# Gamma-Globulin Affinity for Normal Human Tissue of the Central Nervous System

Abstract. *Immunofluorescent staining of the serum and cerebrospinal fluid of patients with and without neurological disease demonstrates an affinity of the gamma-globulin fraction for glial cells and myelin sheaths in normal human nervous tissue. This affinity appears to be specific for the 7S gamma-globulin fraction and is not seen with the other major protein fractions of serum and cerebrospinal fluid or with the fluorescein conjugated antisera alone.*

Antibodies with apparent specificity for rabbit, guinea pig, and human myelin have been demonstrated by immunofluorescence techniques in the sera of animals with experimental "allergic" encephalomyelitis whose nervous tissue was used as the antigen (*1*). Bornstein (*2*), using this technique with whole serum or the globulin fraction from such animals or from patients with acute multiple sclerosis, demonstrated the presence of antibodies to glial cells and myelin sheaths of tissue cultures of rat cerebellum. In the presence of complement, these antibodies produced demyelination in vitro but were not dependent on complement for noncytotoxic fixation.

In a significant proportion of patients with multiple sclerosis there is an elevation of cerebrospinal fluid γ-globulin, particularly the 7*S* component in the presence of normal serum γ-globulin levels (*3, 4*). The source of this increase in 7*S* γ-globulin remains largely unknown, although indirect evidence suggests a site within the central nervous system itself (*3, 5*). By indirect immunofluorescent staining, we have investigated the possible presence of antibodies to human tissue of the central nervous system in the serum and cerebrospinal fluid of patients with multiple sclerosis and other neurological diseases.

We obtained 41 sera and 33 cerebrospinal fluids, mostly autologous, from patients with various neurological disorders, including demyelinating, neoplastic, degenerative, and vascular diseases. In addition, 15 sera and 6 cerebrospinal fluids were studied from normal individuals. Both whole serum and its crude globulin fraction, obtained by precipitation with anhydrous sodium sulfate, were examined. The cerebrospinal fluids were studied with or without concentration by negative-pressure dialysis at 4°C after the γ-globulin concentration had been determined by the immunochemical method of Kabat (*6*). Goat antisera (globulin fraction) to human γ-globulin, 7*S* γ-globulin, 19*S* γ-globulin (β-2 macroglobulin), α-2 globulin, and albumin conjugated to fluorescein isothiocyanate, were obtained commercially (*7*) and absorbed with rat liver powder before use. In addition, an exceptionally pure fluorescein-conjugated duck antihuman 7*S* γ-globulin was used as a control. All sera and cerebrospinal fluids were stored at 4°C.

Normal human cerebrum and spinal cord were obtained at autopsy less than 3 hours after death from eight patients who had died from other than neurological causes. Tissues were presumed to be normal if there was no clinical evidence of nervous system disease, and if there were no histopathologic abnormalities in these tissues as shown by hematoxylin-eosin stains. In addition the central nervous system tissue from one patient with Parkinson's disease and from two patients with amyotrophic lateral sclerosis was similarly obtained. Normal serum from five rhesus monkeys (*Macaca mulatta*) and surgically removed tissue from monkey cerebrum and cerebellum were used as additional controls. All tissues were embedded immediately in egg albumin (*8*), frozen rapidly in a mixture of dry ice and acetone, and stored at −70°C. Sections 4 μ thick were cut in a cryostat and dried overnight at 4°C; they were fixed in 95 percent ethanol for 30 seconds, washed in phosphate-buffered saline (*p*H 7.6), and incubated with cerebrospinal fluid, whole serum, or the albumin and globulin fractions of whole serum, for 30 minutes at room temperature. After washing with buffer, the sections were incubated with one of the fluorescein-conjugated antisera for an equal period of time, washed, mounted in buffered glycerine and studied with a fluorescence microscope.

All sera tested, both human and monkey, produced brilliant fluorescence of myelin sheaths and glial cells throughout the white matter (Fig. 1). The glial cells in the grey matter did not stain, except in the areas adjacent to white matter and along pial surfaces. The fluorescence of glial cells was localized to the cytoplasm of the perikaryon and the glial processes, including the vascular foot plates of astrocytes. There was no fluorescence of glial nuclei. The predominant cell seemed to be the fibrous astrocyte, although occasional oligodendroglia were observed. The brilliant fluorescence of the glial network and myelin sheaths was elicited only when both the globulin fraction of serum or cerebrospinal fluid and the conjugated antisera to 7*S* γ-globulin were present. When the antiserum to 19*S* γ-globulin was used, there was a similar pattern of fluorescence but of minimal intensity. Similar application of the conjugated antisera to α-2 globulin and albumin resulted in minimal fluorescence of an occasional glial cell and isolated myelin sheaths. This minimal fluorescence observed may have represented some contamination with

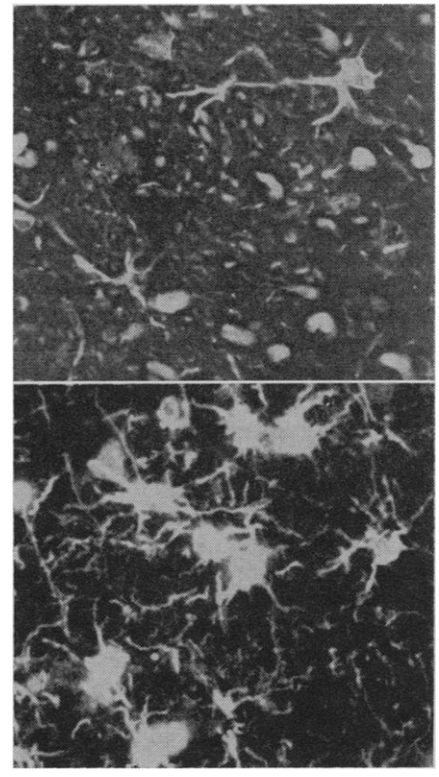

Fig. 1. Normal human cerebral white matter incubated with normal human serum and stained with fluorescein-conjugated goat antiserum (globulin fraction) to human 7*S* γ-globulin. (Top) Fluorescence of scattered myelin sheaths and of a glial cell with its processes. (Bottom) Fluorescence of glial cells (astrocytes). (About × 300)

