*Proc. Natl. Acad. Sci. USA*
Vol. 75, No. 5, pp. 2521–2524, May 1978
Neurobiology

# Myelin basic protein demonstrated immunocytochemically in oligodendroglia prior to myelin sheath formation

(peroxidase antiperoxidase/differential interference microscopy/electron microscopy)

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*Communicated by Edward V. Evarts, March 6, 1978*

ABSTRACT **A specific antibody to myelin basic protein has been used to localize the protein in developing rat oligodendroglia and myelin. Basic protein is found in the oligodendroglial cytoplasm of anterior commissures of 5- and 7-day old rats before the beginning of myelination. Staining of basic protein in oligodendroglia increases, becoming most intense during early myelination; it decreases during rapid myelination. Staining intensity of oligodendroglia is dependent upon age, brain region, and nervous tract studied. In myelin, reaction of basic protein with antibody decreases when large compact sheaths are present, unless tissue sections are first treated with alcohol.**

Myelin basic protein (MBP) comprises about 30% of the myelin proteins of the central nervous system (CNS) and is unique to the myelin membrane. Morphological evidence indicates that the myelin membrane is a spirally wrapped extension of the oligodendroglial surface membrane (1). During development of rat CNS myelin, radioimmunoassay measurements of initial MBP appearance in CNS tissue homogenates correlate with the morphological detection of compact myelin in spinal cords of 5-day-old rats. The rate of MBP accumulation can be related to the rate of myelin synthesis (2). Polyacrylamide gel electrophoresis of myelin proteins has also been used to correlate amounts of MBP with the rate of myelin synthesis (3). MBP has been found in isolated oligodendroglia of myelinated fetal bovine brain (4). However, interpretation of these results is uncertain because of probable absorption of soluble MBP present in the tissue suspension (5). Thus, the relationships among MBP synthesis, oligodendroglia, and myelin formation are not well defined.

Recently, we used a light-microscopic immunocytochemical method to show that MBP is present in myelin-forming oligodendroglia of spinal cords and brains of newborn and immature rats (6). Here, we present immunocytochemical and electron microscopic evidence demonstrating that oligodendroglia in the developing CNS contain MBP before myelin sheaths are formed. As development proceeds, the amount of MBP in oligodendroglia increases as myelination begins and subsequently decreases during rapid myelin formation.

## MATERIALS AND METHODS

Newborn to 25-day-old Osborne–Mendel rats were anesthetized with chloral hydrate and fixed by intracardiac perfusion for 10 min with a solution containing 76 ml of $HgCl_2$ (saturated at 0°) and 20 ml of 37% (vol/vol) formaldehyde. Brain and body weights of littermates were measured. Cervical spinal cord, medulla oblongata, pons, midbrain, diencephalon, and anterior commissure were dissected and fixed for an additional 2–3 hr at 4°. Saggital midline sections of the anterior commissure, 20 μm thick, were cut on a Vibratome. All other regions were sectioned coronally.

Sections were stained immunocytochemically by the peroxidase–antiperoxidase method (7). The staining procedure and antiserum preparation have been described (6). Antiserum to MBP was diluted 1:500 to 1:8000 and then applied to the tissue. This was followed by application of (*i*) sheep anti-rabbit IgG, (*ii*) peroxidase–antiperoxidase, (*iii*) hydrogen peroxide and 3,3′-diaminobenzidine tetrahydrochloride, and (*iv*) 2% (wt/vol) $OsO_4$. The sections were infiltrated with glycerol, mounted on glass slides, and examined with a Zeiss differential-interference contrast (Nomarski) microscope (8). Some sections were postfixed in 95% (vol/vol) ethanol before addition of antibody. Specificity of staining was shown by incubating sections with either preimmune serum or absorbed antiserum in which specific antibodies were removed by precipitation with purified MBP in amounts sufficient to remove all reactivity of antiserum against MBP detectable by radioimmunoassay.

