Chapter 9

CEREBROSPINAL FLUID ANALYSIS

Formation

Produced primarily (70%) from secretions of the four ventricles of the brain by the choroid plexus; small amount from ependymal cells

A selective secretion of plasma, not an ultrafiltrate

Cerebrospinal fluid (CSF) has higher levels of sodium (Na), chloride (Cl), and magnesium (Mg) than does plasma

CSF has lower concentration of potassium (K), calcium (Ca), and protein than does plasma

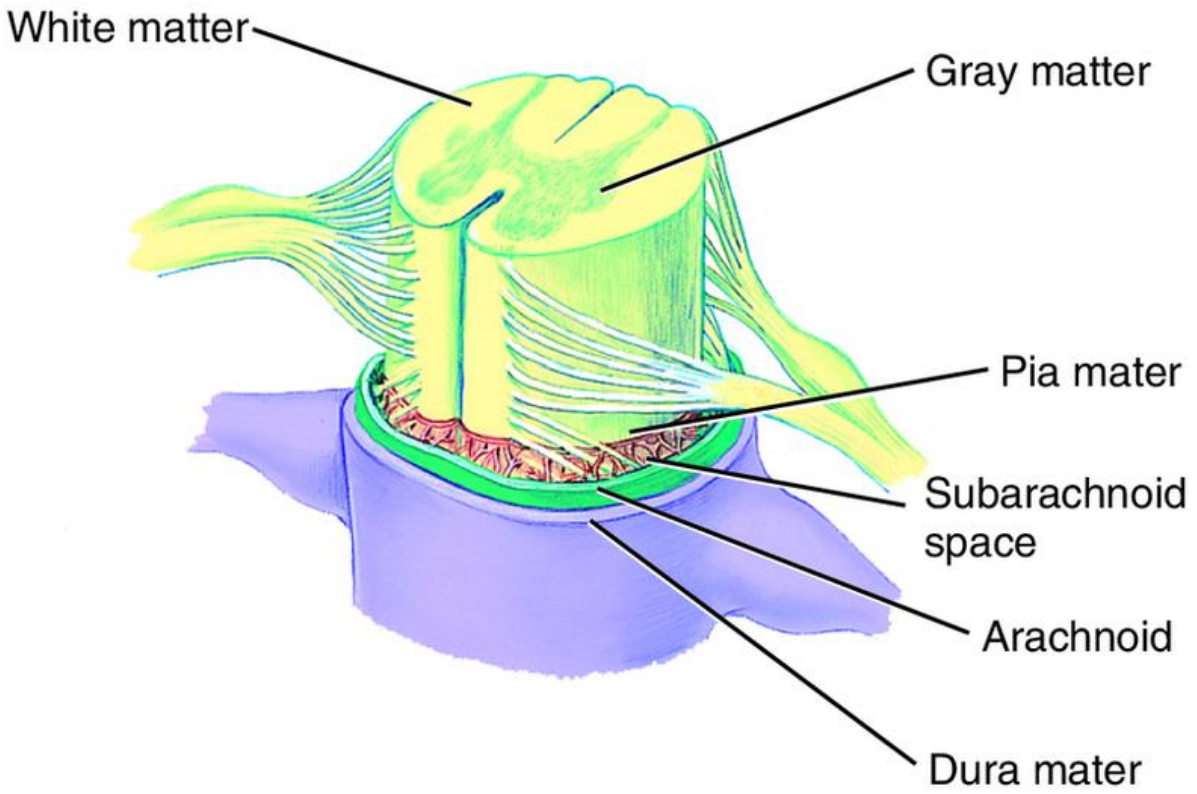
Structures

Brain and spinal cord surrounded by three membranes called *meninges*:

- Dura mater—outer layer next to bone
- Arachnoid mater—middle layer resembling a spiderweb
- Pia mater—innermost layer adhering to surface of neural tissues