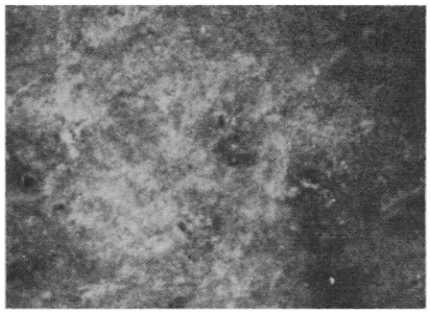

Fig. 2. Normal human cerebral white matter stained with fluorescein-conjugated goat antiserum to human 7*S* γ-globulin alone. No specific fluorescence noted. (About × 300)

the antiserum to 7*S* γ-globulin during the preparation of the conjugated antisera. Heating the serum to 56°C for 30 minutes did not alter the staining pattern suggesting that this reaction is not dependent upon complement as was shown by Bornstein (*2*).

Concentration of cerebrospinal fluid of 5 to 100 times resulted in a pattern of fluorescence not significantly different from that of serum except that it was less intense, and fewer glial cells and myelin sheaths were stained. Such fluorescence was seemingly solely dependent on the concentration of γ-globulin in the cerebrospinal fluid and was not observed with samples of whole cerebrospinal fluid except in two instances when the γ-globulin concentration was greater than 4 mg/ml. Concentrated samples produced detectable fluorescence at approximately 0.8 mg/ml which preliminary studies had suggested as the approximate concentration needed to demonstrate fluorescence with diluted normal serum.

The fluorescence of glial cells and myelin sheaths produced by both serum and cerebrospinal fluid was markedly diminished by the application of noncein-conjugated antisera to 7*S* γ-globubefore staining with the conjugated antiserum. The application of fluorescein-conjugated antisera to 7*S* γ-globulin and the other globulins alone elicited no fluorescence of glial cells or myelin sheaths and elicited only mild fluorescence of blood vessel walls, pial connective tissue, and neuronal nuclei (see Fig. 2).

Except for the tissue from the two patients with amyotrophic lateral sclerosis all human tissues tested reacted in the same manner. In these two instances the region of the lateral funiculus of the spinal cord, representing areas of pyramidal tract involvement, revealed rare, pale myelin sheaths surrounded by a dense intensely fluorescing network of glial fibers. Similar fluorescence of glial fibers was observed after application of the conjugated antisera alone. The significance of this observation in these two cases is not apparent. The reactions of human serum and cerebrospinal fluid with monkey tissue were similar to those described, as were the reactions between monkey serum and the homologous monkey tissue, even though fluorescein-conjugated antimonkey globulin was not used.

The fluorescence of glial cells and myelin sheaths produced consistently by all the serum and cerebrospinal fluid samples tested suggests that this is not an autoantibody in the strict sense. There appears to be, as yet, no significant qualitative difference between the γ-globulins from either cerebrospinal fluid or serum or among the various conditions as studied by this technique. This absence of discernible differences in the staining pattern raises the question of nonspecific protein interaction. Such interactions have been shown to result from electrostatic forces between the fresh frozen tissue and the serum proteins (*9*) or may be related to the inherent pinocytotic properties of glial cells during life (*10*) as has been shown for granulocytes and histiocytes (*9*).

Our studies suggest that the affinity of 7*S* γ-globulin for the glial cells of white matter and for myelin sheaths may represent more than a nonspecific interaction. Such specificity was not seen with the other major protein fractions of serum and cerebrospinal fluid or with the fluorescein-conjugated antisera alone. This affinity may in some way be related to glial cell metabolic functions in normal, and perhaps, pathological states or may simply represent cross relationships between existing antibodies.

The concomitant fluorescence of myelin sheaths with glial cells is of note in view of the concept that the sheath is derived from modified glialcells and myelin sheaths may be peof 7*S* γ-globulin for primate glial cells and myelin sheaths may be peculiar to this highest animal order since this phenomenon has not been described in such experimental animals as the rat, mouse, guinea pig, or rabbit. Such an affinity must be explained for the normal state in order to assess properly the presence or significance of autoantibodies in the human demyelinating diseases and experimental "allergic" encephalomyelitis.

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**References and Notes**

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## DNA from Bacteriophage Lambda: Molecular Length and Conformation

Abstract. *The length of the DNA molecules from wild-type λ bacteriophage is 17.2 μ, corresponding to a molecular weight of 33 million in the B form. The molecules from a double-deletion mutant of λ are 23 percent shorter. Both types of molecules join ends at 60°C to form circular molecules or polymers. The point of junction cannot be distinguished by any irregularity in the uniform duplex molecule.*

We have measured the lengths of unbroken DNA molecules from lambda bacteriophage as seen in electron micrographs, calculated their true length, and related this length with the molecular weight of DNA from the same bacteriophage as judged by other kinds of