Formation and Physiology

First recognized by Cotugno in 1764, cerebrospinal fluid (CSF) is a major fluid of the body.[1] CSF provides a physiologic system to supply nutrients to the nervous tissue, remove metabolic wastes, and produce a mechanical barrier to cushion the brain and spinal cord against trauma.

The brain and spinal cord are lined by the ***meninges***, which consists of three layers: the dura mater, ***arachnoid***, and pia mater. The outer layer is the dura mater that lines the skull and vertebral canal. The arachnoid is a filamentous (spider-like) inner membrane. The pia mater is a thin membrane lining the surfaces of the brain and spinal cord.

CSF is produced in the ***choroid plexuses*** of the two lumbar ventricles and the third and fourth ventricles. In adults, approximately 20 mL of fluid is produced every hour. The fluid flows through the ***subarachnoid space*** located between the arachnoid and pia mater (Fig. 10-1). To maintain a volume of 90 to 150 mL in adults and 10 to 60 mL in neonates, the circulating fluid is reabsorbed back into the blood capillaries in the ***arachnoid granulations***/villae at a rate equal to its production. The cells of the arachnoid granulations act as one-way valves that respond to pressure within the central nervous system (CNS) and prevent reflux of the fluid.

The choriod plexuses are capillary networks that form the CSF from plasma by mechanisms of selective filtration under hydrostatic pressure and active transport secretion. Therefore, the chemical composition of the CSF does not resemble an ultrafiltrate of plasma. Capillary walls throughout the body are lined with endothelial cells that are loosely connected to allow passage of soluble nutrients and wastes between the plasma and tissues. In the choroid plexuses, the endothelial cells have very tight-fitting junctures that prevent the passage of many molecules. This tight-fitting structure of the endothelial cells in the choroid plexuses is termed the ***blood-brain barrier.***

Maintaining the integrity of the blood-brain barrier is essential to protect the brain from chemicals and other substances circulating in the blood that could harm the brain tissue. In contrast, the junctures also prevent the passage of helpful substances including antibodies and medications. Disruption of the blood-brain barrier by diseases such as meningitis and multiple sclerosis allows leukocytes, proteins, and additional chemicals to enter the CSF.

Specimen Collection and Handling

CSF is routinely collected by lumbar puncture between the third, fourth, or fifth lumbar vertebrae. Although this procedure is not complicated, it does require certain precautions including measurement of the intracranial pressure and careful technique to prevent the introduction of infection or the damaging of neural tissue.

The volume of CSF that can be removed is based on the volume available in the patient (adult vs. neonate) and the opening pressure of the CSF taken when the needle first enters the ***subarachnoid space***. Elevated pressure requires fluid to be withdrawn slowly, with careful monitoring of the pressure, and may prevent collection of a large volume.

Specimens are collected in three sterile tubes, which are labeled 1, 2, and 3 in the order in which they are withdrawn.

