The results from *A. marginata* suggest that most of the phenolics are polymerized with high tanning ability. M. A. Ragan and A. Jensen (*16*) and A. Temple [*J. Chem. Ecol.* **8**, 1289 (1982)] critique different methods of assaying for phenolic compounds in brown algae and terrestrial plants, respectively.
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Expression of Glial Fibrillary Acidic Protein in Immature Oligodendroglia

Abstract. *In the human fetal spinal cord at 15 to 16 weeks, glial fibrillary acidic protein (GFAP) was demonstrated within the cytoplasm and processes of cells having the cytological, ultrastructural, and immunocytochemical features of oligodendrocytes—including processes that extend into and contribute to the formation of myelin sheaths. By 17 to 18 weeks, however, GFAP immunoreactivity was no longer evident within such cells. Thus GFAP is expressed by myelin-forming oligodendroglia early in their development.*

Although oligodendrocytes were first discovered and described by del Rio Hortega more than 60 years ago (*1*), unequivocal demonstration of their role in myelin sheath formation was possible only with the advent of electron microscopy and immunocytochemistry (*2–4*). The cell of origin and mode of differentiation of oligodendrocytes in the developing central nervous system, however, are still uncertain. The prevailing view seems to be that oligodendrocytes are derived from "glioblasts," the nature of which has never been clearly defined (*5, 6*).

We showed earlier (*7*) that astrocyte-specific glial fibrillary acidic protein (GFAP) is present within radial glial cells in the developing human fetal spinal cord (HFSC) by 8 to 10 weeks. These cells are the first distinguishable neuroglial element among the cells within the ventricular zone. Correlative electron microscopic, Golgi, and immunocytochemical studies have suggested that radial glia are transformed into astroglial cells in the HFSC, human fetal cerebrum and cerebellum, and fetal monkey telencephalon (*8*). More recently, we showed, by electron microscopy and by immunocytochemical analysis under light microscopy, that "transitional forms" between astroglia and oligodendroglia may exist, and we suggested that radial glia may give rise to both astroglial and oligodendroglial cells (*9, 10*).

We tested this hypothesis further by light and electron microscopic immunocytochemical studies on serial sections (1 μm) of the subpial and marginal zones of the ventral columns of the HFSC obtained from 17 aborted fetuses at an ovulation age of 8 to 20 weeks. Alternating deeponized sections were processed for immunocytochemical determination of GFAP and myelin basic protein (MBP), respectively (*11*). At age 6 to 8 weeks the subpial region of the HFSC is relatively cell free. By 9 to 10 weeks, however, there is a significant increase in the population of cells within this region. Most of these cells show the cytological, ultrastructural, and immunocytochemical features of astroglial cells (*4*). At 11 to 12 weeks, when myelin formation begins, most of the cells still have the characteristics of astroglia, although a few show the features of oligodendrocytes (*6, 12*). By 16 weeks, however, almost all of the cells within this region are oligodendrocytes, as indicated by their morphology and by their intimate association with well-developed myelin sheaths.

Figure 1 shows representative immunocytochemical preparations of adjacent 1-μm sections of the HFSC at 16 weeks. At this stage, immunoreactivity to MBP is localized primarily within myelin sheaths and within the processes of cells with which they appear to be closely associated (Fig. 1A). Nearly all of these cells contain immunoreactive GFAP within their cytoplasmic processes, some of which appear to encircle thinly myelinated axons (Fig. 1B).

To investigate more fully the relation between GFAP-positive cells and myelin sheaths, we processed large numbers of vibratome sections for immunocytochemistry and examined under electron microscopy. At 13 to 15 weeks, many cells exhibited the features of oligodendroglia, the cytoplasm and processes of which, however, were strongly immunoreactive for GFAP (Fig. 2A). These processes often extended into myelin sheaths which, as judged from their thinness and the small number of lamellae, were relatively immature (Fig. 2B). By 17 to 18 weeks most of the axons within this region were surrounded by multilay-

