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Gliogenesis: Historical Perspectives, 1839–1985

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With 39 Figures

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Cover Portraits
(Top) Rudolf Virchow (1821–1902) Discoverer of "glia" in the central nervous system.
(Bottom) Theodor Schwann (1810–1882) introduced the "Cell Doctrine" in 1839.

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Abstract

This historical review of gliogenesis begins with Schwann's introduction of the cell doctrine in 1839. Subsequent microscopic studies revealed the cellular structure of many organs and tissues, but the CNS was thought to be different. In 1864, Virchow created the concept that nerve cells are held together by a "Nervenkitte" which he called "glia" (for glue). He and his contemporaries thought that "glia" was an unstructured, connective tissue-like ground substance that separated nerve cells from each other and from blood vessels. Dieters, a pupil of Virchow, discovered that this ground substance contained cells, which he described and illustrated. Improvements in microscopes and discovery of metallic impregnation methods finally showed convincingly that the "glia" was not a binding substance. Instead, it was composed of cells, each separate and distinct from neighboring cells and each with its own characteristic array of processes. Light microscopic studies of developing and mature nervous tissue led to the discovery of different types of glial cells—astroglia, oligodendroglia, microglia, and ependymal cells in the CNS, and Schwann cells in the peripheral nervous system (PNS). Subsequent studies characterized the origins and development of each type of glial cell. A new era began with the introduction of electron microscopy, immunostaining, and in vitro maintenance of both central and peripheral nervous tissue. Other methods and models greatly expanded our understanding of how glia multiply, migrate, and differentiate. In 1985, almost a century and a half of study had produced substantial progress in our understanding of glial cells, including their origins and development. Major advances were associated with the discovery of new methods. These are summarized first. Then the origins and development of astroglia, oligodendroglia, microglia, ependymal cells, and Schwann cells are described and discussed. In general, morphology is emphasized. Findings related to cytodifferentiation, cellular interactions, functions, and regulation of developing glia have also been included.

1
Gliogenesis

Glia, like neurons, develop from relatively few cells that form a primitive epithelium. During maturation, glial precursors proliferate, migrate, develop processes, and interact with nearby and more distant cells. In most regions, glial and neuronal precursors could not be distinguished during early stages by morphological, physiological, or cytochemical criteria. Distinguishing features appeared during subsequent cytodifferentiation. Primitive glia and neurons became identifiable by differences in cellular size, shape, and content of nucleus and cytoplasm. Differences in cytoplasmic constituents, surface membrane components, and physiological properties also became evident. Junctional complexes differing in structure and function appeared and were thought to have an important role in intercellular exchange and the development of permeability barriers. Relatively late in development, oligodendroglia and Schwann cells could be identified. They became associated with neuronal perikarya and axons and some of them formed all of the myelin sheaths found in central and peripheral nervous tissue.

Gliogenesis can therefore be considered as a set of developmental processes that provides several varieties of glial cells with morphological, biochemical, and physiological properties that characterize them in the mature nervous system. In addition to developing their own individuality as cells, immature glia may, by forming frameworks, aggregates, or chains, influence subsequent patterns of nervous tissue growth and regional specialization. The evidence available in 1985 indicated that glial precursors appeared early and provided a primitive framework for subsequent migration and aggregation of postmitotic neuroblasts. As neurites elongated, glial processes helped guide them to their targets. As synapses formed and began to function, glia participated in the remodeling process that eliminated superfluous neurites and perikarya. Finally, normal nervous system function requires glial ensheathment of many axons and the formation of myelin sheaths by oligodendroglia in the CNS and Schwann cells in the peripheral nervous system (PNS). This complex process occurs during later stages of gliogenesis. Even though glia outnumber nerve cells by a substantial margin, much less was known about their origin and cytodifferentiation. Lack of markers and other morphological, biochemical, and physiological properties specific for glial precursors and each type of postmitotic glial cell made it difficult to define early patterns of division, growth, and cellular interaction, and what regulated them in intact nervous tissue.

Still, almost a century and a half of study produced substantial progress in our understanding of glial cells and their development. As in other areas of science, major advances were associated with the discovery of new methods. These are summarized first. Most of this account describes development of glia in the CNS. We begin with astroglia because of their known presence and significance in early embryonic stages. Some of Åström's unpublished evidence is included in this section. After consideration of oligodendroglia, microglia, and ependymal cells, we describe the development of Schwann cells, the glia found in the PNS.

In general, morphological observations of developing mammalian nervous tissues (mostly rodent) are emphasized; we have also tried to include findings relevant to cytodifferentiation, interactions, and the functions and regulation of developing glia. Of course, much has been learned about glial development by studying other species and a variety of experimental models, such as tissue culture systems, grafts, and mouse mutants. Selected aspects of these subjects are also included.

1.1 Early History

A new method is a key to open the door in a wall, which cannot be climbed, and behind which scientific treasures are found.
F. Weigert
The method is everything.
C. Ludwig

In 1839, Theodor Schwann introduced the cell doctrine (Schwann 1847) and it became a cornerstone in biology and medicine. It also opened up a new epoch in histological research. It was soon shown that all organs, except the CNS, were made up of cells that were specific to each organ. However, it would take 50 years until Cajal (1909) finally showed that cells in the CNS, like other cells in the body, were separate units. And, as we will see, it took decades for scientists to understand that the cells in the nervous system were of two kinds, neurons and glial cells. With today's knowledge in mind one can appreciate the difficulties that confronted investigators in 1839. They had to study an organ with innumerable cells of various shapes, some of which had very long extensions in three dimensions. These cells also were tightly packed and united in complicated networks. A major obstacle was the lack of adequate methods. Around 1839 the main method was microdissection, in which cells and fibers were teased out of hardened tissues, and studied in microscopes with poor optics. During the following 50-year period many improvements were implemented. Preparative methods were developed for fixing, staining, and cutting thin sections of specimens (Bracegirdle 1987). Microscopes were constructed for scanning, focusing, and transillumination of sections, and the power of resolution of lenses was increased to the limits of physical laws for light.

In 1846 Virchow discovered a new type of tissue in the CNS, which he first called "Kitte" and later "neuro–glia." In the 1863 English translation of his 1858 book *Cellular Pathology As Based upon Physiology and Pathological Histology* he wrote: "Now it is certainly of considerable importance to know that in all nervous parts, in addition to the real nervous elements, a second tissue exists … which is allied to connective tissue" (Virchow 1858). He found that it was soft, fragile, and contained some cells and nuclei, and believed that it was a binding material, which held the nervous elements together, and isolated nerve cells, fibers, and blood vessels from each other. During the following years, scientists who followed in the steps of Virchow confirmed and amplified his observations of glia in numerous publications that were reviewed by Kölliker (1863) and von Lenhossek (1895). However, none of them contested his

notion of a "binding substance." It is also noteworthy that all authors considered this substance to be a variety of connective tissue, i.e., of mesodermal origin.

Deiters, a pupil of Virchow but working at the beginning of a new era, claimed that the early ideas concerning neuroglia were a result more of heavenly inspiration than of stringent proofs. He studied cells that had been teased out from hardened pieces of CNS and then stained with carmine. His manuscript was published posthumously as a book (Deiters 1865). This book became famous for its observations and also for the beauty of its lithographic illustrations. He described and illustrated with utmost clarity neurons with dendrites, axon hillocks, and axis cylinders. He also identified other cells that, like neurons, had processes in starlike arrangements. These cells, which lacked axons, were astrocytes. His method had limitations; it showed only single cells, which had been plucked out of their environments. Many of the cells' processes had been broken and many were probably missing. Nevertheless, his observations were accepted. Astrocytes were subsequently called Deiter's cells, and Cajal (1909) considered him to be the discoverer of glial cells. In conclusion, Deiters' work was a major milestone. He helped to define the morphology of astrocytes. His findings set aside the concept of a binding substance in the CNS. Finally, his evidence and concepts were among the first to support what later became the neuron doctrine (Shepherd 1991). Clearly, Deiters and his work created a road that would later lead to a revolution in neurohistology.

When the silver impregnation method was discovered (Cajal 1895; Golgi 1894) it was possible to see neuroglial cells clearly for the first time. Entire cells could be visualized in situ, and since it was easier to stain embryonic and developing CNS, the Golgi method was used extensively in studies of gliogenesis. Early neurohistologists also recognized the existence of neuroglial fibrils in the CNS, especially its white substance. Weigert (1895) using a method he developed, demonstrated the ubiquitous presence of these fibrils and amply documented his observations in a monograph. Cajal (1913a) and his pupils subsequently developed methods for metallic impregnation of different types of neuroglial cells. Their preparations demonstrated beautifully the appearance of these cells and their general relations to neurons, axons, myelin sheaths, blood vessels, and pial surfaces, but left many important questions unanswered.

The introduction of electron microscopy in the 1950s and the subsequent development of methods for tissue processing, thin sectioning, freeze fracturing, scanning electron microscopy, and quantitative image analysis led to many important discoveries. They permitted investigators to obtain high-resolution micrographs (1–10 nm, depending on technique) that revealed for the first time the subcellular structure and organization of nervous tissue. The fine structure of cells, their nuclei, cytoplasmic organelles, and processes were examined and described (reviewed in Peters et al. 1976). Astroglia were identified as the cells that contained the fibrils, each representing a bundle of filaments with a characteristic subunit structure. Central and peripheral myelin sheaths were found to be compact, spirally wrapped extensions of oligodendroglial and Schwann cell plasmalemmal membranes. Electron microscopic studies of synapses and surface membrane relationships of neighboring cells

provided the final definitive proof for the validity of the cell doctrine in the nervous system. Moreover, they showed that there is no "nervenkitte" in the CNS and that the "interstitial material is the cells themselves" (Peters et al. 1976). Biochemical and immunocytochemical methods also offered important new approaches for studies of glia. In the early 1970s, Eng and his collaborators (Eng et al. 1971) isolated a protein from multiple sclerosis plaques that they called glial fibrillary acidic protein (GFAP). These plaques were greatly enriched in fibrillary astrocytes. Large amounts of GFAP also were found in normal fibrillary astrocytes; protoplasmic astrocytes contained less. In many immunocytochemical studies, antisera raised against GFAP were shown to stain astrocytes in normal and pathological tissue with a high degree of specificity [for example see Bignami et al. (1972) and Uyeda et al. (1972)]. Subsequent biochemical and immunocytochemical investigations established that GFAP is one of several proteins found in subunits of the 10-nm intermediate filaments that differ in composition in different cell types (Eng and DeArmond 1980; Bignami et al. 1980; Eng and Bigbee 1978; Eccleston and Silberberg 1984). Astroglial intermediate filaments contained GFAP and vimentin (Franke et al. 1978; Hynes and Destree 1978). GFAP was found to be specific for astrocytes while vimentin was present in other cell types, especially mesenchymal cells. Neuronal filaments (neurofilaments) did not contain GFAP or vimentin; they had different protein constituents that were only found in neurons. Other filamentous proteins, such as keratin and desmin, were not found in either neurons or astroglia.

Since immunocytochemical methods can be highly sensitive and specific, they were also used to study expression of proteins and other substances found in different types of glial cells during development. The first detection of a substance thought to be specific for a cell type was useful for cell identification; it indicated an important early stage in cytodifferentiation and helped define a cell's functional properties.

Thus, intensive research from 1839 through 1985 showed that neuroglia are cellular units in the mature nervous system. Many of their morphological and some of their biochemical properties were described with great precision. Although the picture was far from complete, one could safely say that in 1985 we had a fairly comprehensive understanding of these once mysterious elements. As noted above, much less was known about gliogenesis, especially the early stages.

2
Astroglia

2.1
Epithelial Cells and Neural Tube Development

Initially, epithelial cells on the surface of the embryo proliferate focally and form the neural groove. Continued multiplication of these cells and their elongation from a flat to a cuboidal or columnar shape results in the formation of the neural tube by a process called neurulation. During this process the sequestered neuroepithelium

forms the wall of a centrally located canal or cavity, which contains enclosed amniotic fluid (Fig. 1A). At this stage (e.g., in rat fetus), the surface ectoderm and the neural wall have the same basic appearance. Both structures consist of a single layer of cells that are joined to each other near their luminal ends by junctional complexes. Their opposite ends rest upon a basement membrane that separates them from the mesenchymal tissue. Even at this early stage, the mesenchyme contains blood vessels that later will invade the wall of the neural tube.

The simple shape of the early neural tube is soon modified locally along the neuraxis. Investigators studied changes in cell morphology, arrangement, and number to learn what roles different types of developing cells have in creating these regional differences. These modifications appear early and reflect the future cellular organization of the CNS.

Some years ago, one of us (K.E.Å.) developed methods to reduce preparative artifacts in embryonic rat CNS processed for light and electron microscopy (Åström and Webster 1990). The results from a series of studies on the early development of the telencephalon are summarized and illustrated here as examples of gliogenesis and as a basis for discussion of the identification and function of early glial cells. Our studies strongly suggest that epithelial cells, which are the main cellular constituents of the early neural tube wall, are astroglial precursors. Before neuroblasts are recognizable, these primitive glial cells form frameworks resembling major CNS subdivisions in size and shape. For example, in rats at embryonic day E12 (Fig. 1B), major subdivisions of the cerebrum are visible including the hemispheres, lateral ventricles, foramina of Monroe, the interhemispheric fissure, and precursor cells of the choroid plexus. Nevertheless, at this stage these structures contain few if any nerve cells or their precursors. The subdivisions are formed by

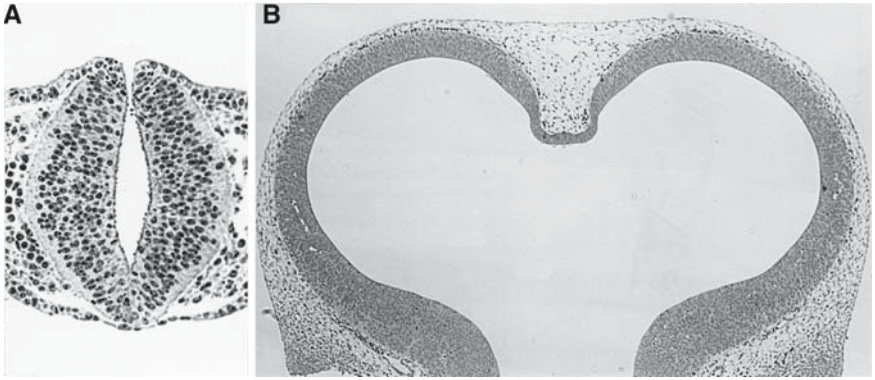

Fig. 1 **A** Phase micrograph, neurulation in a 9-day-old rat embryo. The surface epithelium in the *upper part* of the picture is continuous with the pseudostratified epithelium of the neural tube. The central canal encloses amniotic fluid. **B** Phase micrograph, coronal section through the forebrain of a 12-day-old rat embryo. Expanding hemispheric walls are joined by the area choroidea in the midline

glial scaffolds that give shape to the early nervous system and will help neuroblasts migrate to specific destinations.

In light and electron micrographs of epoxy-embedded tissue, the early neural wall contains two morphologically distinct types of epithelial cells: (1) mitotic cells, spherical in shape, are located along the ventricular surface, and (2) epithelial (columnar) cells constitute the rest of the neural wall.

Of course, developmental patterns in CNS differ depending on the species and region examined. Still, a century of studies identified important features of early development shared by most regions in many species. In the following description of the development of glial cells we have arbitrarily identified three consecutive stages.

2.2 CNS Development

2.2.1 Stage I: Columnar Cells

The dorsolateral part of the neopallial wall of the rat contains only epithelial (columnar) and mitotic cells by E10–E12 (Fig. 1B). During the latter part of this period, the first neuroblasts appear and blood vessels penetrate into the neural wall from the surrounding mesenchymal tissue.

At early stages of development, the nuclei of the epithelia–columnar cells are evenly distributed across the neural wall (Figs. 1, 2, and 3). The epithelium is said

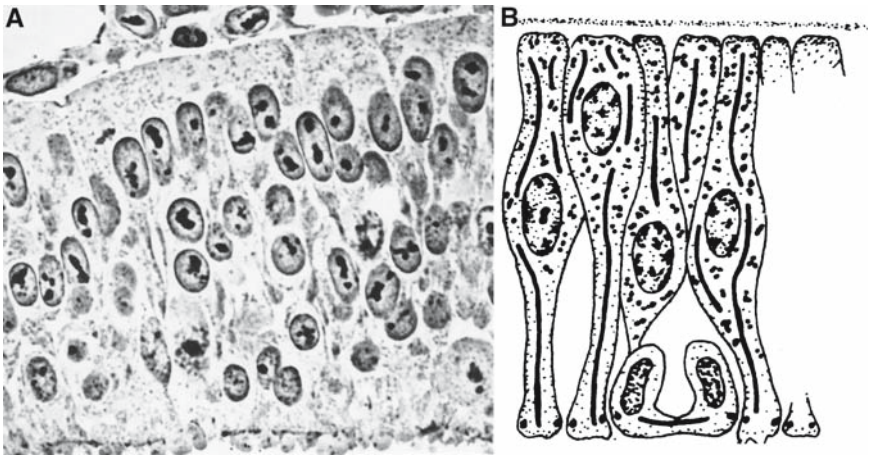

Fig. 2 **A** Phase micrograph of the neural wall in an 11-day-old rat embryo. The outer (pial) and inner (ventricular) aspects of the neural wall are shown at the *top* and *bottom* of this and the following figures. Connective tissue with blood vessels covers the pial surface. **B** The diagram illustrates the shape and general appearance of cells in the early neural wall. Microtubules (*solid lines*) have an axial orientation in the epithelial cells, which span the entire width of the wall. The inner ends are connected by junctional complexes (*dots*) and the outer ends are covered by basement membrane

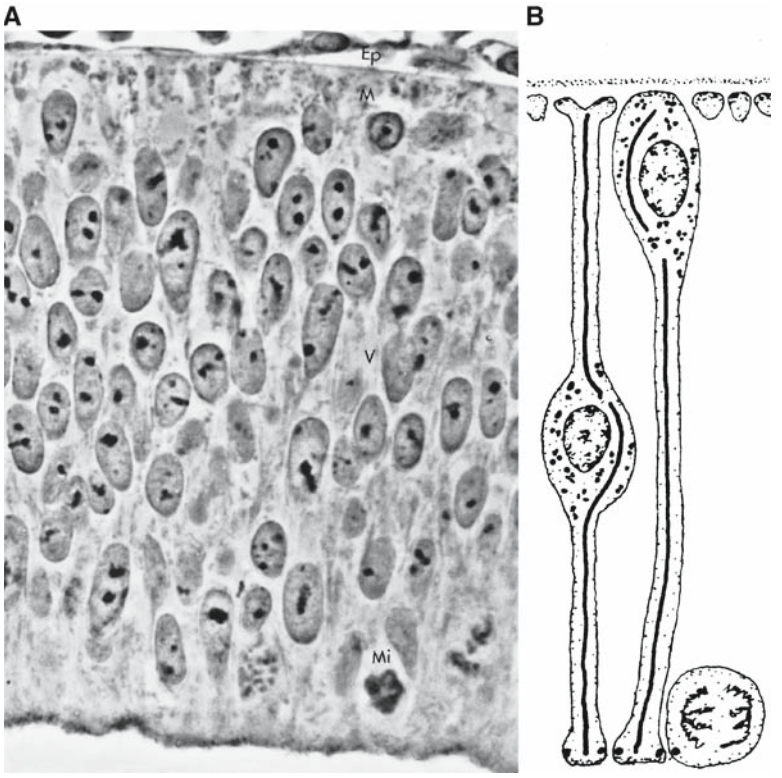

Fig. 3 **A** Phase micrograph of pseudostratified epithelium in the telencephalic wall of a 12-day-old rat embryo. *Ep*, surface epithelium; *M*, marginal zone; *Mi*, zone with cells in mitotic division. **B** Diagram showing bipolar shape of columnar cells that make up the pseudostratified layer of cells. Their general appearance is similar to that of the epithelial cells at an earlier stage of development (Fig. 2). Elongation of cells causes thickening of the neural wall

to be pseudostratified since it has the appearance of being stratified but in reality is composed of a single layer of cells (Sauer 1935b). As the cortical plate and intermediate zone [terminology of the Boulder Committee (1970)] develop and grow in thickness, the nuclei of columnar and the subsequently formed radial glial cells become more concentrated in the subventricular zone (compare Figs. 3 and 4A with 6).

At this early stage of development, the neural wall has only two zones: the ventricular zone (Fig. 3) with mitotic cells and the zone with perikarya of the columnar cells. There is no mantle zone or cortical plate as yet. Up to E12, the telencephalic wall in the rat is made up of columnar cells exclusively, except for the mitotic elements (Fig. 3). Late in E12 or early E13, cells with different morphological features appear in the superficial marginal zone; these cells are the first in the telencephalic wall that can be identified as neurons (Fig. 4). They are rounded or oval, develop bipolar short processes, and have an orientation that is parallel with the outer surface and perpendicular to that of the columnar cells. They will develop into neurons and form layer I in the 6-layered cortex (Åström 1967).

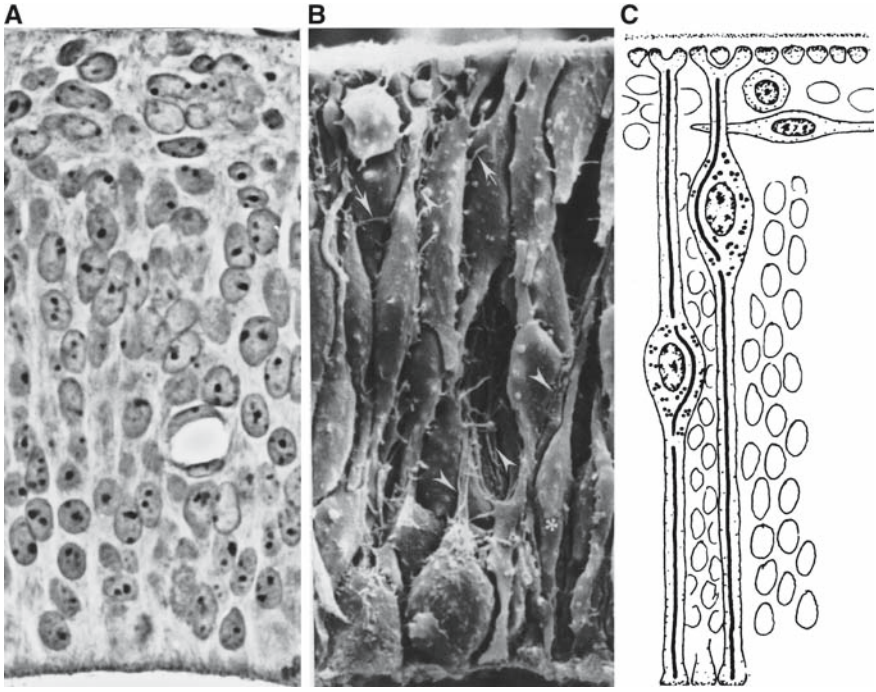

Fig. 4 **A–C** Phase micrograph (**A**), scanning electron micrograph (**B**), and diagram (**C**) of the telencephalic wall in a 13-day-old rat embryo. Neuroblasts that will form the first layer in the isocortex are interposed between endfeet of columnar cells at the pial surface and the perikarya of columnar cells in the ventricular zone of the telencephalic wall. [Panel **B** is from Seymour and Berry (1975) and is reprinted with permission]

Scanning electron micrographs of fixed, fractured cerebral vesicles from rat embryos at this stage of development also showed that the main part of the neural wall still is made up of the perikarya of columnar cells (Fig. 4B; Seymour and Berry 1975). Their inner processes were seen between the mitotic cells near the ventricle. The marginal zone was occupied by radially directed outer processes of columnar cells that were molded and shaped by neurons in the incipient cortical plate.

In the earlier stages of development, most epithelial cells are wedge-shaped. Their inner processes are thinner and extended between mitotic cells to the ventricular surface (Fig. 2). Later, as the neural wall thickens, epithelial cells elongate; they become bipolar and more columnar in shape (Fig. 3). Outer and inner processes extend in opposite directions from the central region that contains the nucleus. The radial orientation of these bipolar cells is apparent. Their long axes are perpendicular to the ventricular and pial surfaces. Because of their shape at this and later stages, we call them columnar cells. Light and electron microscopic observations have suggested that columnar cells are derived from and are homologous to the epithelial cells, the main constituent of the early neural tube wall.

Light microscopic studies of tangential sections at the ventricular surface revealed that columnar cells form a mosaic pattern in which there are no apparent

openings (Sauer 1935a, 1937). Junctional complexes, which were called terminal bars by the light microscopists, connect mitotic cells and the apical ends of the inner processes of columnar cells (Fig. 11A).

After mitotic cell division, one or both daughter cells grow centrifugally until they reach the pial surface. At the same time they remain anchored at the ventricular surface. Hence, the ventricular lining remains intact during the multiplication of cells.

At the neural wall's outer surface, columnar cells terminate in one or several branches with conically shaped endings (Fig. 4C and Fig. 5). The outer surface of these "end knobs" are flattened against and covered by the basement membrane (Fig. 5). They contain ribosomes, smooth endoplasmic reticulum, and microfilaments but no microtubules. Terminal processes of columnar cells and the basement membrane form the outer surface of the developing brain and thus constitute an early pial–glial membrane. There are no junctional complexes between the terminal endings here, suggesting that the external surface is the main route for penetration and exchange of substances required for development of cells in the early neural wall.

The early nerve cells in the marginal zone (Fig. 5) are never in direct contact with the surface of the brain; they are separated everywhere from this by a layer of columnar cell endfeet. Furthermore, columnar cells provide a template in which neurons can be positioned and begin cytodifferentiation after having migrated from the mitotic zone.

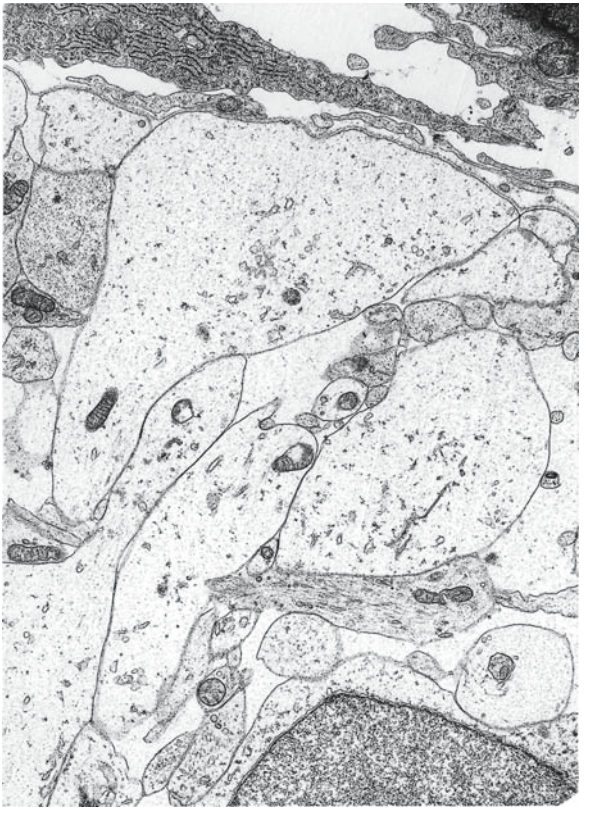

Fig. 5 Outer part of the telencephalic wall in a 12-day-old rat embryo. Conically shaped endfeet of columnar cells form a limiting layer at the pial surface that separates neuroblasts from the basement membrane

Other aspects of the fine structure of columnar cells are similar to those of radial glial cells, which are described in the next section.

2.2.2 Stage II: Radial Glial Cells

The major event in this stage is the appearance of postmitotic neurons. They migrate from their site of origin near the ventricles toward the periphery where in telencephalon they form the cortical plate (Fig. 6). There they develop processes (neurites) that will become dendrites and axons. The latter will fill the intermediate zone (Fig. 6, I) of the neural wall, become myelinated, and form the "white matter" of the developing telencephalon. A subventricular zone (Fig. 6, S) appears beneath the ependyma and the neural wall is penetrated by blood vessels. As these changes occur, the columnar cells described above become radial glial cells now identified as primitive astroglia by several lines of evidence.

This stage occurs in midgestation in vertebrates. It starts at E13–E14 in fetal rat (gestation time 21 days) and E40–E45 in sheep (Åström 1967). In the brain of a fetal monkey, the migration of neurons lasts from about E40–E100 (Rakic 1974). Total

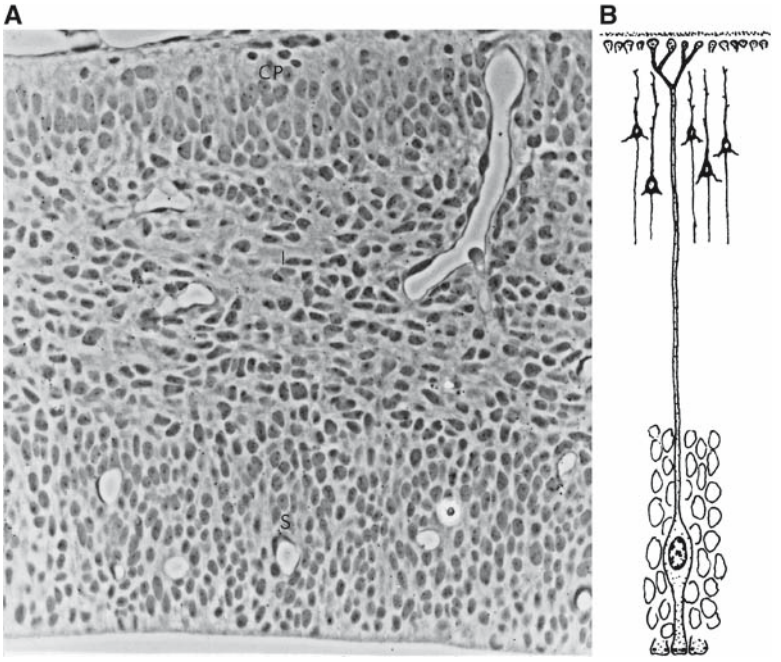

Fig. 6 **A, B** The telencephalic wall in a 16-day-old rat embryo. **A** This phase micrograph shows an intermediate zone (*I*) interposed between the cortical plate (*CP*) and the subventricular zone (*S*). Several blood vessels in mesenchymal tissue covering the pial surface have penetrated the telencephalic wall. **B** Diagram showing the perikaryon of a radial glial cell located in the subventricular zone. Its outer process penetrates the telencephalic wall, branches, and terminates in knobs at the pial surface

gestation times for sheep and monkeys are 150 and 165 days respectively. In human fetal brain, migration starts after 6 weeks and ends at 18–26 weeks (Ikuta et al. 1979).

The cellular picture during this stage is dominated by bipolar cells with long slender processes that penetrate the neural wall. They can only be seen in their entirety in preparations stained according to Golgi's silver impregnation methods. They have been studied in different species during the past 100 years. Pioneering work was carried inter alia by His (1889), Retzius (1893, 1894), von Lenhossek (1895), Kölliker (1896), and Cajal (1909). Among the names given to these bipolar cells were: spongioblasts, matrix cells, radial cells, epithelial cells, and fetal ependymal cells. A special form was called a tanycyte. Due to their structure, orientation, identification as glia, and function, Rakic (1971) called them radial glial cells. That term is used here.

Thus, early anatomists provided excellent comprehensive descriptions of long, bipolar, radially oriented cells in mammalian and submammalian species during embryonic and postnatal development (Fig. 7). Their precise and detailed observations are supplemented by awe-inspiring illustrations. Also, many of their conclusions regarding radial glial cell function have been substantiated by more recent observations.