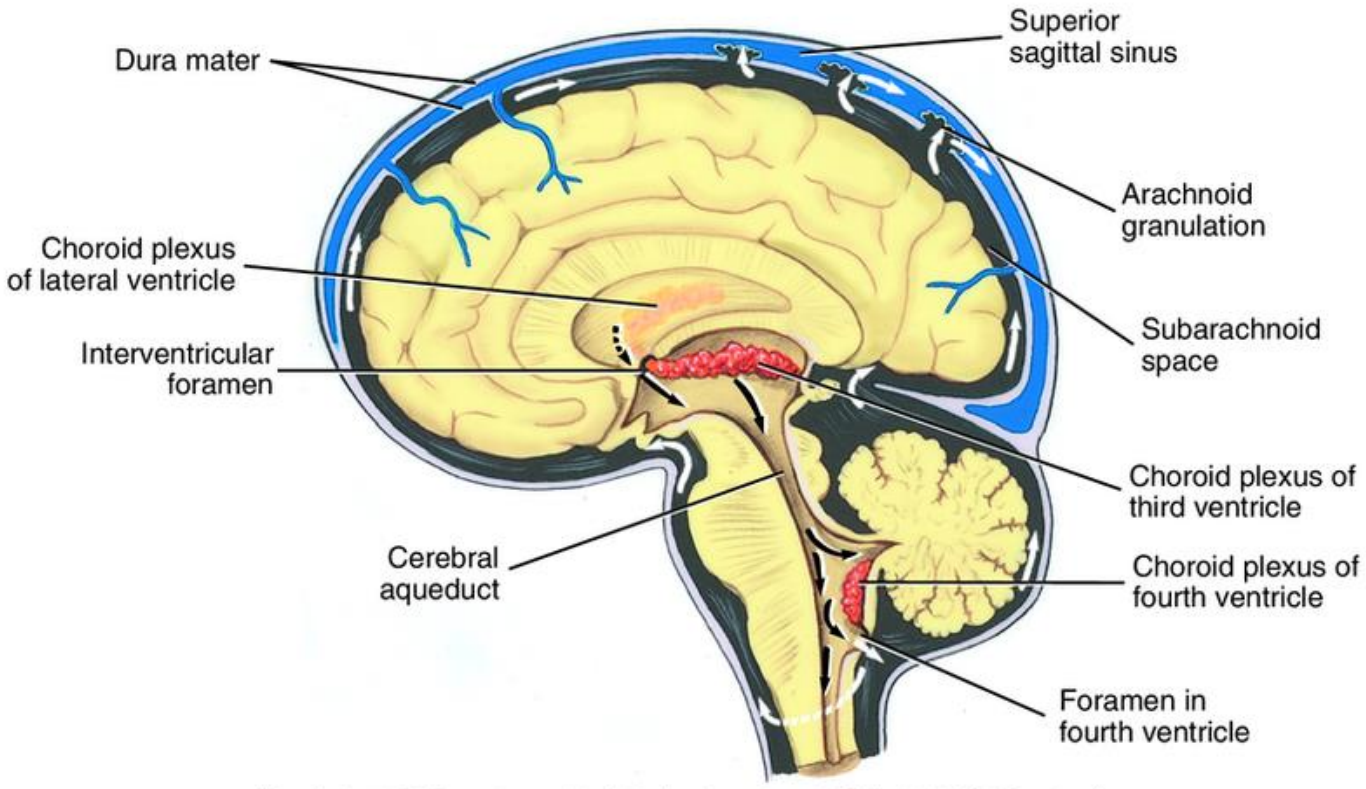
CSF flows in subarachnoid space between arachnoid mater and pia mater, where it bathes and protects brain and spinal cord

Figure 9-1. A schematic representation of the spinal cord and the meninges that surround it. (From Applegate E: *The anatomy and physiology learning system*, ed 4, Philadelphia, 2011, Saunders.)



(From Applegate E: *The anatomy and physiology learning system*, ed 4, Philadelphia, 2011, Saunders.)

Figure 9-2. A schematic representation of the brain and spinal cord, including the circulation of the cerebrospinal fluid. (From Applegate E: *The anatomy and physiology learning system*, ed 4, Philadelphia, 2011, Saunders.)



(From Applegate E: *The anatomy and physiology learning system*, ed 4, Philadelphia, 2011, Saunders.)