Staining intensity of oligodendroglia was measured with an Optomax image analyzer (Micro Measurements) attached to a Zeiss microscope. The response of the image analyzer to different densities was tested by measuring background values with a series of grey filters inserted in the light path. A plot of the logarithms of values obtained against the optical-path densities of the filters was linear. The optical density of cells was measured with brightfield illumination. The background of a stained section was used as zero density. The oligodendroglia selected for measurement were those that were sectioned through an unstained nucleus and did not have stained myelin sheaths above or below the cell being measured. Measurements were made of the highest density of cytoplasmic staining and the highest density of the unstained background. Staining intensity was defined as

$$\text{OD cytoplasm} - \text{OD background}$$

The data were expressed as the mean OD of 10 cells − background readings ± the standard error of the mean.

To relate the distribution of immunocytochemical staining to the cytology of the developing nervous system, we studied the light and electron microscopic appearance of the same regions in littermates. The fixative used for these perfusions contained 1.5% (vol/vol) glutaraldehyde and 0.5% (vol/vol) formaldehyde in 80 mM phosphate buffer. Fixation was continued overnight and then the spinal cord and brain were dissected, processed, thin-sectioned, and stained by conventional techniques.

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Abbreviations: MBP, myelin basic protein; CNS, central nervous system.

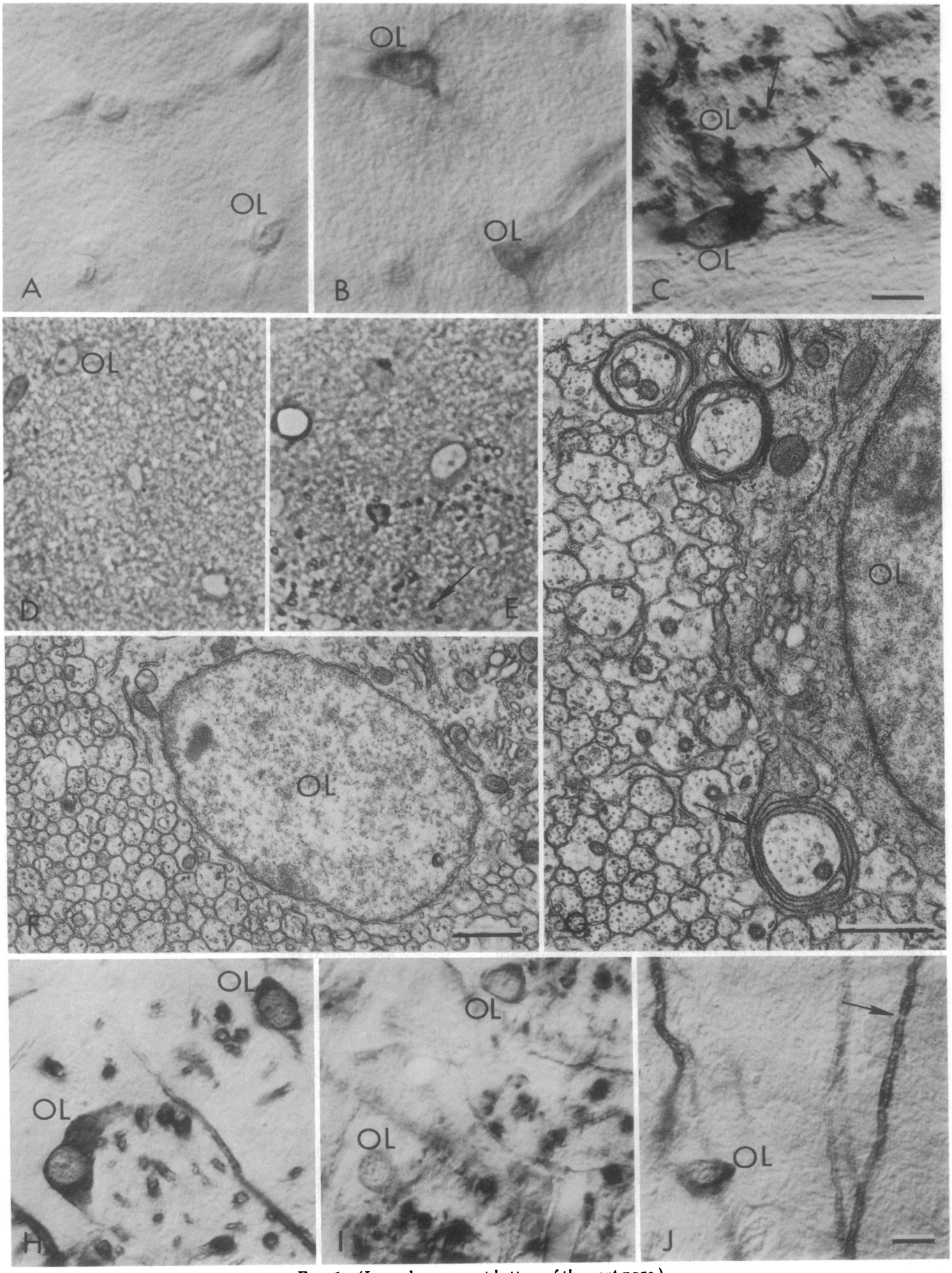


FIG. 1. *(Legend appears at bottom of the next page.)*

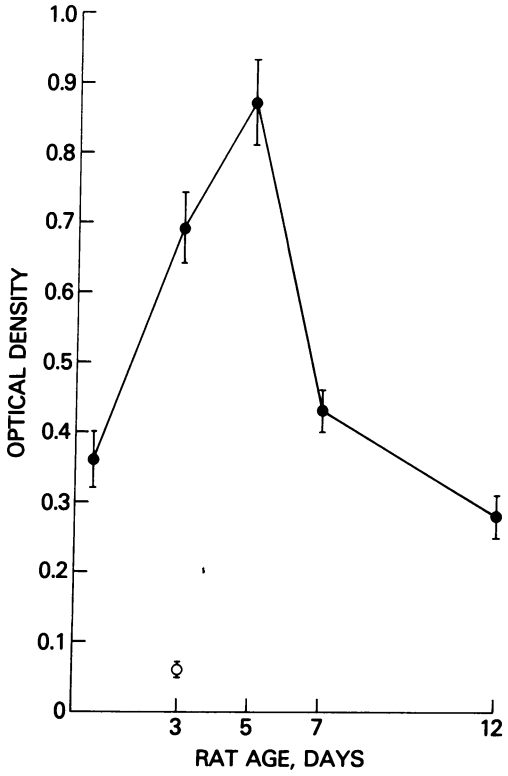


