METHOD OF DEMONSTRATION OF DEMYELINIZATION IN NERVE TISSUE IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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The contradictory nature of existing views on the mechanism of demyelinization is to some degree attributable to the absence of any method of studying relations between periaxonal and inflammatory processes on the same preparations over sufficiently wide areas. This situation has led some workers to deny not only any connection between demyelinization and cellular inflammatory infiltration [1, 2, 5, 6, 9], but also a role of the periaxonal process itself in the pathogenesis of experimental allergic encephalomyelitis (EAE) [8, 10-12]. The need has thus arisen for the development of a light-optical method of combined detection of demyelinization and of cellular inflammatory infiltration in nerve tissue. Marchi's method was chosen as the basis for such a development, for it is the most sensitive method of detecting injury to the myelin sheath.

The aim of the investigation was to discover conditions of processing of nerve tissue which would result in optimal detection of myelin breakdown products and would not affect the ability of the cells to be electively stained by aniline dyes.

EXPERIMENTAL METHOD

The method (granted an author's certificate with respect to claim No. 3536152/14, dated December 30, 1985) of diagnosis of demyelinization was developed in 42 noninbred guinea pigs with EAE. The disease was caused by a single subcutaneous injection of 1 μg of basic myelin protein (BMP) fraction with Freund's complete adjuvant [4]. The BMF fraction was isolated from bovine spinal cord by column chromatography [7]. Animals with neurologic manifestations of EAE were killed by inhalation of ether. CNS material (brain and spinal cord at all levels, with spinal ganglia) was treated by Marchi's method [3]. Another part of the material was kept in a 3% solution of potassium bichromate and a 1% solution of osmic acid for 2.5 days, respectively. Nerve tissue was embedded in celloidin or paraffin wax, or histological sections were cut with a freezing microtome and stained with toluidine blue.

EXPERIMENTAL RESULTS

Results obtained by treatment of nerve tissue by various methods are given in Table 1.

It will be clear from Table 1 that simultaneous detection of myelin breakdown products and cells in nerve tissue is possible only if the material is treated by the modified Marchi method. Under these circumstances, hematogenous cells and gliocytes, responsible for phagocytosis of the osmiophilic fragments of the destroyed myelin sheath, are clearly visible in foci of demyelinization (Fig. 1a). By contrast, with Marchi's original method it is impossible to identify hematogenous and glial cells at sites of myelin breakdown (Fig. 1b).

Most histological methods of diagnosis of demyelinization (Spielmeyer's, Loewit's, Barrera and Klüver, etc.) are based on detection of disturbance of continuity of myelin fibers when they are damaged, and not of myelin breakdown products. The methods mentioned above likewise can reveal only sufficiently marked destruction of myelin sheaths in the late stages of the process, so that they cannot be regarded as methods of choice for the study of the mechanism of demyelinization.

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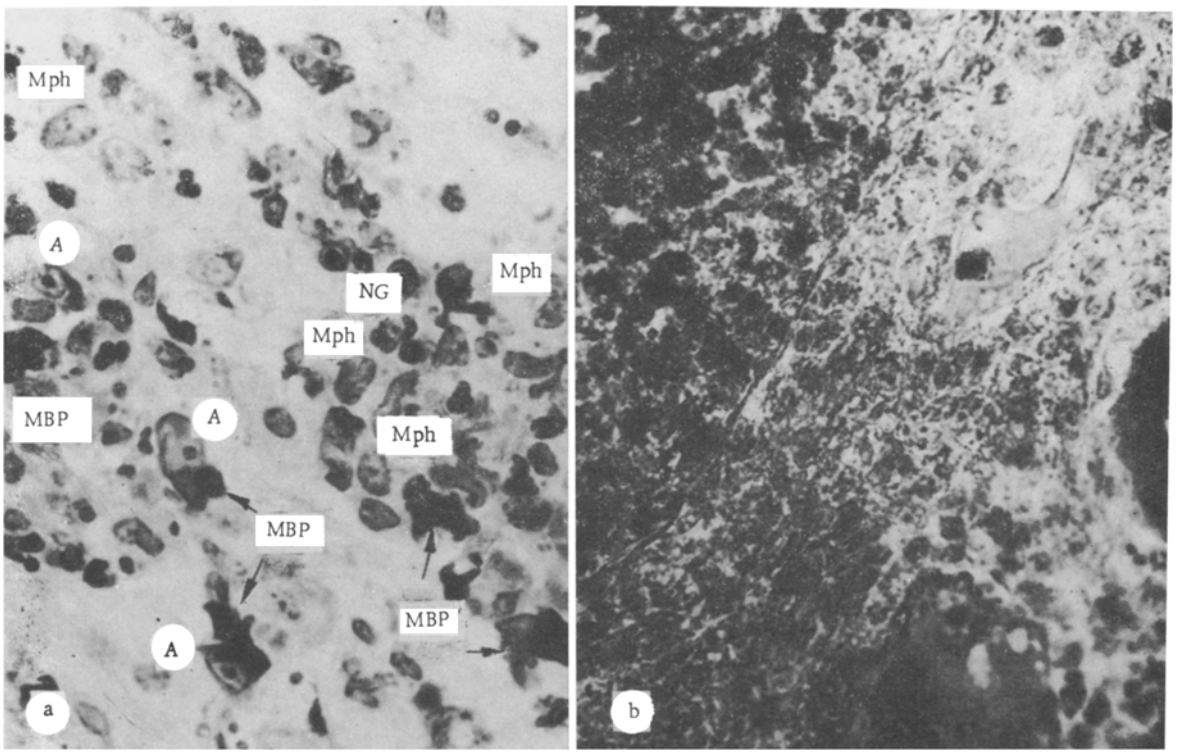