Normal Cerebrospinal Fluid (CSF) Processes

CSF forms, circulates, and is reabsorbed into blood, dynamically turning over 20 mL each hour

If reabsorption process is blocked, CSF builds up causing hydrocephalus

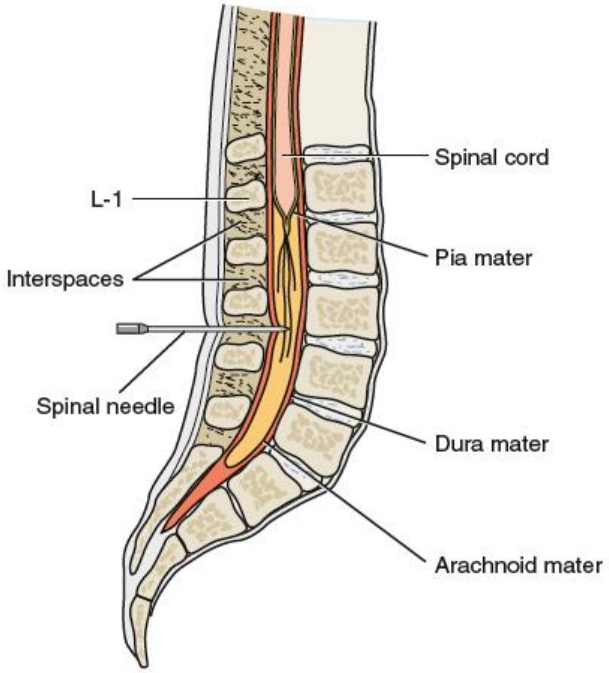
Normal adult volume 85 to 150 mL

Interface between blood and CSF called blood-brain barrier

Only certain substances can cross blood-brain barrier: differences of electrolytes, proteins, and other solutes

Specimen Collection

Collected by aseptic lumbar puncture in third or fourth lumbar interspace with local anesthesia



Testing

Initial or opening pressure taken with manometer attached to spinal needle

Normal recumbent pressure is 50 to 180 mm Hg

Closing pressure taken after collection of sample and should be 10 to 30 mm Hg less

Both pressure values and amount collected are recorded: up to 20 mL with normal pressure

If the CSF pressure is less than or greater than normal, only 1 to 2 mL should be removed

Three sequentially labeled tubes:

- #1—chemistry
- #2—microbiology
- #3—cell counts

Physical Examination

Normal CSF is clear and colorless with viscosity like water

Clarity or turbidity related to number of cells

- Pleocytosis = increased number of cells in CSF
- Cloudy CSF associated with white blood cells (WBCs) greater than 200 cells/mL or red blood cells (RBCs) greater than 400 cells/mL
- Microorganisms or increased protein can cause cloudy CSF

Xanthochromia—abnormal color of CSF, usually yellow, orange, or pink due to various conditions

Presence of Visible Blood

Must differentiate between traumatic puncture and subarachnoid or intracerebral bleeding

Traumatic tap

- Greatest amount of blood in tube 1, least in tube 3
- After centrifugation, colorless supernatant

Hemorrhage

- Consistent amount of blood in all three tubes
- After centrifugation, xanthochromic supernatant
- Macrophages with phagocytosed RBCs
- Macrophages stain positive for hemosiderin and may include hematoidin crystals

Microscopic Examination

In adults, normal cell count is 0 to 5 white blood cells per microliter (WBCs/µL), specifically lymphocytes and monocytes

RBCs not normally present

Cell counts performed immediately to prevent lysing of WBCs; lysing slowed at 4°C

Cell counts performed with hemacytometer on undiluted specimen counting all nine squares on both sides

If dilution needed, use normal saline

White Blood Cell (WBC) Counts

Increased in diseases of central nervous system (CNS) and variety of other conditions

CSF diluted with 2% acetic acid to lyse RBCs

Number of mononuclear and polymorphonuclear cells are noted, and total of two counted

Differential performed on cytospin slide stained with Wright's stain

Pleocytosis

Neutrophils

- In bacterial meningitis, up to 90% of WBCs can be neutrophils
- Early viral, fungal, tuberculosis (TB), or parasitic infections
- Some noninfectious conditions

