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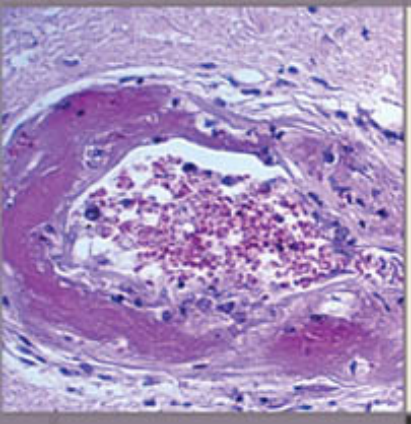
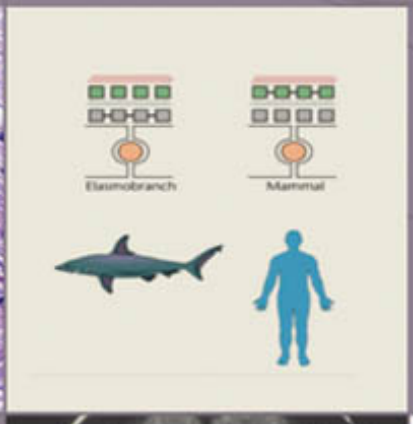
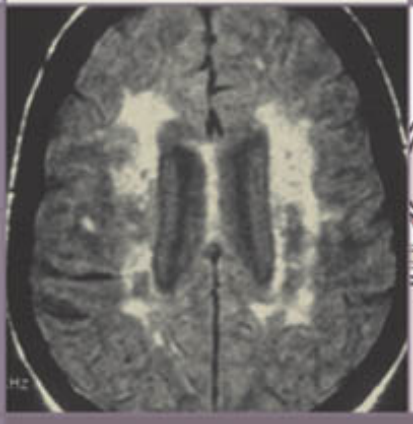
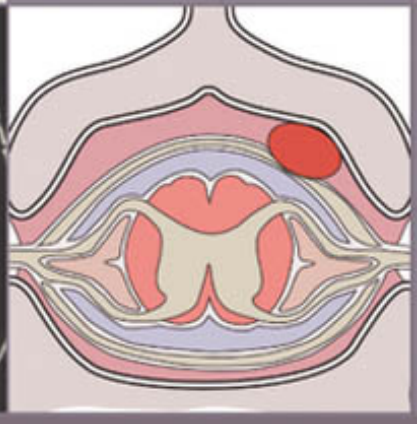
MOLECULAR PHYSIOLOGY AND METABOLISM OF THE NERVOUS SYSTEM

A CLINICAL PERSPECTIVE









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Preface

The neurosciences and clinical neurology have undergone dramatic changes in the past 25 years brought about by major advances in molecular biology and neuroimaging. Clinical practice has remained grounded in ideas and concepts that were first enunciated decades ago, but the advances made in the laboratory are beginning to impact the clinician. In the 1970s, the invention of mathematical equations for tomography opened an era of neuroimaging using x-rays for computed tomography (CT), radionuclear isotopes for positron emission tomography (PET), and magnetic resonance for magnetic resonance imaging (MRI). This ability to visualize brain pathology prior to autopsy profoundly changed the practice of neurology. At around that same time, advances in molecular biology began to penetrate the neurosciences, and have now exploded with the elucidation of the human genome, gene chip technology, and, more recently, the findings of proteomics and metabolomics.

Clinicians and neuroscientists beginning to grapple with this profusion of information are faced with the need to learn older physiological concepts that are relevant to patient care. But knowing the physiology, which used to be sufficient, is no longer adequate. It must be combined with the molecular biology to form a new science of molecular physiology. To be successful in clinical care as well as in clinical or basic neurosciences, multiple concepts and techniques need to be mastered. No longer is it sufficient to be well versed only in one of the major branches of neuroscience, such as neuroanatomy, neurophysiology, neuropathology or neurochemistry; it is now necessary to combine and use them all at some point. To do this successfully, scientists and clinicians need to work as a team; each person in the collaboration brings a unique skill to the project. Each person on the team has a set of skills and a group of words that he or she understands best, but it remains mainly one person's work, and for that individual to lead the effort, an understanding of the others' areas of expertise is required. Learning to do that person's part of the project usually is possible, and having a common language is the key to true teamwork. This is true not only for the complex scientific project but even more so for the clinician, who on a daily, even hourly, basis is involved in a large team.

