An Electron Microscopical Comparison of Primary and Secondary Demyelination in the Rat Central Nervous System

J. I. Howell and M. Kidd
Department of Pathology, Maida Vale Hospital

Received November 15, 1968

Summary. Primary demyelination in rats was investigated by means of experimental allergic encephalomyelitis (EAE). In addition, a portion of cerebral cortex was removed from normal rats in order to produce a Wallerian-type (secondary) degeneration. The criteria for this secondary type of demyelination, established from a study of the latter group of animals, were then compared with those for the demyelination which occurred in the rats with EAE. A moderate amount of secondary demyelination was found in rats with EAE, even in animals with a mild attack of the disease, whereas primary demyelination was rare, only isolated fibres being affected.

A direct cellular attack on the myelin sheath did not seem to be necessary for producing demyelination and myelin was rarely seen to be invaded by inflammatory cells in EAE. An artefactual "demyelination" was observed in normal controls and it is suggested that this was the result of preparation damage to the myelin sheath at the node and the Schmidt-Lanterman cleft. Degenerating myelin, whether resulting from primary or secondary causes, was generally found initially to form spheroids and in the case of primary demyelination, was usually phagocytosed in this form.

Introduction

The first ultrastructural report on experimental allergic encephalomyelitis (EAE) was by Condie and Good (1957) using guinea pigs. Since then two more groups of workers have investigated this condition in guinea pigs (Field and...
RAINE, 1966; LAMPERT and KIES, 1967). One such study on rabbits has been described (LUSE and McDOUGAL, 1960) and several on the rat (BUBIS and LUSE, 1964a, 1964b; LEVINE et al., 1965; LAMPERT and CARPENTER, 1965; LAMPERT, 1965, 1966, 1967). The "hyperacute" form of this disease was described electron microscopically by LEVINE et al. (1965) and later reproduced by LAMPERT (1967). (This form of EAE, first elicited by LEVINE and WENK (1965), is produced in high susceptibility rat strains when an aqueous mixture of pertussis vaccine is used in conjunction with the antigen preparation.)

Electron microscopical observations on the nature of EAE have differed. It has been suggested by some workers (CONDIE and GOOD, 1957; FIELD and RAIN, 1966) that changes in axons generally precede those in myelin sheaths but others (LAMPERT and KIES, 1967; LAMPERT, 1967) have proposed that axon changes are secondary to myelin breakdown. An invasion of myelin sheaths which enclosed normal axons, was observed by BUBIS and LUSE (1964a), LAMPERT and CARPENTER (1965), LAMPERT (1965, 1966, 1967) and LAMPERT and KIES (1967). LAMPERT and his colleagues also described a "peeling off" of myelin lamellae by the direct action of mononuclear cells, but FIELD and RAIN (1966) reported that they never observed mononuclear cells within the myelin sheath "despite careful search".

Since there is still controversy over the nature and sequence of events in EAE, it was felt that a further study of this disease would be of value, together with an examination of artefact in normal controls. Emphasis has been placed on demyelinating changes and their relationship to inflammatory cells. In order to distinguish between primary and secondary demyelination, Wallerian-type degeneration was produced in a group of normal rats and the demyelination which resulted was compared with that found in animals with EAE.

Material and Methods

For the EAE study, 76 albino Wister rats between the ages of 7—22 weeks were used. The nervous tissue homogenate was prepared from equal volumes of a suspension of central nervous white matter from rats or guinea pigs and Freund's incomplete adjuvant (Difco) which was mixed with killed and dried bacteria. This was prepared according to the method of LEVINE and WENK (1961), except that to make complete adjuvant, 4 mg per ml of mycobacterium butyricum were added, instead of the same weight of tubercle bacilli, as used by these authors. The emulsion was administered into the hind footpad, either as a single injection of 0.1 ml or as two 0.05 ml injections, one in each pad. Cerebral biopsies were carried out on 6 paralysed rats and 10 that had not become paralysed and cord biopsies were performed on 2 paralysed rats and 10 non-paralysed rats; 5 cerebral and 7 cord biopsies of normal animals of the same age group and living under the same conditions were used as controls. Animals with EAE were biopsied at intervals from 12—54 days after sensitization.

In the Wallerian-type degeneration series, 15 albino Wistar or Lister hooded rats of 12—16 weeks old were used. A disc of parietal bone was removed under sterile conditions and a small portion of cerebral cortex sucked out, until the corpus callosum was exposed. At periods of 2, 4, 7, 10, 15 and 20 days a contralateral portion of cortex and underlying corpus callosum was removed for electron microscopy.

Tissue from both series of experiments were usually fixed by immersion. In these cases, tissue was cut into 1 mm cubes within 4 minutes of death, using a modified electric animal clipper with a razor blade attached. Fixation was usually with 1% veronal acetate buffered osmium tetroxide or occasionally 2.5% phosphate buffered glutaraldehyde, both of which were kept at 0—4°C for 3—4 hours. The rest of the central nervous system was fixed in formalin for light microscopy. In addition, 3 of the animals with EAE were perfused through