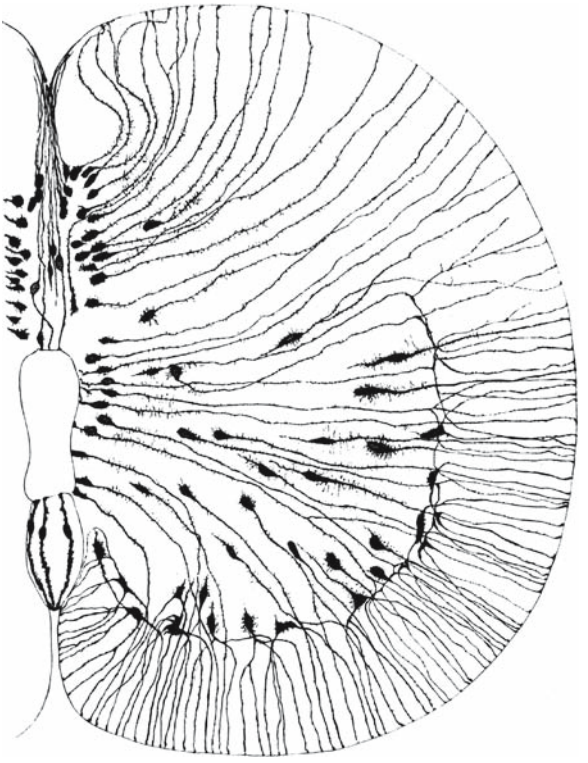

Fig. 7 Golgi-stained radial glial cells radiate through the spinal cord of a 12-cm-long dog embryo. Some cells contact both the pial and the ventricular surfaces. Others have lost contact with the ventricular surface. Other branches of radial glial cells penetrate the white matter at the periphery of the cord. [Reprinted from Retzius 1893, Figure X:3]

After an interval of many years, the Golgi method has again been used for the study of radial glial cells. This reflects a renewed interest in these elements as regards their origin, cytodifferentiation, protein expression (mainly GFAP), and function. Thus, the presence of these cells has been studied in fetal specimens of sheep (Åström 1967; see Figs. 8, 9, and 10), rabbit (Stensaas and Stensaas 1968a), rat (Peters and Feldman 1973), and monkey (Schmechel and Rakic 1979a).

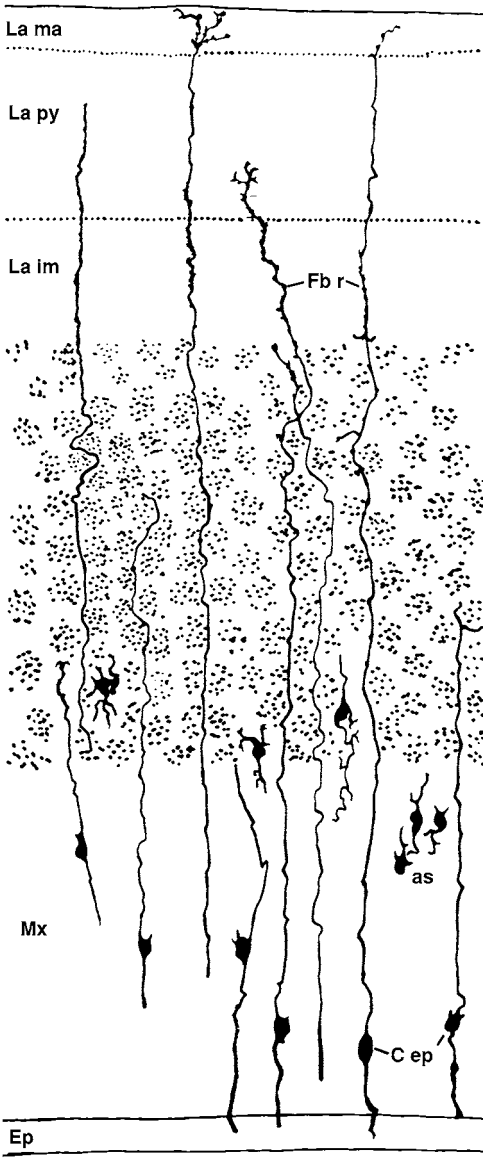

Fig. 8 Golgi-stained radial glial cells (*C ep*) in the telencephalic wall of lamb (estimated fetal age, 48 days) have perikarya in the subventricular zone. Their outer processes (*Fb r*) wind among bundles of axons in the intermediate zone and terminate in branches forming "bouquets" at the pial surface. Astroblasts (*as*) are present also. (Reprinted from Åström 1967, with permission)

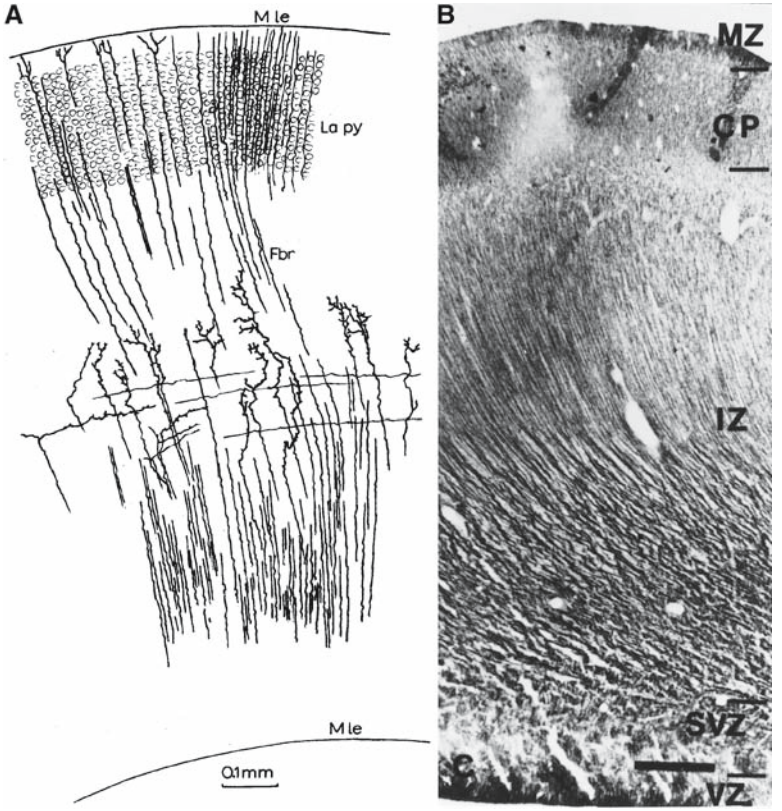

Fig. 9 **A** Golgi-stained radial glial fibers (*Fb r*) in lamb (estimated fetal age, 54 days) have an S-like course through the telencephalic wall. Some fibers terminate in the intermediate zone. (Reprinted from Åström 1967, with permission.) **B** Processes of radial glial cells immunostained with GFAP antiserum curve through the cerebral wall of fetal monkey at E70. *CP*, cortical plate; *IZ*, intermediate zone; *MZ*, marginal zone; *VZ*, ventricular zone. (Reprinted from Levitt and Rakic 1980, with permission)

The above Golgi studies showed that in mammals these radial glial cells are present in all parts of the central nervous system during embryonic, fetal, and early postnatal life but not later. In submammalian vertebrates they persist in adult life (Muller 1900). Although some aspects of their appearance may differ, depending on species, CNS region, and developmental stage, the basic structure of these cells is the same and suggests that these cells are homologous to and in part derived from columnar cells, which are described in the previous section.

Early in this developmental stage, most radial glial cells, which dominate the picture in Golgi sections, span the neopallial wall from the ventricular to the pial surface (Fig. 8). Perikarya are situated in the subventricular zone and there is little perinuclear cytoplasm. The relatively thick, inner processes vary

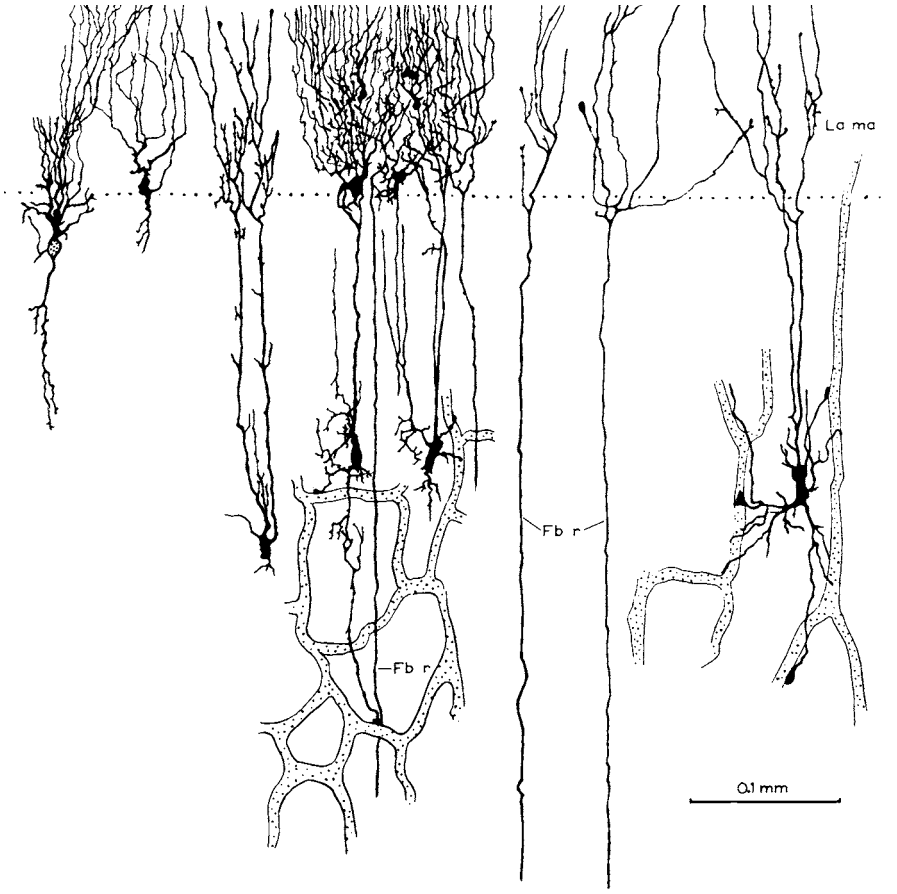

Fig. 10 Golgi-stained developing astrocytes connected to blood vessels in the cerebral cortex of lamb at an estimated fetal age of 62 days. Their outwardly directed processes have the same general orientation as radial glial fibers (*Fb r*). Perikarya of astroglia with a shrub-like appearance are located at the border (*dotted line*) of the second layer of the cortical plate and the marginal zone where all cells terminate in a profuse network of branches. (Reprinted from Åström 1967, with permission)

in caliber, have a rough surface, and extend to the ventricular surface. The outer processes are thinner, often take a tortuous course between bundles of axons in the intermediate zone, and reach the outer surface. There they form a group of terminal branches with growth cone-like knobs located on the inner surface of the pial basement membrane. Some outer processes have short collateral branches and various protrusions. The radial fibers later become covered with increasing numbers of lamellate expansions and small branches, which give them a hairy appearance. This is seen especially in the intermediate zone, the deep part of the cortical plate, and the marginal zone. Still later, the terminal branches increase in

number, forming "bouquets" in the marginal zone of the cortical plate. The radial glial fibers (Figs. 9 and 14) become elongated due to the increased thickness of the neopallial wall and curved while coursing through the wall thickened by an expanded cortical plate (Dickson et al. 1983; Rakic 1971; Schmechel and Rakic 1979a). According to Schmechel and Rakic (1979a), in the occipital lobe of fetal monkeys, the maximal length of radial fibers may be 20 mm—the longest glial cell described in any species.

Using an immunocytochemical method to demonstrate GFAP, Levitt and Rakic (1980) assessed astroglial populations in different regions of the CNS in fetal monkeys. In the visual cortex of the telencephalon, the radial glial fibers were first seen around E47. The density of stained fibers then increased and reached a peak around E70 when about 17,000 fibers were estimated to pass through a 1-mm^2 area at the boundary between the cortical plate and the intermediate zone (Fig. 9B). This peak coincides with the major wave of neuronal migration. The development of radial glial fibers and generation and migration of neurons occur primarily during the middle part of gestation. Eventually, when the neural wall has reached its optimal thickness and the migration of nerve cells has ceased, the radial glial cells become transformed into astrocytes and ependymal cells.

The fine structure of radial glial cells is similar to that of columnar cells and has been described by Wechsler and Mellor (1967), Stensaas and Stensaas (1968a), Hinds and Ruffett (1974), Rakic (1971), Peters and Feldman (1973), Henrikson and Vaughn (1974), Seymour and Berry (1975), Meller and Tetzlaff (1975), Raedler and Sievers (1976), Choi and Lapham (1978), Choi (1981), and K.E. Åström (unpublished observations). It is summarized here.

Apical surfaces of inner processes that line the ventricle are flat or somewhat elevated (Fig. 11A). Cilia project from some of them and their number increases as development proceeds (Fig. 11C). Microfilaments are concentrated near the ventricular surface, where they form bands or collar-like arrays around the apical ends of the inner processes (Fig. 12). The arrays of microfilaments are clearly seen in electron micrographs (Paterson et al. 1973) but it should be remembered that Sauer, on the basis of his light microscopic studies, concluded much earlier that a "network of fibrils," which he called the terminal web, is located beneath the terminal bars.

Junctional complexes connect mitotic cells and the inner ends of radial glial cells (Fig. 11A). In our electron microscopic studies, the spaces between these cells appear to be completely obliterated in spots (Fig. 11D). There are also belt desmosomes in association with the collars of microfilaments. The data, as well as experimental observations, suggest that the junctional complexes form a relatively impermeable barrier and prevent penetration of substances and fluid from the ventricles to extracellular spaces in the embryonic CNS. On the other hand, in the cerebral vesicles of 13-day-old mouse embryos, Hinds and Ruffett (1974) saw junctions with different gap widths but no tight junctions or spot desmosomes in the terminal bars. To clarify this point, permeability has to be studied after an electron dense marker has been injected into the cerebral ventricle.

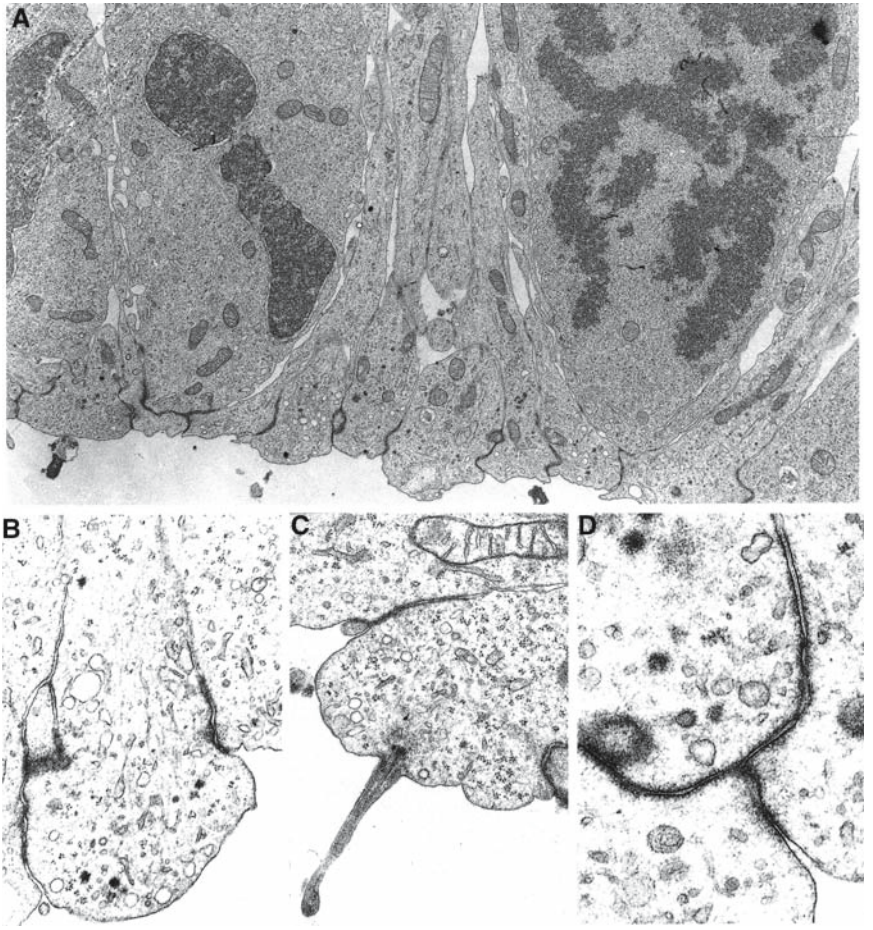

Fig. 11 **A–D** Electron micrographs of the ventricular part of the telencephalic wall in a 13-day-old rat embryo. **A** Slender processes of radial glial cells are interposed between two cells in mitotic division. All cells are joined by junctional complexes. **B** Ventricular ends of radial glial cells' inner processes contain microtubules, ribosomes, vesicles, and profiles of smooth endoplasmic reticulum. **C** A cilium protrudes into the ventricular cavity. **D** Junctional complexes are present between inner ends of radial glial cells. The space between adjacent cells is completely obliterated in spots

The radial glial cell nucleus is oval, contains finely dispersed chromatin, and usually has a prominent nucleolus. The Golgi apparatus (Fig. 12) is always found between the nucleus and the ventricular lumen. Elongated, branching mitochondria are located in all cytoplasmic regions. The perinuclear cytoplasm and inner processes contain a few cisternae of granular endoplasmic reticulum and most of the free ribosomes. The remaining ribosomes are found in varicosities along the surfaces of the outer processes.

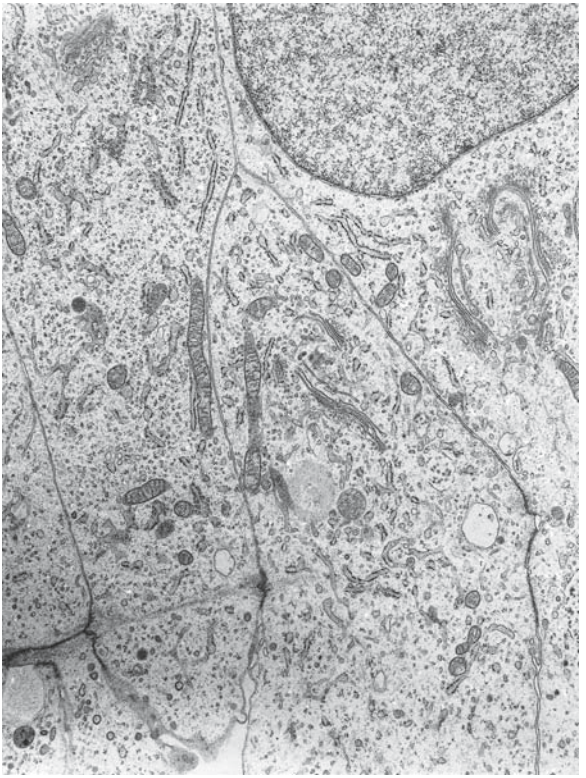

Fig. 12 Electron micrograph of inner parts of radial glial cells in the telencephalon of a 16-day-old rat embryo. The cytoplasm contains elongated mitochondria, ribosomes, vesicles, and profiles of granular endoplasmic reticulum. The Golgi apparatus is always located between the nucleus and the ventricular surface. Junctional complexes and the terminal web of microfilaments (best seen on the *left side* of the figure) form the terminal bars described in light microscope preparations

Columnar and radial glial cells contain longitudinally oriented microtubules that extend from the ventricular surface (Fig. 11B) around the nucleus into the outer processes but not into the terminal branches at the pial surface. Microtubules with a similar orientation and structure have been seen in spinal cord cells of mice as early as E102, i.e., shortly after the formation of the neural tube (Herman and Kauffman 1966). Microtubules have also been noted in the developing motor neurons of the chick (Lyser 1964, 1968).

Compared to earlier stages, the terminal knobs at the pial surface are now more electron-lucent (Fig. 13). Together they constitute an external limiting layer of protoplasm which prevents nerve cells from having direct contact with the surface. The predominant organelles in the outer processes are profiles of smooth endoplasmic reticulum, clusters of intermediate filaments (8–9 nm in diameter) and occasional

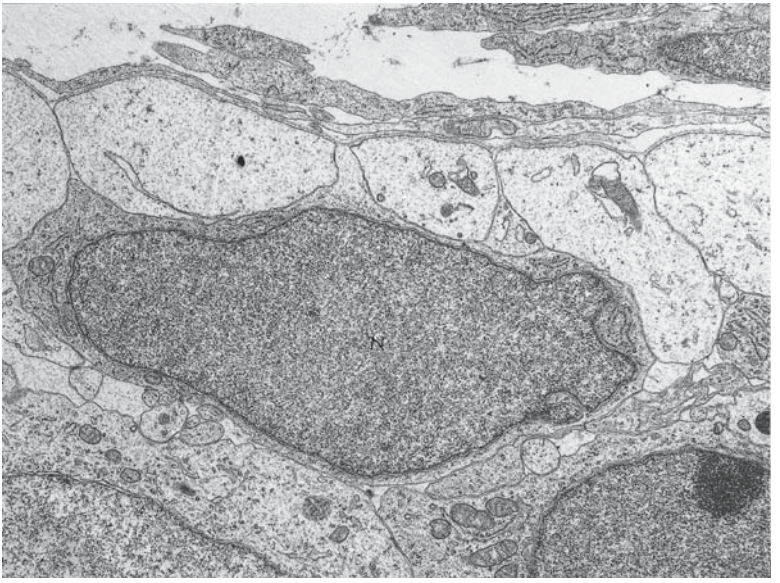

Fig. 13 Electron micrograph of the cortical surface of a 16-day-old rat embryo. Electron-lucent conical ends of radial glial cells are pressed against the basement membrane. They form templates for neuroblasts (*N*) and prevent them from contacting the surface

glycogen granules. The endfeet are PAS-positive. As noted by Choi and Lapham (1978), outer processes of radial glial cells are best identified by the presence of a relatively electron-lucent matrix, 8- to 9-nm filaments, and glycogen granules. As mentioned earlier, microtubules are not present.

2.2.3 Stage III: Astroblasts, Astroglia

After the migration of nerve cells has ceased, the neurons and their dendrites, axons, and axon collaterals continue to grow and develop in situ and establish connections with other neurons during the remainder of the fetal period. In the neopallium, the cortical plate and intermediate zone increase in thickness, the subventricular zone decreases, and the ventricles become smaller (Fig. 14).

The shape of radial glial cells is modified during this stage (an incipient change was already noted in the latter part of stage II) and some of them now have features of astroblasts. Eventually the radial glial cells become transformed into astroglia as first suggested by Cajal (1909). Astroblasts with stellate perikarya and short processes appear in all layers of the neural wall except the marginal and ependymal zones (Fig. 8). Many processes are attached to blood vessels. Astroblasts first appear in fetal sheep around E58, in monkeys at E64, and in man at the end of the first trimester. During the second half of gestation and the first postnatal

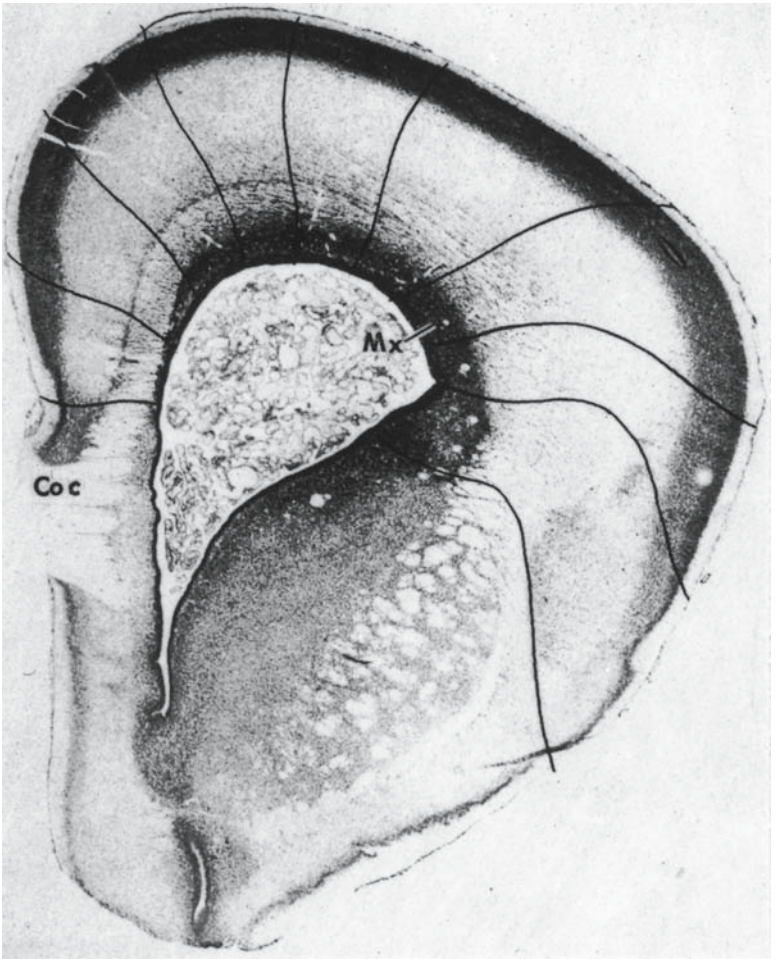

Fig. 14 Light micrograph, cresyl violet-stained coronal section through the brain of a lamb (estimated fetal age, 57 days). The course of radial glial fibers is indicated by *lines*. The cortical plate and intermediate zone have increased and the subventricular zone has decreased in comparison with earlier stages (see Fig. 6). (Reprinted from Åström 1967, with permission)

weeks, astroblasts increase in number and are transformed into astroglia as radial glial fibers and transitional forms decrease in number. The radial glial cells disappear during the first postnatal week in man (Cajal 1911; Retzius 1893) and between postnatal day (P)20 and P50 in the monkey (Schmechel and Rakic 1979a). Postnatally, the numbers of astroblasts and astrocytes increase dramatically (Ichikawa and Hirata 1982; Parnavelas et al. 1983).

Studies using the Golgi method suggest that the transformation of radial glial cells occurs as illustrated diagrammatically in Fig. 15 and as described below. Many radial glial cells are successively detached from the pial surface and their processes

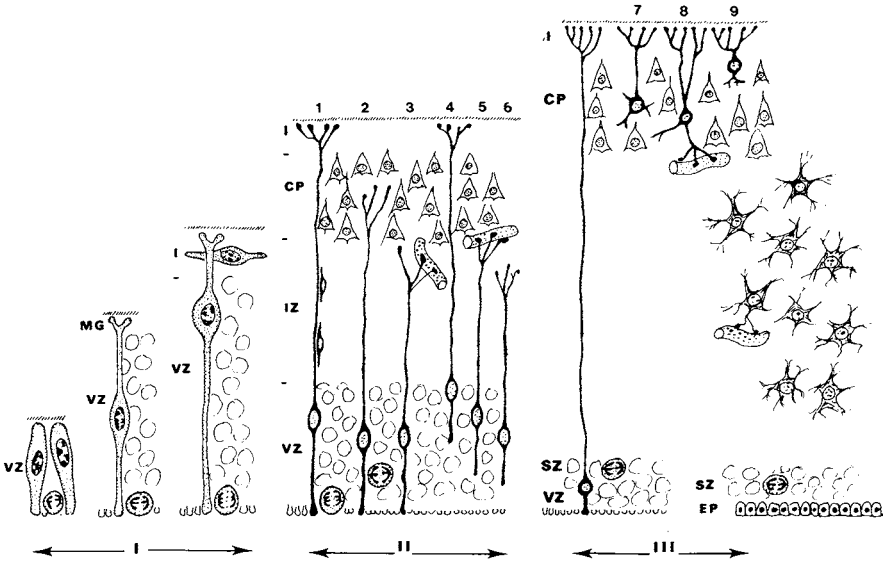

Fig. 15 Diagram illustrating the development of astroglial cells. Epithelial cells elongate and become columnar cells during stage I, and single neuroblasts appear in the marginal zone at the end of this stage. Columnar cells become radial glial cells during stage II when the main migration of neuroblasts occurs. The radial glial cells gradually become transformed into astroglia during stages II and III (see text). Other astroglia develop directly from glioblasts during the latter part of gestation and during early postnatal development. *CP*, cortical plate; *EP*, ventricular epithelium; *IZ*, intermediate zone; *MG*, marginal zone; *SZ*, subventricular zone; *VZ*, ventricular zone

with ramifying branches are pulled toward the cell bodies (Fig. 15; Abney et al. 1983). The distal parts terminate in the cortical plate or in the intermediate zone (Figs. 8 and 9). Some of them are connected to blood vessels and others seem to end freely in the parenchyma (of course, the possibility exists that some of the latter are growing and are on their way toward the pial surface). In older fetuses, the terminal arborizations are more extensive; their branches and endfeet form sheaths around blood vessels.

Other radial glial cells are freed from contact with the ventricular surface (Fig. 15). Their cell bodies are "pulled" outwards or, more likely, their nuclei migrate within the cytoplasm in a centrifugal direction. The inner detached processes contract and trail after their cell bodies (Fig. 8). They may disappear later. The outer ends of these cells end freely in the parenchyma or contact blood vessels, the pial surface, or both.

Many cells with attachments to the pial surface have cell bodies in the cortical plate (Fig. 15; Altman 1963; Altman and Bayer 1978; Anders and Brightman 1979). The majority of them are similar to the radial glial cells in that they have a radial orientation and terminate in multiple branches (Fig. 10). Each cell body emits two or more outer processes that divide at the border between the marginal zone and

the second stratum of the cortical plate. Shorter processes connect the cell body to adjacent blood vessels. Other cells with perikarya at the inner border of the marginal zone emit many shrub-like processes.

A glance at Fig. 10 shows that these cells and the radial glial cells that still remain at this stage of development all terminate in a profuse network of branches that fills a large part of the marginal zone. The end knobs of the branches form the outer surface, the glial cuticle of the brain, and the meshes give support to and isolate the axons that run parallel to the surface (not shown in the figure).

The transition from radial glial cells to protoplasmic and fibrillary astrocytes continues. Eventually, the radial glial cells disappear. The mature cells have no similarities with their cells of origin.

The ventricular zone constitutes the main part of the early telencephalic wall. It is homogeneous and contains the perikarya of the epithelial and columnar cells. They form a pseudostratified epithelium and span the entire width of the wall (Figs. 2–4). These cells are the progenitors of all neurons and neuroglial cells. After the appearance of the intermediate zone and cortical plate during stage II, the ventricular zone occupies a relatively small part of the wall (compare Figs. 4 and 6). Up to a certain point of development, this zone is still homogeneous and only contains the perikarya of the radial glial cells, the homologs of the columnar cells. Radial glial cells, like the columnar cells, stretch across the neural wall (Figs. 6B and 15; Åström 1967). Then, cells of a new type appear in the outer part of the ventricular zone where they form the subventricular zone. These proliferating cells have a stable position and remain rounded in interphase. Although the radial glial cells eventually become astrocytes, most astroglia seem to emanate from cells in the subventricular zone. These proliferating cells also are progenitors of neurons and oligodendroglia. The ventricular zone disappears as the radial glial cells are converted into astrocytes during stage III, but the subventricular zone remains through the early postnatal period. This zone, called the subependymal layer by early light microscopists, is especially prominent around the lateral ventricles in the vertebrate brain. More recent studies by light and electron microscopy, and by autoradiography (reviewed by Privat 1975), show that the zone is densely populated by small, dark, rounded cells, many of which incorporate ^{3}H-thymidine. The cells generally have an immature appearance; an irregular nucleus is surrounded by scanty cytoplasm that contains many ribosomes, few cisternae of endoplasmic reticulum, few microtubules, and no filaments. The multiplication of cells in the subependymal zone is balanced by migration. In rats, the zone is reduced during the first postnatal month, but a few of these immature cells are still found in adults.