Working with residents and graduate students over the past years has taught me the importance of incorporating the newer molecular insights into the care of patients. While we now know the patterns of most neurological diseases from intensive work of neuroimagers, along with many of the genes involved, we remain far behind in developing treatments. The challenge we now face is to relate the imaging to the molecular studies and to understand the underlying physiological role of the specific molecules in the injury cascades. Once that knowledge is available, we will need to translate it into novel therapies.

Translational research attempts to accelerate the movement of information from the basic sciences to the clinic and to take the insights gained from caring for patients back to the laboratory for further study. Drug screening can be done with high-throughput systems; blood tests can identify arrays of genes; clinical trials can be done by large consortia; information learned on one continent can be quickly conveyed to another group far away by the Internet. This acceleration of information transfer has resulted in remarkable advances in treatment. For that to occur, a new type of investigator is needed, one who is equally comfortable working in the worlds of brain physiology and molecular neurochemistry. My goal in writing this book is to combine the important insights into brain physiology gained by early investigators with the new knowledge being obtained on genes and proteins in order to understand the impact of these substances in the living animal.

The goal of this book is explain the basic physiological concepts about the brain fluids, cerebral blood flow, and the blood-brain barrier and the quantitative approaches to their study. This is the topic of the first part of the book. The second part is more concerned with metabolic pathways and aspects of transport. Pathological aspects of the brain fluids and metabolism are introduced where

appropriate in both of these parts but are more extensively discussed in the final part on hypoxia, ischemia, brain edema, and inflammation. I have tried to emphasize the commonalities among the various aspects of fluid balance and metabolism in the different diseases.

The information in this book will aid students, trainees in neurology and neurosurgery and research neuroscientists in the understanding of the basic concepts of physiology and molecular biology that apply to clinical practice and translational medicine.

— G. A. R
Albuquerque, New Mexico 2011

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Contents

PART 1

Physiology of Brain Fluids and the Blood-Brain Barrier

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Chapter 1

Anatomy of Fluid Interfaces that Protect the Microenvironment

HISTORICAL PERSPECTIVE

Brain fluid studies entered the modern era with the work begun at the beginning of the twentieth century by the physiologist Louis Weed and the neurosurgeon Harvey Cushing. They understood that the brain, lacking lymphatics, used the cerebrospinal and interstitial fluids (CSF and ISF) as lymph, calling this a *third circulation*[1] (Figure 1–1). At around the same time, there was growing awareness that the brain was sequestered from the systemic circulation. Ehrlich was the first to show this in 1885; he injected a blue dye into the blood, which stained all organs except the brain. In 1913, Goldmann observed that trypan blue dye injected into the CSF stained brain tissue. These two experiments were a dramatic demonstration that proved that the brain was isolated from the blood but that, once inside the skull, fluids had free access to brain parenchyma via CSF/ISF. This isolation became known as the *blood-brain barrier* (BBB).

Camillo Golgi discovered the method of silver staining. Cajal used this method to describe the close relationship between astrocytes and cerebral blood vessels in the human brain. Silver staining shows only a small population of astrocytes, which fortuitously provided a clearer picture of the connections between glial cells and blood vessels, showing that the astrocytic foot processes expanded as they contacted the cerebral vessels. This prescient observation lost favor for many years as the endothelium assumed the dominant role as the site of the BBB. Recent studies have revived the concept of a functional unit including vessels and astrocytes as the regulator of permeability. Golgi's illustrations were remarkably accurate considering the state of neuroanatomy at the beginning of the twentieth century (Figure 1–2). Comparison of a recent confocal micrograph of astrocytes attached to capillaries and one of Golgi's hand-drawn figures demonstrates this accuracy.[2]