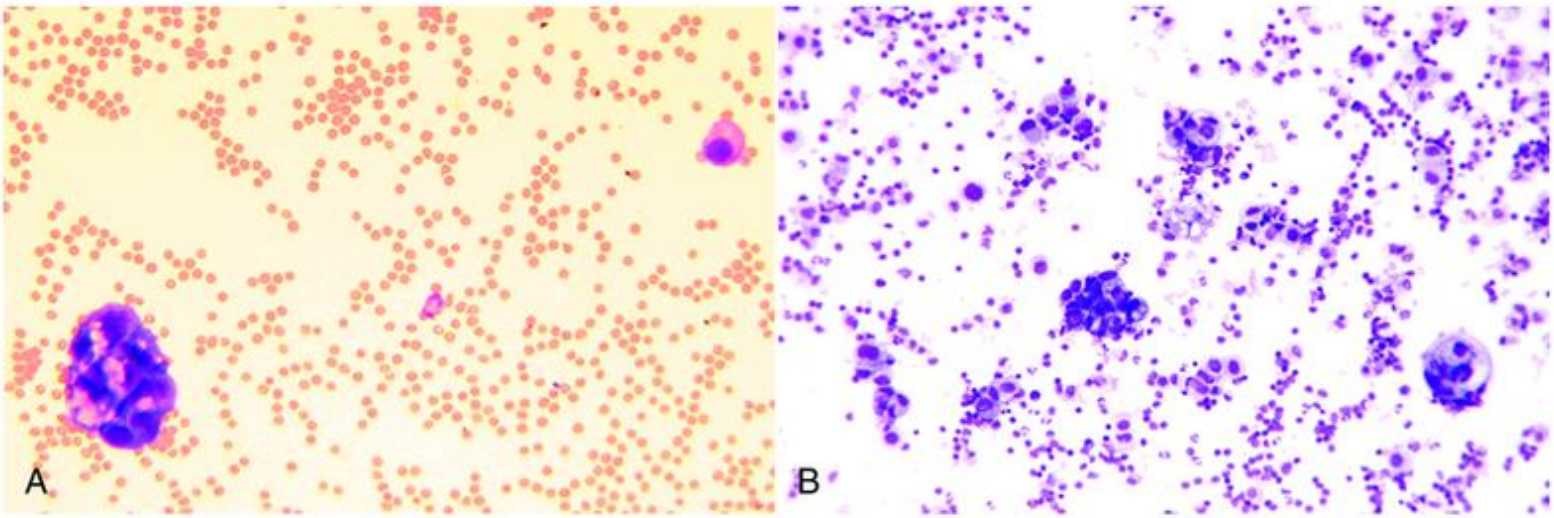
Lymphocytes

- Increased in viral, TB, fungal, or syphilitic meningitis particularly in later stages

Plasma cells

- Are abnormal when seen in multiple sclerosis and acute viral and chronic inflammatory conditions

Figure 9-4. Low-power fields of view of cerebrospinal fluid with tumor cell clumps. (A) Rare tumor clump with numerous red blood cells. (B) Numerous cells with rare tumor clump. (Courtesy Charlotte Janita.)



(Courtesy Charlotte Janita.)

Other Cells

Monocytes

- May be increased in a mixed cell pattern such as TB or fungal meningitis, chronic bacterial meningitis, or rupture of cerebral abscess

Eosinophils

- 10% or greater with parasitic, fungal, or allergic reactions
- Following injection of radiographic contrast media or medications
- Can also result from an allergic reaction to malfunctioning intracranial shunts