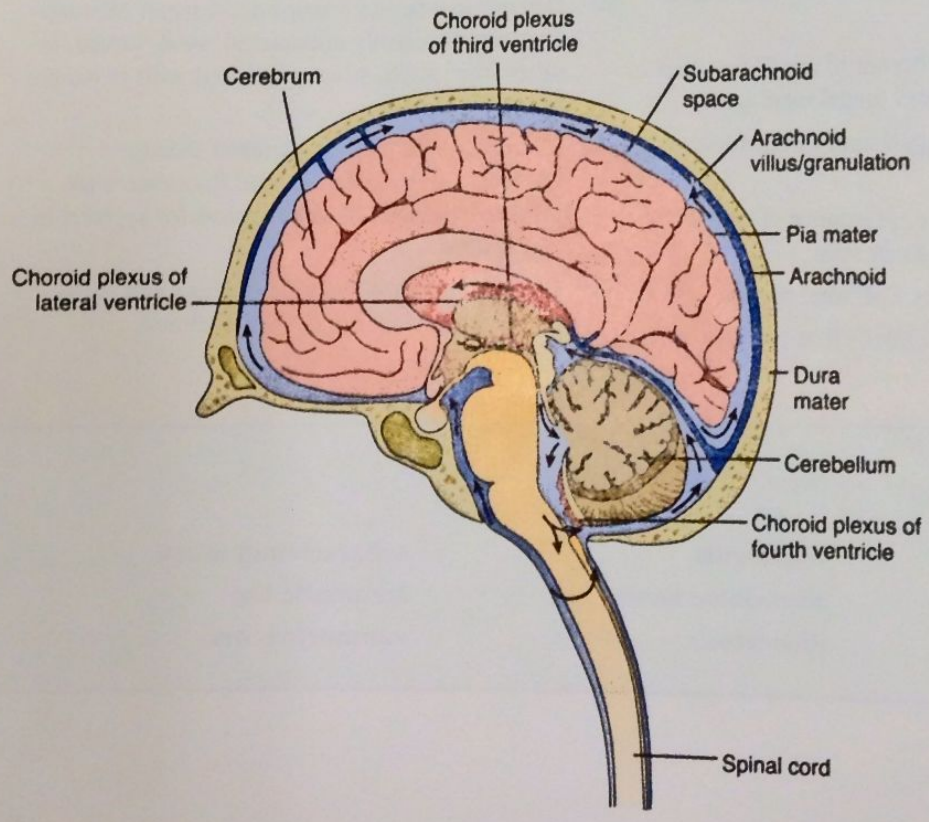


Figure 10–1 The flow of CSF through the brain and spinal column.

Tube 1 is used for chemical and serologic tests because these tests are least affected by blood or bacteria introduced as a result of the tap procedure;

Tube 2 is usually designated for the microbiology laboratory,

Tube 3 is used for the cell count, because it is the least likely to contain cells introduced by the spinal tap procedure

A fourth tube may be drawn for the microbiology laboratory to provide better exclusion of skin contamination or for additional serologic tests. Supernatant fluid that is left over after each section has performed its tests may also be used for additional chemical or serologic tests. Excess fluid should not be discarded and should be frozen until there is no further use for it (Fig 10-2).

Considering the discomfort to the patient and the possible complications that can occur during specimen collection, laboratory personnel should handle CSF specimens carefully. Ideally, tests are performed on a STAT basis. If this is not possible, specimens are maintained in the following manner:

- Hematology tubes are refrigerated.
- Microbiology tubes remain at room temperature.
- Chemistry and serology tubes are frozen.

Appearance

The initial appearance of the normally crystal clear CSF can provide valuable diagnostic information. Examination of the fluid occurs first at the bedside and is also included in the laboratory report. The major terminology used to describe CSF appearance includes crystal clear, cloudy or turbid, milky, xanthochromic, and hemolyzed/bloody (Fig. 10-3). A cloudy, turbid, or milky specimen can be the result of an increased protein or lipid concentration, but it may also be indicative of infection, with the cloudiness being caused by the presence of WBCs. All specimens should be treated with extreme care because they can be highly contagious; gloves must always be worn and face shields or splash guards should be used while preparing specimens for testing. Fluid for centrifugation must be in capped tubes.

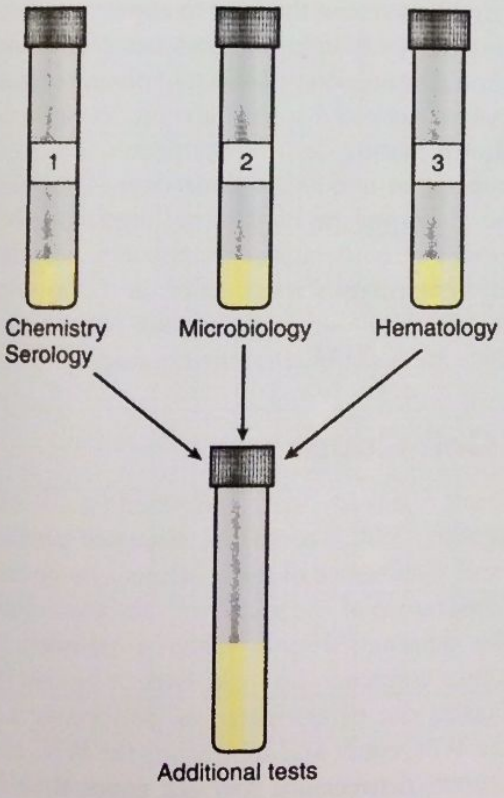


Figure 10–2 CSF specimen collection tubes.

Figure 10–3 Tubes of CSF. Appearance left to right is normal, xanthochromic, hemolyzed, and cloudy.

Xanthochromia is a term used to describe CSF supernatant that is pink, orange, or yellow. A variety of factors can cause the appearance of xanthochromia, with the most common being the presence of RBC degradation products. Depending on the amount of blood and the length of time it has been present, the color will vary from pink (very slight amount of oxyhemoglobin) to orange (heavy hemolysis) to yellow (conversion of oxyhemoglobin to unconjugated bilirubin). Other causes of xanthochromia include elevated serum bilirubin, presence of the pigment carotene, markedly increased protein concentrations, and melanoma pigment. Xanthochromia that is caused by bilirubin due to immature liver function is also commonly seen in infants, particularly in those who are premature. The clinical significance of CSF appearance is summarized in Table 10–1.

