Allergic Encephalomyelitis as an Experimental Model for Multiple Sclerosis

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• **Proteins isolated from bovine spinal cord exhibit encephalitogenic activity. One of these proteins, of collagen type, was found to be homogeneous. This protein, however, is not considered to be the main encephalitogenic agent; other proteins with different physicochemical characteristics were found to possess higher activity.**

The use of these proteins will make it possible to study the allergic nature of the experimental disease and may lead to disclosure of the underlying mechanism of the pathological process not only in allergic encephalomyelitis but in multiple sclerosis.

MULTIPLE SCLEROSIS is a steadily progressive neurological disease with occasional remissions and exacerbations. Neither its cause nor its cure is known. It is characterized by destruction of the myelin sheath with a relative preservation of the axon. Demyelinated glial patches, so-called plaques, are formed in the white matter throughout the central nervous system. An increase in the gamma globulin content of the cerebrospinal fluid without an active infectious process, an apparent sensitization to neural tissue believed by some investigators to be a basis for its etiopathogenesis,[4] a breakdown of the myelin lipids during the disease process—all these phenomena qualify multiple sclerosis as a basic immunobiochemical research problem.

Multiple sclerosis cannot directly be transmitted from humans to animals; therefore allergic encephalomyelitis, which has certain common features with the human disease, is used as an experimental model. First discovered by Kabat and co-workers[6] in 1946, allergic encephalomyelitis can be produced by injection of brain and spinal cord with so-called Freund adjuvant. Monkeys receiving such injections became paralyzed and their brains showed demyelinating lesions.

It has been stated by some investigators that multiple sclerosis as it exists in humans has never been experimentally reproduced in animals.[11,16] Despite these objections we believe that allergic encephalomyelitis may be used as an experimental model because of its close relationship to multiple sclerosis.[15] The most convincing argument was presented by Uchimura and Shiraki.[17] They compared the histological changes occurring in the brain of persons who died inadvertently as a result of antirabies vaccination, with similar changes in both multiple sclerosis and allergic encephalomyelitis. They contended that since the vaccine for antirabies treatment is prepared by the use of brain tissue, therefore its active ingredient could be the same as the one in allergic encephalomyelitis. They concluded that the similarities among these three conditions are more striking and essential than the differences.

An argument voiced against the close relationship among these three is based on the clinical features of the disease: Multiple sclerosis is a disease with exacerbation and remissions, whereas the conditions resulting from antirabies treatment and allergic encephalomyelitis are characterized by a single attack. This could be explained by the fact that the disease is the result of one injection in allergic encephalomyelitis, and of a number of injections in antirabies treatment; but we may assume that the hypothetical release of antigens from the neural tissue in multiple sclerosis is a continuous process.

The problem is to determine which compound or compounds of the neural tissue cause the encephalitogenic activity. Three different kinds of preparations were found to be active: (1) The ether-soluble lower phase of proteolipids;[18] (2) proteins, among them a collagen-like compound,[1,2,7,13] and (3) a preparation obtained by petroleum ether extraction of the neural tissue.[10] The latter was considered to be a lipid, but since the fresh tissue has a high water content, some of the proteins could have been extracted with petroleum ether.

Experimentation

Experiments done in the present study were carried out on bovine spinal cord. It was found that the lipid-free residue has a higher activity than the organic solvent soluble portion.[7,14] In the search for the allergic encephalomyelitic agent efforts were concentrated on this preparation. Soon it was found that all the active fractions contained protein as the

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main constituent. The first preparations were made from bovine cord, using the lipid-free residue. From this a water extract was made by autoclaving the lipid-free residue under 15 lb. pressure for 8 hours and the extract fractionated with ammonium sulfate and the fraction proven to be active further purified. The details of procedure have been described elsewhere.[13] Ultracentrifugal and electrophoretic measurements indicated that the protein is homogeneous. Investigations also included determination of the isoelectric point, molecular weight and electron microscopic measurements.[12] Amino acid analysis of the hydrolyzed protein revealed that the protein is of collagen type. The results obtained by physicochemical measurements revealed that this compound is very similar to collagen isolated from other tissues, presented in Table 1.

It was found that the activity of this collagen accounts for most of the activity of the hot water extract of the bovine spinal cord.[7] Although it produced maximal disease in guinea pigs, it represents only a fraction of the total activity.

Other procedures were employed for the extraction, using potassium chloride and sodium citrate buffer of low pH. These resulted in very active preparations.

The potassium chloride extract contains seven proteins, as established by paper electrophoretic technique. The chromatographic procedure using diethyl aminocellulose exchanger with high adsorptive capacity with a fraction collector is being adapted for the purposes of the research here reported. To date, only partial purification has been achieved.

