is that $K_P^{-1} = 1$, and that the gelatin in the spheres either bears a tertiary structure which must be disrupted to facilitate binding of the enzyme or is associated to form small "crystalline" regions which are impermeable to the enzyme, and which increase in extent as the temperature is lowered. Thus the amount $\bar{K}_m^g$ of substrate required to form a complex with half of the enzyme (in the gel phase) will be greater than $\bar{K}_m$ and will increase with decreasing temperature. A fact which is nicely consistent with this interpretation is that $\bar{K}_m^g K_E^{-1}/\bar{K}_m$ approaches in a continuous manner the value 1.0 near 27°C, close to the melting point of the gel (28° ± 1°C). A discontinuity in $\bar{K}_m^g K_E^{-1}/\bar{K}_m$ would result if either $\bar{K}_m^g/\bar{K}_m$ or K_P^{-1} differed appreciably from 1.0 at the melting point. We conclude that the differences in structure between free-solution and gel-state gelatin tend to vanish smoothly as the melting point of the gel is approached.

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Multiple Sclerosis: Serum Factor Producing Reversible Alterations in Bioelectric Responses

Abstract. *Serums obtained from patients during acute exacerbations of multiple sclerosis produce a reversible depression of polysynaptic reflex responses when applied to the isolated spinal cord of the frog. Motoneuron discharges initiated by monosynaptic activation through an axosomatic spinal pathway are much less affected than reflex discharges. The active factor in serum appears to depend on the presence of complement.*

Auto-immunity processes have long been suspected to play an important part in the etiology of multiple sclerosis. As early as 1934, Sachs and Steiner claimed, on the basis of an extensive study, that complement-fixing antibrain antibodies could be detected in the serum of patients with this disease (*1*). Although their conclusions were challenged from time to time by other investigators, a strong body of immunological evidence for the presence of circulating auto-antibodies has accumulated since (*2*). In keeping with the hypothesis of an auto-immune pathogenesis for multiple sclerosis, a number of neuropathological analogies have been described between the demyelinative lesions of the central nervous system observed in patients and the lesions produced in animals with experimental "allergic" encephalomyelitis (*3*). More recently, this view received additional support from the demonstration, in the serums from patients (*4*) and animals affected with the experimental disease (*5*), of complement-dependent factors inducing similar patterns of demyelination in cultured fragments of mammalian central nervous tissue.

However, the precise relationship between the immune processes, demyelinative lesions, and clinical symptoms of multiple sclerosis is still far from understood. Indeed, it is usually recognized that the sudden and transient attacks followed by prolonged remissions, particularly during the initial stages of the disease, cannot be accurately correlated with lesions in the central nervous system and be entirely accounted for by the process of demyelination alone (*6*). Therefore, the finding of a circulating factor directly responsible for functional disorders in central nerve cell-bodies or fibers would be of particular significance in elucidating the pathogenetic mechanisms of the disease. In fact, in vitro experiments relevant to this problem have recently been reported by Bornstein and Crain (*7*). These authors found that serums obtained from two patients, during acute exacerbations, produced within a few minutes marked alterations in complex electrical responses of cultured cerebral and spinal cord tissues of the mouse. Of particular interest was the observation that these reversible alterations, resulting presumably from interference with synaptic processes, were due to a complement-dependent serum factor and occurred long before any morphological changes were detected in the cultured fragments.