The lack of neurons and a relatively simple structure has made the optic nerve suitable for studies of gliogenesis (Peters and Vaughn 1967; Skoff 1980; Skoff et al. 1976a, b; Vaughn 1969; Vaughn and Peters 1967; also, see references in the section on tissue culture studies). Initially, the optic stalk has the same structure as the early neural wall; it consists of cells that radiate from a central lumen to the periphery. After the closure of the central canal at E16, the ventricular cells become transformed into astroglial cells that spread throughout the optic nerve.

The proliferation of astrocytes then increases, reaches a peak around P5, and subsides during the following weeks. Oligodendrocytes appear and peak later. On the basis of the uptake of tritiated thymidine and the general morphology as seen in the electron microscope, it is possible to differentiate between astroblasts, oligodendroblasts, and their source, the glioblast. A "blast" cell is proliferating and a "cyte" cell is postmitotic.

The development of neurons and macroglial cells in the CNS differ in several respects (Skoff et al. 1976b). The neurons emerge from undifferentiated cells near the ventricular lumen and their movements along radial gradients are precise in terms of space and time. They do not divide when stimulated. On the other hand, neuroglial cells originate from astroblasts and oligodendroblasts; they can divide one or several times (Meinecke and Webster 1984). The proliferation takes place throughout the neural wall. Macroglial cells can also divide when stimulated, and it is likely that differentiated astrocytes retain the ability to divide during adult life (Kaplan and Hinds 1980).

The astroblasts in the rat optic nerve are rich in organelles (Skoff et al. 1976a, b; Vaughn and Peters 1967). They and the early astrocytes contain microtubules but no visible filaments (Peters and Vaughn 1967). Fewer microtubules and other organelles are evident as glial filaments increase in number. The cytoplasm of mature fibrous astrocytes is mostly filled with bundles of filaments. Differentiation of astrocytes into fully mature cells takes 2–3 weeks (Skoff et al. 1976b).

The maturation rate of astrocytes in the rat visual cortex and optic nerve is similar. The morphology of astrocytes at corresponding stages of development is also similar in the two sets of preparations (Parnavelas et al. 1983).

The presence of glial filaments is generally considered to be the most typical feature of astrocytes, and their appearance during development has been taken as a criterion of differentiation. Other signs of maturation can be found in the plasma membrane. Anders and Brightman (1979) used the freeze-fracture technique to investigate the subpial astrocytic processes in the cerebral cortex of rats. At E18, the cytoplasm contains 8- to 9-nm filaments. The plasma membrane of foot processes adjacent to the basement membrane begins to acquire orthogonal arrays of small particles as a sign of differentiation between E19 and E20. From E20 to maturity, the foot processes will mature by constant rearrangement and addition of these arrays. Other features of the plasma membrane that are distinctive for developing glial cells will be described in the section on tissue culture.

2.3
Glial Filaments: GFAP and Vimentin Expression

During development, glial filaments and their proteins appear in astrocytes as signs of differentiation and maturation. Bignami and Dahl (1973, 1974a, b, 1975) were the first to use anti-GFAP sera in an immunocytochemical method that was used to study the development of astrocytes. In rats, anti-GFAP immunofluorescence appears focally at the pial surface of the neopallium during the first postnatal days

and a continuous layer is seen there by the ninth day (Bignami and Dahl 1974a). Perivascular glial membranes are formed later. In the adult animal, the GFAP-positive elements have an appearance and a distribution similar to that of fibrous astrocytes in adult man as seen in preparations stained according to the Weigert method.

In almost all studies employing well-characterized antisera, GFAP is localized in astrocytic cells. Choi and Lapham (1978) found evidence of GFAP protein in radial glial fibers in the cerebrum of human fetuses aged 10 weeks or more. Electron microscopic examination of these cells in specimens with a fetal age of 7–18 weeks shows features that are typical for astroglia. The cytoplasm of these cells is relatively electron lucent and it contains 8- to 9-nm filaments and some glycogen granules. They form pericapillary investments and terminate in expansions at the pial surface.

In an immunocytochemical study of gliogenesis in fetal monkeys, Levitt and Rakic (1980) found that the structure, density, and distribution of astroglial cells as well as the first appearance of GFAP in them are unique for each CNS region at various stages of development. GFAP first appears in radial glial fibers in the spinal cord and brainstem by E41, in the diencephalon by E45, and in the cerebellum and telencephalon by E47. The development of stained fibers in the telencephalon is described above (Fig. 9B). Other observations of interest in Levitt and Rakic's paper are: GFAP is distributed throughout the main shaft of the radial glial fibers; GFAP staining allows quantitative assessment of glial populations; GFAP staining shows that radial glial fibers are more abundant than is evident in Golgi-stained preparations; radial glial fibers and neural cells co-exist from a relatively early age; and radial glial fibers are present in great numbers during periods of peak migration.

Although GFAPs are present in radial glial fibers of man and monkey, staining has failed in rodents and chickens. Thus, Bignami and Dahl (1974b) were unable to demonstrate GFAP in the medial frontal cortex of newborn rat. However, positive staining could be demonstrated after injury. Likewise, radial glial fibers did not stain for GFAP in the cortex of newborn rats (Dahl et al. 1981) and in the telencephalon of 11-day-old mouse embryos (Schnitzer et al. 1981). Finally, Tapscott and coworkers (1981b) found anti-GFAP immunoreactivity in astrocytes but not in radial glial cells of the chicken CNS.

The presence and distribution of GFAP in developing astroglial cells have also been studied in the cerebellum(Bignami and Dahl 1973), hippocampus (Bignami and Dahl 1974a), and optic nerve of the rat (Dixon and Eng 1981), in the spinal cord of chicken (Bignami and Dahl 1975) and man (Choi 1981), and in the cerebral white matter of children (Takashima and Becker 1983).

The above papers only deal with GFAP (M_r 51,000). Other investigations (Franke et al. 1978; Hynes and Destree 1978) have shown that developing astroglial cells also may contain another protein constituent of glial filaments called vimentin (M_r 58,000).

Schnitzer and coworkers (1981) detected vimentin but not GFAP in radial glial fibers of the telencephalon and in ventricular cells and blood vessels of 11-day-old mouse embryos. However, the cells Schnitzer and collaborators called radial fibers seem to correspond to what we have called columnar cells (development of the CNS is similar

in rats and mice). Astrocytes in adult mice were positive for both vimentin and GFAP. They concluded that vimentin can be used as a marker for early glial differentiation since it can be detected before the onset of GFAP expression in astrocytic and ependymal cells of mouse neuroectoderm (see also Houle and Fedoroff 1983).

Vimentin, but not GFAP, is present in radial glial fibers in the cortex of newborn rats, whereas in mature astrocytes less vimentin is detected than GFAP (Dahl et al. 1981). Immunocytochemical staining of radial glial fibers in mouse cerebral cortex is positive for vimentin and negative for GFAP in 14-day-old embryos but positive for both proteins in newborn animals (Fedoroff et al. 1983). Intermediate filaments with vimentin and GFAP immunoreactivity are also seen in fibrous astrocytes that have been explanted from mouse neopallium and grown in tissue culture for 10–12 days. The authors assume that vimentin is a component of intermediate filaments in astrocyte precursor cells since filaments of this type exist in the cells before anti-GFAP staining is detected. Also, vimentin is a well-known constituent of the intermediate filaments found in other cell types.

Tapscott and coworkers (1981b) found that vimentin is present in cells that extend from the luminal to the pial surfaces in the newly formed neural tube of the chick, 33–38 h after conception (Fig. 16). These are columnar cells in our terminology. Immunofluorescence is especially bright in the endfeet that form a

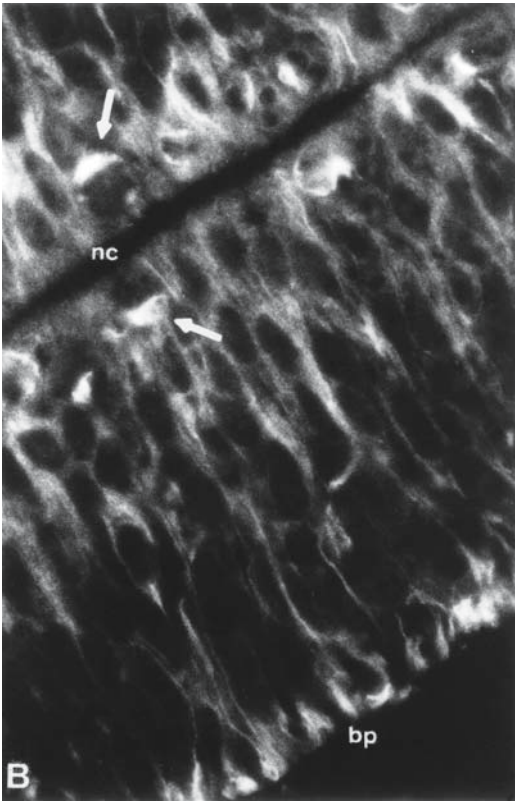

Fig. 16 Micrograph of a longitudinally sectioned stage 12–14 chick neural tube stained by indirect immunofluorescence with antibody to vimentin. Antivimentin binds to cell processes extending radially from the neurocoele (*nc*) to the external limiting membrane. At the luminal surface, spherical cells (presumed to be in mitosis) have a bright fluorescent "cap" in the region basal to chromatin (*arrows*). (Reprinted from Tapscott et al. 1983, with permission)

glial membrane at the pial surface. It is likely that vimentin is a subunit of these filaments since intermediate filaments are found in electron micrographs of areas where the staining is located. During the third day of development, postmitotic neurons appear. Their processes contain neurofilamentous proteins. The neuroepithelial cells, which now have the typical appearance of radial glial fibers, stain for vimentin but never for GFAP. On the other hand, astroglia, which appear in increasing numbers after the 12th day of development (some of which probably emanate from radial glial cells), stain for both vimentin and GFAP. This dual expression is maintained into adult life.

In conclusion, the immunocytochemical studies of filament proteins opened up new possibilities to mark the different types of neuroepithelial cells at early embryonic stages and to study the differentiation and development of these cells. Although the studies were still far from complete in 1985 and differences existed among species, it was possible to draw some preliminary conclusions of a general type.

Vimentin is present in neuroepithelial cells shortly after neurulation. Expression of vimentin is then retained in all cells belonging to the astrocytic lineage, i.e., columnar cells, radial glia, astroblasts, and mature astrocytes [in rats, vimentin is seen mainly in immature glia according to Dahl (1981)]. The expression of GFAP is switched on later. In the mouse and chicken it is first seen in immature glia, and dual expression of vimentin and GFAP is then maintained into adult life. In man and monkey GFAP is already present in radial glial fibers, indicating earlier expression than is seen in the mouse and chicken. The expression of vimentin has not been investigated in these species.

Astroglia are unusual in that the cells in situ contain two filament proteins (Yen and Fields 1981). GFAP is specific for astrocytic cells and is the major constituent of glial filaments in mature cells. Vimentin is not specific for astroglia, and it is not clear whether vimentin and GFAP molecules are subunits of the same or of different filaments. Regardless of their chemical composition, the filaments seem to maintain the same appearance during development.

Intermediate filaments form part of the cytoskeleton. They probably help maintain cell shape and the distribution of organelles (Lazarides 1980). Vimentin may be of special importance for mechanical support and for anchoring the nucleus. Filamentous proteins are present in replicating neuroepithelial cells and may function in division. That is described in a later section. The use of other markers for studying chemical and immunological differentiation during development was reviewed by Varon and Somjen (1979) and some aspects are described in the next section.

2.4 Tissue Culture Studies

Developmental studies of CNS in vivo were impeded in several ways:

1. The neuroepithelial cells are heterogeneous and occasionally are difficult to recognize; they also interact in complicated ways.

2. It is impossible to study single environmental factors that affect their proliferation, growth, and development.
3. Repeated observations of dynamic processes in living tissue cannot be carried out.

Valuable complementary information was obtained from in vitro studies. They made it possible to isolate and aggregate neuroepithelial cells of different types, to manipulate cells and their environment, to study their properties under defined conditions, and to observe sequential changes in living cells under the microscope (De Vellis et al. 1983; Fedoroff and Doering 1980; Trapp et al. 1979a). Due in part to technical improvements in cultivating and marking cells, this field grew rapidly. We cannot provide a comprehensive review here of the very large body of literature. Instead, we have selected some observations to complement our account of gliogenesis.

Of paramount importance in studies of CNS development was the identification of cells. In studies in vivo, classification was based on morphological criteria and immunocytochemical staining of filamentous proteins in astrocytes and neurons (as described above) and myelin basic protein in oligodendrocytes (Sternberger et al. 1978a, b). The preparations were mostly fixed sections of CNS. The need for proper marking of unfixed cells in cultures was and still is especially urgent because, in these preparations, light microscopic identification of cells is more difficult.

Techniques for marking living cultured cells included the use of ligands that bind to the plasma membrane (Bartlett et al. 1981; Miller and Raff 1984; Raff et al. 1979). With their use, specific cell surface antigens could be visualized with immunocytochemical staining procedures in which monoclonal antibodies were employed (Abney et al. 1981, 1983; Bartlett et al. 1981; Dickson et al. 1983; Lagenaur et al. 1980; Miller et al. 1984; Raff et al. 1983a; Solter and Knowles 1978; Sommer et al. 1981; Sommer and Schachner 1983).

These techniques gave precise data about the chemical composition of surface membranes. In conjunction with methods for staining intermediate filaments, they allowed not only identification of major nonoverlapping populations of cells but also recognition of biochemically distinct subclasses within these groups. Furthermore, it was possible to follow in culture subtle changes that occurred in precursors and daughter cells during development. Information about biochemical features that distinguished cells from each other was essential for tracing cell lineages, for understanding relationships between cells at different stages of development, and for the documentation of development in single cells.

Development of neuroepithelial cells in culture was similar to that in intact tissue (Fedoroff et al. 1983, 1984; Honneger and Richelson 1976; Kozak et al. 1978; Trapp et al. 1979a). Abney and coworkers (1981) showed that astrocytes, ependymal cells, and oligodendrocytes appeared in cultures of dissociated cells from 10- and 13-day-old rat embryos at about the same time as in intact animals. They suggested that, after 10 days of gestation, "biological clocks are more important in gliogenesis than positional information."

In classical neurohistology it is customary to distinguish two types of astrocytes (Peters et al. 1976). Fibrous astrocytes have scanty cytoplasm, contain more glial filaments, and are located mainly in the white matter of the CNS. Protoplasmic astrocytes have more voluminous processes, fewer glial filaments, and are located mainly in the gray matter. Glial filaments stain for GFAP in both types of cells.

It appears that these cells correspond to the two types of astrocytes present in cultures of developing optic nerve (Miller and Raff 1984; Raff et al. 1983a, b). Type 1 astrocytes, which are identical to protoplasmic astrocytes, are fibroblast-like cells and are stimulated by epidermal growth factors and pituitary extract. They are stained weakly by GFAP antiserum and bind monoclonal antibodies against a cell surface antigen Ran-2 (Bartlett et al. 1981). However, they do not bind tetanus toxin or the monoclonal antibody raised against A2B5 (Eisenbarth et al. 1979). Type 2 astrocytes, which correspond to fibrous astrocytes, are shaped like neurons or oligodendrocytes. They are not stimulated by epidermal growth factor (EGF) or pituitary extract. They are stained intensely by anti-GFAP, bind tetanus toxin and A2B5 antibody, but are not stained by Ran-2 antiserum. The plasma membranes of type 1 and type 2 astrocytes are different in that they contain gangliosides that bind different groups of ligands. Type 1 astrocytes appear before birth whereas type 2 cells develop postnatally, at least 1 week later than type 1.

The interior of the optic nerve contains mostly cells with the antigenic phenotype of fibrous astrocytes (type 2) whereas the glial limiting membrane is formed by cells with the antigenic phenotype of protoplasmic astrocytes (type 1). The limiting membrane of the optic nerve is probably homologous to the glial membrane at the pial surface of the cerebral cortex. That is derived in part (or perhaps totally) from radial glial cells (Figs. 10 and 15; Anders and Brightman 1979). Type 1 rather than type 2 astrocytes are involved in reactive gliosis in the CNS (David et al. 1984).

Investigations of neuroepithelial cells in vitro gave valuable information about lineages of cells and timing of cell determination which complemented the in vivo studies (see next section and Fig. 18). Raff and coworkers (1983a, b) found that a cell type in a 7-day-old optic nerve developed into a type 2 (fibrous) astrocyte if the culture medium contained fetal calf serum, and into an oligodendrocyte if the medium lacked serum. They concluded that fibrous astrocytes and oligodendrocytes were ontogenetically related and developed from a common progenitor. The choice of the bipotential progenitor was reversible for a period of up to 2 days but not more. It is of interest that this progenitor cell did not develop into a type 1 (protoplasmic) astrocyte or any other known cell type. Experiments with cell-type-specific markers showed that some molecules that exist in the surface membrane of both neurons and immature oligodendrocytes disappeared from oligodendrocytes during development but persisted in neurons during maturation (Abney et al. 1983). Taken together, these observations suggested that fibrous astrocytes and oligodendrocytes are more closely related to neurons than to protoplasmic astrocytes.

In conclusion, Raff and his coworkers (1984a) suggested that two lineages of cells in the rat optic nerve develop at E17 or earlier. One set of precursors develops into type 1 astrocytes around E15–E17. The other set gives rise to oligodendrocytes at P2–P3 or later and to type 2 astrocytes after P7. It is also possible that ependymal cells belong to the first lineage and neurons to the second.

Problems related to proliferation, maturation, and growth of cells are well suited for study in tissue cultures since different cell types can be isolated and the environment controlled. A CNS cell is influenced by contacts with other cells and by diffusible molecules. In the case of developing astroglial cells, the following humoral factors were considered to be of importance.

Glial maturation factor (GMF) (Lim 1985) is extracted from bovine brains. It is an acidic protein that in purified form has a molecular weight of 13,000 or more, depending on the species. It regulates proliferation and differentiation of astroglial cells and stimulates them to secrete lymphokines, which, in turn, may affect glial cells during normal development and in disease (Fontana 1982; Merrill et al. 1984). GMF appears first in the brain at the time of postnatal gliogenesis (Kato et al. 1981). Lim (1985) suggests that GMF acts as an autoregulator in the CNS since both the source and target are located there. Differentiation is stimulated more effectively by extracts from adult brains than from brains of embryonic and newborn rats. Morrison and coworkers (1982) suggested that the CNS possesses growth-stimulating activities that vary at different stages of development.

Astroglial factor (AGF) is extracted from bovine brain. It stimulates proliferation and maturation of astroblasts from newborn rats, which can grow in serum-free medium (Pettmann et al. 1982). It probably is similar or identical to GMF.

Glial growth factor (GGF) has been extracted from bovine brain and pituitary gland (Brockes et al. 1980a; Lemke and Brockes 1984). It is a basic protein with a molecular weight of 30,000 that is mitogenic for astrocytes, Schwann cells, and fibroblasts. The relation between this and the other factors (GMF, AGF) will remain unclear as long as their exact chemical compositions are not known.

Fibroblast growth factor (FGF) is a basic protein (molecular weight 13,000) which is isolated from the pituitary gland (Gospodarowicz 1975). It stimulates proliferation of fibroblasts and astrocytes but not oligodendrocytes (Pruss et al. 1982).

EGF is an acidic polypeptide (molecular weight 6,000) which is purified from murine salivary glands. It binds to specific surface receptors and affects a variety of cultured cells of epidermal, mesenchymal, and neuroectodermal origins. It stimulates proliferation of human glial cells (Westermark 1976). It also binds to cultured astrocytes from early postnatal (Leutz and Schachner 1981) and adult (Simpson et al. 1982) rats and stimulates DNA synthesis. Raff and coworkers found that only type 1 astrocytes respond to EGF and GGF (Raff et al. 1983a).

Other factors that may affect the proliferation, growth, and maturation of glial cells during development are platelet-derived growth factor (Heldin et al. 1980), proteases (Kalderon 1982; Kalderon 1985), and somatomedins, which are a family of growth factors related to insulin (Lenoir and Honneger 1983).

We have seen how neurons with processes and astroglial cells frequently are intimately associated during development. A central question in neuro-embryology was whether the cells in these associations interact and influence the proliferation, growth, and development of each other. This question was addressed in several tissue culture studies.

In cultures of dissociated cells from mouse embryonic and postnatal cerebellum, astrocytes formed a template for neurons (Hatten and Liem 1981). We are here reminded of our observation that columnar cells create niches in which young neurons can settle before further development (Fig. 13). Thus, in vitro and in vivo observations suggest a two-step mechanism during development. First, the young neurons are positioned along glial processes. Then they become differentiated and emit neurites.

Hatten (1985) developed a method for separating and purifying early postnatal cerebellar neurons and astrocytes. When these two types of cells were recombined, astroglial proliferation was slowed and their more primitive flat shape was transformed to the stellate form of a more differentiated astrocyte. She concluded that neurons influence the proliferation and differentiation of astroglia.

Noble and coworkers (1984) found an "adherence hierarchy" in cells from postnatal rat brain. Neurons were more adherent to glia than to other neurons and more adherent to other neurons than to nonglial cells, e.g., fibroblasts. These findings may, inter alia, explain relations between neurons and glia during cell migration and axonal growth. An in vivo study of chick spinal cord showed that a neuron–glia adhesion molecule (Ng–CAM) plays a key role in the attachments of neurons to glia (Thiery et al. 1985).

As described earlier, a progenitor cell (O-2A) in the optic nerve could develop into either a type 1 astrocyte or an oligodendrocyte depending on the presence or absence of fetal calf serum (FCS) in the culture medium (Raff et al. 1983b). FCS acted directly on the progenitor and the differentiation did not require the presence of other cells (Temple and Raff 1985). There was relatively little cell division in the O-2A progenitors and their progeny in vitro, which resulted in depletion of progenitors during the course of differentiation. Division was enhanced when purified type 1 astrocytes were added to the culture (Noble and Murray 1984). Thus, cells from one astroglial lineage (type 1) interacted with those from another lineage (O-2A and progeny type 2 astrocytes+oligodendrocytes). In the natural in vivo situation, astroblasts and oligodendroblasts go through repeated division before the postmitotic cells emerge (Skoff et al. 1976a).

David and coworkers (1984) found a striking decrease in oligodendrocytes, type 1 astrocytes, and their O-2A progenitors after transection of the optic nerve in newborn rats. They concluded that axons are needed for the survival of cells of this lineage at least during the postnatal period.

In conclusion, it is evident that studies of neuroepithelial cells in culture will continue to add information, especially at the molecular level, that will supplement our knowledge and modify our concepts about neural development in the intact organism.

2.5 Astroglial Precursors: Conclusions

We have described the appearance of the following cells: epithelial cells in the neural tube at the time of neurulation; columnar cells in the early neural tube; and radial glial cells in the neural tube after the appearance of neurons. We have postulated that they share certain morphological and functional characteristics that set them apart from other neuroepithelial cells (neurons and oligodendroglia) and that they belong to the astroglial cell line (Fig. 15). Now we will present the arguments for these conclusions.

Epithelial, columnar, and radial glial cells and the transitional forms between them have the following features in common:

1. The main mass of cytoplasm is located around or near the nucleus.
2. They have a bipolar shape and radial orientation, and span the entire neural wall.
3. The inner ends are joined to each other by junctional complexes at the ventricular surface.
4. The terminal parts of the outer processes are subdivided into branches that terminate in knob-like structures at the pial surface.
5. Their cytoplasmic fine structure is similar. Especially noteworthy are the microtubules that extend through entire lengths of these cells. Other important features are the microfilaments at the inner ends of these cells, their content of intermediate filaments that have glial filament immunoreactivity, and the presence of translucent cytoplasm in the terminal end knobs at the pial surface. Also, there is no evidence that these cells ever develop into neurons.
6. Epithelial cells seem to evolve into columnar cells and columnar cells into radial glial cells; the main change is cellular elongation as the neural wall thickens. In order to simplify the description, we have arbitrarily given names to three types of cells, one for each stage of development, as defined earlier.

From this review, it is evident that these cells have the same basic morphology. They also have, in part, the same function (see the next section). Their relation to astroglia is shown by several lines of evidence. The radial glial cells in man and monkey contain GFAP, a protein thought to be specific for astrocytes. Furthermore, radial glial cells eventually develop into astroglia. GFAP is not seen in epithelial cells, columnar cells, or in radial glial cells in many species. Instead, these cells contain another fibrillary protein, vimentin, which also can be detected with GFAP in immature and mature astrocytes.

In conclusion, developmental, morphological, functional, and immunocytochemical data suggest that the epithelial, columnar, and radial glial cells as well as transitional forms are a sequence in the cytodifferentiation of the astroglial cell line (Fig. 15).

The notion that the lineage of astrocytes can be traced back to the earliest cells in the neural tube is not new. In 1909, Cajal concluded that radial glial cells emanate

from "spongioblasts" (epithelial cells in our terminology) on one hand, and develop into astroglia and ependymal cells of adult type on the other (Cajal 1909).

According to a now generally accepted hypothesis, all cells in the early neural wall are germinal cells (Fujita 1963; Sauer 1935a, 1936; Sauer and Walker 1959; Sidman et al. 1959; Berry and Rogers 1965). Hinds and Ruffett (1974) as well as Seymour and Berry (1975) described and illustrated the movement of cells and cytokinesis as follows: The nuclei of the epithelial/columnar cells successively move inwards as they enter the S-phase. Their outer processes are detached from the pial surface and are pulled toward the nuclear region. The cells become spherical at the ventricular surface where all mitoses take place (Figs. 17 and 18). After division, the nuclei of the daughter cells move outwards during the G_1-phase. The outer processes eventually reach the pial surface and the cells acquire a columnar shape before the cycle starts again. During this sequence of changes, progenitor and daughter cells remain anchored to neighboring cells at the ventricular surface.

Sauer's hypothesis implies that the growth and differentiation of the epithelial/columnar cells are interrupted repeatedly by periods of dedifferentiation, contraction, nuclear migration, division, and renewed expansion of daughter cells. Thus, all cells in the early neural wall are both stem cells, from which all neurons and neuroglial cells emanate and, in interphase, differentiated cells with special functions. From a

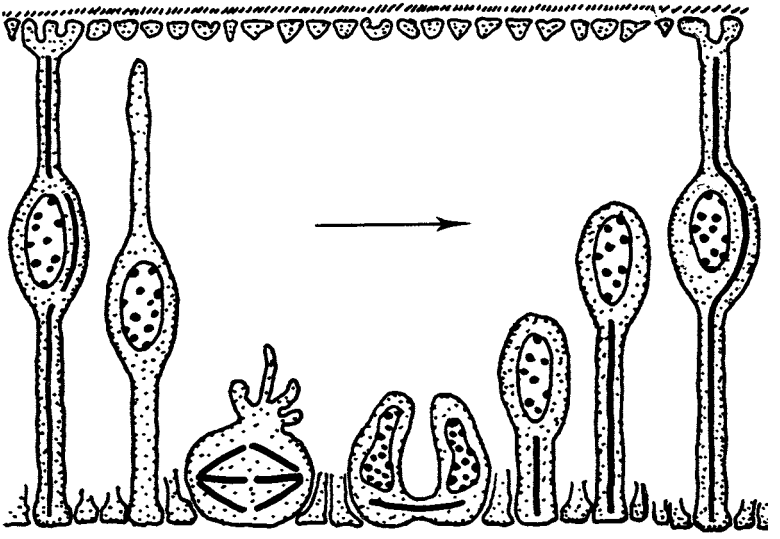

Fig. 17 Diagram illustrating the transformation of a columnar cell during cytokinesis. During the S-phase, the outer process detaches from the pial surface and retracts toward the nucleus. The nucleus moves inwards and divides at the ventricular surface. Daughter cells (only one is illustrated) grow centrifugally during the G_1-phase until they reach the pial surface. The collapse of a columnar cell and the subsequent elongation of the daughter cells is associated with disassociation and reassembly of microtubules (*solid lines*)

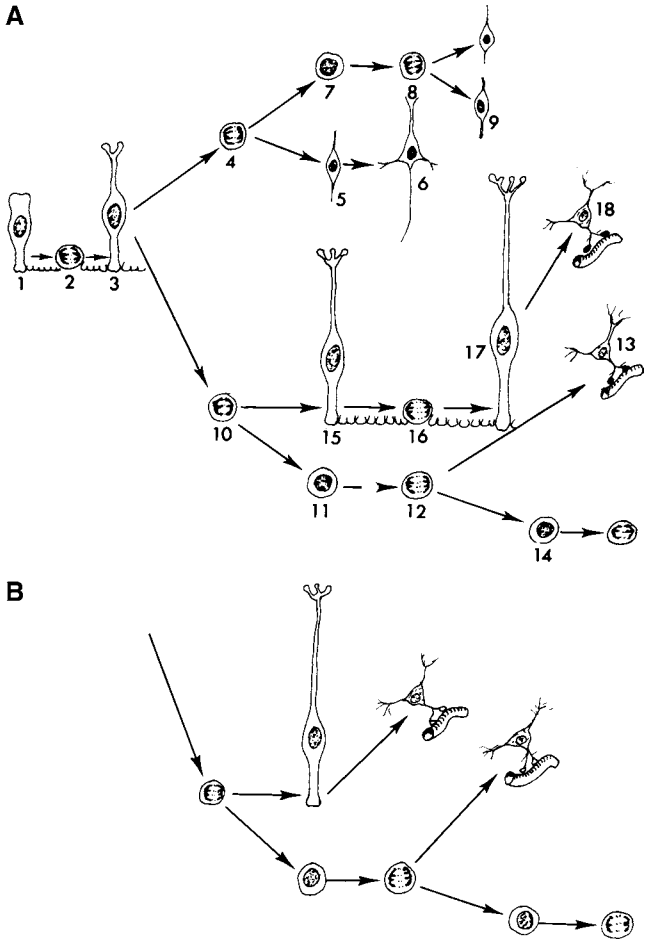

Fig. 18 A, B Diagram illustrating suggested lineages for neuronal (**A**, *4–9*) and astroglial (*10–18*) cells. *1*, epithelial cells; *3*, columnar cells. Alternative interpretations are given in **A** and **B** as described in the text

teleological point of view, one may assume that this arrangement with dual functions is "economical" or, differently expressed, that the early, growing CNS does not have the space for or cannot "afford" to have both structural elements and a separate pool of stem cells.

Investigations with autoradiography in mouse (Sidman et al. 1959), light and electron microscopy in rat (Åström, unpublished observations), and immunocytochemistry in mouse (Schnitzer et al. 1981) and chicken (Tapscott et al. 1981a, b) have shown that the dividing cells in the early neural wall constitute a homogeneous population. These early proliferating cells are the ultimate source of stem cells for all neurons, astroglia, oligodendroglia, and ependymal cells in the CNS.

During the past century, investigators have asked at what stages of development do the lines for the neurons and neuroglial cells diverge from the replicating, presumably uncommitted stem cells in the germinal zone? This problem has been approached from two directions.