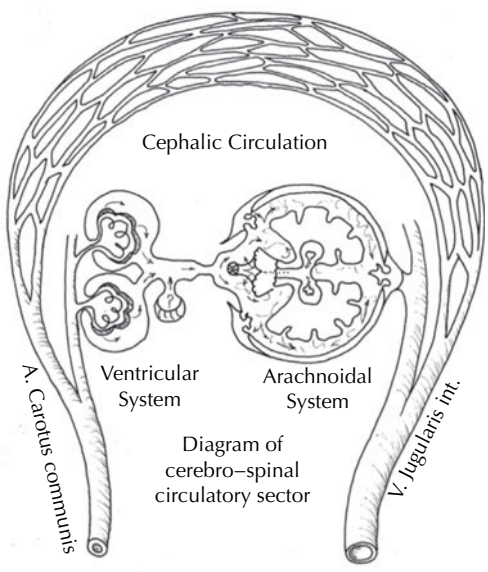

Figure 1–1. Drawing of the third circulation. Blood and lymph are the first and second circulations. Fluid leaving the capillaries and the choroid plexuses moves into the brain's extracellular space and the cerebral ventricles. Removal of CSF and ISF is across the arachnoid granulations. (From Ref. 1.)

CEREBRAL MICROENVIRONMENT

Normal cellular function depends on the microenvironment created around brain cells by the ISF, which is contiguous with CSF (Figure 1–3). Cerebrospinal fluid fills the cerebral ventricles, the subarachnoid space over the brain, and the fluid spaces around spinal cord and pools in the lumbar sac below the ending of the cord at L1-L2. Cerebral ventricles contain about 20 mL, with the remainder of the 140 mL over the brain, around the spinal cord, and in the lumbar sac, where it can be removed by lumbar puncture. Several important neurological illnesses require examination of the CSF for diagnosis. Infection, Guillain-Barré syndrome, central nervous system vasculitis, idiopathic intracranial hypertension, multiple sclerosis, and, more recently, Alzheimer's disease are examples of diseases in which the CSF is an important diagnostic aid (Table 1–1). An imaging study should be performed prior to lumbar puncture to rule out a mass lesion or obstructive hydrocephalus. Occasionally, in critically ill patients suspected of having meningitis or when imaging is difficult, lumbar puncture can be done after careful examination of the fundi to rule out papilledema and close observation after the test to assess for possible herniation. Examination of the CSF should always be considered in the diagnostic workup when infection, subarachnoid hemorrhage or idiopathic increased intracranial pressure is suspected because it is cost-effective and ultimately can contain costs by reducing the need for more expensive tests.

Cerebrospinal fluid and ISF are separated by the ependyma in the ventricles and by the pia in the subarachnoid space. Substances in the CSF can enter the brain across these cell layers because they have gap junctions rather than tight junctions. Continuity of the CSF and ISF means that substances that would be excluded from the brain after injection into the blood can be injected intrathecally where they can access brain cells. Drainage of metabolic products from the brain into the CSF via the ISF provides a lymph-like function, which solves the problem created when the brain tissues were isolated from the body during evolution. Fluids of the third circulation are formed at the cerebral endothelial cells and the ependymal cells of the choroid plexus. At both sites, adenosine triphosphatase (ATPase) pumps provide the energy to create the osmotic gradients that pull water into brain extracellular space and cerebral ventricles. Newly formed ISF from the capillaries and CSF from the choroid plexuses join together in the ventricles before draining back into the blood.

Proteins in the blood circulate into tissues through fenestrations in systemic capillaries. Acting as passive sieves, pores in the capillaries permit these large molecules to move into the tissue to be drained back to the blood by the lymphatic vessels. This essential lymphatic function prevents protein accumulation in the tissues, which would create oncotic pressure, resulting in edema.