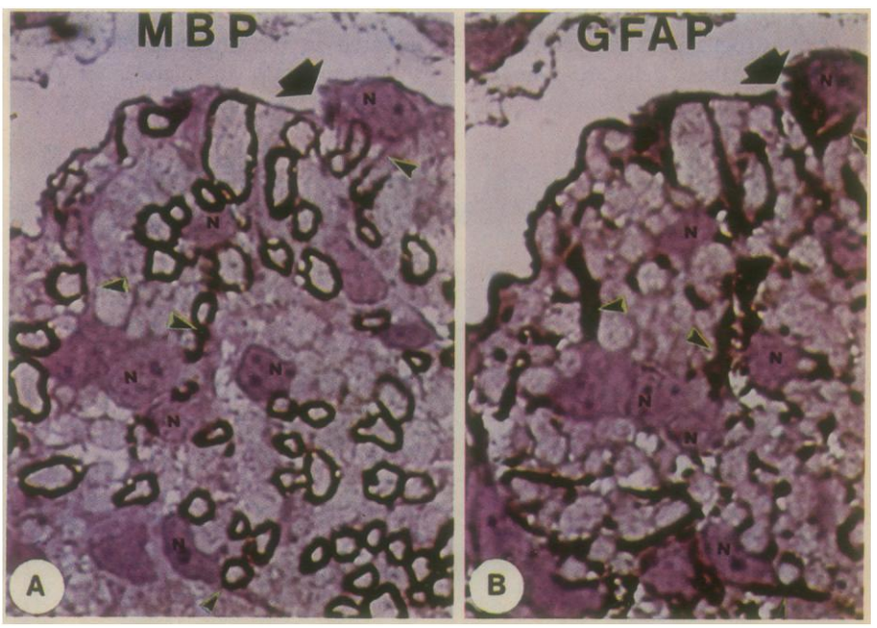

Fig. 1. Photomicrographs showing adjacent 1-μm sections of the ventral column of the HFSC at 16 weeks of age. The sections were prepared for (A) MBP and (B) GFAP immunocytochemistry with the unlabeled antibody enzyme technique. Dark staining represents immune precipitate. Immunoreactivity to MBP is localized primarily within myelin sheaths and within the processes of immature oligodendrocytes, with which they are closely associated. These cells show dense nuclear (*N*) and cytoplasmic staining and contain prominent nucleoli and marginated chromatin. Nearly all of these cells show strong immunoreactivity for GFAP within their cytoplasm and processes (thick arrow and arrowheads). Magnification, ×1350.

ered compact myelin, but the myelin-forming cells no longer showed immunoreactivity to GFAP.

In view of the consensus among myelin researchers that mature central myelin sheaths are formed and maintained solely by oligodendrocytes (*3*, *13*), the GFAP-positive cell processes extending into thin myelin sheaths in the HFSC must represent those of immature oligodendrocytes. Within the developing central nervous system, as we have shown previously (*9*), many cells with the characteristic features of oligodendrocytes under electron microscopy exhibit GFAP immunoreactivity within their cytoplasm. These studies strongly suggest, therefore, that myelin-forming oligodendroglial cells express GFAP early in their development, supporting the hypothesis that such cells, along with astroglial cells, are ultimately derived from radial glial cells.

At least two possibilities may be considered regarding the sequence of cellular events leading to the formation of myelin sheaths in the developing central nervous system. The first is that radial glia or radial glial cell-derived astroglia actually undergo differentiation or transformation into myelin-forming oligodendroglia after the release of some still undetermined signal, presumably arising from the axon. This possibility is supported by the observation that, prior to the onset of myelination, these cells have all of the cytological, electron microscopic, and immunocytochemical features of astroglial cells. A second possibility is that these cells are committed from the start to forming myelin and that during development they express GFAP only transiently. The two possibilities described above are not mutually exclusive. What is important is that either formulation virtually eliminates the need for the use of the cytogenetically imprecise term "glioblast."