Tapscott and coworkers (1981a, b) studied the appearance of filamentous proteins in cultured neuroepithelial cells of the chick spinal cord. A sequence of changes was observed in the neurons and their precursors that appeared after 3 days of incubation. The replicating cells contained only vimentin until the final cell cycle. Even before the final division, production of neurofilamentous proteins started in cells that were destined to become neurons, and was maintained thereafter in the maturing cells. The production of vimentin ceased in the early postmitotic period. The development was different in the astroglial cell line. Vimentin alone was seen in epithelial cells, columnar cells, and radial glial cells. Later, GFAP production was switched on. Immature and mature astroglia both contained vimentin and GFAP but did not stain with antisera raised against neurofilaments or other filamentous proteins. These observations in chicken agree with those found in mouse (Fedoroff et al. 1981; Schnitzer et al. 1981).

Using immunocytochemical methods with antisera against GFAP, Levitt and coworkers (1983) investigated the proliferative ventricular and subventricular zones in the occipital lobe of fetal monkeys. They found that germinal cells could be separated into two classes, GFAP-positive and GFAP-negative cells, as early as E39 and E40. At that time, migration of young neurons to the cortical plate had not yet started. They concluded that the GFAP-positive cells were astroglial precursors and that at least some of the GFAP-negative cells were neuronal precursors. This means that separate cell lines probably are established for neurons and astroglia before any (or more than a few) postmitotic neurons appear. These results in the monkey brain supplement those by Tapscott and coworkers (1981a, b) in the chick spinal cord. The conclusions of the two groups are also in agreement.

In conclusion, on the basis of available data derived from different regions of CNS in different species (Levitt et al. 1981, 1983; Rakic 1981), we suggest the following scheme of development for glial cells. The homogeneous line of epithelial/columnar cells (Figs. 1, 3, 18) bifurcates into neuronal (Figs. 4, 18) and glial (Figs. 10, 18) cell lines shortly before the stage of development when young neurons start migrating toward the mantle zone and cortical plate. The separation is signaled by the expression of new proteins, neurofilament protein, and GFAP (in man and monkey) respectively. In neurons, production of vimentin is stopped early (Fig. 18A, numbers 5, 6, and 9) but is maintained in radial glial cells and later in astrocytes (Fig. 18A, numbers 15–18).

The proliferation of neuronal and glial cells occurs mainly during midgestation (stage II). During the last part of gestation (stage III), the production of neurons ceases, radial glial cells decrease in number, and astroglia multiply rapidly. Astroblasts and astrocytes originate from radial glial cells (Fig. 18A, numbers 17 and 18) and also directly from germinal cells in the subventricular zone (Fig. 18A, numbers 12 and 13).

We have assumed that the early replicating cells are homogeneous up to the point when neuronal and glial cell lines diverge (Fig. 18A, number 3). However, newer methods may reveal differences among cells which now appear to be homogeneous. In that case, the separation can be pushed further back, perhaps to the time of neurulation or even earlier. One is here reminded of His's hypothesis (His 1889) that the early neuroepithelium consists of two classes of cells: the germinal cells that will develop into neurons, and the spongioblasts, which will become glial cells.

The observations of Sauer (1937) and others who have been concerned with this concept have mostly been made in comparatively young embryos in which the neural wall has a simple structure (stage I in our description). The main migration of neurons has not yet started, the neural wall is mainly made up of columnar and mitotic cells, and the division into neuronal and neuroglial cell lines has not yet occurred (Fig. 18A, numbers 1–3).

The replication of cells is more complicated and less well known at later stages, when the structure of the neural wall becomes increasingly complex. The neurons (Fig. 18A, numbers 4–9) and many astrocytes (Fig 18A, numbers 10–13) now seem to emanate from two separate pools of committed germinal cells that are located in the subventricular zone during their entire reproductive cycles. These cells do not develop into columnar/radial cells in interphase.

As regards the replication of radial glial cells, at least three alternatives are feasible. (1) The radial glial cells are premitotic and will divide in the same way as their predecessors, the epithelial and columnar cells (Figs. 18A, numbers 15–18). It is known that differentiated cells can undergo mitosis (Meinecke and Webster 1984; Skoff 1980; Skoff et al. 1976a; Sturrock 1982). This alternative is supported by the work of Schmechel and Rakic (1979b). On the basis of autoradiographic data, they suggest that a group of radial glial fibers in the brains of fetal monkeys stops dividing around E90 and re-enters the mitotic cycle around E140. However, in our view, their data do not constitute a definitive proof for their conclusions. If radial glial cells divide, they will either be cleaved in situ, which seems unlikely, or (like premitotic columnar cells) they will go through a metamorphosis that includes contraction, collapse, and rounding up in the subventricular zone (Fig. 18). After mitosis the daughter cells, as they become transformed into radial glial cells, must find their way through the intricate structure of the neural wall before reaching and establishing contact with the pial surface again. When one considers that a radial glial cell is well differentiated and may have a length of 20 mm or more, this way of replication seems, from a teleological point of view, unnecessarily complicated and wasteful. The next alternative is simpler. (2) The radial glial cells are postmitotic and originate directly from the line of committed germinal cells in the subventricular zone (Fig. 18B). (3) The radial glial cells become postmitotic after having divided once or several times and are thereafter recruited from germinal cells in the subventricular zone. This third alternative is a combination of (1) and (2). In vitro observations described in the preceding section have provided additional information about the lineages of glial cells.

2.6
Functions During Development

Our studies, as well as those of others (Choi and Lapham 1978; Schmechel and Rakic 1979a), strongly suggest that glial cells are the main constituents of the early CNS. These astroglial cells form a framework that supports neuroblasts and other precursor cells during their migration and have an important role in creating the microenvironment for growth and interaction of developing CNS cells and their processes. Indeed, it is inconceivable that neuroblasts could move to predetermined locations, assemble in highly ordered arrangements, and develop into nerve cells that connect to each other in precise, specific patterns without the support and guidance of a temporary supporting structure. This concept differs from that of earlier neuroanatomists who used light microscopic methods extensively for cell identification. Results obtained by many [for example, see Lorente de No (1933)] suggested that glial cells appear and develop after neuroblasts.

Neurulation and the early molding of developing CNS regions are associated with changes in shape of epithelial and columnar cells (Burnside 1971, 1975; Sauer 1937). Microtubules, known to have a role in cell elongation, are more numerous in columnar cells where the neural wall becomes thicker. Microfilaments are especially prominent in inner processes of columnar cells where they form a collar beneath apical processes (Figs. 11 and 12). Their presence here suggests that they could contract like sets of purse strings at the inner surface of the neural wall and could play a role in shaping ventricular surfaces of the cerebral hemispheres (Fig. 1B).

After division, the daughter cells of columnar/radial glial cells grow centrifugally during the G_1-phase (Fig. 17). This growth is presumably achieved through the assembly of microtubules that were disassembled in the premitotic phase.

The terminal branches with end knobs, which are best seen at the pial surface, represent the front (leading portion) of the columnar/radial glial cells as they grow centrifugally. They are reminiscent of the "cones de croissant" of the neuroepithelial cells in the spinal cord of the chick embryo (Cajal 1909; see his Fig. 239) or the growth cones of neurites (Tennyson 1970) or the "ruffling portion" at the leading end of growing astrocytes in the G_1-phase as seen in cultures of tumors, glial scars, and in explants from chick embryos (Ikuta et al. 1979). One can assume that further growth of the columnar/radial glial cells is inhibited after contact has been established between the end knobs and the pial surface.

The fine structure of the columnar cell, its shape changes, junctional complexes, and relationships with germinal cells suggest that it has the following functions:

1. By extending from the ventricular to the pial surface, columnar cells form a radially oriented cellular framework for migration of postmitotic blast cells.
2. The columnar cells provide a template in which young neurons can settle in order to develop after migration has ceased.
3. Since both an increase in columnar cell number and an elongation of these cells occur along with growth in the length, width, and thickness of the neural wall, we

suggest that these glial cells have an important role in determining early cellular growth patterns and the shape of the CNS before the migration of postmitotic neurons begins.

4. Differences in the density and fine structure of columnar cells' junctional complexes at the ventricular and pial surfaces suggest that they participate in the early development of CNS surface barriers with different permeability characteristics.
5. Closely packed inner processes of columnar cells and germinal cells united by impermeable junctional complexes may limit the escape of fluid from the ventricles and create intraventricular pressure sufficient to help shape the growth of the developing brain (Desmond and Jacobson 1977).
6. Outer columnar cell processes, by forming the first pial–glia membrane, probably function in the exchange of nutrients and metabolites between the neural wall epithelium and mesenchymal vessels.
7. The columnar cells insulate the early neurons from direct contact with the pial surface.
8. Columnar cells are germinal cells in interphase.

The radial glial cells have, with some modifications, the same functions as their predecessors, the columnar cells. Their specific and main functions are to facilitate the migration of neuroblasts to the cortical plate, preserve the radial alignment of clonally related neurons in cortical columns, and reproduce the mosaicism of the germinal zone at the cerebral surface (Rakic 1971, 1972, 1978). The lamellated expansions which cover the outer processes of radial glial cells in increasing numbers as development proceeds may help to position young neurons after their migration has ceased and contribute to their spatial organization by separating axons from each other as suggested by Cajal (1909). In the adult CNS, the astrocytes isolate the receptor surfaces of neurons in a very precise manner (Peters and Palay 1965; Peters et al. 1976).

Henrikson and Vaughn (1974) have shown that motor neuron dendrites grow preferentially along the interface between radial glial fibers and axons in the marginal zone of embryonic mouse spinal cord. They also suggest that axoglial contacts in the marginal zone represent a trial-and-error step in synaptogenesis before specific and final contacts have been established. Singer and coworkers (1979) have suggested that radial glial cells direct the growth of axons during embryogenesis and regeneration in the spinal cord of the newt. Fetal glia also guide the growth of axons in the CNS (Silver et al. 1982; Silver and Sapiro 1981).

Finally, it has been suggested that fetal glia release a neurotrophic substance (possibly a nerve growth factor) that stimulates the growth of neighboring neurons (Varon and Somjen 1979), participates in the formation of early CSF and blood–brain barriers (Wechsler and Mellor 1967), mediates molecular transport, and acts as a communication link between the germinal zone and cortical plate (discussed by Schmechel and Rakic 1979a).

2.7 Conclusions

Thus, the epithelial–columnar–radial glial cells form a massive cell system that dominates the early embryonic CNS. They have important mechanical functions and possibly others throughout the embryonal and fetal periods. They give form to and serve as scaffolding for the early CNS and constitute the inner and outer surfaces of the brain and the spinal cord. They guide the migrating neuroblasts and possibly also the growing axons and dendrites. They serve as templates for young neurons and help isolate and spatially organize neurons and their axons.

Although from a pure developmental point of view, epithelial, columnar, and radial cells, and transitional forms are precursors of astroglia, it would be incorrect to call them immature since they have special functions and are mature for the stage of development at which they appear. Their appearances at different stages reflect not only a morphological evolution but also changes in function in relation to the developing CNS. Jacobson (1978) expressed this in the following way: "—one has to interpret the morphology of neuroglia as well as of neurons in relation to the functional role of the neurons or glial cells at the particular times and places at which they are observed, and one should expect the morphology to change under different conditions at different stages of development."

The radial glial cells disappear when they are no longer needed. The function of guidance is obviated after migration of neurons has ceased. Other functions are taken over by more specialized cells: ependymal cells, which will separate the CNS parenchyma from the fluid-filled ventricles and astrocytes, which will form the glial membrane at the pial surface and give strength and support to the CNS through their processes and perivascular attachments. Astroglia also isolate neurons from each other and possibly in other ways assist the neurons in their development, growth, nutrition, and function. Finally, astroglia probably also have a role in the development of oligodendroglia and the formation of myelin.

3 Oligodendroglia

Oligodendroglia were first studied in detail and clearly identified as a separate type of glial cell by del Rio Hortega (1921, 1928). He classified oligodendroglia according to the branching pattern of their processes and recognized four types (Fig. 19). Type I cells, usually located in gray matter, had branches that radiated in all directions. Types II–IV were found in tracts and had a more parallel arrangement. Branches in type II were shorter and more numerous. There were fewer, longer processes in type III, and type IV cells had a single long branch extending from either pole. Del Rio Hortega's illustrations [reprinted in Bunge (1968) and Wood and Bunge

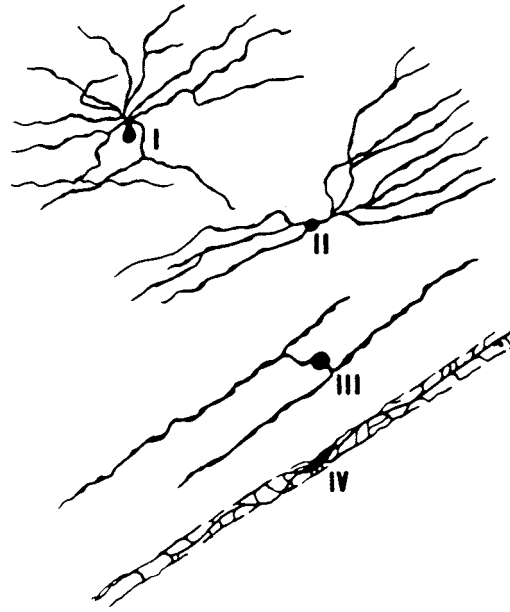

Fig. 19 The four types of oligodendrocyte recognized by del Rio Hortega. Type I presumably is related to many small myelin segments arranged at different angles to each other (as in gray matter). In type II cells, the myelin segments are similar to those in type I in size and number but are parallel to each other (as in a fascicle or tract). Type III is a cell related to a few much larger myelin segments. A type IV oligodendrocyte relates to only one large myelin segment, and Hortega noted the similarity of this oligodendroglial cell type and the myelin-forming Schwann cell. (Reprinted from Wood and Bunge 1984, with permission)

(1984)] clearly show the close relationships of myelin sheaths and oligodendroglial processes, and he thought that the main function of this glial cell was myelin formation and maintenance. His findings have been confirmed by many light and electron microscopic studies (Bunge 1968; Billings-Gagliardi et al. 1983; Puelles 1978; Spacek 1971; Stensaas and Stensaas 1968b; Wood and Bunge 1984). Early light microscopic observations also showed that in gray matter, oligodendroglia were found clustered around neuronal perikarya. These "satellite" oligodendroglia had fewer, thinner processes, did not have obvious connections to myelin sheaths and were thought to function as supporting cells for the neurons they surrounded. More recently, myelin basic protein (MBP) and myelin-associated glycoprotein (MAG) have been detected in some perineuronal oligodendrocytes in both developing and remyelinating CNS (Ludwin 1979; Luduena and Wessels 1973; Sternberger 1984). These observations show that some oligodendroglia, although next to neurons in a "satellite" position, have processes connected to myelin sheaths. Further studies of myelin constituent expression by oligodendroglia shown by electron microscopy to be perineuronal would be of interest, especially if the regions studies lacked myelin. This evidence would help determine if there is a subset of oligodendroglial cells analogous to the perineuronal satellite Schwann cells in dorsal root ganglia that does not form myelin and has different functional properties.

The lack of markers has limited our recognition of oligodendroglia as a specific type of glial cell until relatively late in their development. Even so, many investigators have been interested in these cells and published reviews (Norton 1984) showing that a substantial amount is known about their origin (Wood and Bunge 1984),

in vivo and in vitro development (Pfeiffer 1984; Wood and Bunge 1984), biochemistry (Benjamins 1984; Cammer 1984; Morell and Toews 1984; Pleasure et al. 1984), functional characteristics (Sternberger 1984), and the regulation of their differentiated properties (Weingarten et al. 1984).

3.1 Origin and Development

3.1.1 Origin

Oligodendroglia were thought to originate from germinal cells as a separate cell line and perhaps also from primitive glioblasts (Skoff 1980; Wood and Bunge 1984). Early studies showed that after neuronal production ceases, a new population of glioblast-like cells appears (Fugita and Figita 1964; Smart and Leblond 1961). Whether these cells are progenitors of oligodendroglia and astroglia or whether each cell line arises separately from germinal epithelial cells has not been clarified. Unfortunately, in spite of substantial interest and investigative effort, specific markers for distinguishing early oligodendroglial precursors from progenitors of other glial cell types are not available. Absence of anti-GFAP immunoreactivity was a criterion that has been used by some to help identify oligodendroglial precursors. But this criterion may no longer be valid; anti-GFAP staining has been described in immature oligodendroglia (Choi and Kim 1984; Choi and Kim 1985) and in another ectodermal derivative, the lens epithelium (Hatfield et al. 1984).

In vivo morphological studies provided most of the available evidence on oligodendroglial origins. In spite of their limitations, they still are the basis for a number of conclusions (Caley and Maxwell 1968; Fugita and Figita 1964; Imamato et al. 1978; Ling and Leblond 1973; Mori and Leblond 1970; Parnavelas et al. 1983; Paterson et al. 1973; Philips 1973; Privat and Leblond 1972; Skoff 1980; Skoff et al. 1976a, b; Smart and Leblond 1961; Sturrock 1976; Vaughn 1969; Vaughn and Peters 1971). Depending on the region examined, oligodendroglial types were identified in late embryonic or early postnatal development. They were distinguished by their appearance in light and electron micrographs and their capacity to incorporate ^{3}H-thymidine [the usefulness and limitations of autoradiography were well reviewed by Sidman (1970)]. For each oligodendroglial type, labeled and unlabeled cells were counted during development and the combined data provided the maturation sequence summarized here.

In electron micrographs of developing and mature CNS there are immature ("light"), active ("medium"), and mature ("dark") oligodendroglia that were distinguished by nuclear and cytoplasmic density, size, extent of processes, and the number and organization of cytoplasmic organelles (Fig. 20). Before myelination began, most identifiable oligodendroglia were relatively large, had lightly stained, scanty cytoplasm, few processes, and a homogeneous, lightly stained nucleus readily labeled with ^{3}H-thymidine. This cell was called an oligodendroblast (Fig. 21)

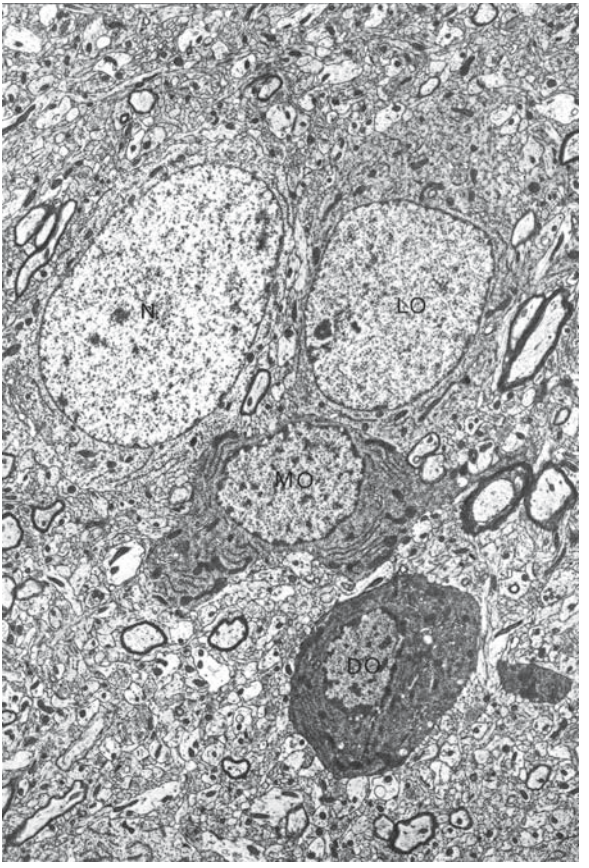

Fig. 20 Electron micrograph of the visual cortex of a 24-day-old rat. A light, medium, and dark oligodendrocyte (*LO*, *MO*, *DO*) and a neuron (*N*) are located in layer V. The three oligodendroglial types differ in size as well as in cytoplasmic and nuclear characteristics (see text). (Reprinted from Parnavelas et al. 1983, with permission)

and was distinguished from more mature oligodendroglia, astroblasts, and astrocytes. During myelination, the proportion of oligodendroglia with more densely stained, smaller nuclei and well-developed, basophilic processes extending to nearby myelin sheaths increased dramatically. In electron micrographs, profiles of granular endoplasmic reticulum, clusters of polysomes, Golgi membranes, mitochondria, and microtubules were found in the perikarya of these cells. Also, surface membranes of their processes were continuous with spirally wrapped, compact lamellae of developing myelin sheaths. As noted in Sect. 3.1.3 below, these cells were actively engaged in biosynthesis and express proteins, lipids, and enzymes that are known to be constituents of CNS myelin. In autoradiographic experiments, fewer of these cells incorporated ^{3}H-thymidine than those with the staining characteristics and electron microscopic appearance of oligodendroblasts

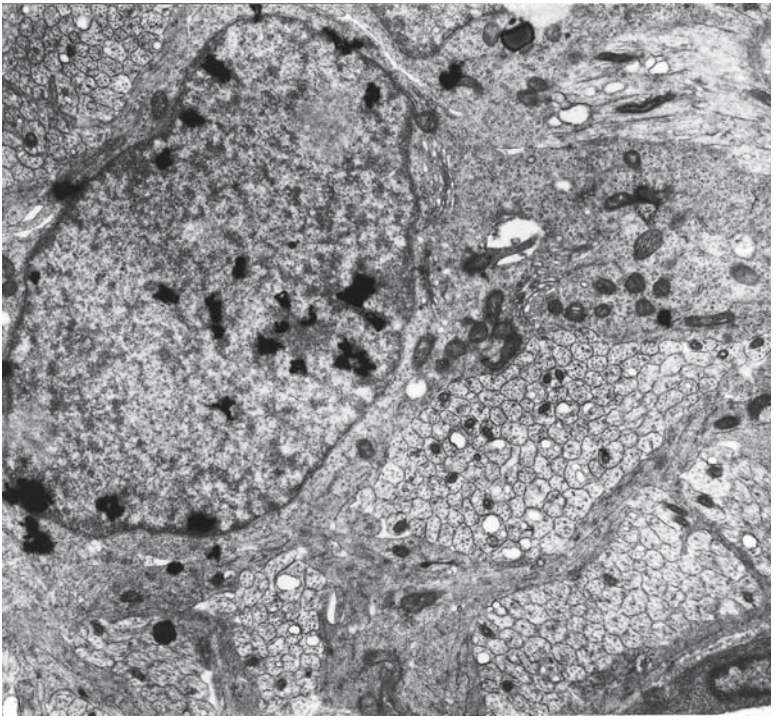

Fig. 21 Electron microscopic autoradiograph of transversely sectioned optic nerve from a 5-day-old rat. The nucleus of an oligodendroblast has been labeled with ^{3}H-thymidine. The cytoplasm contains many organelles, and to the right of the nucleus a cilium projects into an extracellular lumen. (Reprinted from Skoff et al. 1976a, with permission)

and immature oligodendrocytes. Late in development and in the mature CNS, the predominant cell is smaller with a scant, intensely basophilic cytoplasm and slender processes that are thought to be continuous with outer tongue processes of nearby myelin sheaths. Most investigators have reported that few of these cells incorporate ^{3}H-thymidine and concluded that these small, basophilic oligodendroglia represented mature, less metabolically active, postmitotic cells that functioned in myelin sheath maintenance.

The above evidence therefore strongly suggested that oligodendroglia and astroglia are separate cell lines postnatally. There also was general agreement that the immediate precursors of most myelin-forming oligodendroglia were oligodendroblasts (Fig. 21) which appeared in late embryonic or early postnatal development, had distinctive morphological features, and divided rapidly. The origin of oligodendroblasts was not known. More primitive precursors may have arisen as a separate cell line from germinal epithelial cells. They also could have been derived from radial glial cells (Choi et al. 1983) and other primitive glioblasts that then gave rise to both oligodendroblasts and astroblasts.

The presence of glial filaments and expression of GFAP were thought to be criteria for distinguishing astroglia and their precursors from oligodendroglia, but myelin formation by glial filament-containing cells had been described in the rabbit retina (Narang 1977). In addition, Raff and his collaborators (1983b) demonstrated that in vitro, the same glial cells could express GFAP and constituents of CNS myelin. Finally, as noted earlier, Choi and Kim (1985) described anti-GFAP immunoreactivity in developing oligodendroglia in vivo. These results suggested that neither the presence of some GFAP-containing glial filaments nor the occurrence of GFAP or myelin constituent immunoreactivity were sufficient to classify an immature cell as an astroglial or oligodendroglial cell. Another potentially useful approach for studying oligodendroglial precursors was described by Manuelidis (1984). She showed that satellite DNA arrangements differed in granule cells and Purkinje cells of the cerebellum; she suggested that differences may exist in other cell populations in the mature and developing nervous system. Similar studies of nuclear DNA during development might be useful in identifying glial precursors, especially those still capable of dividing.

3.1.2 Division

As noted in the previous sections, a continuum was observed from the large, rapidly dividing oligodendroblast with poorly organized cytoplasm and few processes (Fig. 21) to the postmitotic oligodendrocyte that had formed and was maintaining mature myelin sheaths. Whether more mature oligodendroglia with large processes and well-organized cytoplasm could divide while forming or maintaining myelin sheaths was an important unanswered question. Evidence in favor of this possibility had been provided (Sturrock 1981; Sturrock and McRae 1980). It also was of interest that nuclei of mature oligodendroglia (including those next to myelin sheaths) could incorporate ^{3}H-thymidine (Kaplan and Hinds 1980; Ludwin 1984). On the other hand, when serial sections of mitotic oligodendroglia were examined during development, these cells lacked processes and demonstrable connections to myelin sheaths (Fig. 22; Meinecke and Webster 1984).

Factors inducing and controlling oligodendroglial proliferation during in vivo development were difficult to examine and were not well understood. In an important study, Fulcrand and Privat (1977) showed that axons must be present for a significant population of oligodendroglia to develop. When they removed an eye before the optic nerve contained recognizable oligodendroglia (2–5 days postnatally), they found very few of these cells in optic nerves that they examined at 3 days. Also, enucleation at 8 days, when oligodendroglia comprised 20% of the optic nerve's glial population, prevented further increases in their numbers. In vitro studies have shown that before expressing galactocerebroside, oligodendroglia divide in the presence of axons (Wood and Williams 1984). Thus, constituents of the axolemma may be mitotic signals for oligodendroglial precursors as they are for Schwann cells in the PNS (DeVries et al. 1982; Sobue et al. 1983; Wood and Bunge 1975).

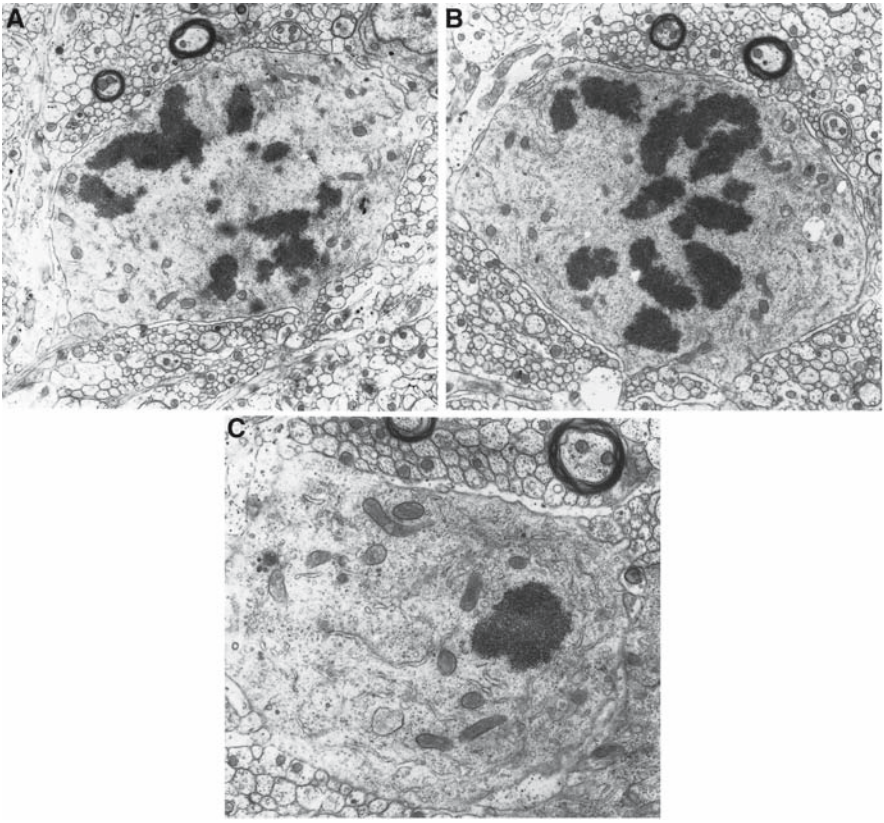

Fig. 22 Electron micrographs of serial sections cut through a metaphase oligodendroblast in the spinal cord of a 5-day-old mouse. A few very small processes project a small distance into the neuropil. Otherwise the surface membrane is smooth and covered by neurites, astroglial processes, or processes of adjacent interphase oligodendroglia. (Reprinted from Meinecke and Webster 1984, with permission)

3.1.3 Early Stages

How oligodendroglia interact with axons in vivo and begin forming myelin is a fascinating problem. Most investigators interested in the maturation of oligodendroglia have found it easier to study their properties and relationships with axons, myelin sheaths, astroglia, and other cells in developing tracts that contain bundles of parallel axons with similar origins, destinations, and functions. As noted above, appropriate numbers of axons that will become myelinated are required for the development of a suitable oligodendroglial population. The mitotic signal is thought to be an axolemmal constituent (Wood and Bunge 1984; Wood and Williams 1984).

Early maturation begins before the end of the proliferative phase of oligodendroglial development. It is characterized by close association with astroglia, enlargement of the perikaryon, a more complex array of cytoplasmic organelles, and growth of processes. Astroglial processes in developing tracts were often sheet-like and frequently extended radially from the perikaryon for long distances. These processes persisted during astroglial mitosis (Meinecke and Webster 1984) and provided a supporting framework for axon-oligodendroglial interactions (Reier and Webster 1974; Rawlins and Uzman 1970b). The importance of astroglia in oligodendroglial maturation was also suggested by in vitro observations (Wood and Williams 1984) and by the presence of numerous astrocyte-to-oligodendrocyte gap junctions (Massa and Mugnaini 1982). Subsequent oligodendroglial enlargement and increased cytoplasmic complexity reflected the beginning of the biosynthesis of myelin constituents. This occurred before the onset of myelination (Figs. 23 and 24; Sternberger 1984; Sternberger et al. 1978, 1979).

Subsequently, rates of synthesis increased and reached maximum levels in large "active" (medium) oligodendroglia. Also, enzymes involved in myelin biosynthesis appeared to be induced simultaneously, not sequentially (Tennekoon et al. 1980).

3.2 Myelin Formation

The biosynthesis and transport of proteins and lipids and their deposition as compact membrane sheaths around axons are among the most dramatic biochemical and morphological processes in CNS development (Braun 1984; Bunge 1968; Davison 1970; Lees and Brostoff 1984; Matthieu 1980; Norton and Cammer 1984; Lund and Bunt 1976; Peters and Vaughn 1970; Suzuki 1980; Waehneldt and Linnington 1980). For example, in rat brain, it begins at about birth and reaches its maximum rate at 20 days (Norton and Poduslo 1973). At this age, oligodendroglia are depositing about 3.5 mg of myelin per day. If there are about $20–40 \times 10^6$ oligodendroglia and if an oligodendroglial perikaryon weighs approximately 50×10^{-12} g, it can be calculated that each cell is making about twice its own weight of myelin per day (Norton and Cammer 1984).