In the experiments described here, the isolated spinal cord of the frog (*Rana temporaria*) was used to investigate the effects of serum from patients with multiple sclerosis on bioelectric responses of a highly organized nerve center. The preparation was mounted in a recording chamber and submitted to a constant flow of circulating Ringer solution maintained at 10°C. The Ringer solution, equilibrated with oxygen, had the following composition (in millimoles per liter): NaCl, 112; KCl, 2; $CaCl_2$, 1.8; $NaHCO_3$, 2.4; glucose, 26. The cord was allowed to stabilize in this solution for 2 hours after dissection. Dorsal and ventral roots were suspended in air on stimulating and recording platinum electrodes. Motoneuron discharges were recorded from the ninth or tenth ventral root; they were evoked either monosynaptically, by stimulation of lateral column fibers establishing axosomatic connections, or through polysynaptic reflex pathways, by stimulation of the ipsilateral dorsal root (*8*). Mean values of peak amplitude of discharge were calculated from at least ten oscilloscope recordings initiated at 3-second intervals. Serums obtained from normal humans and from patients were used, at the concentration of 40 percent, to prepare solutions whose Na, K, Ca, and glucose contents were adjusted to duplicate the composition of the Ringer solution. The preparation was removed from the recording chamber and transferred to beakers for exposure to these media, kept at 10°C, and equilibrated with oxygen. To facilitate the access of applied solutions, the spinal cord was hemisected sagittally in the experiments dealing with the monosynaptic responses, whereas it was sectioned transversely above and below the lumbar enlargement when the segmental reflex activity was investigated.

A series of control experiments showed that exposure to serum from normal humans sustained or even en-

hanced the electrical activity of the isolated frog spinal cord. As compared with the responses recorded after 2 hours equilibration with Ringer solution, the reflex discharge was increased in 12 of 16 preparations treated with the serums from different subjects (Fig. 1, A_2). After stabilization in normal serum, the increase in peak amplitude of discharge ranged, among the preparations showing this phenomenon, from 9 to 84 percent (average, 42). Recordings of the response made after various durations of exposure to serum indicated that this effect developed, in large part, within 30 minutes. Moreover, preparations examined up to 5 hours after their immersion in normal serum revealed no impairment of the reflex response. On the other hand, after the discharge was stabilized in the serum from a given subject, little (less than 8 percent) or no further change was detected when the spinal cord was subsequently treated for up to 3 hours with other normal serums. After exposure to normal serum, the monosynaptic response of motoneurons was also enhanced, although to a lesser extent, in 9 of 14 different preparations. In these 9 preparations, the increase in amplitude of response ranged from 3 to 71 percent (average, 23). In view of these data, the bioelectric responses were always measured at least 30 minutes after transfer of the preparation from Ringer solution to normal serum, and the effect of subsequent treatment with serum from patients was assessed by comparison with these controls.

Exposure of the spinal cord to serums from patients diagnosed as having multiple sclerosis, on the basis of history and clinical examination, resulted in a significant depression of the polysynaptic reflex discharge (Fig. 1, A_3 and C_2). In a first series of experiments, this effect was observed with serums obtained from eight patients during an acute exacerbation (*9*), each serum being tested on a different preparation. Recordings made at various intervals showed that the functional alteration was usually detectable after about 10 minutes and that it increased gradually with prolongation of the exposure to serum. After stabilization of the preparations in serum, usually within 60 to 90 minutes, the decrease in peak amplitude of discharge ranged from 35 to 69 percent (average, 52) of the control values measured after treatment with normal serum. In contrast to this definite effect on polysynaptic activity, serum was either ineffective or exerted a weaker depressant action on the monosynaptic response of motoneurons elicited by lateral column stimulation. The serums from five of the patients were tested on hemisected preparations for their action on this type of response. Two serums exhibited no effect (Fig. 1, D_2), whereas with the three effective serums the amplitude of response decreased by 7, 11, and 31 percent, respectively. Moreover, conduction of impulses in dorsal and ventral root fibers was not affected by exposure of the spinal roots to serum. In addition to this apparent selectivity, a significant feature of the effect of serum on the bioelectric responses was its reversibility. Indeed, exposure of the spinal cord for 30 to 60 minutes to normal serum restored, at least partly, the electrical activity altered by prior treatment with patient's serum (Fig. 1,

