of the Nervous System* (Oxford, London, 1962), p. 534.
2. See S. C. Dyke, *Recent Advances in Clinical Pathology* (Blakiston, Philadelphia, 1947), p. 402.
3. M. B. Bornstein and S. H. Appel, *Ann. N.Y. Acad. Sci.* **122**, 280 (1965).
4. E. J. Field and D. Hughes, *Brit. Med. J.* **2**, 1399 (1965).
5. We thank Mrs. Nadean Abney, Mrs. Camille Campbell, and Mrs. Shirley Walton for their assistance. Supported in part by the Jane Reinking-Killgore Fund for Neurological Research, California Community Foundation.

5 September 1972

A Hidden Antigenic Site Localized to the Constant Region of Light Chains of Immunoglobulins

Abstract. *An antigenic site, which was not immunochemically demonstrable in the intact κ light polypeptide chain, was exposed by enzymatic cleavage of the κ chain into its variant half and constant half. This antigenic site located in the constant region the κ chain was detected immunochemically by several antiserums that had specificity for this site. Treatment of an intact κ chain with a dissociating agent resulted in the exposure of the hidden antigenic site, which was as readily detected in the unfolded polypeptide chain as in the isolated constant half.*

The light chains of immunoglobulins can be cleaved specifically into two halves (*1*), the NH_2-terminal variant half (V_L) and the COOH-terminal constant half (C_L). The V_L and C_L have been characterized structurally and immunochemically (*1*, *2*). The amino acid sequence of the V_L of all light chains appears to be unique for each protein, whereas the sequence of the C_L of all light chains of the same type (κ or λ) is virtually identical (*3*). During our immunochemical studies (*4*), which have confirmed the structurally defined (*3*) subgroups of light chains and have differentiated among proteins of the same subgroup, we found that certain antiserums had specificity for a hidden antigenic site localized to the C_L.

To study the immunochemical relation between C_L and intact Bence-Jones protein, we subjected Bence-Jones proteins to limited proteolysis so that some intact protein remained (*1*). Len, a κ Bence-Jones protein, was cleaved selectively; and three components, the C_L, intact Bence-Jones protein, and V_L, were detected by immunoelectrophoretic analysis (Fig. 1A). Three types of antiserums were used in the identification of the C_L and V_L (*1*). When tested with homologous antiserum against Len, the C_L and V_L were antigenically deficient to the intact protein Len. Absorption of the homologous antiserum with a heterologous κ Bence-Jones protein removed the reactivity with antigenic determinants in the C_L, and the resulting antiserum gave a precipitin reaction of identity between the V_L and the intact protein Len. An antiserum prepared against a heterologous κ Bence-Jones protein recognized only antigenic determinants in the C_L and gave a precipitin reaction of identity between the C_L and the intact protein Len.

With an antiserum against Bence-Jones protein, the more typical and previously demonstrated immunochemical relation between the C_L and the homologous Bence-Jones protein had been that the C_L was antigenically deficient to the intact protein. However, certain homologous antiserums were unusual in their reactivity in that they had major specificity for the C_L. These antiserums recognized an antigenic site that was localized in the C_L and that was not exposed or immunochemically recognized in the native or intact κ chain. Furthermore, examination of the reactivity of our numerous antiserums against κ Bence-Jones proteins with the partially cleaved sample of protein Len revealed that with certain heterologous antiserums the intact Bence-Jones protein was antigenically deficient to the C_L. The specificity of these antiserums for a hidden antigenic site that was exposed by cleavage of the intact Bence-Jones protein could be readily demonstrated. Absorption of either a homologous or heterologous antiserum with