When biochemical methods for isolating purified myelin were discovered (reviewed by Norton and Cammer 1984), many investigators studied the composition of myelin in adult and developing CNS. When isolated from adult CNS, the proportions of myelin's dry weight accounted for by lipids and proteins are about 70% and 30%. The major lipids in rat myelin (in % total lipid weight) are cholesterol (27%), galactolipids (31%), and phospholipids (about 44%). The main myelin proteins are proteolipid protein (PLP, about 50% of myelin total protein, M_r 25,000) and MBP (30%–35%, M_r 18,500). Proteins present in smaller amounts include: (1) Wolfgram proteins [W_2 which has a M_r of 55,000 and co-migrates with tubulin; W_1 a doublet with a M_r of 42,000–50,000 that has the same M_r and amino acid composition as 2′,3′-cyclic nucleotide-3′ phosphohydrolase (CNP)], (2) DM-20 (properties similar to PLP) (3) other basic proteins, and (4) many glycoproteins.

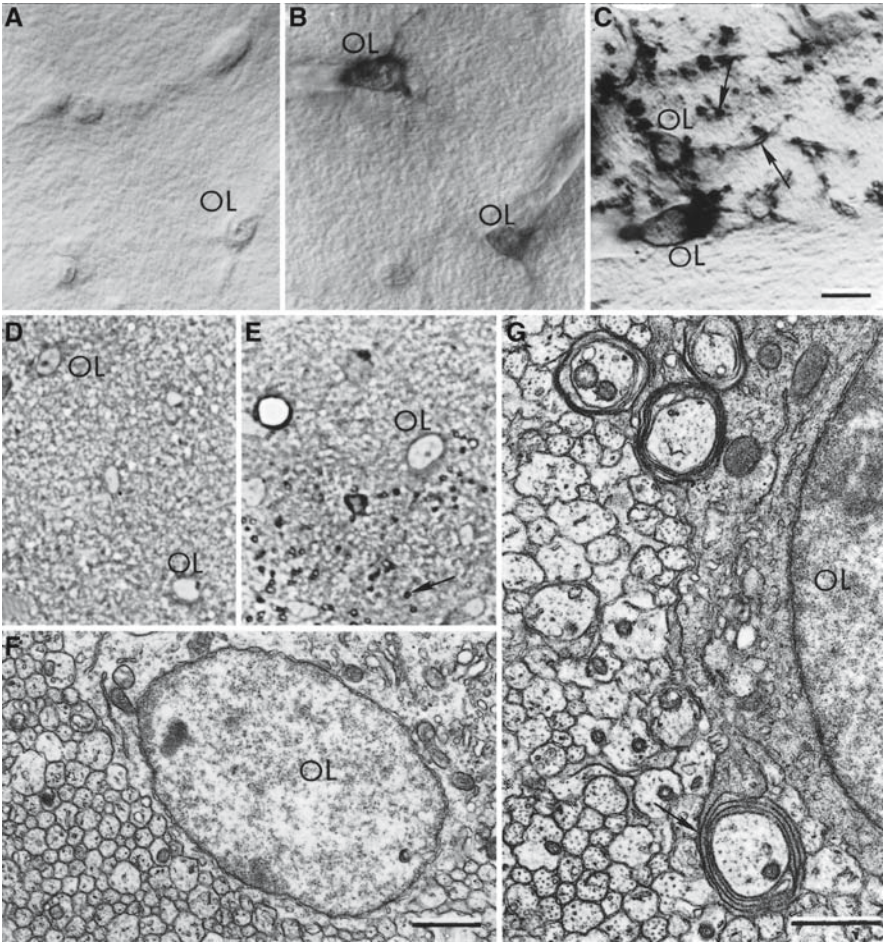

Fig. 23 **A–G** Light micrographs (**A–E**) and electron micrographs (**F, G**) of the anterior commissure of postnatal rats. Sections shown in **A–C** were immunostained with 1:500 myelin basic protein (MBP) antiserum. Immunostaining of oligodendroglia (*OL*) by anti-MBP is absent at 3 days of age, 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**) the oligodendroglia are larger. Their perikarya contain more ribosomes and their processes extend to newly formed myelin sheaths (*arrows*). (Reprinted from Sternberger et al. 1978b, with permission)

Of the glycoproteins, the best characterized was called MAG or myelin glycoprotein (MGP); it has a M_r of about 100,000 and constitutes about 1% of the protein in adult myelin. Enzymes also are present in myelin, and those that are myelin-specific are CNP and cholesterol ester hydrolase. About 20 other enzymes have been found in myelin but they are not myelin-specific. Their functions include biological transport and the metabolism of lipids and proteins.

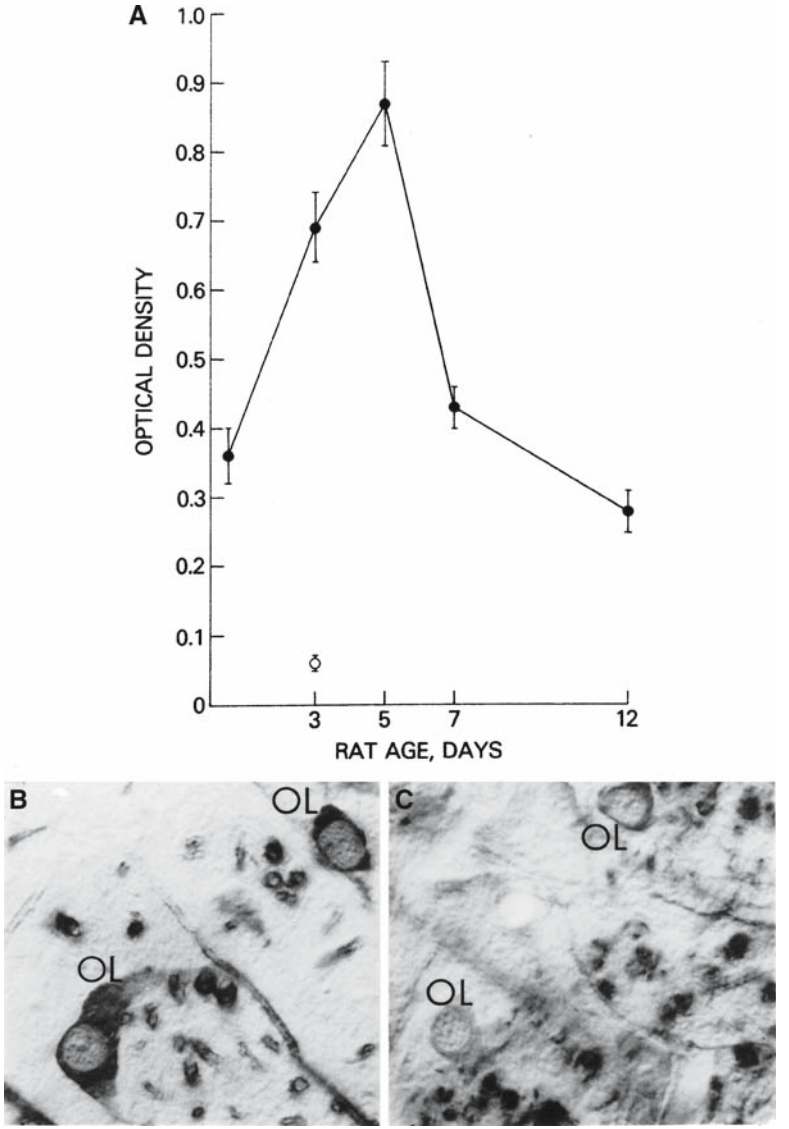

Fig. 24 **A** Optical densities of oligodendroglia immunostained with 1:500 anti-MBP (*dots*) or preimmune control serum (*open circles*) during the early development of the pontine tectospinal tract. In light micrographs, immunostaining of oligodendroglia (*OL*) is much denser at 5 days (**B**) than at 12 days of age (**C**). (Reprinted from Sternberger et al. 1978b, with permission)

Many studies have shown that the lipid and protein composition of myelin changed during myelination (reviewed in Norton and Cammer 1984). As myelin sheaths increased in thickness, length, and number, their galactolipid content rose

and levels of phospholipids decreased, while levels of cholesterol and plasmalogen remained relatively constant. Immature myelin also contained a higher proportion of high molecular weight proteins that were not well characterized. As development proceeded, there was a relative decrease in high molecular weight and Wolfgram proteins and increased amounts of MBP and PLP. Also, myelin density increased, reflecting the increase in the protein/lipid ratio that probably was associated with the decrease in lamellar spacing observed in electron micrographs. Readers can supplement this brief summary by consulting Morell's book about myelin (1984). It contains excellent reviews of the molecular structure and biochemistry of myelin.

Electron microscopic studies of CNS myelination (reviewed in Bunge 1968; Peters et al. 1976; Peters and Vaughn 1970) showed that when an oligodendroglial process surrounds an axon, the opposed pair of membranes (called the mesaxon) enlarges and become spirally wrapped around the axon (Fig. 25). Most investigators agree that this is the first morphological event in the formation of a myelin segment. Then the length and the number of turns in the mesaxon spiral increase rapidly; junctional complexes also appear (Dermietzel et al. 1978; Schnapp and Mugnaini

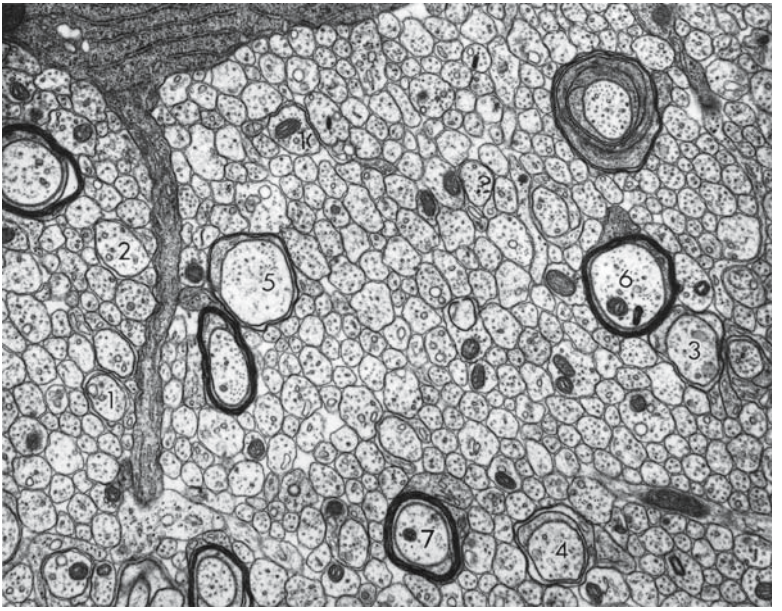

Fig. 25 Electron micrograph of the subcortical white matter of a 15-day postnatal rat. Some of the many axons shown are becoming myelinated. As shown in Fig. 26, some are enveloped by a simple oligodendroglial process (*1*). Dense lines are present in central parts of processes that have lost cytoplasm; these processes have a dumb bell shape (*2*). Part of the sequence of subsequent spiral formation is shown in (*3–5*) and further growth of the compact sheath is seen in (*6*) and (*7*). Part of an oligodendroglial perikaryon is present at the top left and one of its processes extends down among the myelinating and the still unmyelinated axons. (Reprinted from Peters et al. 1976, with permission)

1975; Shinowara et al. 1980; Tabira et al. 1978). When serial sections were examined, there were differences in the shape of the ensheathing process, the content of cytoplasm, and the number of spiral turns along the length of the developing segment (Knobler et al. 1974). While the first few turns were forming, the cytoplasm between turns and along the inner and outer surfaces of the spiral disappeared rapidly. Developing myelin's multiple, spirally wrapped membrane layers achieved a compact arrangement by fusion along their cytoplasmic faces and formed a membrane structure called myelin. The morphology of CNS myelination is shown diagrammatically in Fig. 26. Myelin is birefringent, has a well-defined X-ray diffraction pattern (Kirschner et al. 1984), and has a characteristic appearance in transmission electron micrographs. The compact multilayered spiral is made up of alternating dense and a pair of less dense lines that have a periodicity of about 11.5 nm and are continuous with the cytoplasmic and extracellular leaflets of the oligodendrocyte's surface membrane (reviewed in Peters et al. 1976). In biochemical studies, subcellular fractions might contain fragments of myelin (often called "compact myelin") that had the same lamellar arrangement and spacing noted above.

3.2.1 Mechanisms for Myelin Membrane Growth: Pereyra-Braun Hypothesis

As noted in Sect. 3.1.1, myelin lamellae become compactly arranged when they surround small axons early in development. During subsequent axonal growth, the membrane forming each myelin segment enlarges very rapidly in the transverse plane. The number of layers and both the inner and outer circumferences increase dramatically. The very rapid dimensional changes observed morphologically have been correlated with the dynamics of myelin assembly by Pereyra, Braun, and coworkers (Pereyra and Braun 1983a; Pereyra et al. 1983b). Their findings indicate that previously suggested mechanisms for enlargement of the compact spirally arranged layers do not account for the observed growth rate or their biochemical findings. They therefore proposed a model for myelin membrane growth within the planes of outer myelin layers. It includes the following sequence and is illustrated in Fig. 27: (1) myelin lipids and proteins are transported to outer tongue processes and form vesicular aggregates, (2) vesicular aggregates fuse and form "premyelin" endomembranes, (3) outer surfaces of endomembranes and cytoplasmic faces of myelin lamellae become aligned. Apposition of endomembrane inner surfaces produces a spacing that corresponds to myelin's less dense lines, and (4) "promyelin" endomembranes and myelin membranes fuse at specific sites.

This sequence could account for three important geometric features of developing CNS myelin. The previously described alignment of outer and inner tongue processes (Peters 1964) is maintained. Cytoplasmic compartments and interlamellar junctional complexes (Dermietzel et al. 1978; Tabira et al. 1978) keep their relative positions in the transverse plane as lamellae increase in number and circumference. Longitudinal growth of myelin layers produces the observed arrangement of paranodal loops with successive loops covering their predecessors as myelination

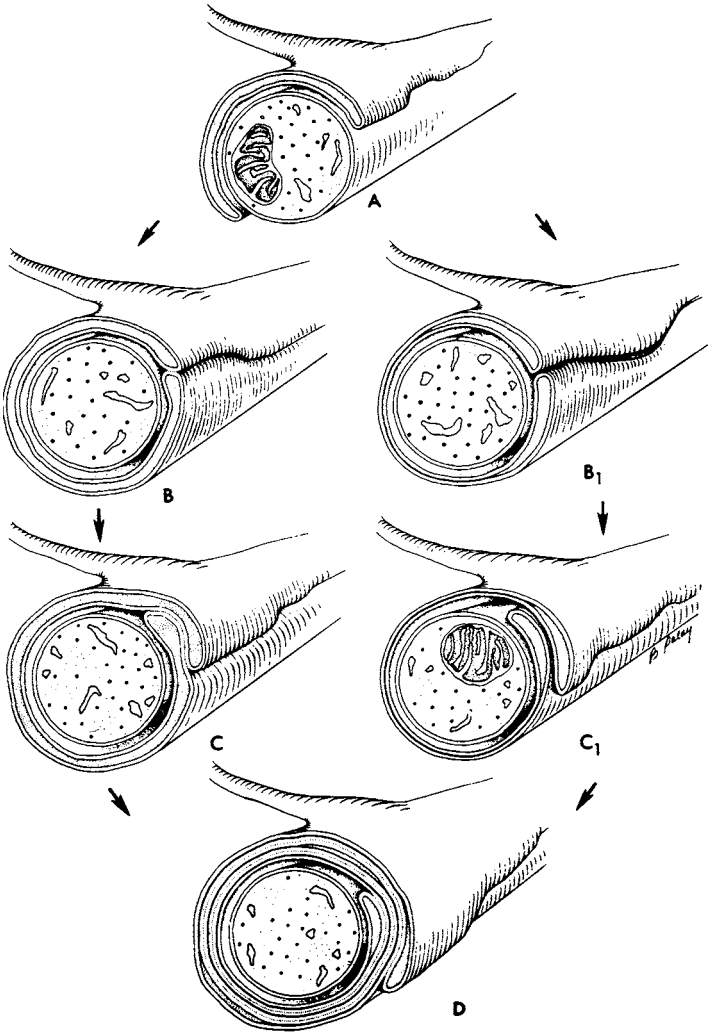

Fig. 26 A–D Diagrammatic representation of CNS myelin formation. First (**A**) an axon is surrounded by an oligodendroglial cell process, and where the process lips meet a mesaxon is formed (**B**). Cytoplasm may be present all along the enveloping process (**B**) or may be lost where cytoplasmic membrane faces have fused and formed a major dense line. The oligodendroglial process elongates (**C** and **C1**) and a compact spiral is formed that grows in thickness and length (**D**). Apposition of extracellular membrane faces produces paired less dense lines that alternate with dense lines in the compact spiral found in mature sheaths. (Reprinted from Peters et al. 1976, with permission)

proceeds. The model also suggests that microtubules and filaments found in tongue processes and in myelin's interlamellar cytoplasmic pockets may have important functions. There they could form a framework for transport and ordered aggrega-

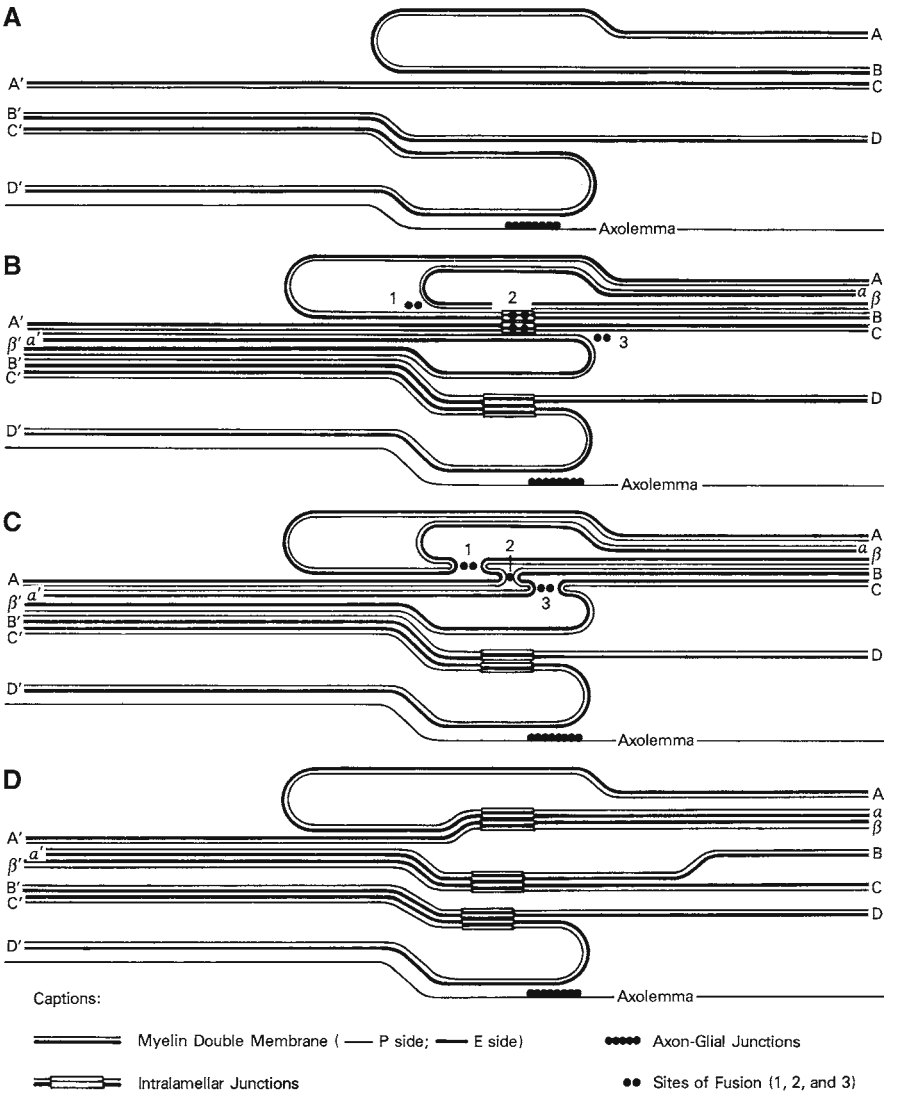

Fig. 27 **A–D** Diagram representing the Pereyra-Braun hypothesis for the addition of new lamellae to CNS myelin sheaths by endomembrane formation, alignment, and then fusion with existing lamellae (see text). An enlarged section of a transversely sectioned sheath is shown in **A**. It includes an outer tongue process (*A*, *B*), its continuation as the second spiral turn (*A′*, *B′* on the *left*; *C*, *D* on the *right*) and as the inner process (*C′*, D′) next to the axolemma. In *B–D*, an endomembrane (α, β on the *right* and α', β' on the *left*) become aligned and fuses so that a new lamella is formed without displacing tongue processes or junctional complexes. (Diagram by P.M. Pereyra)

tion of myelin constituents and their assembly into "promyelin" endomembranes that then could be aligned and inserted rapidly into existing lamellae. Microtubules and filaments in these locations could also have an important role in remodeling rapidly growing sheaths and in the turnover of myelin constituents.

Relatively little evidence was available to assess this hypothesis. There were observations that supported aspects of the model and indicated that further studies are needed. They included:

1. Tongue processes sometimes contain small endomembrane profiles of the proposed type [for example, see Figs. 11 and 26, in Raff et al. (1984b)].
2. When tadpoles that are forming optic nerve myelin were cooled rapidly, large numbers of vesicles appear in outer and inner tongue processes. Similar collections of vesicles were produced by exposing the nerves briefly to cycloheximide, an inhibitor of protein biosynthesis. These changes were reversible (Cullen and Webster 1977).
3. During myelination, MBP was detected on microtubules in perikarya and tongue processes by ethanolic phosphotungstic acid staining (Tabira and Webster 1979).
4. Bundles of 6- to 8-nm filaments were present in an electron micrograph of an oligodendroglial inner tongue process during myelination (Choi and Kim 1985). The composition of these filaments had not been studied. However, their dimensions suggest that they could contain actin.
5. When isolated under certain conditions, myelin contained proteins found in cytoskeletal elements (Braun et al., unpublished observations).

As each myelin segment grows in thickness and length, its irregularities and level-to-level variations decrease. At different levels along the mature sheath, the number of lamellae becomes quite uniform. Except for a few small, isolated pockets, oligodendroglial cytoplasm is also reduced to a thin, continuous strip located along the outer, inner, and paranodal edges of the compact myelin membrane. As noted already, there also are interlamellar junctional complexes in CNS myelin (Tabira et al. 1978) that correspond to the radial component described earlier (Peters 1961a). Otherwise, in tissue processed by standard methods such as those described by Palay and Chan-Palay (1974), the pattern of alternating major dense and less dense lines is similar to that found in the PNS. However, in the CNS, the distance between major dense lines is much less (Karlsson 1966) and this probably is due to CNS–PNS differences in myelin protein composition. During development, the lamellar spacing of myelin increases (Hedley-Whyte and Kirschner 1976); this increase probably is associated with the developmental changes in myelin composition noted above.

3.2.2
Expression of Myelin Constituents

The expression of myelin constituents was reviewed by Sternberger (1984). In the first immunocytochemical studies that compared distributions and relative concentrations of myelin proteins during myelination, she and her collaborators showed

that anti-MBP immunoreactivity was diffusely distributed in oligodendroglial perikarya and processes; its presence was also dependent on age and region examined (Sternberger et al. 1978a, b). This pattern of cytoplasmic staining was confirmed in an electron microscopic study that demonstrated reaction product on and near free ribosomes (Omlin et al. 1982) where MBP is synthesized (Carson et al. 1983; Yu and Campagnoni 1982). Also, Sternberger and her collaborators (1978b) showed that optical densities of anti-MBP staining of oligodendroglial perikarya were detected before myelination began, increased rapidly and then decreased before the rate of myelin deposition reached high levels (Figs. 23, 24; Sternberger et al. 1978b). Electron microscopic immunocytochemical observations showed that MBP is localized in CNS myelin's dense line regions (Herndon et al. 1973; Omlin et al. 1982), confirming results obtained in biochemical experiments (Golds and Braun 1976) and with ethanolic PTA staining (Tabira and Webster 1979).

The other major protein constituent of CNS myelin, PLP, was also detected in developing oligodendroglia and myelin (Agrawal et al. 1977; Hartman et al. 1982). A more granular pattern of staining was observed in oligodendroglial perikarya than with BP antiserum, suggesting localization in Golgi membranes and a different transport mechanism to insertion sites; this evidence was consistent with biochemical observations (Bizzozero et al. 1984). In comparative observations, PLP appeared in oligodendroglia after MBP (Hartman et al. 1982). These authors also found that in adjacent sections, anti-MBP stained large myelin sheaths more intensely than anti-PLP and that PLP was more readily detected in small sheaths. This suggested that myelin sheath size might have a role in determining relative concentrations of PLP and MBP.

Myelin's major protein constituents were also studied immunocytochemically during development. The Wolfgram proteins, CNP (thought to correspond to the Wolfgram doublet) and MAG could be detected in oligodendroglia before or just after myelination began. For MAG, Sternberger and her collaborators observed a granular staining pattern in perikarya and processes, suggesting localization in the Golgi complex and in vesicles that are found in processes. Compared to MBP, optical densities of MAG staining increased more slowly, reached maximum levels later, and declined more slowly (Sternberger 1984). In light microscopic studies, anti-MAG and anti-MBP immunoreactivities had the same localization in developing myelin. But in the adult, the localizations were different. Anti-MAG was only been found in periaxonal regions, suggesting that MAG might be localized in periaxonal membranes of oligodendroglia and function in axon–glial interactions needed for myelin maintenance (Itoyama et al. 1980; Sternberger et al. 1979). However, anti-MAG reaction product was not detected on these membranes in electron microscopic immunocytochemical studies (Favilla et al. 1984; Webster et al. 1983a). Instead, anti-MAG immunoreactivity was located on compact myelin in both developing and mature CNS. Because of these differences in the light and electron microscopic observations, the localization of MAG needed further study.

Distributions of two myelin-associated enzymes, CNP and carbonic anhydrase (CA) were studied immunocytochemically (Cammer et al. 1985; Kumpulainen and Korhonen 1982; Kumpulainen and Nystrom 1981; Nishizawa et al. 1981; Korr 1980; Spicer et al. 1979; Sprinkle et al. 1983). CNP immunoreactivity was present in some oligodendroglia before myelination began and later, in mature myelinated fibers, there was staining of periaxonal regions similar to that described for MAG by Sternberger and her colleagues (1979). There was general agreement that CA is localized in oligodendroglia. Low levels, near detection threshold, may also have been present in immature astroglia. Although Kumpulainen and Korhonen (1982) described intense staining of human cerebellar white matter with anti-CA, most other studies described staining limited to a faint ring around axons.

Thus, as Sternberger (1984) pointed out, many studies showed that myelin constituents could be detected in oligodendroglia at the time that myelination began, but not much earlier when their detection might help separate them from other immature glia that resembled each other according to other criteria. Also, myelin constituents that had been studied did not serve as satisfactory markers for either oligodendroglial perikarya or their periaxonal processes, a functionally important region for axonal interactions needed for myelin formation and maintenance. Finally, immunocytochemical detection of substances in myelin was difficult due to its compact lamellar structure and the problems associated with achieving adequate reagent penetration in well-preserved tissue. Differences in technique, species, and relative biochemical maturity of the myelinated region being examined may also have explained some of the differences that had been described.

3.2.3 Regulation of Myelination

Del Rio Hortega's observations (del Rio Hortega 1928) and more recent evidence (Blakemore 1981; Blakemore et al. 1984; Peters and Vaughn 1970; Sternberger et al. 1978a) suggested that the branching pattern of an oligodendrocyte's processes relates to the number, spatial distribution, and size of myelin segments to be formed. How is the pattern achieved? Blakemore (1981) suggested that myelin induction signals on developing axon surfaces interacted with receptors on oligodendroglial processes (Fig. 28). The signal density on the axolemma would tell the oligodendrocyte how much of its total myelin-producing capacity to commit to the segment that would be formed there. The highest signal density would limit an oligodendrocyte to the formation and maintenance of one very large myelin segment. An oligodendrocyte responding to lower signal densities would form smaller segments on more axons (Fig. 29). This hypothesis seemed consistent with measurements of growing axons and myelin segments (Blakemore et al. 1984) with numbers of segments per oligodendroglial cell (Peters and Vaughn 1970) and with different thicknesses of two sheaths connected to the same oligodendroglial process (Friedrich and Mugnaini 1983).

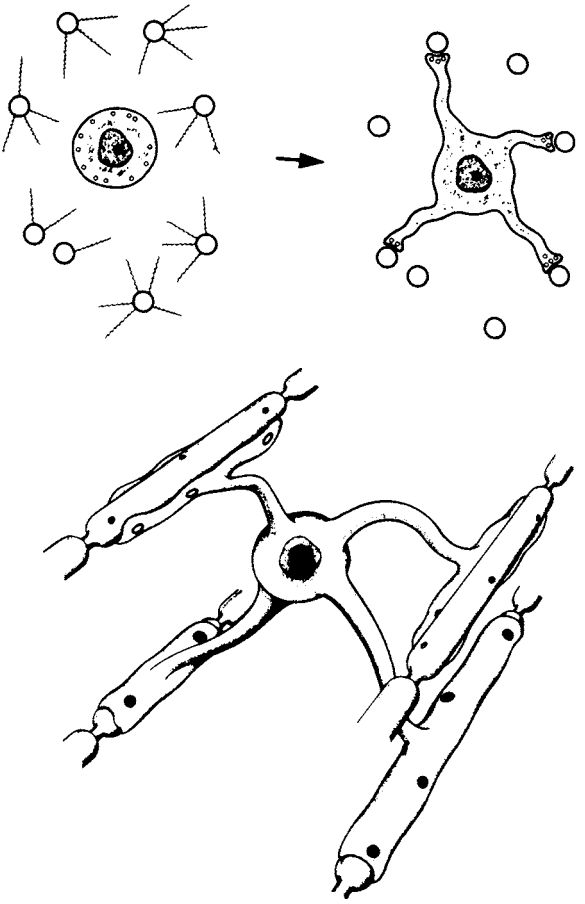

Fig. 28 Diagram illustrating the consequences of an oligodendrocyte responding to axonal signals. An oligodendrocyte with 12 units of ensheathing potential (*small open circles*) is surrounded by axons sending out signals of varying strength depending on their final size. On receipt of a signal, the oligodendrocyte commits ensheathing potential to the axon in the form of ensheathing receptors. Their density is proportional to the axonal signal received, as shown in the second stage of the diagram. Matching of axonal signals and oligodendroglial ensheathing receptors (*open circles*) produces myelin internodes of differing length. (Reprinted from Blakemore 1981, with permission)

Does the hypothesis also seem applicable to developmental situations in which myelin sheaths are remodeled? This occurs as the optic nerves of *Xenopus* tadpoles shorten during metamorphosis (Cullen and Webster 1979). The Blakemore hypothesis (Blakemore 1981) would allow temporary axonal shortening, with an associated change in the density of myelin-forming signals and their interactions with receptors, to help initiate and control the myelin remodeling process that Cullen and Webster (1979) described.