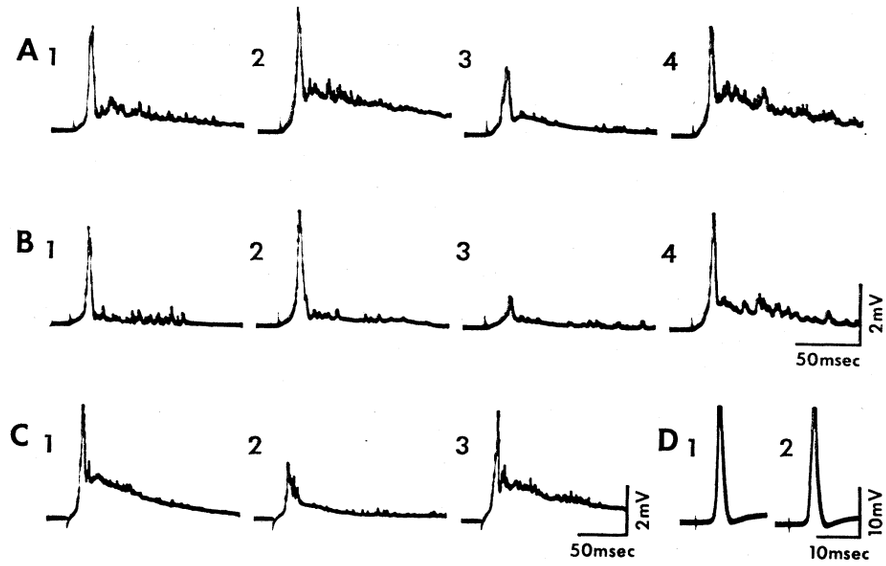

Fig. 1. Effects of serums from patients with multiple sclerosis on bioelectric responses of the isolated spinal cord of the frog. *A*, Oscillographic recordings of motoneuron discharges evoked by polysynaptic reflex activation. Single supramaximal stimuli (0.1-msec duration) were applied to the ninth dorsal root, and the responses were recorded from the ipsilateral ventral root. The stimulating electrodes were 1 mm apart and 6 mm distant from the site of entry of the root in the spinal cord. The proximal and distal recording electrodes were 2 and 8 mm distant from the spinal cord, respectively. The capacitance-coupled amplifier had a time constant of 0.5 second. A_1, Motoneuron discharge recorded after equilibration of the preparation with Ringer solution for 2 hours. A_2, Increase in amplitude of discharge after exposure to normal human serum for 30 minutes. A_3, Partial block of the response after 30 minutes' exposure to serum from a patient. A_4, Almost complete restoration of reflex activity, 30 minutes after return to normal serum. In this experiment, as in the following, normal and patients' serums were used at the concentration of 40 percent of the medium. *B*, Other preparation. The stimulating and recording conditions were similar to those shown in *A*, and the same patients' and normal serums were used. B_1, Motoneuron discharge recorded after equilibration of the spinal cord with Ringer solution for 2 hours. B_2, Increase in amplitude of discharge after 30 minutes' exposure to patients' serum heated at 56°C for 30 minutes. B_3, Marked depression of the response, 30 minutes after addition of 20 percent normal human serum to the heated serum. B_4, Restoration of normal reflex activity, 30 minutes after return to normal serum. The time and voltage calibrations apply to all eight records (*A* and *B*). *C*, Blocking effect, observed in a different preparation, of serum obtained from another patient with multiple sclerosis. The stimulating and recording conditions were identical with those in *A* and *B*, except that the responses were recorded from the tenth ventral root. C_1, Motoneuron discharge recorded after exposure of the spinal cord to normal serum for 75 minutes. C_2, Partial block of the response after 75 minutes' exposure to patient's serum. C_3, Restoration of normal reflex activity, 60 minutes after return to normal serum. The calibrations apply to all three records. *D*, Motoneuron discharges evoked by monosynaptic activation after application of single supramaximal stimuli (0.05-msec duration) to the lateral column of the spinal cord. The stimulating electrodes rested on the lateral surface of the preparation between the third and fourth segment, and responses were recorded from the ninth ventral root by electrodes located as in *A*. The serums used were the same as in experiments *A* and *B*. D_1, Control response recorded after exposure to normal serum during 30 minutes. D_2, Response unchanged after 30 minutes exposure to patient's serum. The calibrations apply to both records.

Table 1. Depressant effect of serums from patients with multiple sclerosis on reflex responses in different spinal cord preparations. Each serum was tested on four different preparations (P_1–P_4).