By contrast, cerebral vessels restrict transport between the blood and the brain of most substances except lipophilic molecules. Specialized proteins that self-assemble to form tight junctions fill the clefts between cerebral capillaries. Tight junction proteins restrict the movement of charged or large protein molecules. Another unique feature of the brain capillary is the requirement for an energy source to power sodium-potassium ATPase exchange pumps, which maintain a constant flow of ISF

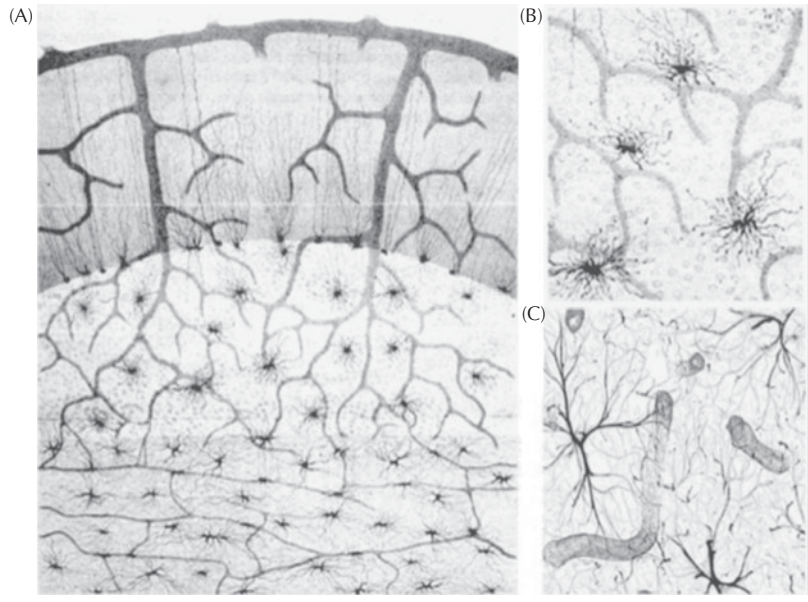

Figure 1–2. Camillo Golgi (1843–1926) described the close relationship between astrocytes and cerebral blood vessels in the human cerebellum as follows: "the connection between glia and vessels is either direct, the cell bodies being applied on the vessel walls, of which they seem to be part of, or occurs through protrusions more or less pronounced, which exhibit a small expansion at the point of contact." (**A**) Drawing from table XII of Golgi's book. (**B**) Enlargement of (**A**) showing the relationships between astrocytes and blood vessels. (**C**) Confocal image in which astrocytes were double-labeled with aquaporin 4 and glial fibrillary acidic protein (GFAP). The similarities between Golgi's drawing and the confocal image obtained more than a century later are striking. (From Ref. 2.)