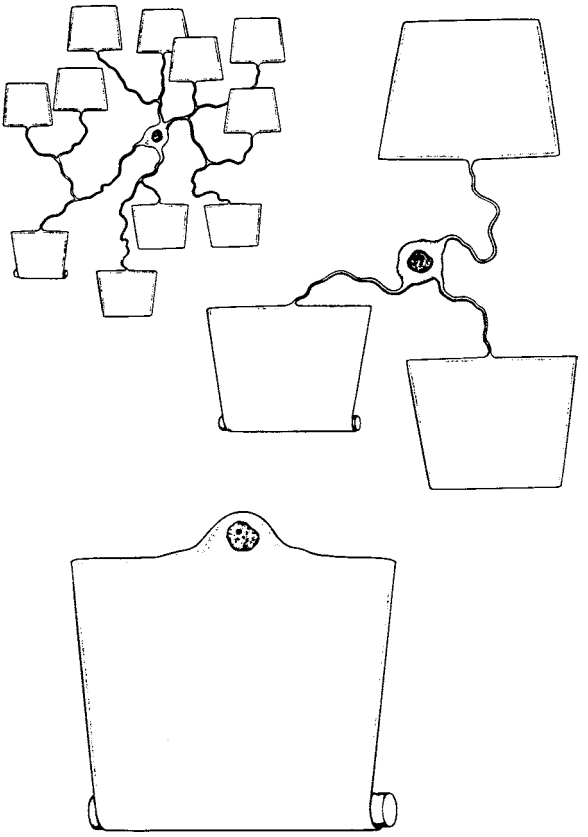

Fig. 29 Diagram illustrating the relationship of oligodendrocytes with axons based on calculations of internodal axonal areas and myelin segment volumes for axons of differing diameters. The three oligodendrocytes shown are associated with the same total area of axonal membrane. However, the total volume of myelin supported by each cell is different as this is determined by the relationships existing at the individual internodes. (Reprinted from Blakemore et al. 1984, with permission)

In the initial stages of myelin formation, oligodendroglial processes may extend to ten developing sheaths and may be 5–38 micrometers (μm) long (Fig. 30; Sternberger et al. 1978a). Sternberger and her associates (1978a) observed that processes were attached to sheaths near their midpoints, suggesting that longitudinal growth of developing myelin segments is a fairly symmetrical process. Lengths of the segments formed by an oligodendrocyte may differ and the axons being myelinated could be in different tracts. This suggested that an oligodendrocyte can myelinate axons that differ in origin, destination, and function. Also, for a given cell, the myelin segments that it formed and maintained could differ in size and spatial distribution. Presumably, the onset of myelination and the size of the segment to be formed are determined by specific focal

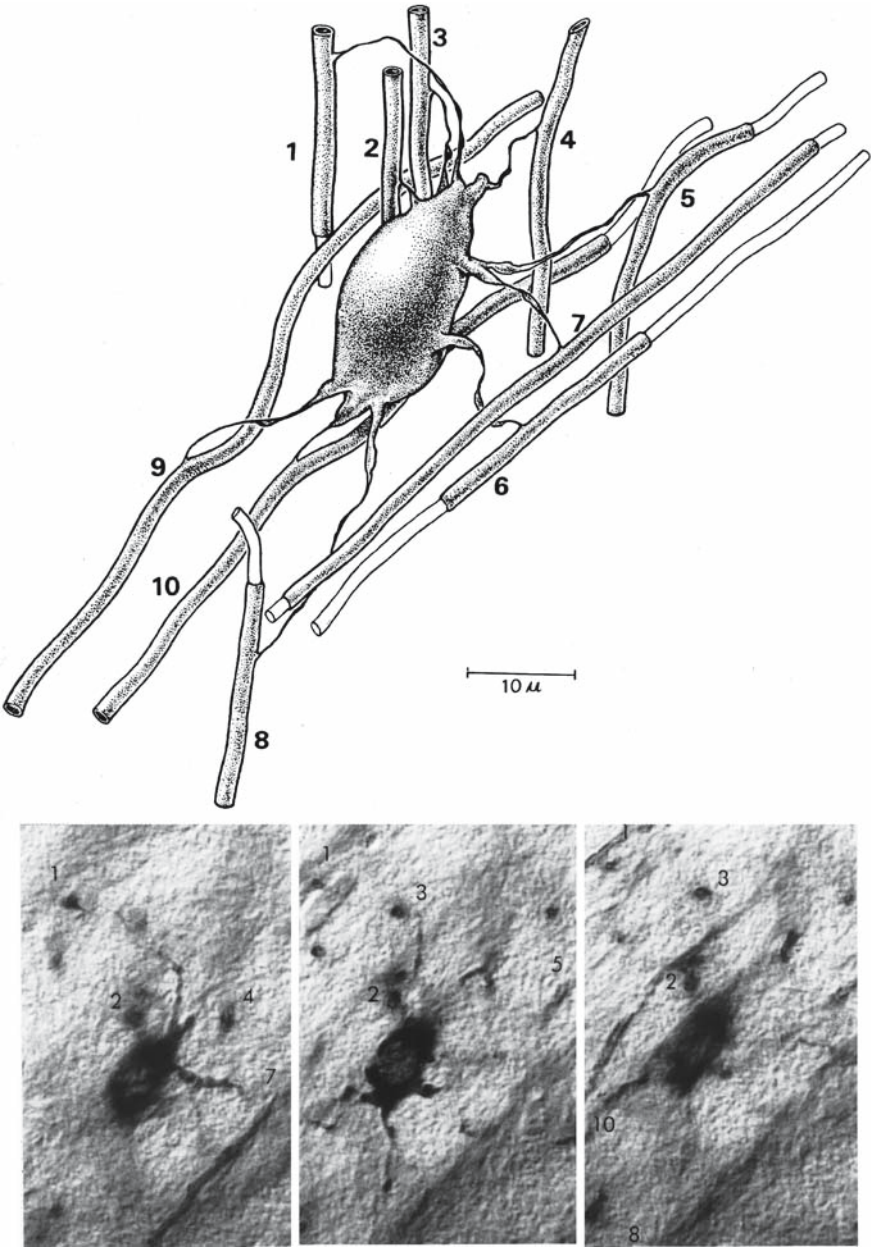

Fig. 30 Reconstruction of ten myelin sheaths being formed by an oligodendrocyte in the tectospinal tract of a 5-day-old rat. Six that are transversely sectioned belong to the tectospinal tract and the other four that are longitudinally oriented probably surround pontocerebellar fibers. The same oligodendrocyte is shown below in micrographs taken at three levels of focus using Nomarski optics. (Reprinted from Sternberger et al. 1978a, with permission)

interactions of an oligodendroglial process with the axon that will become myelinated. However, for all processes of a given cell, axonal interactions and subsequent myelin formation probably do not start at the same time and progress uniformly. Instead, they appear to be asynchronous and, as noted above, they are not tract-specific.

Although process branching patterns and dimensional changes during development clearly show the general importance of axons in the initiation and progression of myelination, there are a few compact sheaths that have the periodicity of myelin that do not surround axons. These are uncommon and most of them represent uncorrected developmental errors; they include large, closely apposed loops, other irregularities, and occasional thin sheaths that may surround neuronal perikarya (Rosenbluth 1966) or dendrites (Pinching 1971; Remahl and Hildebrand 1985). Another example of oligodendroglial myelin formed and maintained in the absence of axonal contact are outer lamellae of the very large sheaths that surround Mauthner cell axons in *Xenopus*. In these sheaths, multiple oligodendroglia make lamellae for each segment and many have no surface membrane contacting the axolemma.

If interactions of axonal signals and oligodendroglial process receptors generally initiate and regulate myelination, do they begin at the perikaryon and progress distally to axon terminals? Is the gradient centripetal or is still another pattern evident? This was carefully examined in optic nerves of rats and rabbits (Skoff et al. 1980). Patterns in rats and rabbits differed but in both, myelination did not begin at either the retina or the optic chiasm, nor did it progress proximally or distally. Instead, it began in several regions at once where the vascular density was high. This suggested that vascular density and/or focal differences in hormone levels, nutrients, and perhaps other factors in the microenvironment could influence the cellular interactions that lead to myelin formation (Skoff et al. 1980). Supporting evidence includes the observed reduction in oligodendroglia and myelin associated with hypothyroidism (Bass and Young 1973; Clos et al. 1982; Legrand 1980), adrenalectomy (Preston and McMorris 1984), and undernutrition (Fuller and Wiggins 1984; Wiggins 1979; Wiggins 1982; Yu and Campagnoni 1982).

3.2.4
In Vitro Studies

In 1957, Hild reported that explanted oligodendroglia would develop and produce myelin in vitro (Hild 1957). The use of Maximow chambers (Bornstein and Murray 1958) allowed long-term serial observations at relatively high resolution and provided investigators with an important new technique that allowed them to explore many aspects of oligodendroglial development and myelinogenesis that could not be studied in vivo. Since then, explant methods have been modified and many new culture systems have been described. Pfeiffer (1984) has written a comprehensive review of this very large subject, and more specialized aspects are included in other reviews (Billings-Gagliardi and Wolf 1982; Weingarten et al. 1984; Wood and Bunge 1984). Here, only a few of the highlights can be included.

In explant cultures, morphological, biochemical, and histochemical studies showed that the maturation of oligodendroglia and the formation of myelin sheaths paralleled that seen in vivo. Because of the high degree of tissue organization produced, these cultures were extremely useful in characterizing abnormalities due to toxic substances (including sera that induce demyelination). They were also useful in defining changes associated with variations in nutrient media and in characterizing myelination inhibition and remyelination in vitro. In other studies, cell types were separated and then recombined to study axon–glial interactions (Wood and Williams 1984). Wood and Williams (1984) described early fasciculation of axons by astroglia, oligodendroblasts that incorporated ^{3}H-thymidine and did not express galactocerebroside (GalC), and oligodendroglia that expressed GalC about 7 days before their newly formed myelin sheaths were detected morphologically. Their observations were additional evidence favoring a role for astroglia in the interactions that lead to myelination. Dissociated-reaggregated cultures were also useful models for studies of oligodendroglial development and myelin formation (Matthieu and Honegger 1979; Matthieu et al. 1980; Schmidt 1975; Sheppard et al. 1978; Trapp et al. 1982). The advantages of this system were that aggregates from the same flask could be harvested for biochemical and morphological study of development at sequential time points. In general, tissue organization achieved was less than that observed in explant cultures. Oligodendroglial maturation was slower and less myelin was produced. Levels of constituents synthesized were lower, the synthetic rates were lower, and even after 30 days in vitro (DIV), the composition was that of relatively immature myelin. However, as in explant cultures (Bornstein 1973), raising media concentrations of glucose substantially increased myelin production (Trapp et al. 1982). In a correlative immunocytochemical, electron microscopic, and biochemical study, Trapp and his collaborators found that very few myelin sheaths were found at 15 DIV. Some oligodendroglia were stained by anti-MBP; anti-MAG immunoreactivity was detected in fewer oligodendroglia, suggesting that both MBP and MAG were expressed before myelination began and perhaps in these cultures MAG was expressed later than MBP. At this interval, low levels of CNP were detected biochemically but no MBP or MAG was found by radioimmunoassay (Trapp et al. 1982). At later intervals, numbers of myelin sheaths and oligodendroglia immunostained by anti-MAG and anti-MBP increased dramatically. The distribution of MAG and MBP in developing sheaths was the same, indicating that both were constituents of developing compact myelin sheaths containing 4–22 lamellae (Trapp et al. 1982).

Dissociated-attached cultures are those in which dissociated cells are allowed to attach to and grow on a solid substrate. Studies using this system and its variations yielded a substantial amount of new information on the biochemical maturation of oligodendroglia, their nutritional requirements and regulation as well as the capacity of these cells to express myelin constituents in the absence of axons and myelination. Synthesis of sulfatide and other myelin lipids was studied by many investigators (Pfeiffer 1984; Pleasure et al. 1984). It began during the first week in vitro and peaked at about 20 DIV (Pfeiffer et al. 1981), a time course that corresponded to that observed in vivo. Oligodendroglia also accumulated and showed

surface membrane expression of the sulfatide GalC. Because synthesis and plasmalemmal localization occurred early in development, anti-GalC immunoreactivity was a very useful marker for oligodendroglia in culture preparations (Raff et al. 1978). It is of interest that oligodendroglia could differentiate and become GalC-positive in a serum-free, hormone-supplemented medium (Eccleston and Silberberg 1984). Thus, Barbarese and Pfeiffer (1981) found that oligodendroglia began accumulating MBP at 20 DIV. Progressively higher levels of MBP in the cultures at later intervals were due primarily to increasing numbers of oligodendroglia that contained MBP rather than higher amounts per cell.

Some culture studies of oligodendroglia explored their origins and division patterns. Schachner and coworkers (1981) developed monoclonal antibodies that recognize cell surface antigens of oligodendroglia. Two of these, designated 03 and 04, identified oligodendroglia-like cells before they became positively stained by anti-GalC. These studies were extended by showing that most of the 0-positive cells had electron microscopic features of immature (light) oligodendroglia (Berg and Schachner 1981). Another important contribution was the discovery that composition of the culture medium could determine whether a glial progenitor cell became an oligodendrocyte or an astrocyte (Raff et al. 1983b). Finally, to assess the number of oligodendroglial precursor cells in 20- to 21-day fetal rat CNS, Barbarese and her colleagues (1983) cultured single cell suspensions at several concentrations for 22 days and determined the number of oligodendroglia expressing MBP. Limiting dilution analysis of the results led them to conclude that there were about 300–500 oligodendroglial precursors in 20- to 21-day embryonic rat CNS and that each progenitor cell divided about 11 times during postnatal development (Barbarese et al. 1983).

3.2.5 Mutations Affecting Myelination

This field of research began in the 1960s when neuropathological and biochemical observations of the mouse mutants *jimpy* and *quaking* were described by Sidman, Dickie, and Appel (Sidman et al. 1964). These findings and the catalog of neurological mutations published a year later (Sidman et al. 1965) evoked wide interest. Since then, many investigators have used these and other mutants to investigate gliogenesis and myelination (see, for example, Baumann 1980; Billings-Gagliardi and Wolf 1982; Hogan and Greenfield 1984). A comprehensive review of this large subject is not possible here. However, a summary of the major findings from a few studies of *jimpy* (jp) and *shiverer* (shi) mice is included to indicate the importance of mutants as models for analyzing the development of myelin-forming cells.

In the mutants that have been studied, hypomyelination of the CNS is most severe in *jimpy*, and there now is substantial morphological evidence that oligodendroglia are severely affected (Hirano et al. 1969; Meier and Bischoff 1975; Sidman et al. 1964; Skoff 1976). Although their time of origin was similar to that observed in controls, the life span of oligodendroglia was greatly reduced and survivors continue to proliferate (Skoff 1982). Astroglial changes also were present before myelination began and

may reflect abnormalities in the early interactions that lead to axonal ensheathment and myelin production by a large population of postmitotic oligodendrocytes (Skoff 1976; Omlin and Anders 1983). Retarded axonal growth also was described (Robain and Mandell 1974; Webster and Sternberger 1980) but no changes were observed in the distribution of particles on axolemmal faces in freeze-fracture preparations (Robain and Mandell 1974; Webster and Sternberger 1980). This latter observation suggests that axonal abnormalities, if present, do not have a major role in producing the severe hypomyelination. Additional evidence strongly supporting a primary defect in oligodendroglia was the capacity of normal oligodendroglia to myelinate *jimpy* axons in vitro (Billings-Gagliardi et al. 1983). Early biochemical studies showed that reduced levels of protein and lipid constituents were consistent with the degree of hypomyelination found in *jimpy*. Subsequent analyses showed that the reduced incorporation of 21.5k MBP was less severe than the reductions observed in the 18.5k, 17k, and 14k forms. PLP was not detected at any age (Kerner and Carson 1984). The authors suggested that the gene for PLP may be directly affected and that after 21.5k MBP was inserted into the developing myelin membrane, the *jimpy* mutation blocked myelin assembly before PLP was incorporated (Kerner and Carson 1984).

Compared to *jimpy*, the hypomyelination found in the mutant *shiverer* (shi) was less severe. The *shiverer* mutation was of special interest because it produced a known biochemical defect, an almost complete lack of MBP in the CNS and PNS (Dupouey et al. 1979). CNS myelin sheaths produced by *shiverer* in vivo (Privat et al. 1979) and in vitro (Billings-Gagliardi et al. 1984) were thin and their lamellar structure was abnormal. Dense lines were not present and oligodendroglial cytoplasm separated the loosely wrapped lamellae. To identify the location of the primary defect, Billings-Gagliardi and her colleagues exposed explants of *shiverer* cerebellum to normal oligodendroglia from "injected" optic nerves and discovered that *shiverer* axons were surrounded by two ultrastructurally and immunocytochemically distinct types of myelin. The first type was MBP negative *shiverer* myelin (described above) and the second was MBP positive with the dense lines, compact structure, and lamellar spacing found in normal CNS myelin (Billings-Gagliardi et al. 1984). These results showed that the genotype of the oligodendrocyte (*shiverer* vs normal) controlled which type of myelin was made around this mutant's axons. They also added significantly to the evidence demonstrating the importance of MBP in the creation and maintenance of CNS myelin's compact structure.

3.3 Summary

Even though much remained to be learned and understood, it was clear that oligodendroglial development is a remarkable series of events. These cells, which became recognizable relatively late in development, may arise as a separate cell line and/or from glial precursors. Relatively few oligodendroglia were present before birth. Their numbers then increased rapidly and the mitotic signal was thought to be an axolemmal constituent. Astroglia and their processes organized

developing axons into bundles and provide a supporting framework for oligodendroglia. The presence of gap junctions that connect astroglia and oligodendroglia indicate that astroglia may also influence oligodendroglial division, process formation, and the interactions with axons that lead to myelin formation. Postmitotic oligodendroglia probably form nearly all CNS myelin sheaths, and the onset of this process in tracts such as the optic nerve is influenced by local factors, such as vascular density. Myelin formation requires oligodendroglial synthesis of protein and lipid constituents, their transport along processes, and perhaps their assembly into "promyelin" endomembranes before insertion into the rapidly growing myelin membrane spiral. Focal interactions between an axon and the ensheathing oligodendroglial process appear to control the size of the myelin segment that is formed. Myelin sheaths are dynamic metabolically active membranes with a highly ordered compact molecular structure that depends on the presence of MBP. During development, they are remodeled; few irregularities are observed in the mature CNS.

Thus, it is clear that myelin formation is the main function of oligodendroglia during development. As noted in the introduction to this section, early light microscopic studies also identified perineuronal oligodendroglia and suggested that they had important roles in neuronal function. Biochemical studies of oligodendroglia surrounding large neurons in Deiter's nucleus by Hyden and his collaborators (reviewed in Hyden 1967) provided strong support for this concept. However, electron microscopic studies of these neurons showed that their surfaces were covered by axon terminals and processes of astrocytes, not oligodendroglia (Sotelo and Palay 1968). Although the suggested symbiotic relationships are of interest, the existence of a separate population of perineuronal satellite oligodendroglia with different functions has been questioned (Peters et al. 1976; Wood and Bunge 1984).

4
Microglia

Microglia constitute a small minority (about 4%–18%) of the cells found in the normal CNS, and their numbers depend on the region examined. In early studies, Cajal (1913b) included small cells that lacked prominent processes in a group of cells that he called the "third element." He thought that some of them might originate from mesodermal elements. Del Rio Hortega modified available silver methods and clearly identified two species of small cells with different morphological characteristics. He called them oligodendroglia (described in Sect. 3) and microglia (reviewed by del Rio Hortega 1932). In light microscopic sections of mature CNS, typical microglial cells are more abundant in gray matter, have polymorphic darkly stained nuclei, and rather scanty perinuclear cytoplasm. Their processes, which vary in number, length, and thickness, are covered with fine twigs that give microglia a distinctive spiny appearance. Cells with all of these features are readily identified as microglia. But there are small cells that may resemble microglia and

also have morphological characteristics of oligodendrocytes or macrophage-like cells. In early electron microscopic studies of CNS, the range of morphological appearances reported for small cells was an added source of confusion. Better preparative methods were used in subsequent studies, and in these publications a typical microglial cell had an oval or elongated nucleus with large, marginally located clumps of chromatin and cytoplasm containing prominent long profiles of granular endoplasmic reticulum and a variable number of dense polymorphic inclusions (Peters et al. 1976). However, as in light microscopic sections, there were small cells with an appearance intermediate between this and the one described for other cells. Additional problems were the lack of specific markers and the substantial regional and species differences in microglial morphologies. These problems added significantly to the uncertainties of establishing microglial identity during development. They also partially explain why less was known about the genesis of microglia than other glial types.

4.1 Cytodifferentiation and Origin

In his preparations of developing CNS, del Rio Hortega (1932) did not observe cells that resembled mature microglia until several days after birth. Late in embryonic development, he found clusters of small cells at meningeal–CNS interfaces and subependymally; he identified them as microglial precursors. Because these cells were closely associated with vessels, and ameboid macrophage-like cells were found in nearby neonatal white matter, he thought that microglia had a mesodermal origin. Many have accepted and quoted this view (Cammermeyer 1970; Peters et al. 1976). But others, such as Rydberg (1932), felt that microglia were derived from neuroectoderm and originated, like other glia, from precursors in the subependymal zone. This origin was supported by evidence in reports by Fujita (1965), Vaughn and Peters (1971), and Fujita (1973). Also, round and ameboid microglia had been described in neonatal rabbit CNS by Stensaas and Reichert (1971), but they thought that their origin and fate were not clear. Other investigators (Baron and Gallego 1972; Sturrock 1974) concluded that microglia were morphologically well differentiated when they appeared in the CNS and that they were derived from pericytes.

Later, rat corpus callosum and the adjacent subependymal zone were used to reexamine microglial origins because this region contains all the cells thought to be possible precursors of microglia (glioblasts, ameboid macrophage-like cells, and pericytes). In a radioautographic study, Imamoto and Leblond (1978) found that cells with electron microscopic features of microglia appeared at about 12 days of age and doubled in number during the next week. At 5 days of age, when ^{3}H-thymidine was administered, the corpus callosum contained many glioblasts and 6%–7% of phagocytic "ameboid" cells. Cell counts, labeling indexes, and electron microscopic observations showed that 2 h after administration, label was only present in glioblasts and ameboid cells, none of which

resembled microglia. When results at later intervals were assessed, the labeling peak for ameboid cells occurred at 12 days. At this age, typical microglia were numerous and most of them (78%) were labeled. Cells thought to represent transitions between ameboid cells were identified and as numbers of microglia subsequently increased, ameboid cells disappeared (Imamoto and Leblond 1978). These observations and the earlier labeling peak for glioblasts (9 days of age) led Imamoto and Leblond (1978) to conclude that most microglia in the rat corpus callosum originate from ameboid cells, a group of phagocytic cells they thought were derived from monocytes. Additional evidence favoring this concept was the demonstration of nonspecific esterase activity in these ameboid cells (Ling et al. 1982) and their presence in the corpus callosum prenatally (Tseng et al. 1983) and postnatally (Ling 1976).

As noted above, microglia are more numerous in gray matter and their development was studied quantitatively in postnatal rat visual cortex by Parnavelas and his collaborators (1983). From birth until 6 days of age, only a few microglia were identified. They had relatively electron-lucent cytoplasm, some long profiles of granular endoplasmic reticulum, cytoplasmic inclusions containing phagocytosed material, and few other organelles. From ages 6–14 days (Fig. 31), numbers of immature and mature-appearing microglia increased rapidly. Both had marginated heterochromatin, polymorphic dense bodies, and stringy profiles of granular endoplasmic reticulum in a relatively large volume of cytoplasm. Compared to mature forms, immature microglia contained more free polyribosomes, smaller Golgi profiles, more phagocyte-like inclusions, and a less prominent ring of peripheral heterochromatin. Immature-appearing microglia were not observed after the second postnatal week. At that time, cells with electron microscopic features of mature microglia were found evenly distributed in all layers. They increased in number, reached adult levels by about 20 days of age, and thereafter constituted 8%–12% of all glial cells in the visual cortex (Parnavelas et al. 1983). Macrophage-like cells and ameboid microglia resembling those found in the corpus callosum were not observed. Instead, the authors only described the two classes of microglia noted above. They discussed their origins and concluded that many uncertainties still existed. They also indicated that their microglial cell population could be one line of morphologically diverse cells that was differentiating to form mature microglia. However, these investigators suggested in addition that microglia with immature and mature morphological features might be derived from different cell lines. They considered the features used to identify immature microglia to be relatively nonspecific and thought that these cells may be immature macroglia (glioblasts) functioning briefly in the neonatal cortex as phagocytes. If so, the abrupt subsequent appearance of morphologically mature microglial cells suggested that they could originate elsewhere, migrate into the CNS, and belong to a different cell line. However, no evidence for migration was found and the authors concluded that microglia are intrinsic CNS elements with an uncertain origin and lineage (Parnavelas et al. 1983).

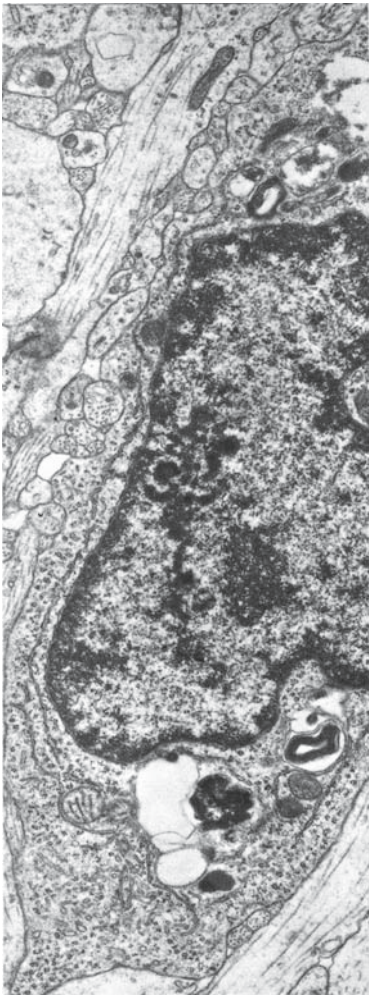

Fig. 31 Electron micrograph of an immature microglial cell in the visual cortex of a 6-day-old rat. Note the dark nucleus as well as the numerous polyribosomes, the stringy profiles of granular endoplasmic reticulum, and the large debris-filled inclusions in the cytoplasm. (Reprinted from Parnavelas et al. 1983, with permission)

4.2 Conclusions

Even though the precursors and functional properties of microglia still have not been clearly defined in the developing CNS, studies for 50 years after their discovery produced substantial progress. There was general agreement on criteria that identified mature microglia in sections prepared for light and electron microscopy. Identifying properties shared by microglia and macrophage-like cells was aided by tests for the presence of enzymes (nonspecific esterase, acid phosphatase) and cell surface components that react with specific antibodies or with lectins. Additionally, many studies of rodent CNS showed that cells fulfilling these morphological

criteria appeared abruptly in the second postnatal week, multiplied rapidly, and achieved adult numbers and distribution in the next few weeks. In the corpus callosum, observations of Imamoto and Leblond (1978) showed that many microglia originated from ameboid cells that were present prenatally (Tseng et al. 1983) and had properties of monocytes and macrophages (Ferrer and Sarmiento 1980; Ling et al. 1982; Valentino and Jones 1981), but similar ameboid cells were not observed in the developing visual cortex where the density of microglia reached substantially higher levels than it did in the corpus callosum. Futhermore, ameboid macrophage-like cells were not identified as microglial precursors in the optic nerve, another white matter region where gliogenesis had been studied extensively by autoradiography and electron microscopy. As others have noted, these rather different findings may reflect regional morphological variables in developing microglia and their precursors. They also indicated that the origins and functional properties of microglia in many regions of developing CNS were uncertain and needed further study.

5
Ependymal Cells

The ependyma is the epithelium that lines the ventricles and the central canal of the spinal cord. Cells forming this epithelium include the ependymal cells, tanycytes, and a few other cells. Ependymal cells are the most numerous and form the epithelial lining of the central canal and ventricular system except for regions in the third ventricle that border the hypothalamus. Tanycytes are found in these areas. A few other cells, such as CSF-contacting neurons, neurosecretory neurons, and glandular epithelial cells, are occasionally seen; they are most frequent in the third ventricle, have been reviewed by Knowles and Anand Kumar (1967), and are not described further here.

5.1
Origin and Division

There is general agreement that ependymal cells originate from the epithelium that forms the primitive neural tube (see sections on astroglia: epithelial cells and columnar cells during CNS development). Experiments using ^{3}H-thymidine demonstrated labeling of ependymal cells as early as E10 or E11 in rats and mice (Korr 1980; Rakic and Sidman 1968). In rat brain, labeling was seen first in the ependyma of the fourth ventricle with subsequent rostral progression (Das 1979); there were regional variations in labeling peaks, and in each area studied labeling always was observed in ependymal cells before it occurred in other glial cell types. Also, Das (1979) found occasional cells of the choroid epithelium labeled along with ependymal cells and concluded that genesis of the ependyma and of the choroid plexus had a similar time course. Ependymal cells continued to proliferate postnatally, and occasional labeled cells were observed in adult animals (Altman 1963).

5.2
Maturation and Protein Expression

As noted in the description of early CNS development in the section on astroglia (Sect. 2), the cells forming the neural tube epithelium in the rat have a pseudostratified arrangement and a similar appearance at E13–E15 when studied by light and electron microscopy [also, see observations by Rutzel and Schiebler (1980)]. Most of the organelles were concentrated at the ventricular surface except for multivesicular bodies and phagosomes. These appeared to be more numerous near the external (pial) surface. Rutzel and Schiebler (1980) found that at E16–E17, primitive ependymal cells could be identified in the developing median eminence by their cilia, their microvilli—which also protruded into the ventricular lumen—and their content of glycogen. Subsequently, their organelles included more profiles of granular endoplasmic reticulum, dense bodies, multivesicular bodies, and filaments; the density of organelles increased and their organization became more complex. As prenatal development progressed, junctional complexes between ependymal cells also increased in number.

The early postnatal development of ependymal cells in the third ventricle of rats was studied comprehensively by Walsh, Brawer, and Lin (Walsh et al. 1978). In newborn animals studied by scanning electron microscopy, ependymal cell cilia were shorter and there were patches of nonciliated cells in regions of ciliated ependymal cells. By transmission electron microscopy, many ciliated ependymal cells contained perinuclear filaments and the adult complement of organelles. Other cells lacked perinuclear filaments seen in the adult and contained numerous apical cilia or large collections of fibrous granules and multiple subapical centrioles; these latter are known to be associated with ciliogenesis, suggesting an early postnatal transformation from nonciliated to ciliated ependymal cells (Walsh et al. 1978).

Compared to studies of other glial cell types, there were relatively few studies of protein expression in ependymal cells (see review by Bruni and coworkers 1985). Roessmann and his collaborators (1980) showed that GFAP could be detected transiently in human fetal ependymal cells at the time of ciliogenesis. At that stage and subsequently, ependymal cells contained intermediate filaments. These investigators wondered if transient expression of GFAP was due to a change in intermediate filament antigenicity or reflected different detection thresholds for the immunocytochemical method that was used (Roessmann et al. 1980). Immunocytochemical methods had not been used to look for other intermediate filament constituents in developing ependymal cells.

6
Tanycytes

Tanycytes are thought by most investigators to be a special variety of ependymal cell that has a long basal (tail) process that extends radially through the neuropil for a substantial distance (reviewed in Peters et al. 1976). They occur in clusters and are most numerous in the floor and ventral walls of the posterior third ventricle

where relatively short distances separate the ventricular and pial surfaces of the brain. Endings of tanycytes contact blood vessels, neurons, and the pial surface. When described in Golgi preparations and electron micrographs, tanycytes had a somatic portion that lies in the ependyma and contains the nucleus, a neck region that extends into the subependymal neuropil, and a tail process. Regional differences in the morphology of tanycytes and the distribution of their endings were described and related to functions suggested for this interesting cell type (Altman and Bayer 1978; Walsh et al. 1978).