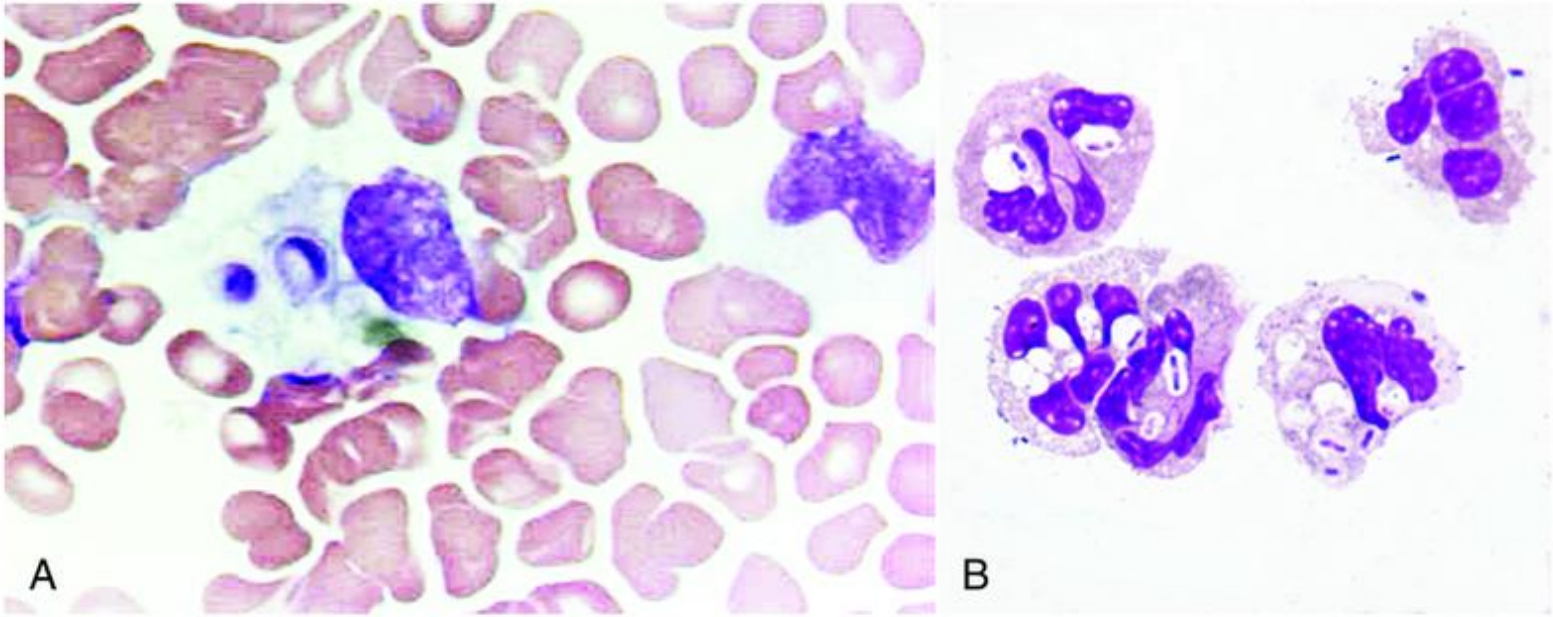
Macrophages

- Often found after hemorrhage because of phagocytic ability

Other cells

- Ependymal, choroid plexus, or malignant cells in clumps; need to distinguish between malignant cells and others

Figure 9-5. (A) Macrophage with intracellular yeast (cerebrospinal fluid [CSF], ×1000). (B) Bacteria engulfed by neutrophils (CSF, ×1000). (A, Courtesy Charlotte Janita. B, From Carr JH, Rodak BF: *Clinical hematology atlas*, ed 3, St. Louis, 2008, Saunders.)



(A, Courtesy Charlotte Janita. B, From Carr JH, Rodak BF: *Clinical hematology atlas*, ed 3, St. Louis, 2008, Saunders.)

Figure 9-6. Eosinophilia in cerebrospinal fluid. (Courtesy Charlotte Janita.)