Activity Measurements

The various proteins prepared from spinal cord have been tested on guinea pigs and evaluated by a scale set up by Alvord and Kies.[7] A disease index of 0-10, depending on the severity of the neurological and histological reaction, was assigned. Ten guinea pigs were used for each level of antigen tested. The average disease index was calculated for each group and plotted against the dose injected on a logarithmic scale. The maximum disease index was found to be around 8; a half maximum, 4 (equal to 50 per cent of effective dose). A disease index of 4 was chosen as one unit of the activity and the specific activity expressed as units per milligram of dry weight.

On the basis of this disease index scale, the specific activity of the collagen was 40 units[7] in comparison with the lyophilized cord which showed a specific activity of 18. Much higher activity than in the collagen was found in our KCl and citrate extracts[8] (see Table 2). The smallest quantity applied was .004 mg. of the KCl and .001 mg. of the citrate

TABLE 1.—*Comparison of the Properties of Collagen from Bovine Spinal Cord with Collagen from Other Tissue.*

	Some Properties of	
	Collagen from Bovine Cord	Collagen from Other Tissue
Total nitrogen (per cent)	17.61	18.6
Glycine—Hydroxyproline ratio in molar quantities	6:2	6:2
Mean residue weight	95.0	92.6-93.7
Apparent minimal molecular weight	39077	38730
Ultracentrifuge measurement molecular weight	38000	.....
Isoelectric point (calculated from mobility)	4.6 pH	4.3 pH
Isoelectric point (precipitation with sodium dodecyl-sulphate)	4.5 pH	.....
Hexose (grams per 100 gm.)	1.45	1.0
Hexosamine (grams per 100 gm.)	0.22	0.33
Carbohydrates identified	Glucose Galactose Mannose Eucose	Glucose Galactose Mannose

TABLE 2.—*Encephalitogenic Activity of Protein Fractions Prepared from Bovine Spinal Cord*[7,8,9]

Preparation Tested	Specific Activity Units per Mg., Dry Weight
Lyophilized cord	18
Cord acetone powder	22
Collagen-like protein	40
KCl-extracted proteins	200
Citrate-extracted proteins	1000

soluble preparations. These results indicated that the preparations are highly active. Since these protein fractions are not yet homogeneous, we may expect that after further purification the activity will rise.

DISCUSSION

An attempt will be made to give a unified theory for allergic encephalomyelitis, for the condition resulting from antirabies treatment and for multiple sclerosis, based on the following considerations. It has been shown that proteins of the nerve tissue are encephalitogenic and that the disease is produced by injection of these proteins with killed tubercle bacilli containing adjuvant. Furthermore, since in the preparation of the antirabies vaccine, brain tissue is used, it can be assumed that the active compound here is the same as in allergic encephalomyelitis.

If the underlying mechanism of the disease process in multiple sclerosis is similar to the conditions mentioned, then the following chain of events may take place: an infective agent (previous infections on subclinical level) enters through the blood brain barrier and combines with the protein of the brain. This modified protein becomes then antigenic and produces antibodies in the central nervous system. This particular reaction would not require the pres-

ence of a living organism; a residual cell component of a bacterium or of a virus may be sufficient. It has been reported that a single injection of a lipopolysaccharide fraction of E. coli changed the permeability of the central nervous system vasculature.[3] There is, however, no proof yet that the action of the killed tubercle bacilli in the production of allergic encephalomyelitis is associated with an alteration of the blood brain barrier. But it is noteworthy that the activity of tubercle bacilli does reside in a compound which contains a high concentration (45 per cent) of polysaccharide. Colover[1,2] reported that a chromatographically pure compound prepared from tubercle bacilli was active when used together with the protein preparations made by the methods herein described. Assuming that a similar polysaccharide from an earlier infection remained in the human body and passed into the general circulation then through the blood brain barrier into the central nervous system and there combined with a particular protein of the brain and spinal cord, then this modified protein might act as a complete antigen, producing antibodies in the central nervous system. As a matter of fact, in both situations, in allergic encephalomyelitis and in multiple sclerosis, the gamma globulin content of the cerebrospinal fluid is greatly increased.

Autosensitization against the brain proteins, which are combined with exogenous substances, is offered then as the basis for the unified theory.

It should be mentioned here that as early as 1937 Ferraro[5] considered multiple sclerosis an infectious allergic reaction of the central nervous system. As recently as 1958 he reaffirmed this concept although admitting that he did not know under what biochemical or immunobiological circumstances the combination of an infectious or toxic agent and partial antigens occurs.

Considerable progress has been made. Recent work in experimental encephalomyelitis has shown that the disease can be produced by substituting for whole tubercle bacilli and whole brain, purified fractions of tubercle bacilli and purified fractions of whole brain. With the use of a single homogeneous protein it may be possible to find out whether or not antibodies are directly related to the disease process.

In view of the close relationship between allergic encephalomyelitis and multiple sclerosis, it seems supportable that allergic encephalomyelitis may serve as a laboratory model for multiple sclerosis.

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