In addition, it is a common observation that glial neoplasms of either astroglial or oligodendroglial lineage in man often contain a mixture of both cell types. Many "pure" oligodendrogliomas contain GFAP-positive tumor cells (*14*). The perspective gained by viewing immature oligodendroglial cells either as "differentiated" astroglial cells or as having the capacity to express GFAP may enhance our capacity to study the biological behavior of these neoplasms. The interconvertibility of either normal or neoplastic astroglial and oligodendroglial cells has been suggested by the work of others. Hirano and Zimmerman (*15*) observed the formation of glial filaments within the cytoplasmic portions of myelin sheaths after implantation of vinblastine into rat cerebrum. Parker *et al.* (*16*), using enzymatic markers presumed to be specific for either astrocytes or oligodendrocytes, remarked on the potential for "transdifferentiation" among murine C6 glioma cells after serial passage in culture. Raff *et al.* (*17*), with the aid of immunocytochemical markers, reported the identification of a cell type in 7-day-old rat optic nerve with the capacity to differentiate into an astrocyte if cultured in the presence of fetal calf serum and into an oligodendrocyte if cultured in its absence. Van Alstyne *et al.* (*18*), exposed GFAP-positive adult rat brain cells in culture to dibutyryl adenosine 3′,5′-monophosphate and observed an increase in the proportion of cells containing oligodendrocyte-specific galactocerebroside. All of the foregoing observations suggest that the relation between astroglial and oligodendroglial cells is closer than previously believed, and this may account in part for the admixture of cell types frequently seen in human glial neoplasms.

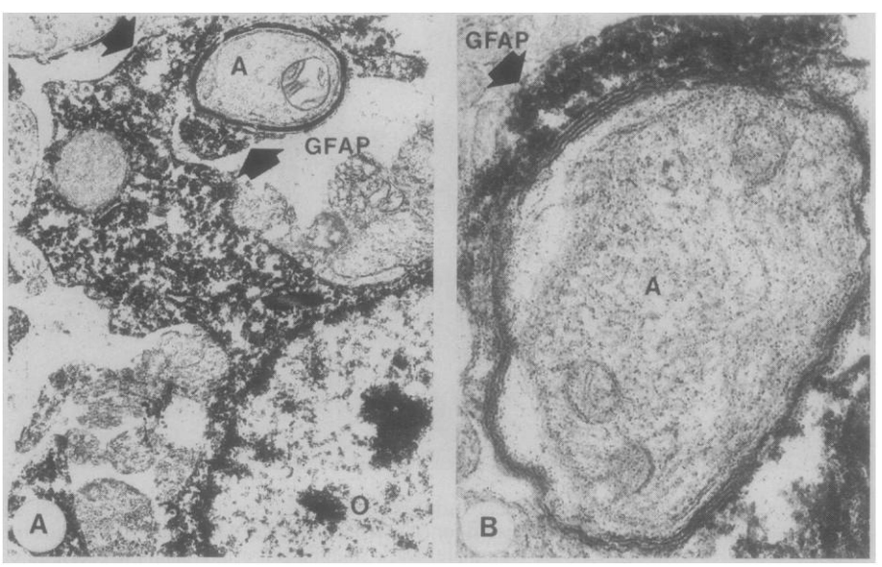

Fig. 2. Electron micrographs showing electron-dense GFAP immunoprecipitate of GFAP within the cytoplasm of (A) an immature oligodendrocyte (*O*) in the ventral column of the HFSC at age 15 weeks. The reaction product is present (A and B) within processes (arrows) that extend into immature myelin sheaths surrounding axons (*A*). Magnification: (A) ×15,400; (B) ×37,500.

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Prenatal Exposure to Carbon Monoxide: Learning and Memory Deficits

Abstract. *Exposing pregnant rats to carbon monoxide (150 parts per million) produced only minor reductions in the birth weights of the pups and gave no evidence of overt teratogenesis. However, behavioral evaluation of learning and memory processes in a two-way avoidance task suggested a functional deficit in the central nervous system of the exposed offspring. Multiple dependent measures and specific control groups confirmed that this deficit was independent of nonassociative or motivational alterations.*

The toxic and physiologic effects of carbon monoxide exposure have been well documented since the pioneering investigations in the late 19th century by Haldane (*1*). However, little is known of the consequences of chronic exposure to low concentrations of CO, particularly with respect to specific populations such as fetuses (*2*). Mild prenatal CO exposure is possible as a result of maternal cigarette smoking or industrial and ambient air sources (*3*). Animal models have revealed that after maternal exposure, CO readily crosses the placenta and decreases fetal oxygen partial pressures (*4*). Prenatal CO exposure throughout gestation increases the incidence of minor skeletal alterations in mice (*5*) and may be teratogenic in rabbits under certain conditions (*6*). Depressions in birth weight and reduced growth patterns may also be observed after CO exposure throughout gestation (*6, 7*). The reduced locomotor activity, an attenuated neurochemical response to L-dopa administration, and delays in development of two landmark behaviors observed in prenatal rats shortly after birth (*7*) represent the only functional evidence of central nervous system toxicity. We now report (i) that chronic exposure to CO restricted to the prenatal period disrupts the acquisition and retention of a conditioned avoidance response in juvenile rats, (ii) that this impairment is associative and does not reflect changes in nonassociative or motivational processes, and (iii) that this effect was observed in the absence of any overt toxicological or teratological effect.