The origin and division pattern of tanycytes was explored in autoradiographic experiments. Labeling of these cells with ^{3}H-thymidine occurred late in embryonic development and reached a peak postnatally (Altman and Bayer 1978). Depending on the region examined, cytodifferentiation of tanycytes may continue for several weeks postnatally; among the last to achieve adult form are those adjacent to the dorsal zone of the ventromedial nucleus of the hypothalamus (Walsh et al. 1978). GFAP expression began later in development in tanycytes than in ependymal cells, was found postnatally in many cells, and was detected in adult tanycytes (Hajos and Basco 1984; Levitt and Rakic 1980; Roessmann et al. 1980).

6.1 Conclusions

The ependyma is not formed by a single population of cells with a uniform pattern of development. Compared to other glial cells, less is known about the genesis and cytodifferentiation of ependymal cells and tanycytes (see review by Bruni et al. 1985). Both cell types originate from the primitive epithelium in the ventricular zone and the latter cells, because of their GFAP expression and long tail processes, are grouped by some authors with radial glia in the astrocyte cell line. However, others who have investigated the highly specialized structure of tanycytes in different regions of the third ventricle suggest that functions of these cells may include secretion, uptake and transport of humoral substances, transport of substances between CSF, extracellular and vascular compartments, and perhaps receptor activity (Walsh et al. 1978).

7 Schwann Cells

7.1 Origin and Development

7.1.1 Origin

The cells described by Schwann have a long and interesting history (Causey 1960; Jacobson 1978; Schwann 1847) and a substantial amount was known about how they acquired their differentiated properties during development. In the 1920s, Harrison

(1924) had shown that the neural crest provided most of the Schwann cells for developing nerve fibers. Although this conclusion was disputed, it was reexamined when autoradiographic techniques made it possible to label neural crest cells, transplant them, and follow their subsequent differentiation. Both Johnston (1966) and Noden (1975) showed that virtually all satellite cells that surrounded neurons, their processes, and terminals in the cranial portion of the PNS were derived from the neural crest. It was clear that neural crest cells also participated in the formation of bone, cartilage, connective tissue, and skeletal muscle. Finally, these experiments also documented the great migratory capacity of neural crest cells, a characteristic exemplified by the occasional presence of spinal ganglion neurons in the sciatic nerve (Metz et al. 1958). Since Schwann cells began migrating early and proliferated extensively before differentiating, their origin was reexamined with a new biologic marker that is not diluted during cell division (LeDouarin 1973). Cells in the chick and Japanese quail are compatible and could be distinguished by differences in chromatin pattern that continued to replicate. This technique provided additional, convincing evidence for the neural crest origin of Schwann cells and has also been useful in identifying other neural crest derivatives (Johnston et al. 1974; LeDouarin et al. 1981). Later observations showed that development of dorsal root and sympathetic ganglia (including their Schwann cells) depended on the survival and differentiation of somite-derived structures (Teillet and LeDouarin 1983). Teillet and LeDouarin showed that excision of the neural tube and notochord produced rapid death of somitic cells. Dorsal root and sympathetic ganglia failed to develop; development of cephalic and enteric ganglia was unaffected. The neural tube permitted normal development of dorsal root and sympathetic ganglia after notochord removal, but if the neural tube was removed and the notochord was left intact, only the sympathetic ganglia developed normally. These interesting results showed that cephalic, enteric, and peripheral (dorsal root and sympathetic) ganglia have different requirements for development and that the latter ganglia are differentially sensitive to the presence of the neural tube and notochord (Teillet and LeDouarin 1983).

7.1.2 Development of Perineuronal Satellites in Ganglia

Schwann cells that become perineuronal satellites in dorsal root ganglia were studied by Pannese (1969, 1974) during the embryonic development of chicks. His electron microscopic observations showed that neuroblasts could be identified before Schwann cells. When the latter appeared, they were few in number, were star-shaped, and their processes related to several nearby neuroblasts (Fig. 32). Nuclei contained dispersed chromatin; few organelles were present in the cytoplasm and surface membranes were relatively smooth. As development proceeded, the Schwannian satellites increased in number and each related to only one neuron. Their nuclei became increasingly dense, organelles filled the cytoplasm, and their processes became sheet-like with thin, overlapping expansions that often were linked by gap and adherent junctions.

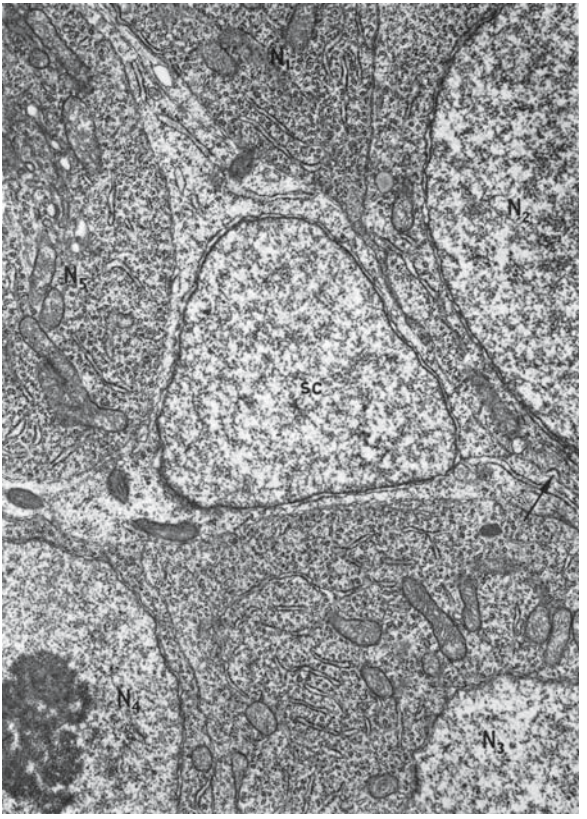

Fig. 32 Electron micrograph of an immature star-shaped satellite cell with processes contacting five neuroblasts in the spinal ganglion of a chick embryo. The *arrow* indicates a pinocytotic vesicle on the surface of neuroblast 2. (Reprinted from Pannese 1974, with permission)

Observations on the postnatal development of spiral ganglion cells of the rat identified the emergence of two identifiable types of neurons (Schwartz et al. 1983). Initially, both were surrounded by a few uncompacted membrane lamellae of satellite cells. Between P8 and P14, satellite Schwann cells formed multilayered compact myelin sheaths around the type 1 neurons, while the sheaths formed around the type 2 cells consisted only of satellite cell processes and a few loosely arranged lamellae (Schwartz et al. 1983). These observations, as well as earlier results in kittens (Romand and Romand 1982; Romand et al. 1980), showed that a compact myelin sheath that surrounds a spiral ganglion neuron is formed by more than one Schwann cell. Also, the overlapping arrangement of Schwann cells that produced these sheaths suggested that direct interactions with the neuronal surface membrane may be less important in regulating the formation and growth of these myelin sheaths than those formed around axons.

7.1.3 Migration and Early Association with Axons

Experiments using explants of dissociated neurons added to the evidence showing that neither glia nor Schwann cells were required by neurons during the elongation of their processes or the formation of functional endings. Nevertheless, in the developing PNS, outgrowth of neurites is soon followed by the appearance of Schwann cells. Their behavior in vivo was described in detail by Speidel (1932), who later correlated his own observations with in vitro and electron microscopic findings of others (Speidel 1964). In the transparent tailfins of lightly anesthetized tadpoles, Schwann cells migrated out along pioneer nerve sprouts that were growing toward the skin. Actively migrating cells moved about 60 μm a day; however, the rate was highly variable and some Schwann cells remained stationary for days. Mitotic division occurred as Schwann cells moved distally along pioneer sprouts, which provided paths for the "myelin-emergent" axons that usually grew out later. As migration and proliferation continued, transfer of Schwann cells to "myelin-emergent" fibers began. Usually this occurred within the same small fascicle of axons. However, some Schwann cells developed small pseudopods before migrating laterally to establish contact with a fiber in a different bundle. The preferential attraction of "myelin-emergent" axons for Schwann cells seemed to be quite specific, since transfer from these fibers to those that remained unmyelinated was rarely observed.

These observations were extended by correlating in vivo observations with the electron microscopic appearance of developing nerve fibers in tadpoles (Billings-Gagliardi et al. 1974; Webster and Billings 1972). When examined with the differential interference (Nomarski) microscope, Schwann cells seen moving freely between fibers were ovoid in shape and had several long processes ending in blunt expansions. Other observations by Billings-Gagliardi (1977) showed that Schwann cells moved "inchworm-style" along axons. Locomotion was sporadic with up to about 5 min of rapid movement (5 μm per minute) followed by longer periods of little or no movement (Fig. 33). In the electron microscope, Schwann cells moving between fibers did not have a basal lamina (Billings-Gagliardi et al. 1974). Ruffled areas were found along the plasmalemma, but there was no conspicuous microfilamentous sheath beneath it. The organelles were similar to those found in Schwann cells that had settled down and had begun to spread along or on several axons. During spreading, they became spindle-shaped and acquired a basal lamina, a morphological sign, perhaps, of a more permanent axon–Schwann cell relationship. In the material of Billings-Gagliardi, individual axons that could be followed for long distances were almost always accompanied by one or several others. Schwann cell nuclei were easily located in vivo but their cytoplasmic margins and the branches of each axon could not be traced with certainty. Thus, it was not possible to characterize the dynamic geometry of the relationships between individual Schwann cells and axons in these small fascicles before myelination. They are, however, probably more complex than Speidel's descriptions and diagrams indicated.

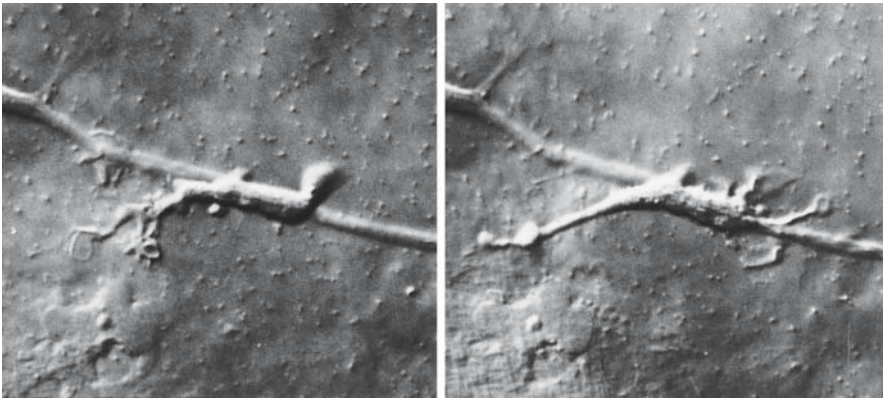

Fig. 33 Photomicrographs illustrating the migration (from the *left panel* to the *right panel*) of a Schwann cell along a small axon bundle during a 22-min interval. The nucleus of a sedentary Schwann cell at the *left* in each micrograph serves as a reference point. During this period, the Schwann cell extended a number of slender pseudopodia to its right along the axon bundle. At later time intervals (not illustrated) the cell body was rapidly translocated to the right and the blunt trailing process was shortened as the entire cell hitched along "inchworm style." Living *Xenopus* tadpole tailfin preparation. Nomarski optics. (Micrographs courtesy of S. Billings-Gagliardi. Reprinted from Webster and Favilla 1984, with permission)

Although tissue culture methods had been used for many years to study the pattern of neurite outgrowth and Schwann cell migration, there were few observations that characterized their relationships before myelination. Peterson and Murray (1955) showed that Schwann cells that surrounded axons were long, thin, and veil-like. Prior to myelin formation their attachment seemed firm and their position along axons relatively fixed. Occasionally, small refractile granules were seen in the perinuclear region. Myelination was observed in these cultures and a subsequent light and electron microscopic study showed that after long-term maintenance in vitro, the Schwann cell–axon relationships were similar to those found in mature nerves (Bunge et al. 1967). Later, it was possible to culture neurons, Schwann cells, and fibroblasts separately. Contributions of this more recent work are summarized in the section on in vitro studies.

The structural basis of migratory motion had been studied in dissociated glial cells derived from embryonic chick dorsal root ganglia (Spooner et al. 1971). These cells, some of which resembled Schwann cells, contained a sheath of actin-like microfilaments parallel to the direction of movement. When cultures of these cells were treated with cytochalasin B, migration ceased; the microfilaments were disrupted while other organelles, including microtubules, remained intact. Similar sheaths or aggregates of microfilaments may be present in actively migrating Schwann cells within developing nerves or cultures of dorsal root ganglia.

7.1.4
Early Schwann Cell–Axon Relationships

In developing nerves, the relationships between Schwann cells and axons change rapidly. During this complex sequence of events, axons are sorted into a population of fibers that becomes myelinated and into another that remains unmyelinated. During the 1960s, the surface interactions of the cells were not well defined and existing concepts were based in part on electron microscopic observations.

Prestige and Wilson (1980) in a detailed quantitative study of how nerve fibers are delivered to the developing limb, showed that initially, naked neurites grew out in nonparallel patterns. Later, Schwann cells appeared and while migrating, extended processes that collected axons and transferred them inward. In this manner, rows of Schwann cells formed primitive sheaths around neurite bundles to maintain parallel growth. Counts of axons in successive, more distal transverse levels of a nerve identified a site of branching. Since this branching site appeared earlier and was located proximal to the nerve's bifurcation, Prestige and Wilson (1980) suggested that branching was part of a trial and error mechanism of neurite pathway choice. As also shown by Peters (1961b), newly formed nerves in amphibian limb buds contained small naked axons, all of which were surrounded by a single layer of Schwann cells. Then, larger axons appeared, and Schwann cell processes began invading the central core of axons. A similar appearance was described in the digital nerves of rat embryos (Peters and Muir 1959). Schwann cell processes separated all of the nerve's axons into large bundles that were subsequently subdivided as the Schwann cells multiplied rapidly. Some of the larger axons were segregated in separate furrows of Schwann cell cytoplasm. Single Schwann cells surrounded other larger axons and this 1:1 relationship preceded myelin formation. Nuclear counts in this study showed that mitoses were numerous and that the increase in the nerve's Schwann cell population before birth could have occurred by division of those already present in the nerve at 16.5 days of gestation. This initially high rate of Schwann cell division and its decrease during myelination also was demonstrated autoradiographically (Asbury 1967). In neonatal mouse sciatic nerves, Schwann cells were dividing every 24 h and about 25% of them stopped dividing during the cycle that Asbury studied (Asbury 1967).

Some geometric and quantitative aspects of these changing relationships were examined in skip serial sections of a fiber population found at the margin of the sciatic nerve's posterior tibial fascicle (Fig. 34; Webster et al. 1973). At birth, none of this region's axons was myelinated and almost all of its transverse area was occupied by "Schwann cell families," a term used to describe all of the axons and processes of different Schwann cells located in the center of each family. Larger axons were found more commonly at the edge of a bundle, segregated in a separate furrow, or in a 1:1 relationship with a Schwann cell located on the family's outer surface. This concentric arrangement, which persisted during axon bundle subdivision and the onset of myelination, suggested that radial sorting of axons destined to become myelinated occurred in sheaths formed by longitudinal columns

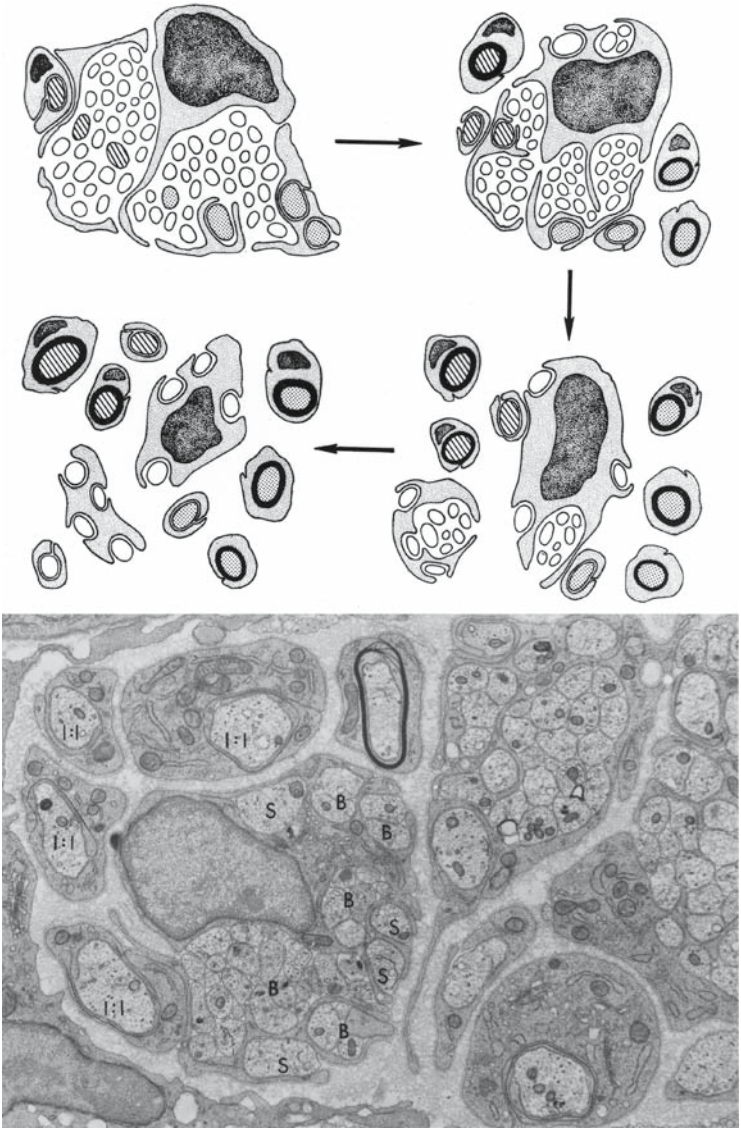

Fig. 34 The diagram (*top panel*) shows radial sorting by developing Schwann cells during axon bundle subdivision (see text). Axons to be myelinated progress radially from a bundle to a segregated furrow and then to a 1:1 relationship at the outer margins of Schwann cells. When sorting is completed, chains of individual Schwann cells form the sheaths that surround myelinated and unmyelinated axons. (Reprinted from Webster et al. 1973, with permission). The *lower panel* is an electron micrograph of a 7-day-old rat sciatic nerve illustrating this radial sorting process (see *top right* part of diagram). The Schwann cell family shown at the *left* contains five axon bundles (*B*) and four segregated axons (*S*). The family's third Schwann cell surrounds a single axon at the *lower left* (*1:1*). Other axons (*1:1*) and their Schwann cells probably were part of this family at earlier stages of this sorting process. (Reprinted from Webster 1971, with permission)

of Schwann cell families (Fig. 34). The sorting sequence included initial surface contact with a Schwann cell process, segregation in a separate furrow of a family sheath, Schwann cell division, and establishment of a 1:1 relationship with one of the daughter Schwann cells, which then became isolated from the family sheath before myelination began (Webster et al. 1973).

Counts during the week after birth also showed that Schwann cell families did the sorting in this population of fibers and permitted us to estimate rates for axon bundle subdivision, segregation of larger axons in separate furrows, and establishment of 1:1 relationships. With time, families decreased greatly in size and increased in numbers. These changes were associated with a dramatic decrease in the size of the axon bundles. Each family, however, continued to contain about the same number of bundles and larger axons in segregated furrows or in a 1:1 relationship. Since approximately half of the Schwann cells that surrounded axon bundles also enveloped larger axons in separate furrow, this process of segregation was thought to be an essential intermediate step in the establishment of the 1:1 relationship that preceded myelination (Webster et al. 1973).

In order to understand better the geometry and dynamics of this sorting process, the relationships of dividing Schwann cells were examined also (Martin and Webster 1973). In newborn rat sciatic nerves, virtually all of the mitotic Schwann cells were located in the family sheaths described above (Fig. 35). As mitosis began, the radial extent of the processes that surrounded axons decreased. By the end of prophase, the Schwann cell was spindle-shaped and remained so through metaphase and much of anaphase. The axis of mitosis was parallel to the long axis of the cell, and cytoplasmic division occurred between daughter nuclei that had formed proximally and distally to each other in the nerve. In anaphase, cytoplasmic outgrowth was thought to begin with the appearance of two new slender axial processes that contained longitudinally oriented microtubules. These processes originated at the level of each spindle pole, arched over, and grew longitudinally beside and beyond the dividing nucleus in opposite directions as shown by Martin and Webster (1973). These axial processes were thought to play a role in reestablishing the longitudinal symmetry of the daughter cells. Radial processes that surrounded axons reappeared in telophase and extended along processes of neighboring interphase Schwann cells or the basal lamina that enclosed the family. The rapid reduction in surface membrane area associated with radial process retraction was accompanied by the appearance of numerous 100-nm vesicles in the cytoplasm from late prophase to telophase. They probably were derived from the surface membrane since they disappeared as the radial processes were reextended. The nature of the Schwann cell–axon interactions that were associated with this sorting process are still not well defined, but the shape changes that occur during mitosis may help increase the contact rate. Also, glycoproteins (Pleasure et al. 1982; Shuman et al. 1983), including the one called myelin-associated glycoprotein (MAG) (Figlewicz et al. 1981; Quarles et al. 1973; Sternberger et al. 1979), was thought to possibly have a role in the cellular recognition and segregation of axons to be myelinated and in the subsequent development of myelin sheaths.

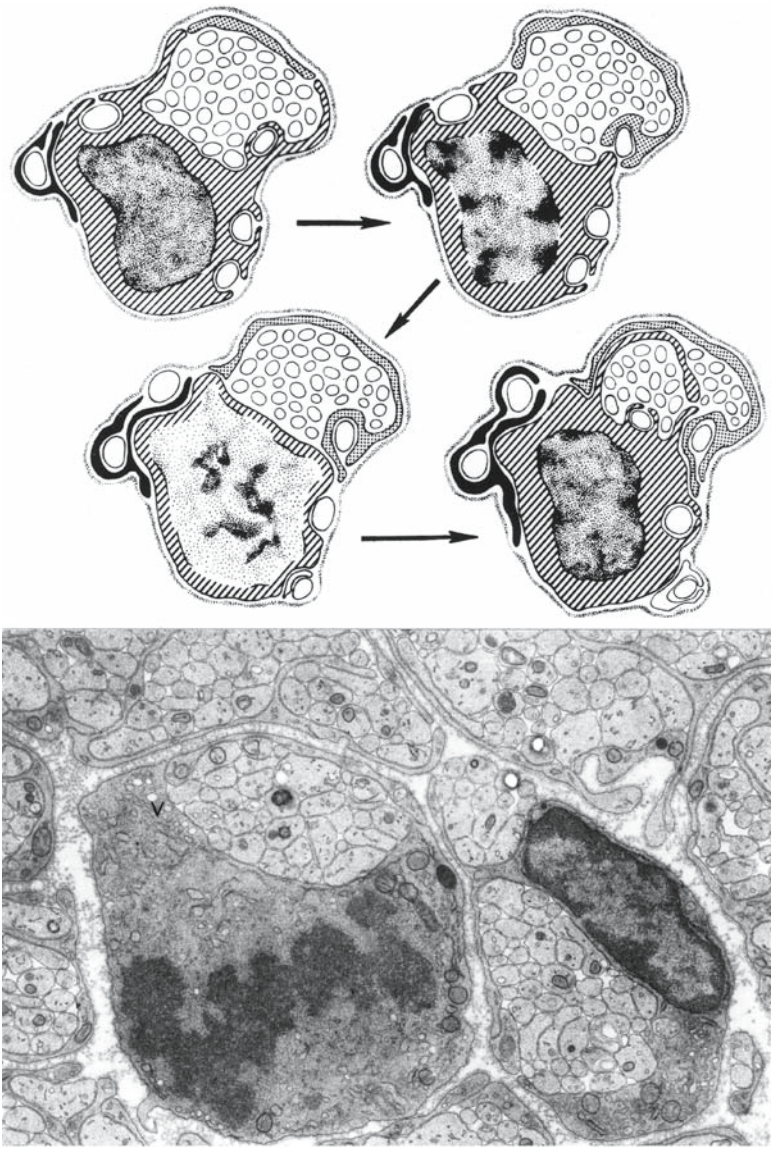

Fig. 35 Diagram (*top panel*) of relationships between a Schwann cell family and its axons during Schwann cell division. In prophase and early metaphase, radial processes that surround axons retract. Some axons are temporarily enclosed by processes of other Schwann cells in the family sheath that later guide the mitotic cell's processes as they reextend during telophase. (Reprinted from Martin and Webster 1973, with permission.) *Below*, an electron micrograph of newborn rat sciatic nerve illustrates the stage shown diagrammatically at the *lower left*. The Schwann cell in metaphase has no radial processes surrounding axons; it contains a few vesicles (*v*). (Reprinted from Webster 1974, with permission)

7.1.5 Formation and Growth of Myelin Sheaths

Although the resolution of microscopes of the time was insufficient to demonstrate and trace membranes, the basic relationships between Schwann cells, myelin sheaths, and axons were known over a century ago (Ranvier 1878). While living nerve fibers were developing in vivo or in vitro, thin segments of myelin were found first in the perinuclear regions of Schwann cells (Peterson and Murray 1955; Speidel 1932; Speidel 1964). How these segments were formed remained obscure until the electron microscopic observations and hypothesis of Geren (1954) clearly established the basic morphologic parameters of peripheral myelination.

The mesaxon, which is continuous with the Schwann cell surface membrane, grows and forms a spiral sheet around the axon. Further growth and apposition of the spiral layers occur as the myelin sheath matures. Geren's observations were confirmed and extended by many investigators (Robertson 1962; Sjostrand 1963; Peters and Vaughn 1970; Peters et al. 1976). Additional references of interest included those by Matthews (1968), Friede and Samorajski (1968), and Uzman and Hedley-Whyte (1968). In general, they agreed that the Schwann cell, or part of its surface, moves around the axon during growth of the myelin spiral. Two other observations that could not be explained by any simple rotation mechanism had also been discussed. The contour of the myelin spiral was not uniform along the internode; complex variations occurred (Rosenbluth 1966; Webster 1964; Webster and Spiro 1960). Second, after the compact sheath was formed, its internal circumference increased to accommodate the growing axon (Friede and Samorajski 1968; Geren 1956; Robertson 1962; Rosenbluth 1966). Since little was known about the geometry and dimensions of the myelin spiral as it formed, these parameters were studied in skip serial sections of a well-defined fiber poulation by Webster (1971). Since all of these fibers were unmyelinated at birth, the onset and extent of myelination were easily established. At appropriate intervals, approximate dimensions of the bundle and its largest fibers were measured and calculated at the same relative level in litter mates' nerves. These data showed that the myelin membrane's area and transverse length increased exponentially with time; the growth rate increased rapidly during the formation of the first four to six spiral layers and remained relatively constant during the subsequent enlargement of the compact sheath. As others had noted, the minimum diameter of axons that Schwann cells began myelinating was about 1 μm (Fig. 36; Duncan 1934; Matthews 1968). During the formation of the first spiral turn, the mesaxon's length and configuration varied when they were studied at different levels in the same Schwann cell (Fig. 36). The position of the mesaxon's termination shifted while its origin, at the Schwann cell's surface, remained relatively constant. Along myelin internodes composed of two to six spiral turns, there were many variations in the number of lamellae and their contour; near the mesaxon's origin, longitudinal strips of cytoplasm separated the myelin layers (Fig. 36). Thicker sheaths were larger in circumference, more circular in transverse sections, and more uniform at different levels. Separation of lamellae

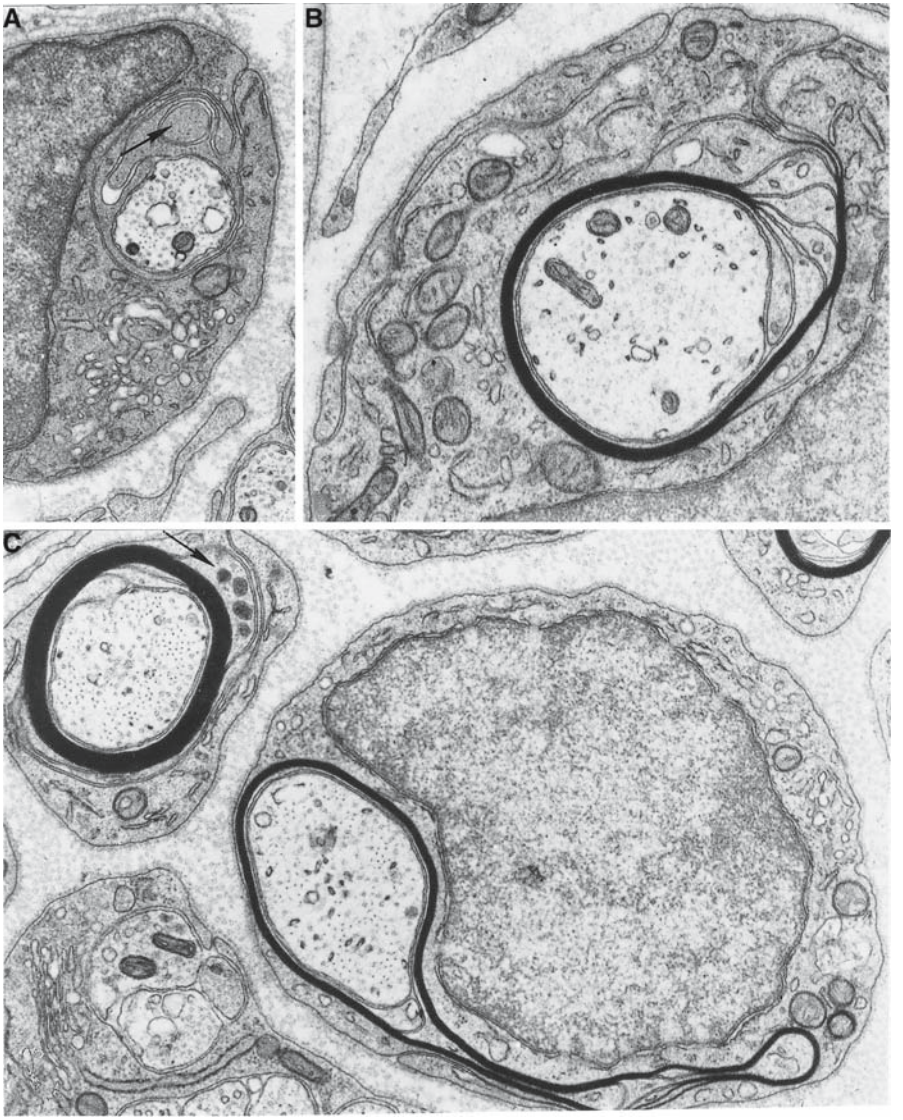

Fig. 36 A–C Electron micrographs of a 7-day-old rat's sciatic nerve. In **A** there is a large loop in the upper myelin sheath. There is a thicker, more circular myelin sheath at the *lower right*. Four dense bodies (*arrow*) that resemble lysosomes are present in a patch of cytoplasm inside this sheath's outer layer. In **B**, cytoplasm containing organelles separates the layers of compact myelin near the mesaxon's origin. When traced at other transverse levels, these cytoplasmic zones form continuous longitudinal strips. Part of this Schwann cell's surface is also covered by a thin band of projecting cytoplasm. In **C** there is a redundant loop in the mesaxon's first spiral turn. It partially surrounds a projection (*arrow*) of the Schwann cell's surface. (Reprinted from Webster 1974, with permission)

by cytoplasm was discontinuous and generally occurred at Schmidt-Lanterman clefts. Variations in sheath contour, similar to those described earlier by Webster and Spiro (1960), were confined to the paranodal region. Junction-like complexes similar to those located at nodes of Ranvier were also occasionally found along mesaxons or the outer or inner myelin lamellae (Webster 1971).