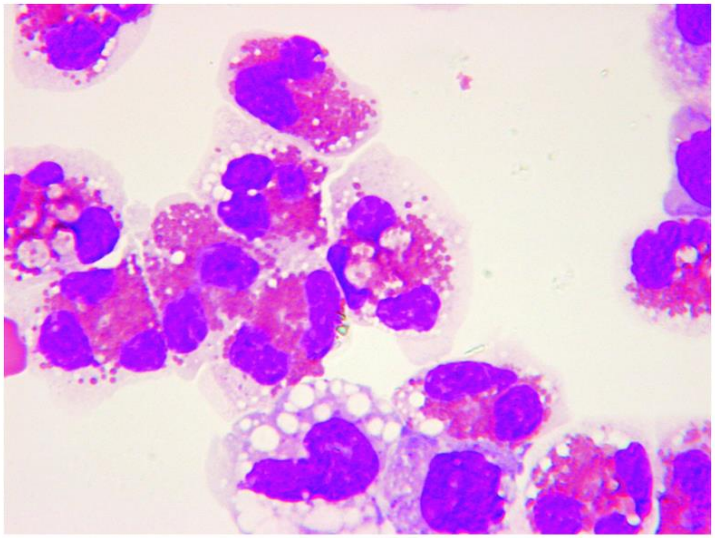
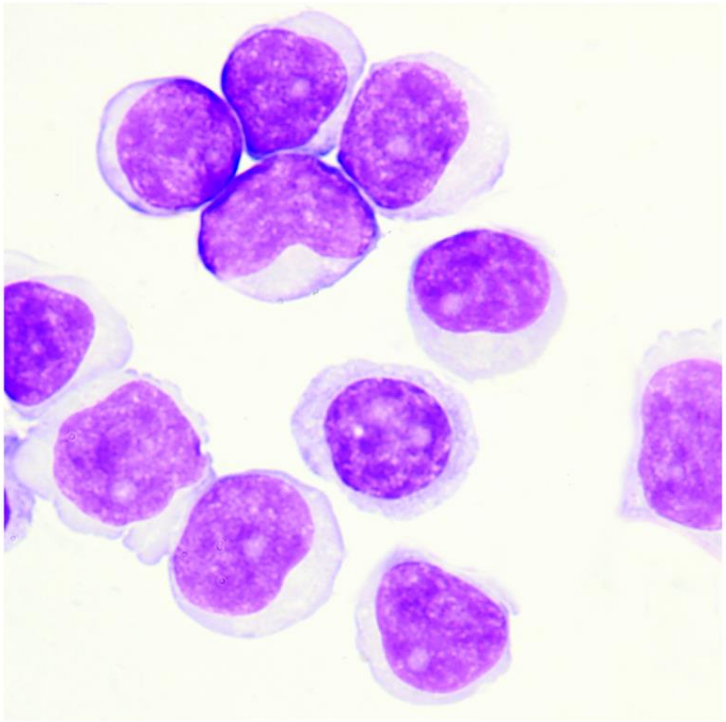


Figure 9-8. Reactive lymphocytes (cerebrospinal fluid [CSF], ×1000). (From Carr JH, Rodak BF: *Clinical hematology atlas*, ed 3, St. Louis, 2008, Saunders.)



(From Carr JH, Rodak BF: *Clinical hematology atlas*, ed 3, St. Louis, 2008, Saunders.)

A monocyte *(red arrow)*, a small lymphocyte *(black arrow)*, many neutrophils and red blood cells. Cerebrospinal fluid, Wright stain, ×1000.