FIG. 2. Optical densities of oligodendroglia in the pontine tectospinal tract during early development immunostained with antiserum to MBP (●) or preimmune control serum (○). All antiserum dilutions were 1:500.

## RESULTS

The anterior commissure is a tract well suited for studying MBP in oligodendroglia before and during the onset of myelination (9). Saggital midline sections of the anterior commissure from 3-, 5-, 7-, and 12-day-old rats were treated with antiserum to MBP. At 3 days a few cells resembling oligodendroglia were present in the anterior limb of the anterior commissure but no reaction with antiserum against MBP was detected (Fig. 1*A*). At 5 and 7 days, oligodendroglia with many fine processes reacted with the MBP antiserum but no stained myelin sheaths were seen (Fig. 1*B*). In phase (Fig. 1*D*) and electron (Fig. 1*F*) micrographs of the anterior commissure at 5 and 7 days, oligodendroglia were present but none of the axons was myelinated. By 12 days the number of MBP-containing oligodendroglia in the anterior limb had increased to 52 per section (from 13 and 20 per section at 5 and 7 days, respectively) and the staining was more intense than at earlier ages (Fig. 1*C*). In addition, some axons were surrounded by a collar of staining, and the presence of myelin sheaths was confirmed in phase (Fig. 1*E*) and electron (Fig. 1*G*) micrographs.

A semiquantitative analysis of oligodendroglial MBP staining during early development of the pontine tectospinal tract is shown in Fig. 2. Some stained myelin was found at all ages studied but rapid accumulation of myelin was not observed before 7 days of age. In the newborn, staining by antiserum against MBP was found in oligodendroglia; it increased to maximum levels at 3–5 days and then decreased as myelination proceeded. Fig. 1 *H* and *I* illustrate the difference in oligodendroglial staining intensity at 5 and 12 days.