The observations in the above study suggested that the spiral form and initial enlargement of the myelin sheath could be explained, in part, by developmental events that imposed limits on the geometry of the rapid membrane growth that occurred in these Schwann cells (Webster 1971). In the cellular columns that surrounded and subdivided axon bundles, the arrangement of processes was consistent with a relatively free pattern of surface membrane growth. After mitosis, a daughter cell in a 1:1 relationship acquired a complete basal lamina and was surrounded by endoneurial collagen, both of which probably limited radial spread and favored growth along and around the axon. Cellular elongation predominated until proximal and distal Schwann cells met at nodes of Ranvier. Then the major site for membrane expansion shifted from the Schwann cell's external and axonal surfaces to the membrane pair that connected them, the mesaxon. Initially the position of its outer edge was relatively fixed while its inner edge remained free to rotate around the axon. The conditions seemed consistent geometrically with a situation that would favor spiral growth of the mesaxon regardless of where new membrane components were added. Periaxonal movement of the Schwann cell nucleus may occur as Murray (1965) observed, but it did not seem to be an essential prerequisite for spiral formation. The mesaxon's growth rate increased rapidly. The spiral sheet enlarged, became more compact, and the relative area of myelin membrane that was covered by cytoplasm began to decrease. As these surface relationships changed, the rate of membrane growth leveled off and the geometry of the sheath became more regular.

The greatest increase in myelin membrane area probably occurs at a relatively constant rate while the sheath is a compact lamellar spiral. Its length and internal circumference become larger; the number of turns also increases. How this happens was poorly understood. Our data indicated that the mesaxons, the incisures, and the sheath's outer and inner layers provided large enough interfaces for the addition of new membrane material from the cytoplasm at the rate required for the sheath's growth. As others have suggested, the positions of the layers and their components probably change continuously to achieve the best packing arrangement for the changing molecular constituents of the growing sheath (Friede 1972; Friede and Samorajski 1968; Robertson 1962; Rosenbluth 1966). As the number of spiral turns increased, the origin of the external mesaxon and the nucleus probably moved around the axon. Rotation, in the opposite direction, of the sheath itself and the internal mesaxon may also have occurred. During growth of the compact sheath, the periodicity increased (Hedley-Whyte and Kirschner 1976). This increase was related to developmental changes in myelin composition. The developmental remodeling of myelin described by Webster (1971) suggested that sheaths interacted dynamically with adjacent Schwann cell cytoplasm. Later, Mugnaini and his collaborators (1977) studied the distribution of cytoplasm in mature Schwann cells

and showed that a network of cytoplasmic channels bordered on and extended into compact areas of myelin. These channels and the large numbers of vesicles (caveolae) they described along the outer mesaxon and plasmalemma probably were thought to have an important role in sheath maintenance and the turnover of myelin constituents.

The location of Ranvier nodes on axon membranes probably is determined before compact myelin is formed. In freeze-fracture replicas, patches of particles characteristic of nodes appeared in the axolemma near ensheathing Schwann cell processes (Wiley-Livingston and Ellisman 1980), and soon afterwards axon membranes in developing paranodal regions contained rows of dimeric particles that were thought to have a role in saltatory conduction (Wiley and Ellisman 1980). During early stages of myelin formation, cytochemical properties of nodes also appeared in regions of axolemma between adjacent Schwann cells (Waxman and Foster 1980). As development proceeds, all myelin segments do not survive (Berthold and Carlstedt 1977). Sheath remodeling and removal probably also help segments along maturing fibers achieve dimensions appropriate for their function.

From a functional standpoint, the three most important dimensions of a myelinated nerve fiber are the axon diameter and both the thickness and length of its myelin sheath. Are developmental changes in these dimensions related to each other, and are similar patterns of change found throughout the PNS? Generally, elongation of internodes parallels growth of the part that contains the nerve fibers (Schlaepfer and Myers 1973; Thomas 1955; Vizoso and Young 1948). Although longer myelin sheaths are usually thicker and surround larger axons in mature nerves, developmental increases in axon caliber and myelin sheath thickness do not parallel body part growth throughout the PNS.

In a series of studies, Friede and his collaborators examined relationships of axon caliber and both the onset and rate of myelin formation (Friede 1972; Friede and Martinez 1970; Friede and Miyagishi 1972; Friede and Samorajski 1967; Friede and Samorajski 1968). Their results suggested that axon size had a major role in the induction of myelination and the regulation of myelin sheath size. However, the model that they describe and the growth rates discussed were not consistent with data obtained from more uniform fiber populations. When axon diameters and myelin sheath thicknesses in developing sensory and motor fibers were compared, different growth patterns were found (Williams and Wendell-Smith 1971). From birth to age 8 weeks (the period of marked limb growth) the diameters and growth rates of axons in rabbit sural (sensory) and medial gastrocnemius (motor) nerves were similar, but the myelin sheaths surrounding the motor axons were thicker at each interval. After 8 weeks the sensory axons stopped growing while the motor axons and their sheaths continued to enlarge. Furthermore, a statistical analysis of developing anterior root fibers has shown that myelination began around fibers that varied greatly in circumference (Fraher 1972). A linear relation between axon caliber and myelin sheath thickness was not found during the initial stages of myelination and was not clearly established until age 17 days, when more than half of the sheaths examined had 30 turns or more. In later studies, Fraher (1978a, b)

examined a series of central and peripheral myelin segments along developing motor neuron axons. When individual axons were measured, they were smaller in the CNS than peripherally. Still, myelin appeared simultaneously (at birth) on both parts of the same axon. Centrally, there was greater variation in axon caliber, myelin thickness, and internodal length than peripherally. Along the same fiber, Fraher found no association between patterns of longitudinal variation in axon caliber and sheath thickness. In developing peripheral fibers studied in dorsal root transition zones, a linear relationship between axon diameter and internodal length could only be demonstrated in smaller fibers (Carlstedt 1980). The available evidence therefore indicated that in most fibers—up to a certain size—axon caliber, myelin sheath thickness, and internodal length were related after growth patterns were established. But, axon diameter certainly was not thought to be the major determinant of the onset of myelination, the sheath's growth rate (in thickness or length), or the final area of myelin's compact spirally wrapped membrane sheet.

7.2 Biochemistry of Peripheral Myelination and Distribution of Myelin Constituents

In general, peripheral myelin was more difficult to isolate and purify than myelin from the CNS. Therefore, less was known about its composition and metabolism during development. The available data suggested that myelin was not made de novo [see reviews by Davison (1970), Benjamins and Smith (1984) and Benjamins and Morell (1978)]. Instead, the initial turns of the mesaxon spiral probably are similar biochemically to the Schwann cell's plasmalemma.

In peripheral myelin, the major protein constituent is a glycoprotein called P_0 and it has a molecular weight of 30,000 (Everly et al. 1973; Brostoff et al. 1975). It can be detected immunocytochemically in newborn rat sciatic (Brockes et al. 1980b) and trigeminal (Trapp et al. 1981) nerves but had not been identified with more conventional biochemical methods until the day after birth (Wood and Engel 1976). At age 5 days, it was 3% of the total protein in sciatic nerves and levels increased to 13% by age 15 days (Wiggins et al. 1975). Wood and McLaughlin (1975) employed a peroxidase labeling technique to visualize binding sites of a plant lectin (concanavalin A) in polyacrylamide gels and in sections of myelinated sciatic nerve fibers. In gels, the P_0 band stained intensely. Since the same labeling method produced dense deposits along myelin's intraperiod lines, the authors concluded that the carbohydrate portion of P_0 probably was located at this site. This localization was supported also by the labeling of intraperiod band material by lactoperoxidase (Peterson and Gruener 1978) and by X-ray diffraction data (Blaurock and Nelander 1979). Gould (1977) studied sites of glycoprotein synthesis and insertion into myelin by injecting tritiated fucose into sciatic nerves of rat pups and preparing autoradiographs at intervals following injection. After 1 h, newly formed product (primarily P_0) was present in juxtanuclear areas of Schwann cell cytoplasm that contained many Golgi profiles. After longer labeling periods, grain counts were lower in Schwann cell cytoplasm and higher over myelin sheaths. To

extend their earlier observations (Trapp et al. 1979b), Trapp and his collaborators (1981) immunostained semithin Epon sections of developing rat nerves with P_0 antiserum and traced the distribution of cytoplasmic staining on electron micrographs of the same Schwann cells in adjacent thin sections. They showed that very thin myelin sheaths were detected more easily in semithin sections stained with P_0 antiserum than with other cellular stains such as toluidine blue or paraphenylenediamine. In addition, anti-P_0 staining was present in the cytoplasm of developing and mature myelin-forming Schwann cells and it was located in regions occupied by Golgi profiles (Trapp et al. 1981; Webster et al. 1981).

Peripheral myelin contains two basic proteins. The first, called P_1, has a molecular weight of 18,500 and is similar if not identical to myelin basic protein (MBP) isolated from the CNS. Anti-MBP immunostained myelin-forming Schwann cells and sheaths in immature trigeminal ganglia (Sternberger et al. 1978a). MBP also has been demonstrated in myelin sheaths of newborn rat sciatic nerves by immunofluorescence (Brockes et al. 1980b) and with the peroxidase-antiperoxidase method (Trapp et al. 1980; Webster et al. 1981). When Trapp and his colleagues treated serial Epon sections with antisera to P_0 and P_1, the latter stained fewer myelin sheaths. This may reflect the differences in concentration of the two proteins in newly formed myelin, in the onset of their insertion into myelin lamellae, or in conditions required for the detection of immunoreactivity.

The second basic protein, P_2, has a molecular weight of 13,500; the P_1/P_2 ratio varies in different regions of the PNS; there also are variations among different mammalian species. When myelinated fibers from dorsal roots, trigeminal ganglia, and sciatic nerves of rats were treated with P_0, P_1, and P_2 antisera, all sheaths were stained by anti-P_0 and anti-P_1. But anti-P_2 did not stain all sheaths; those that were stained generally were larger (Trapp et al. 1979b) and a similar pattern of selective P_2 distribution has also been observed in developing sheaths (Trapp et al. 1980; Winter et al. 1982). However, later densitometric measurements of anti-P_2 immunoreactivities in developing sixth cranial nerves suggested that differences in detection and in observed intensities probably reflected relative amounts of this minor PNS myelin constituent that could be detected by the method rather than selective expression of P_2 by certain myelin-forming Schwann cells (Hahn et al. 1987).

Among the high molecular weight proteins in peripheral myelin is MAG. It is present in newly formed peripheral myelin (Fig. 37), and in mature sheaths it appears to be concentrated in Schmidt-Lanterman clefts, paranodal areas, and periaxonal regions of myelinated fibers (Schober et al. 1981; Trapp and Quarles 1982). Even though there is substantially less MAG in peripheral than in central myelin, its presence peripherally has been confirmed by biochemical experiments (Figlewicz et al. 1981).

Electron microscopic autoradiography has been used to study sites of incorporation and subsequent distribution of precursors and lipid constituents of myelin. When myelinating dorsal root ganglion cultures were exposed to tritiated choline, radioactivity appeared all along the internode (Hendleman and Bunge 1969). The distribution within the developing spiral could not be determined, and there was no preferential site of initial incorporation in the Schwann cell. When tritiated cholesterol was injected into 5-day-old mice and their sciatic nerves were examined,

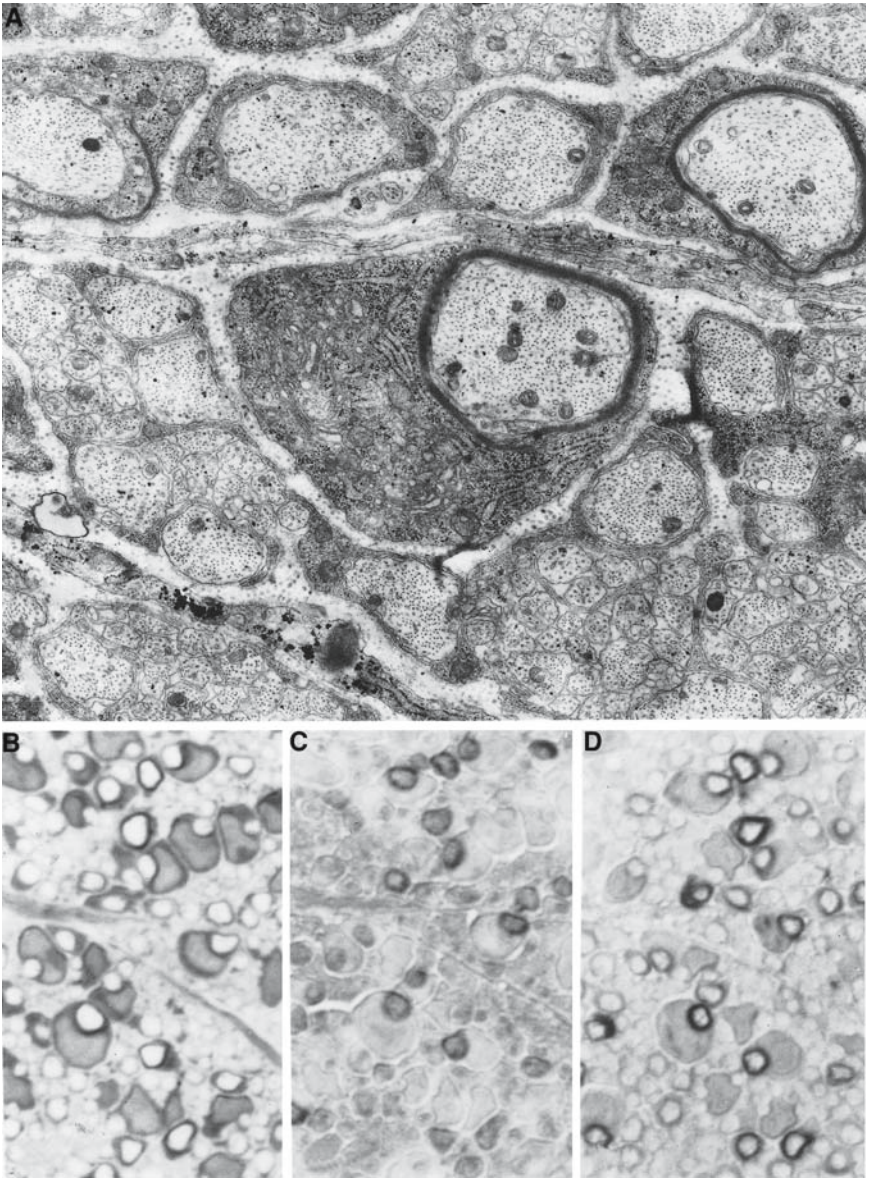

Fig. 37A–D Distribution of myelin protein P_0 and myelin-associated glycoprotein (MAG) in developing nerve fibers of the trigeminal ganglion in a newborn rat (**A**). Semithin serial Epon sections of the same area were stained with toluidine blue (**B**), MAG antiserum (**C**), and P_0 antiserum (**D**). Myelin sheaths are readily identified in **B**, **C**, and **D**. In addition, punctate perinuclear anti-P_0 staining was found in some myelin-forming Schwann cells. Electron micrographs of these cells in serially cut thin sections showed that the staining was localized in regions containing Golgi membranes (cell in center of panel **A**, also, see Trapp et al. 1981). (Reprinted from Webster and Favilla 1984, with permission)

the labeled cholesterol was found predominantly in myelin (Hedley-Whyte et al. 1969). The growing sheaths were uniformly labeled in a radial direction after 3 h, 24 h, and 7 weeks. The diffuse distribution of radioactivity suggested that incorporation also occurred at multiple sites and that there was continuous exchange of cholesterol along and between the spiral layers during myelination.

Peripheral myelin biosynthesis was studied in vitro by Pleasure et al. (1982). When incubated in a simple medium, sciatic nerves of chick embryos incorporated labeled sulfate into myelin sulfatide. They also found that a transport lipoprotein was required to transfer the labeled sulfate from the nerve's microsomal subfraction into myelin.

Winter and her collaborators (1982) subsequently used immunofluorescence and specific antisera to compare the appearance and distribution of myelin proteins and glycolipids in freshly dissociated Schwann cell suspensions, sciatic nerve sections, and dissociated Schwann cell cultures from newborn and day-old rats. Galactocerebroside and sulfatide were detected on surfaces of many Schwann cells before myelination started. Since their appearance preceded detection of myelin proteins P_0, P_1, and P_2, it was thought to be an early event in myelin formation (Winter et al. 1982).

7.3 Experimental Alterations Affecting Schwann Cells and Myelination

Undernutrition and inhibitors of cholesterol biosynthesis also selectively affect Schwann cells and myelination. In the former condition, the Schwann cell mitotic rate, the relationships associated with the initiation of myelination, and axonal growth were normal. However, the growth of myelin sheath thickness was slower than that found in controls (Clos and Legrand 1970; Hedley-Whyte and Meuser 1971). Inhibitors of cholesterol biosynthesis delayed the initiation of myelination in addition to slowing the growth of those cells formed prior to drug administration (Rawlins and Uzman 1970a, b). In contrast to the foregoing conditions, neonatal hypothyroidism produced little change in the early development of myelinated fibers (Clos and Legrand 1970; Reier and Hughes 1972). Quantitative data in the latter study, however, showed that the growth rate of unmyelinated axons and the Schwann cells that surrounded them decreased, slowing the rate of axon bundle subdivision (Reier and Hughes 1972). These changes, which could be reversed by thyroid hormone treatment, suggested that Schwann cells might be especially sensitive to hormone deficiency at specific stages in their relationships with axons during the sorting process that precedes myelination.

7.4 Studies Using Grafts and Mouse Mutants

Although morphological, biochemical, and immunocytochemical studies have helped define axon–Schwann cell relationships during development, they have provided relatively little information about how these processes are regulated. To

study how axons, Schwann cells, fibroblasts, and other endoneurial constituents interact, Aguayo, Bray, and their collaborators removed nerve segments from normal and mutant mice and transplanted them to another site in the same host or to sites in normal and mutant recipients. To illustrate how they have used grafts and mutants to explore the regulation of these interactions, a few of their experiments are summarized here. Readers interested in this approach should consult reviews by Aguayo and coworkers (1979), Bray and coworkers (1981), and by Aguayo and Bray (1984). Their use of primary mouse chimeras and in vitro experiments to study glial differentiation also are described.

The method of nerve grafting used by Aguayo and collaborators (Aguayo et al. 1979) is shown diagrammatically in Fig. 38. After a donor segment is grafted into a host nerve, axons in the host distal nerve and in the grafted segment degenerate. After axons regenerate from the proximal stump, responses of donor Schwann cells and endoneurium to regenerating host axons can be studied in the graft and compared to those that the same axons produce more distally in host Schwann cells and endoneurium.

Early experiments investigated interactions of normal axons and Schwann cells after nerve segments with unmyelinated axons were transplanted into myelinated nerves and after myelinated segments were transplanted into nerves with unmyelinated axons. The results showed that the axon determined whether a Schwann cell will differentiate into a cell that will ensheath multiple unmyelinated axons or a cell that will relate to a single axon and form its myelin sheath (Aguayo et al. 1976). Comparable results were reported after cross-anastomosis of myelinated and unmyelinated nerves (Aguayo et al. 1976; Weinberg and Spencer 1975, 1976).

Similar graft experiments identified the primary defect responsible for hypomyelination in several mouse mutants and also led to a better understanding of other factors that influence axon–Schwann cell interactions that initiate myelination (Aguayo and Bray 1984). For example, trembler mice have a dominantly inherited disorder characterized by thin, poorly compacted, or absent PNS myelin segments; unmyelinated fibers and their Schwann cells are normal morphologically. In graft experiments, trembler hypomyelinated nerve segments transplanted

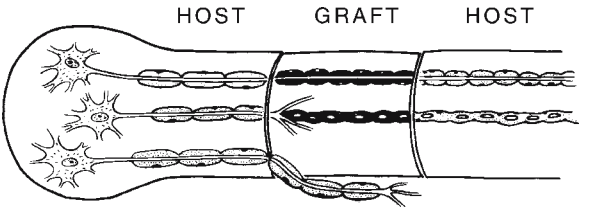

Fig. 38 Diagram of an experimental nerve graft. Regenerating axons from the host proximal stump are ensheathed by donor Schwann cells in the distal stump. Regenerating axons that grow outside the graft are ensheathed by host Schwann cells that migrate from the proximal stumps. (Reprinted from Aguayo et al. 1979, with permission)

into normal host nerves showed the same abnormalities after regeneration as had been described in intact trembler nerves; normal myelinated fibers were present proximally and distally (Aguayo et al. 1979; Fig. 39). When a normal segment was grafted into a trembler nerve and examined after regeneration, it contained normal myelinated fibers. Proximal and distal stumps showed the abnormalities characteristically seen in trembler nerves (Fig. 39). Finally, if a segment containing trembler unmyelinated axons and their Schwann cells was transplanted into a heavily myelinated nerve of a normal mouse, the characteristic trembler abnormalities were found in the segment after regeneration (Perkins et al. 1981a). These studies clearly showed that all trembler Schwann cells have a primary genetic defect that was expressed during interactions and stages of differentiation needed for myelin sheath formation. Earlier interactions, including those required for ensheathment of unmyelinated axons, appeared to be unaffected (Aguayo and Bray 1984). Another interesting conclusion from these graft experiments was that Schwann cells probably had a significant role in determining the size of myelinated axons during

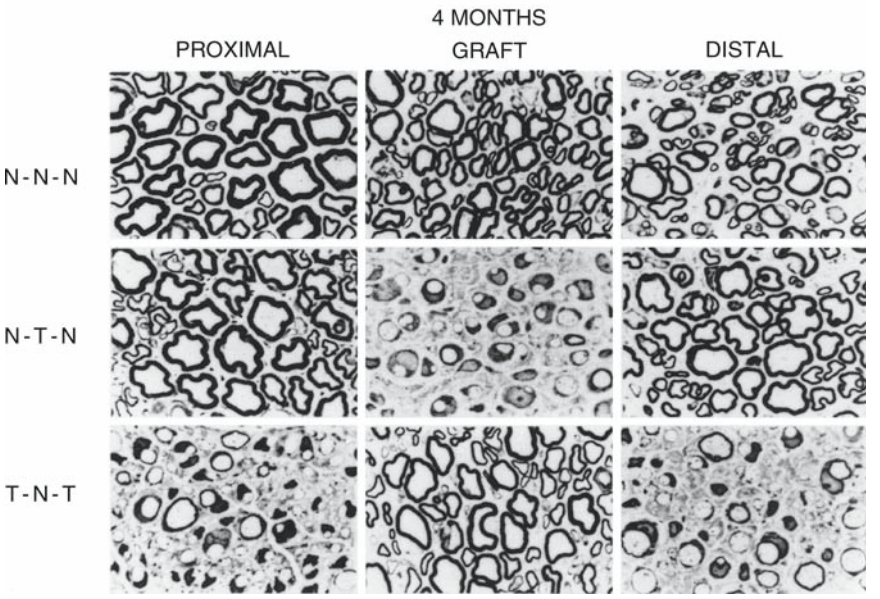

Fig. 39 Proximal stumps, graft segments, and distal stumps of regenerated nerve grafts 4 months after grafting. In normal–normal combinations (*N-N-N*), the grafts and distal stumps contain many regenerated myelinated fibers that resemble those in the proximal stump. When trembler mouse nerves are grafted into normal nerves (*N-T-N*), the proximal and distal stumps appear normal, but fibers in the graft lack myelin or are hypomyelinated. Normal nerves grafted into trembler mouse nerves (*T-N-T*) show deficient myelination in the proximal and distal stumps, whereas the grafted portion of the same nerve is normally myelinated. (Reprinted from Aguayo et al. 1979, with permission)

development and regeneration (Aguayo and Bray 1984). In Fig. 39 it is clear that hypomyelinated axons in a trembler proximal stump are smaller than normal; in the graft these same axons are ensheathed by normal Schwann cells and are normal in size; further distally—when again ensheathed by trembler Schwann cells—these axons are abnormally small.

In the mouse mutant called dystrophic, there are discontinuities in the basal lamina of Schwann cells and they are associated with focal and more generalized changes in Schwann cell ensheathment of axons. In electron micrographs, some Schwann cells next to unensheathed or partially ensheathed axons have an undifferentiated appearance. When studied in graft experiments, dystrophic roots transplanted into normal nerves were normally myelinated after regeneration. This indicates that the undifferentiated Schwann cells found in this mutant had the capacity to ensheath and myelinate axons located in normal endoneurium (Perkins et al. 1981b). Further investigation showed that altering constituents of the endoneurium could partially or completely reverse the defects in basal lamina, axon ensheathment, and myelination found in dystrophic PNS. Alterations used to reverse the defects included: (1) the production of more endoneurial collagen by inducing Wallerian degeneration and regeneration in vivo (Bray et al. 1983), and (2) the addition of normal fibroblasts to cultures of dystrophic Schwann cells (Bunge et al. 1982; Cornbrooks et al. 1983). The conclusions from these and other experiments are that fibroblasts, collagen, and perhaps other endoneurial constituents have important functions during early axon–Schwann cell interactions that are prerequisites for axonal ensheathment and subsequent myelin formation (Aguayo and Bray 1984).

7.5 In Vitro Studies

Tissue culture studies also substantially increased our understanding of Schwann cells' requirements for the development of differentiated properties and the interactions needed for basal lamina formation, axon ensheathment, and myelin formation. These investigations involved the development and use of methods to isolate, maintain, and then—at defined stages of development—co-culture Schwann cells, neurons, and fibroblasts. The brief summary included here is selective; much more extensive reviews have been published by Mary Bunge and her collaborators (Bunge et al. 1983) and Richard and Mary Bunge (Bunge and Bunge 1984).

Generally, investigators used differential adhesion properties, antimitotic agents, and adjustment of media constituents to prepare neuron cultures. Methods for culturing Schwann cells included procedures to remove fibroblasts followed by steps to enhance Schwann cell proliferation. The Wood method (Wood 1976) began with explantation of dorsal root ganglia. Antimitotic agent treatment produced neuronal cultures by killing the dividing Schwann cells and fibroblasts. After outgrowth, microdissection created separate preparations of neuronal perikarya and of neurites suitable for either co-culture experiments or biochemical study. This

method was used to study neurite membrane fractions and their mitogenic effects on cultured Schwann cells (Salzer and Bunge 1980; Salzer et al. 1980a, b). If explants were treated briefly with antimitotic agents and transplanted to a new culture dish, and then the neurons were excised, the remaining cells were Schwann cells. These cells could then ensheath and myelinate sensory neuron axons in co-culture (Wood 1976). Trypsin digestion of sensory and autonomic ganglia was used in the above method to obtain populations of neurons and Schwann cells that could be used in co-culture experiments. Cultured populations of Schwann cells also were prepared by digesting neonatal sciatic nerves with trypsin. Then these neuron-free mixtures of Schwann cells and fibroblasts were treated with the mitotic inhibitor cytosine arabinoside, with antibodies to Thy-1, (a rodent antigen expressed by fibroblasts but not by Schwann cells) and with known Schwann cell mitogens (Brockes et al. 1977, 1980b). These methods, as well as some others described in the reviews mentioned above, were exploited to explore the control of Schwann cell proliferation, the expression of Schwann cell and fibroblast constituents, and the sources of extracellular matrix needed for normal nerve development.

Use of the above methods showed that in colonies of Schwann cells there is proliferation in regions of direct axonal contact (Wood and Bunge 1975) and that Schwann cell multiplication is most intense along the distal, unensheathed, immature growing tip of each developing neurite (Salzer and Bunge 1980). Subsequent studies showed that the mitogenic stimulus is present in particulate fractions prepared from isolates of these neurites (Salzer et al. 1980a, b). Axolemmal fractions (Sobue et al. 1983), a glial growth factor isolated from pituitary (Brockes et al. 1980a; Lemke and Brockes 1984), and certain tissue culture substrate characteristics (Cassel et al. 1982; Dubois-Dalcq et al. 1981) also promoted Schwann cell division.

In cultures derived from neonatal sciatic nerve, an antibody named Ran-1 specifically identified Schwann cells; these cells then expressed myelin basic protein and galactocerebroside during the first few days of in vitro maintenance (Brockes et al. 1977, 1979, 1980b). However, continued Schwann cell expression of these myelin constituents required contact with axons (Mirsky et al. 1980). In more recent studies combining use of these cultures and tissue preparations, Mirsky and her collaborators showed that monoclonal antibodies raised against two cell surface proteins of astrocytes called Ran-2 and A5E3 could distinguish nonmyelin-forming from myelin-forming Schwann cells (Jessen and Mirsky 1984; Mirsky et al. 1985; Mirsky and Jessen 1983). These antigens were co-expressed with GFAP (Jessen and Mirsky 1984) suggesting that CNS and PNS glia sharing the phenotype *Ran-2*±, *A5E3*±, *GFAP*± (non-myelin forming Schwann cells and astroglia) might have a common function.

The sources of the extracellular matrix found in the endoneurial compartment of nerves were investigated by using co-cultures of neurons, Schwann cells, and fibroblasts in different combinations then analyzing them morphologically, immunocytochemically, and biochemically. Electron microscopic examination of neuron–Schwann cell cultures showed that the matrix includes basal lamina and thin

collagenous fibrils; the perineurium does not develop (Bunge et al. 1980). Observations in this and subsequent studies (Bunge et al. 1980; Carey and Bunge 1981; Carey et al. 1983) showed that these cultures contained laminin and types I, III, IV, and V collagen; the results also suggested that neurons have a role in regulating the secretion of procollagen IV by Schwann cells but do not influence laminin production. These investigators concluded that a sequential release of proteins and a series of complex Schwann cell, neuron, and fibroblast interactions are needed for the formation of the extracellular matrix found in the endoneurial compartment of mature peripheral nerves (Bunge and Bunge 1984).

7.6 Conclusions

The use of an impressive array of new techniques has substantially advanced our understanding of the events that lead to the association of Schwann cells, neuronal perikarya, and axons. Direct contact with growing, immature axons stimulates Schwann cell proliferation and there now is substantial evidence suggesting that an axolemmal constituent is the mitogen. In other early interactions, Schwann cells ensheath neurons and axons and, in the presence of fibroblasts, help form the extracellular matrix for the endoneurial compartment. Neurons and their axons determine whether a given Schwann cell remains an ensheathing cell that is closely related to neuronal perikarya or to unmyelinated axons. These Schwann cells express surface markers shared by astrocytes (GFAP, Ran-2, A5E3). The development of the other Schwann cell phenotype is different. It relates to one axon, expresses myelin constituents, and assembles them into a myelin sheath. An important goal in subsequent research was to define the steps in this assembly process and to show how sheath formation and growth are regulated. The peripheral myelin sheath is a highly ordered, compact, multilayered spiral extension of the Schwann cell's surface membrane. The axon that the sheath surrounds is required for its survival and also has a role in determining its thickness and length. It is of interest that Schwann cells deprived of appropriate axonal contacts rapidly cease expressing myelin constituents and their sheaths degenerate. The degenerative process is rapid and if new axons are provided in cultures or by regeneration in vivo, new myelin sheaths are formed relatively quickly. As noted earlier, the situation is quite different in the CNS. Isolated oligodendroglia can synthesize CNS myelin constituents in vitro for several weeks in the absence of axons. Degeneration of CNS myelin sheaths is a slower process and oligodendroglia are much less effective than Schwann cells in myelin sheath regeneration.

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