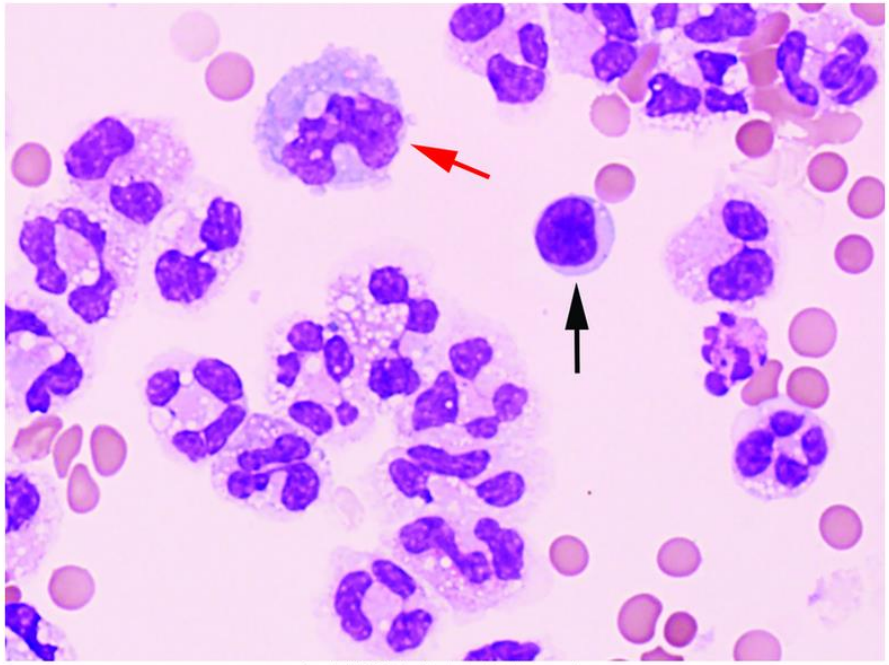
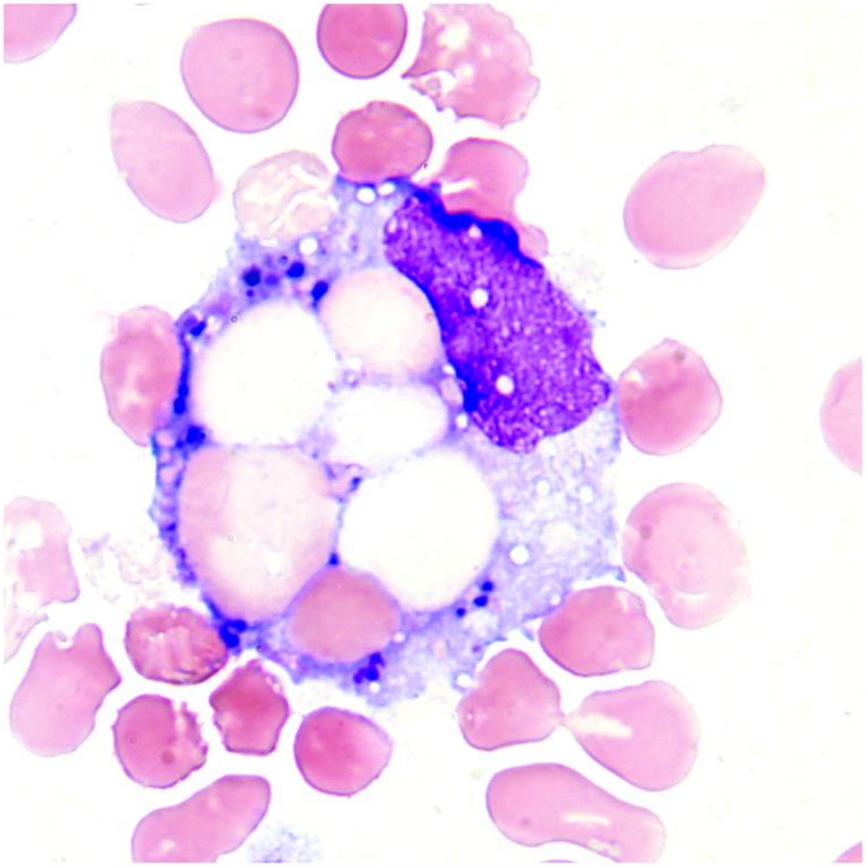


Figure 9-10. Macrophage with engulfed (intracellular) red blood cells; can also be called an *erythrophage*. (From Carr JH, Rodak BF: *Clinical hematology atlas*, ed 3, St. Louis, 2008, Saunders.)



(From Carr JH, Rodak BF: *Clinical hematology atlas*, ed 3, St. Louis, 2008, Saunders.)

Total Protein in Cerebrospinal Fluid (CSF)

Normal is approximately 15 to 45 mg/dL; higher in infants and older adults

Increased in CSF from:

- Contamination with blood during traumatic tap
- Change in blood-brain barrier
- Decreased reabsorption into venous blood
- Increased synthesis in CNS

Associated Conditions

Increased protein seen in numerous disorders

- Bacterial, viral, and other forms of meningitis
- Cerebral infarction
- Hemorrhage
- Endocrine disorders
- Multiple sclerosis
- Obstruction of CSF flow
- Trauma

Decreased protein

- Increased reabsorption because of increased intracranial pressure
- Loss of fluid because of trauma or invasive procedures

Cerebrospinal Fluid (CSF)/Serum Albumin Index

Used to assess permeability of blood-brain barrier

Less than 9 is normal; 9 to 14 minimal impairment; 15 to 100 moderate to severe; exceeding 100 complete breakdown of barrier

$$\text{CSF/serum albumin index} = \frac{\text{Albumin}_{\text{CSF}}\,(\text{mg/dL})}{\text{Albumin}_{\text{Serum}}\,(\text{g/dL})}$$

Cerebrospinal Fluid (CSF) Immunoglobulin G (IgG) Index

$$\text{CSF IgG index} = \frac{\text{IgG}_{\text{CSF}}\,(\text{mg/dL})}{\text{IgG}_{\text{Serum}}\,(\text{g/dL})} \times \frac{\text{Albumin}_{\text{Serum}}\,(\text{g/dL})}{\text{Albumin}_{\text{CSF}}\,(\text{mg/dL})}$$

A typical reference interval for the IgG index is 0.30 to 0.70

Values greater than this range are associated with increased intrathecal production of IgG

Values less than this range indicate a compromised blood-brain barrier

CSF IgG Index (Cont.)