Adult female Long-Evans hooded rats were maintained in the laboratory with continuous access to food and water, a diurnal light cycle (12 hours of light and 12 of darkness), and room temperature of 18° to 22°C. The rats were bred and, after a sperm-positive vaginal smear, transferred to exposure chambers for the duration of gestation. Chamber CO concentrations were monitored electrochemically so that aside from weekly weighing or cage cleaning, or both, the dams were left undisturbed throughout pregnancy (*8*). Average daily chamber CO concentrations (mean ± standard error) were, respectively, 149 ± 2 parts per million (ppm) and 154 ± 2 ppm during the two experiments reported below. In a third experiment, we determined that average daily CO concentrations of 148 ± 2 ppm produce maternal carboxyhemoglobin (HbCO) concentrations of 15.6 ± 1.1 percent (N = 28) relative to control subjects exposed to air (0.5 ± 0.5 percent, N = 20). By comparison, human cigarette smokers show HbCO concentrations ranging from about 1 to 16 percent (*9*).

Within 12 hours after birth, the subjects were removed from the exposure chambers and placed in a normal air environment. All litters were culled to eight pups; an equal number of each sex were left whenever possible.

Among the offspring of 16 dams exposed to CO and 16 to air, we noted a slight but nonsignificant depression in birth weight of experimental animals. We observed no differences in initial growth of the dams, number of pups per litter, sex ratio, or mortality on day 1 (Table 1); no evidence of gross structural deformities was seen in either group.

We investigated the influence of prenatal CO exposure on the functional ontogeny of learning and memory for a two-way conditioned avoidance response (*10*). Three male and three female offspring were randomly chosen from each of eight randomly selected dams from each group. Each pup received 100 acquisition trials in the conditioned avoidance response task at 16, 23, or 30 days of age. After a 24-hour retention interval, subjects were administered a second session of 100 trials (*11*).

The results of the avoidance task (Fig. 1A), analyzed by a 2 by 3 (exposure condition by age) analysis of variance, indicated that acquisition improved with age ($P < 0.001$). However, an interaction of the exposure treatment with age [$F(2, 89) = 3.3$, $P < 0.05$] resulted from an impairment for the prenatal CO-exposed offspring in acquisition of the two-way avoidance contingency. Among the offspring of dams exposed to air, acquisition was minimal in 16-day-old pups but significant by 30 days ($P < 0.01$) (*12*). In contrast, 30-day-old offspring of dams exposed to CO failed to perform the avoidance task any more successfully

Table 1. Consequences of CO (150 ppm) or air exposure on pregnant rats and their offspring. All data are expressed as means ± standard errors.

Prenatal treatment	Litters (No.)	Weight gain during gestation (%)			Gestation period (days)	Litter size (No.)	Sex ratio (M:F)	Birth weight (g)
		Week 1	Week 2	Week 3				
				Experiment 1				
Air	16	8.8 ± 1.1*			21.9 ± 0.2*	12.8 ± 0.7	1.4 ± 0.3	6.30 ± 0.12
CO	16	8.5 ± 1.2*			22.2 ± 0.1*	11.2 ± 0.6	1.0 ± 0.1	5.87 ± 0.10
				Experiment 2				
Air	18	12.7 ± 0.8	13.5 ± 1.0	23.2 ± 0.9	22.0 ± 0.1	13.4 ± 0.5	1.0 ± 0.1	5.91 ± 0.13
CO	18	10.9 ± 0.9	12.8 ± 1.1	23.6 ± 1.2	21.9 ± 0.1	12.4 ± 0.7	1.0 ± 0.1	5.73 ± 0.12

*Data available for only 12 litters.