Chapter 34

Problems Related to the Protein-Eliciting Experimental Allergic Encephalomyelitis*

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INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease with central nervous system (CNS) involvement. The compound responsible for the condition is a basic protein and derives from the CNS myelin. This has been established by localization of the encephalitogen through immunofluorescent studies of Rauch and Raffel(41) and by actual isolation of the encephalitogenic protein(s) from the myelin. (13,18,27,29)

The two most crucial questions related to the problem of EAE are: (1) Is there more than one encephalitogen in the myelin? (2) Why is this particular myelin protein encephalitogenic? An attempt will be made to answer these questions.

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IS THERE MORE THAN ONE ENCEPHALITOGEN IN THE MYELIN?

The idea of multiple encephalitogens is based on reports that encephalitogenic proteins have been isolated with molecular sizes ranging from 3000 to 50,000.\(^{(3,5,6,11,23,27,30,36,37)}\)

The divergence in reported molecular size of the encephalitogenic protein may be attributed to several factors:

1. To the mode of preparation, especially the initial steps consisting of selection of organic solvents for removal of the lipids and extraction of the encephalitogen with salt or acid of the lipid-free residue. The pH of the acid used for the extraction also plays a role.
2. To proteolysis due to the action of an endogenous enzyme resulting in an active fragment(s).
3. To selection of particular methods used for measuring the molecular weight.
4. Notwithstanding the validity of points 1–3, we do not exclude the possibility that there is more than one encephalitogenic protein present in the neural tissue.

Influence of the Mode of Preparation

Prior to extraction of the encephalitogen the lipids are removed with organic solvents. Acetone and petrol ether were used by Einstein et al.\(^{(11)}\) Nakao et al.,\(^{(26)}\) Kibler and Shapira,\(^{(23)}\) Lumsden et al.,\(^{(30)}\) and Carnegie et al.\(^{(3)}\) A chloroform–methanol mixture was first employed by Folch and Lees\(^{(21)}\) for the extraction of proteolipids. This solvent mixture was also used very successfully for the removal of lipids by Kies\(^{(25)}\) and by others including ourselves. For extraction of the encephalitogen two types of chemicals have been used: salts, such as potassium chloride, by Einstein et al.,\(^{(10,11)}\) citrate by Roboz (Einstein) and Henderson\(^{(42)}\) and Kibler and Shapira,\(^{(23)}\) and acetate by Eng et al.\(^{(18)}\) Acid extraction with dilute of H\(_2\)SO\(_4\) or HCl, a method started by Kies,\(^{(25)}\) at present is used by a majority of investigators. It should be mentioned that it is not necessary to remove the lipids before extraction of the encephalitogen. The two operations may be carried out together.\(^{(13,49)}\)

We have found differences in the immunological response to the encephalitogens, depending on whether initially the neural tissue was extracted with acid or salt. Although these encephalitogens are very similar in their physicochemical properties and both are highly encephalitogenic, the one prepared by salt extraction elicits a precipitating antibody, while the