About 90% of patients with multiple sclerosis have an IgG index greater than 0.70

- Also increased in inflammatory disorders of CNS

Protein Electrophoresis

Four bands predominate in CSF: transthyretin, albumin, and two distinct transferrin bands

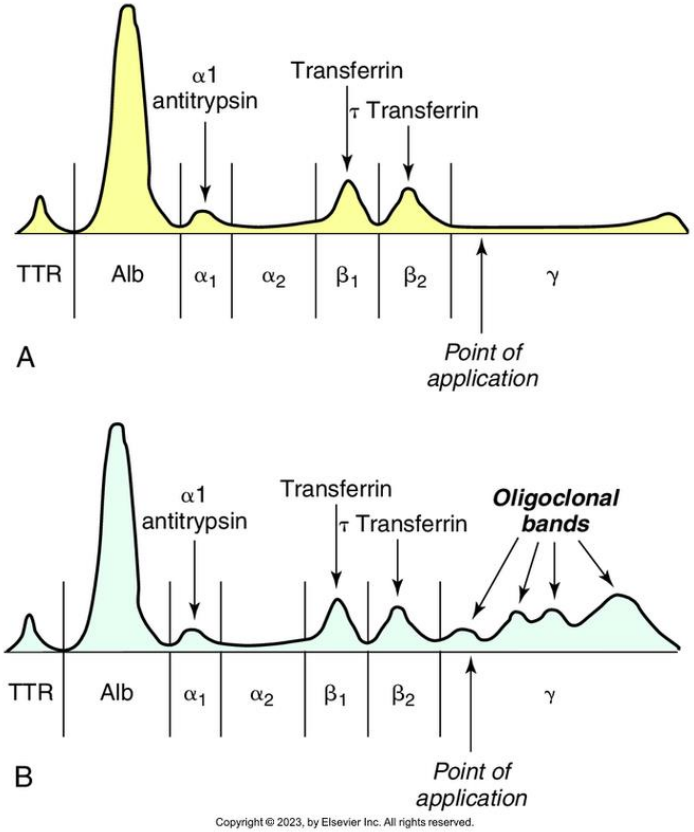
The second band of transferrin, known as “tau protein,” is specific for CSF

Faint bands of α_1 antitrypsin and IgG possible

Electrophoresis is performed to identify oligoclonal bands that are present in CSF but absent in serum in multiple sclerosis

Other conditions with oligoclonal bands in CSF have them in serum also

Figure 9-18. Cerebrospinal fluid protein patterns using high-resolution electrophoresis. (A) A “normal” cerebrospinal fluid protein pattern. In the β2 region, the presence of τ transferrin, a protein unique to cerebrospinal fluid, is noteworthy. (B) An “abnormal” cerebrospinal fluid protein pattern demonstrating the presence of oligoclonal bands in the γ region. These bands will not be present on electrophoresis of the patient’s serum. TTR, Transthyretin (previously called prealbumin).



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Other Tests

Myelin basic protein

- Seen in multiple sclerosis and other demyelinating diseases

Glucose

- Value reflects plasma value 30 to 90 minutes before CSF collection; decreased glucose associated with many conditions including meningitis

Lactate

- Increased in conditions impairing blood supply or transport of oxygen to the CNS; viral meningitis normal; others elevated

Microbiological Examination

Gram stain—for bacteria and fungi

India ink—for *Cryptococcus neoformans*

Acid-fast stain—for TB meningitis

Wright's stain—for ameba *Naegleria fowleri*

Culture—to detect bacterial cause

Detection of microbial antigens—to assist in identifying bacterial and fungal antigens in CSF

Figure 9-19. Cerebrospinal fluid from patient with bacterial meningitis. (A) Gram stain of an unconcentrated smear showing many gram-negative rods. (B) Wright-stained cytospin preparation that reveals bacteria, both intracellular and extracellular, as well as marked cellular degeneration. Hematin crystals are present in the *arrowed* macrophages.