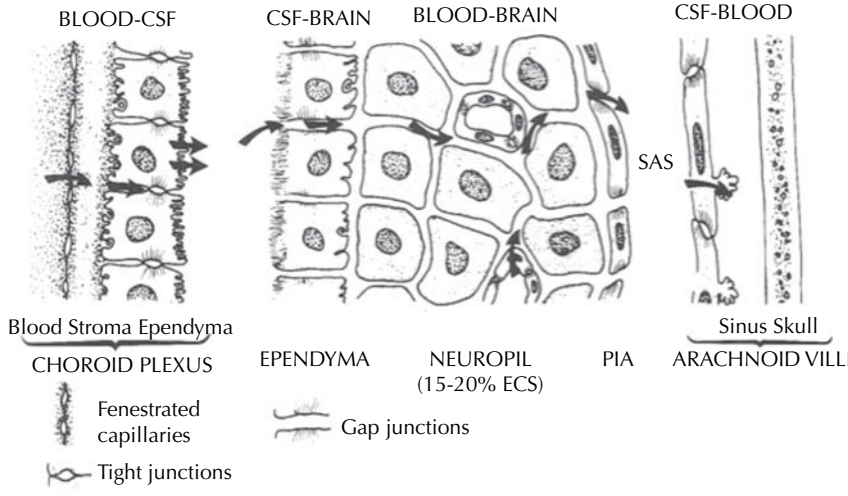

Figure 1–3. Illustration of the third circulation with the regions of tight and gap junctions. In the choroid plexus, substances in the blood leave fenestrated capillaries to enter the stroma beneath the ependyma, where apical tight junctions prevent them from entering the CSF. Once in the CSF, molecules can pass through the gap junctions of the nonchoroid plexus ependyma. Extracellular space makes up 15% to 20% of the brain, and ISF delivers substances to the cells in the neuropil. Interstitial fluid leaves the neuropil to enter the subarachnoid space before returning to the blood across the arachnoid villa. The arachnoid has tight junctions. Within the brain, the capillaries also have tight junctions.

Table 1–1 **Cerebrospinal Fluid Examinations Essential in the Diagnosis of Several Neurological Disorders**

CSF test for central nervous system infection
Cellular and protein content of CSF are helpful in distinguishing the various types of meningitis
Protein elevation without cells is diagnostic of Guillain-Barré syndrome
CSF pressure is diagnostic test for idiopathic intracranial hypertension
Large number of cells in central nervous system vasculitis, which can separate it from multiple sclerosis
Demyelinating profile in multiple sclerosis diagnosis (myelin basic protein, oligoclonal bands, IgG synthesis)

that leaves the capillaries along osmotic gradients created by ion pumps on the abluminal surface. Similar mechanisms of fluid formation are found at the choroid plexus.

Water can readily cross membranes, which is anomalous behavior that was poorly understood until the recent discovery of a family of pore-forming molecules called *aquaporins*. These molecules form channels through which water molecules move passively along pressure and osmotic gradients.[3] Astrocytic endfeet are rich in aquaporin molecules, and when edema fluid forms from movement of water out of the capillaries into the extracellular space, the astrocytic endfeet swell. The flow of ISF that is formed by capillaries occurs through the extracellular spaces by either passive diffusion or bulk flow. White matter tracts permit the unidirectional movement of ISF, while gray matter has random flow through a dense neuropil.

Table 1–2 **Circulation of Brain Fluids Controlled by Types of Junctions Between Cells**

Blood and lymph form the first and second circulations
CSF and ISF form the third circulation
CSF and ISF are formed at the choroid plexuses and cerebral capillaries
CSF and ISF are contiguous across ependymal and pial surfaces
Drainage back into the blood occurs at arachnoid granulations

Fluid formed by brain capillaries and choroid plexus flows through interstitial spaces, delivering nutrients and removing waste, eventually draining into the ventricles for removal over the convexities via the arachnoid granulations in the superior sagittal sinus and down along the spinal cord, where arachnoid granulations located in the nerve root sleeves perform a similar function (Table 1–2).

DEVELOPMENT OF THE BRAIN-FLUID INTERFACES

Neural Tube, Ependymal Cells, and Stem Cells

The nervous system develops from a region in the middorsal line of the embryo. A thickened plate of ectoderm folds in to form the neural groove, which, once closed, becomes the neural tube (Figure 1–4). The cephalic part begins to dilate to form the brain and the ventricular system, while the caudal segment that will be the spinal cord maintains a uniform diameter. An internal limiting membrane on the inner surface forms next to the cells that will become the ependyma. At the outer surface is mesenchyma that is separated from the ectoderm by an external limiting membrane. Germinal cells are found between the inner and outer membranes. The mantle layer becomes the gray matter, composed of glia and neurons, and the marginal layer becomes white matter. Ciliated epithelial cells line the neural tube, and cilia persist in some regions of the adult human ependyma.