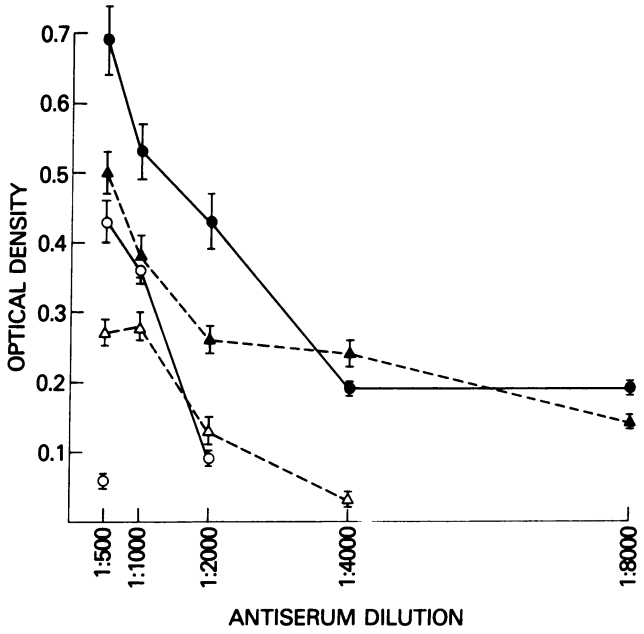


FIG. 3. Effect of antiserum dilution on optical densities of immunostained oligodendroglia in the 3-day pontine tectospinal tract (●—●); 12-day pontine tectospinal tract (○—○); 12-day midbrain medial lemniscus (▲---▲); and 12-day pontine medial lemniscus (△---△). A 1:500 dilution of preimmune control serum is indicated by the open circle at OD = 0.06.

The relative amounts of MBP present in oligodendroglia during different stages of development are shown in Fig. 3. Oligodendroglia in the 3-day pontine tectospinal tract were stained by all dilutions of antiserum tested. The intensity of staining and number of cells stained decreased with increasing dilution. By 12 days, oligodendroglia in the same tract stained less intensely and staining could not be detected at antiserum dilutions greater than 1:2000. A similar comparison of different levels of the medial lemniscus in the 12-day rat showed that in the midbrain, oligodendroglia stained more intensely than in the pons where myelination was more advanced. MBP could also be detected in oligodendroglia of the midbrain at higher dilutions of anti-MBP than could be detected in the pons.

Myelin sheath staining also changed with increasing age (Fig. 1 *H* and *I*, and Fig. 4). From birth to 12 days, staining intensity increased initially as sheaths grew thicker and longer. Nodes of Ranvier (Fig. 1*J*) could be identified between adjacent myelin segments. After 12 days, reaction of antiserum with MBP in larger compact sheaths decreased. By 25 days, myelin staining was variable and often faint (Fig. 4*A*) unless the sections were briefly treated with 95% ethanol (Fig. 4*B*). No staining was observed in sections reacted with preimmune serum or anti-MBP serum absorbed with MBP.

FIG. 1 (*on preceding page*). Saggital midline sections of the anterior commissure (*A–G*) and coronal sections of the pontine tectospinal tract (*H* and *I*) and the anterior column of the spinal cord (*J*). Sections shown in *A–C* and *H–J* were immunostained with 1:500 dilution of MBP antiserum and photographed with differential interference contrast optics. Immunostaining of oligodendroglia (OL) is absent at 3 days (*A*), present at 7 days (*B*), and much denser at 12 days (*C*) when myelin sheaths (arrows) also are heavily stained. At 7 days, oligodendroglia are present in phase (*D*) and electron (*F*) micrographs; axons are small and none is myelinated. At 12 days (*E* and *G*), oligodendroglia (OL) are larger. Their perikarya contain more ribosomes and their processes extend to newly formed myelin sheaths (arrows). Immunostaining of oligodendroglia (OL) in the pontine tectospinal tract is much denser at 5 days (*H*) than at 12 days (*I*). Heavily stained myelin sheaths are present at both ages, and in favorable sections (*J*) nodes of Ranvier (arrow) can be identified. (*A–E* and *H–J*, scale bar = 10 μm; *F* and *G*, scale bar = 1 μm.)