Serum No.	Decrease in peak amplitude of responses (%)*			
	P_1	P_2	P_3	P_4
1	15	28	33	24
2	82	55	55	69
3	25	54	28	62
4	39	28	43	43
5	40	44	38	45

* Given as percentage of control values measured in each preparation after treatment with normal human serum.

A_4, B_4, and C_3). Complete functional recovery was observed in about half the preparations.

Another series of experiments was performed with the serums from five other patients experiencing an active stage of the disease. Each serum was tested for its effect on the reflex responses of four different spinal cord preparations. The following procedure was routine. The initial stabilization period in Ringer, first step in the preceding experiments, was omitted and the isolated preparations were immediately soaked in normal serum for 3 hours. Control recordings were made after this period, and the spinal cords were next exposed for 1 hour to patient's serum to which 10 percent normal serum was added (to ensure an adequate amount of complement). A depression of reflex activity was regularly observed, although the intensity of the effect was subject to variations from preparation to preparation within each group (see Table 1). Subsequent exposure to normal serum restored, at least partly, the reflex responses in all these preparations.

The relationship between the active factor demonstrated by these observations and immunologic agents present in serum of multiple sclerosis patients was further examined. In five experiments, heating the serum at 56°C for 30 minutes abolished its depressant effect on the reflex discharge. In fact, the heated serums showed properties similar to those of normal serum, in that they enhanced the bioelectric responses in four of five preparations (Fig. 1, B_2). On the other hand, subsequent addition of 15 to 20 percent normal human serum to heat-inactivated serum restored its ability to block the reflex responses (Fig. 1, B_3), presumably by bringing to the medium an adequate amount of complement. In these experiments, the decrease in amplitude of response ranged from 44 to 81 percent (average, 65) of the values recorded after exposure to heated serum.

These results, obtained on a nerve center with a high degree of organization, can be compared with those obtained by Bornstein and Crain on culture fragments of central nervous tissues (*7*). In both sets of observations, a complement-dependent factor present in multiple sclerosis was particularly effective in blocking, reversibly, bioelectric potentials transmitted through multineuronal pathways. However, the present data indicate that exposure to multiple sclerosis serum produces a definite alteration in polysynaptic reflex activity of the frog's spinal cord, contrasting with inconstant and minor changes in the monosynaptic response of motoneurons to lateral column stimulation. This suggests that multiple sclerosis serum affects relatively less the function of motoneuron cell-bodies and axons and that major alterations should be looked for in other spinal structures participating in reflex activity. At this stage, however, one can only speculate on the possible mechanism and localization of the observed functional disturbance. It may be inferred, from most recent studies on the amphibian spinal cord (*8, 10*), that dorsal root volleys lead to excitation of the motoneurons through activation of internuncial neurons, synapsing predominantly with their extensive dendritic arborizations. Thus, the bioelectric effect of multiple sclerosis serum could possibly result from interference with electrogenesis or synaptic processes in internuncials, although the hypothesis of a functional alteration restricted to the subsynaptic membrane of motoneuron dendrites cannot be dismissed. Moreover, the observation that conduction of impulses is unimpaired in dorsal root fibers does not rule out the possibility that the active factor in multiple sclerosis serum interferes with the release of transmitter from afferent terminals. On the other hand, the specific gliotoxicity of such serum which has been demonstrated in vitro (*4, 11*) should prompt a search for eventual primary alterations in spinal glial cells, resulting in a disturbance of the neuron-glia relationship.

Although more work, along different lines of investigation, will be needed to obtain precise information on the site of the blocking effect, it is hoped that the preparation used in the present experiments will provide a useful model for further study of the pathogenesis of multiple sclerosis. Preliminary tests have shown that serums obtained from five patients with other destructive lesions of the spinal cord (syringomyelia; Charcot-Marie-Tooth type of muscular atrophy; amyotrophic lateral sclerosis; hereditary spastic spinal paralysis; Friedreich's disease) did not produce the typical functional block we have described. Definite conclusions concerning the specificity of the effect of multiple sclerosis serum on the frog spinal cord must, however, await the outcome of a survey of neurological cases (*12*).

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