The neural tube is formed from neuroepithelial cells that extend from the internal to the external limiting membranes.[4] Nuclei synthesizing DNA are found near the external limiting membrane and migrate toward the inner limiting membrane. Once DNA synthesis is complete, these cells become the neuroblasts that form the mantle layer. When neuroblasts mature into the neuronal cells of the adult, they lose their ability to divide. Neurons establish synapses with specific nuclear groups, probably on the basis of chemical affinities (Figure 1–5). After neuroblast differentiation has ceased, future glial cells are formed from neuroepithelial cells that have differentiated with glioblasts. Ependymal cells are formed along

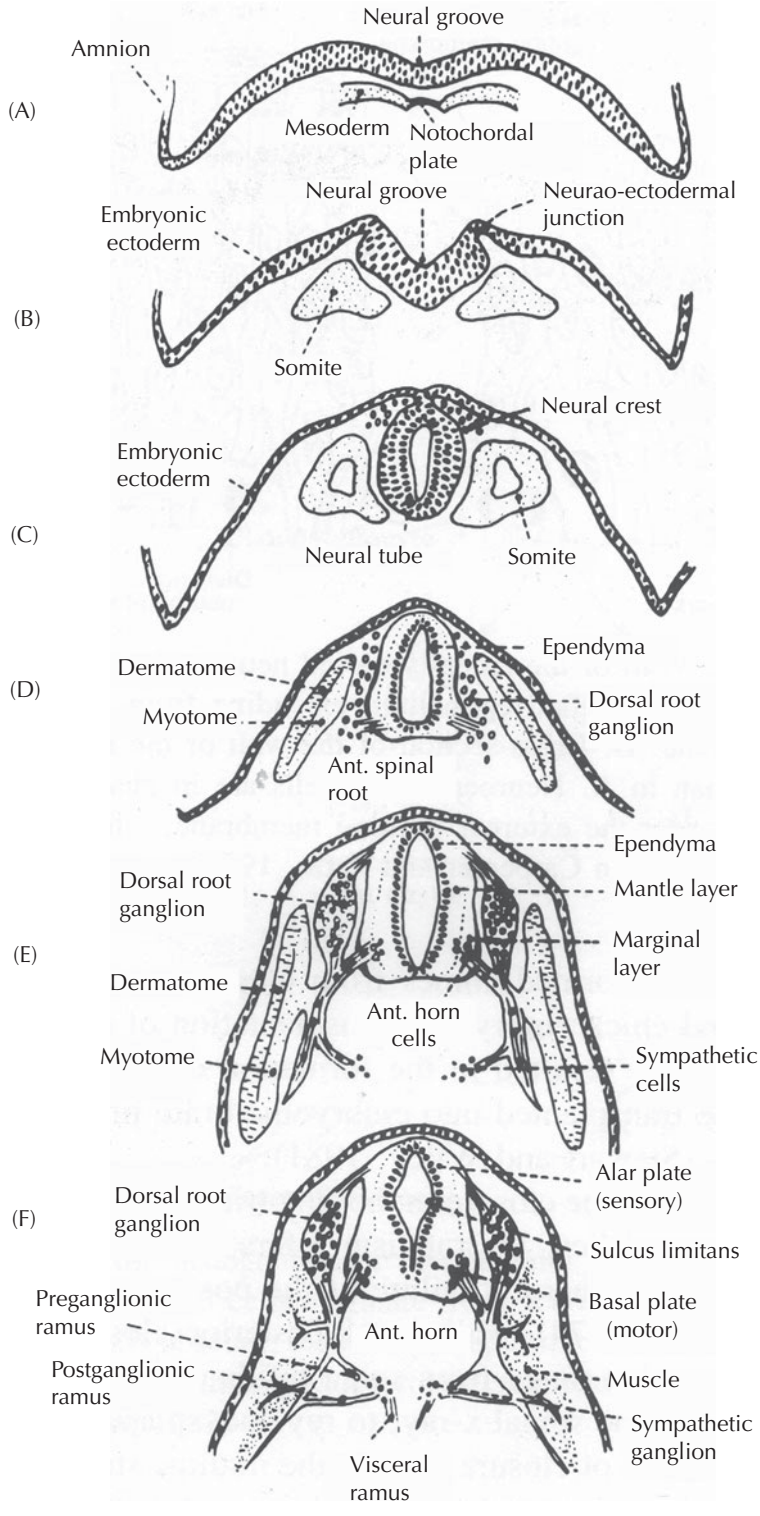

Figure 1–4. Stages in the development of the neural tube from the neural plate with subsequent formation of the spinal cord. (From Ref. 29.)