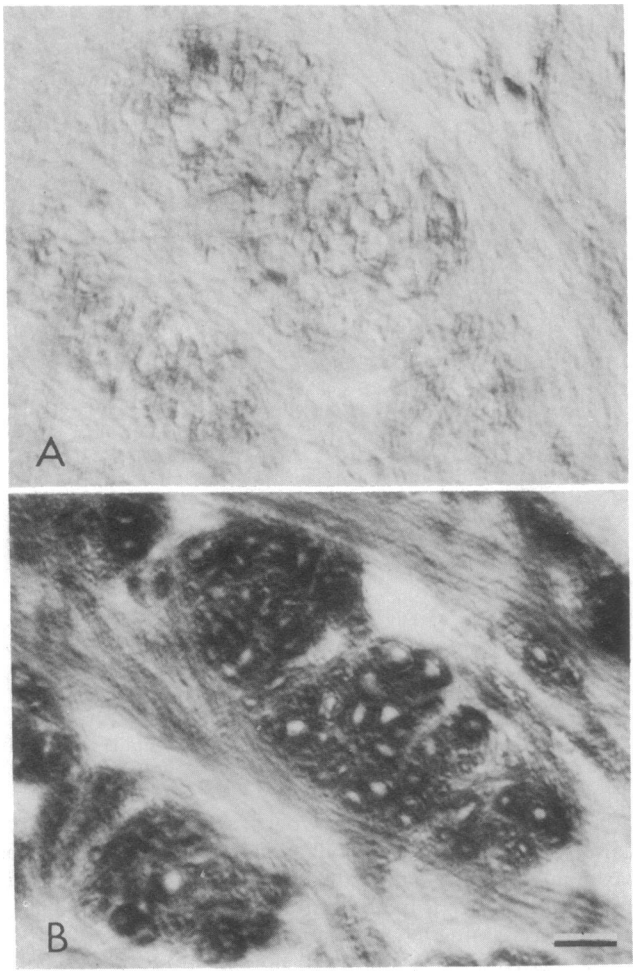


FIG. 4. Coronal sections of the medulla oblongata, medial longitudinal fasciculus, age 25 days, immunostained with 1:500 dilution of MBP antiserum. In *A*, myelin sheaths are lightly stained. Much heavier staining is present in *B*, a section that was immersed in 95% ethanol before immunostaining. (×800, scale bar = 10 μm.)

## DISCUSSION

Our results clearly show that MBP can be detected immunocytochemically in oligodendroglia before myelin sheath formation begins. Semiquantitative estimates of oligodendroglial staining intensity strongly suggest that the MBP content of oligodendroglia rises rapidly as the cells enlarge and extend processes to surround and myelinate axons. Thus, at age 5 days, when oligodendroglial staining in the pontine tectospinal tract reached maximum levels, processes of single oligodendroglia were 4–38 μm long and were attached to as many as 10 myelin sheaths (6). Later, myelin sheaths increased greatly in number, thickness, and length. During this rapid phase of myelin formation, oligodendroglial perikarya decreased in size, and processes extending to myelin sheaths were much thinner; MBP staining also decreased and, in the adult, oligodendroglia were unstained.

We believe that the differences in intensity of oligodendroglial staining reflect changes in MBP content that precede and accompany myelination. In the tectospinal tract the MBP content of oligodendroglia was higher at 3 days than at 12 days. This was indicated by the greater intensity of stain with a given dilution of antiserum and also by the observation that MBP could be detected at higher dilutions of antiserum in the 3-day oligodendroglia. At 12 days, myelination of the medial lemniscus was significantly more advanced in the pons than in the midbrain. Optical densities of pontine oligodendroglia were significantly lower at all dilutions than those measured in the midbrain.

The finding of MBP in oligodendroglia before myelin synthesis begins and in the initial wrapping of axons by oligodendroglial processes (6) suggests that MBP is one of the first components incorporated into the developing myelin sheath. As myelin sheaths increased in thickness and length, dense staining of entire segments was observed initially. Later, however, the intensity of the reaction decreased unless the tissue was pretreated with ethanol. This suggests that during myelin maturation, MBP in myelin sheaths becomes less accessible to antibodies. Adult peripheral nervous system myelin must also be treated with alcohol in order to obtain reaction with MBP antiserum (10). In contrast, we find that the reaction of antiserum with oligodendroglia was not enhanced by pretreatment with ethanol. Oligodendroglial MBP appears to be accessible at all stages of development.

We thank Mrs. Kathryn Winchell and Ms. Sue Larrick for excellent assistance. We also thank Dr. Robert D. Allen and Mr. Michael Huns for help and advice with the quantitative measurements.

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