Fig. 1. Anterior gray columns of lumbar segments of spinal cord of animal with EAE, 20 days after inoculation with encephalitogenic mixture. a) Hematogenous macrophages (Mph), astrocytes (A), and neutrophilic granulocyte (NG) among myelin breakdown products (MBP). Modified Marchi method was stained by toluidine blue. 580 ×; b) MBP in white matter of spinal cord in zone of inflammatory infiltration. Marchi method with counterstaining by toluidine blue. 480 ×.

TABLE 1. Detection of Myelin Breakdown Products and Cells after Treatment of Nerve Tissue by Classical and Modified Marchi Method

Method of histologic treatment	Length of exposure, days		Detection of		Thickness of histologic sections, μ	Reason for inability to obtain thinner sections
	in 3% solution of potassium bichromate	in 1% solution of osmic acid	myelin breakdown product	cells		
Marchi	28	31	+	—	15—20	Fragility of material
Modified Marchi method	2,5	2,5	+	+	2—3	Limit of strength of celloidin

One of the most promising methods for studying the pathology of myelin is electron microscopy. However, in the early stages of the process pathological changes in the ENS arise in circumscribed areas and at widely separated points in space and time. Accordingly the need arises to investigate areas of nerve tissue much greater in area than is possible by electron microscopy.

The most appropriate method of diagnosis of demyelinization at the light-optical level is Marchi's method. Its high sensitivity is due not only to its specificity (reaction for choline esterase), but also to the very principle of the method: the discovery of dark osmiophilic granules against a light background. This means that myelin breakdown can be detected even in single nerve fibers, i.e., the earliest stages of the process can be diagnosed. How-

ever, counterstaining of the cells in this case is unsatisfactory and requires long exposure of the histological sections to the dyes. As will be evident, this is because of the long time which the nerve tissue undergoes treatment in potassium bichromate solutions.

The method now developed not only enables demyelinization to be detected and the state of the various cells in nerve tissue to be studied in the same preparations, but it also enables the time spent on preparing the material for histological investigation to be greatly reduced (for sealing in celloidin to one month, for embedding in paraffin wax to 1.5 weeks, and for freezing to 5-6 days), i.e., by 2.5 and by more than 10 times, respectively compared with the classical method. In addition, when the suggested method is used it is unnecessary to impregnate the nerve tissue in potassium bichromate, which leads to increased fragility of the material and makes it impossible to obtain sufficiently thin sections. Histological sections obtained in this way (2-3 μ thick) can be examined with immersion magnifications of the light microscope.

The suggested method of simultaneous detection of myelin breakdown products and of cells in demyelinized nerve tissue opens up new prospects for the study of the pathogenesis of demyelinization. It can be used to investigate the process of destruction of the myelin fiber of varied etiology, including for the rapid diagnosis of periaxonal changes in morbid anatomical practice.

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HETEROGENEITY OF BASEMENT MEMBRANES REVEALED IN HUMAN TISSUES BY MONOCLONAL ANTIBODIES TO LAMININ AND ENTACTIN

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The glycoproteins laminin and entactin are components of basement membranes (BM) of varied origin [3, 8, 13]. The presence of these glycoproteins has been demonstrated with the aid of polyclonal antibodies (PA) in virtually all BM of organs studied [5, 7]. The appearance of monoclonal antibodies (MA), with narrow specificity, has demonstrated heterogeneity of BM with respect to several components, including to type IV collagen and laminin [6, 10, 15], i.e., besides MA which, like PA, reveal laminin of BM, there are also others which react with by no means all BM. There is no unanimity at present on whether entactin is a universal component of BM. According to some data [3] entactin was revealed by PA in all rat tissue BM studied. However, in [8], in which the distribution of entactin was studied

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