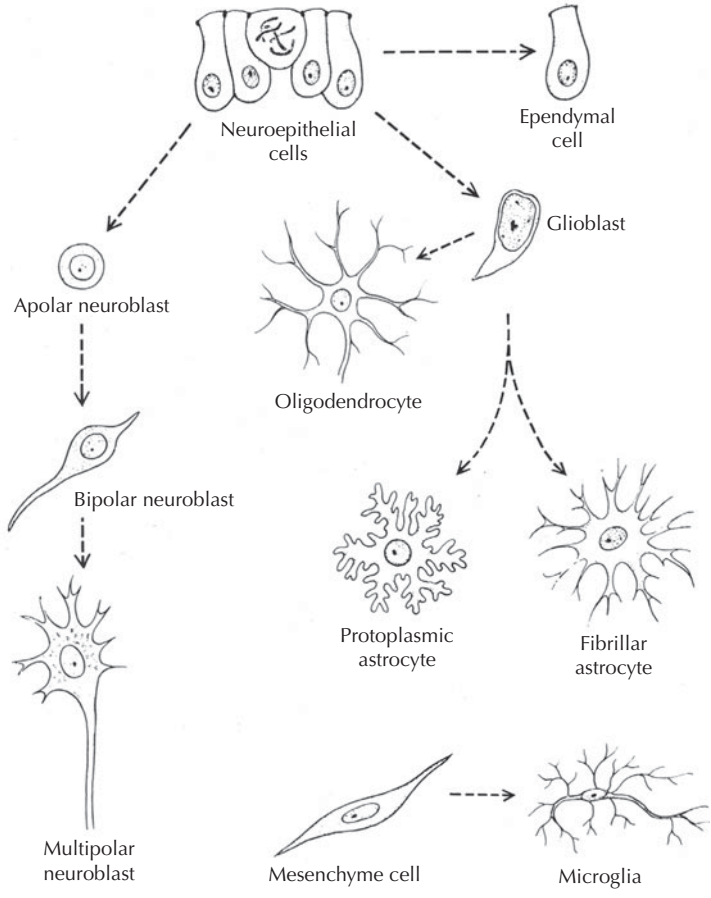

Figure 1–5. Diagram of the histogenesis of neurons and neuroglial cells. Neuroblasts, glioblasts, and ependymal cells originate from neuroepithelial cells. The origin of the oligodendrocyte is obscure, but both protoplasmic and fibrillary astrocytes are derived from glioblasts. The microglia are considered to arise from mesenchyme. (From Ref. 30.).

with glioblasts. Ependymal and subependymal cells form a separate unit loosely attached to the outer limiting membrane.

Cells in the subependymal zone of the lateral wall of the lateral ventricles continue to divide throughout life and proliferate after an injury to participate in the repair process. Stem cells differentiate into a wide variety of cells but mainly form glial cell types including astrocytes and oligodendroglial cells.[5] The hippocampus is another region that has plasticity based on stem cells. The discovery that there is continued growth of brain cells in adults was made by studies of patients dying of terminal cancer who had been injected with a molecule incorporated into dividing cells prior to death; their brains were studied after death. Human brain tissue was obtained postmortem from patients who had been treated with the thymidine analog bromodeoxyuridine (BrdU), which labels DNA during the S phase. Using immunofluorescent labeling for BrdU and for one of the neuronal antigens, that is, a marker for mature neurons—NeuN (neuronal nuclei), calbindin, or neuron specific enolase—the researchers demonstrated that new neurons, as defined by these markers, are generated from dividing progenitor cells in the dentate gyrus of adult humans, showing that human hippocampus retains its ability to generate neurons throughout life.[6] The field of stem cell biology has grown dramatically since this seminal observation.[7,8]

Cilated Ependymal Cells and CSF Movement

Ependymal cells that line the walls of the ventricular system in the adult brain are ciliated epithelial cells. These polarized epithelial cells are