Traumatic Collection (Tap)

Grossly bloody CSF can be an indication of intracranial hemorrhage, but it may also be due to the puncture of a blood vessel during the spinal tap procedure. Three visual examinations of the collected specimens can usually determine whether the blood is the result of hemorrhage or a *traumatic tap*.

Uneven Distribution of Blood

Blood from a cerebral hemorrhage will be evenly distributed throughout the three CSF specimen tubes, whereas a traumatic tap will have the heaviest concentration of blood i

Table 10–1 Clinical Significance of Cerebrospinal Fluid Appearance

Appearance	Cause	Major Significance
Crystal clear		Normal
Hazy, turbid, milky, cloudy	WBCs	Meningitis
	Microorganisms	Meningitis
	Protein	Disorders that affect blood-brain barrier
		Production of IgG within the CNS
Oily	Radiographic contrast media	
Bloody	RBCs	Hemorrhage
		Traumatic tap
Xanthochromic	Hemoglobin	Old hemorrhage
		Lysed cells from traumatic tap
	Bilirubin	RBC degradation
		Elevated serum bilirubin level
	Carotene	Increased serum levels
	Protein	Disorders affecting blood-brain barrier
	Melanin	Meningeal melanosarcoma
Clotted	Protein	Disorders affecting blood-brain barrier
	Clotting factors	Introduced by traumatic tap
Pellicle	Protein	Disorders that affect blood-brain barrier
	Clotting factors	Tubercular meningitis

tube 1, with gradually diminishing amounts in tubes 2 and 3. Performing RBC counts on all three tubes to measure decreasing or constant blood is not always reliable.[2] Streaks of blood also may be seen in specimens acquired following a traumatic procedure.

Clot Formation

Fluid collected from a traumatic tap may form clots owing to the introduction of plasma fibrinogen into the specimen. Bloody CSF caused by intracranial hemorrhage does not contain enough fibrinogen to clot. Diseases in which damage to the blood-brain barrier allows increased filtration of protein and coagulation factors also cause clot formation but do not usually produce a bloody fluid. These conditions include ***meningitis***, ***Froin syndrome***, and blockage of CSF circulation through the subarachnoid space. A classic web-like pellicle is associated with tubercular meningitis and can be seen after overnight refrigeration of the fluid.[3]

Xanthochromic Supernatant

RBCs must usually remain in the CSF for approximately 2 hours before noticeable hemolysis begins; therefore, a xanthochromic supernatant would be the result of blood that has been present longer than that introduced by the traumatic tap. Care should be taken, however, to consider this examination in conjunction with those previously discussed, because a very recent hemorrhage would produce a clear supernatant, and introduction of serum protein from a traumatic tap could also cause the fluid to appear xanthochromic. To examine a bloody fluid for the presence of xanthochromia, the fluid should be centrifuged in a microhematocrit tube and the supernatant examined against a white background.

Additional testing for differentiation includes microscopic examination and the ***D-dimer*** test. The microscopic finding of macrophages containing ingested RBCs (***erythrophagocytosis***) or hemosiderin granules is indicative of intracranial hemorrhage. Detection of the fibrin degradation product, D-dimer, by latex agglutination immunoassay indicates the formation of fibrin at a hemorrhage site.

Cell Count

The cell count that is routinely performed on CSF specimens is the leukocyte (WBC) count. As discussed previously, the presence and significance of RBCs can usually be ascertained from the appearance of the specimen. Therefore, RBC counts are usually determined only when a traumatic tap has occurred and a correction for leukocytes or protein is desired. The RBC count can be calculated by performing a total cell count and a WBC count and subtracting the WBC count from the total count, if necessary. Any cell count should be performed immediately, because WBCs (particularly granulo-

cytes) and RBCs begin to lyse within 1 hour, with 40% of the leukocytes disintegrating after 2 hours.[4] Specimens that cannot be analyzed immediately should be refrigerated.

Methodology

Normal adult CSF contains 0 to 5 WBCs/μL. The number is higher in children, and as many as 30 mononuclear cells/μL can be considered normal in newborns.[5] Specimens that contain up to 200 WBCs or 400 RBCs/μl may appear clear, so it is necessary to examine all specimens microscopically.[6] An improved Neubauer counting chamber (Fig. 10-4) is routinely used for performing CSF cell counts. Traditionally, electronic cell counters have not been used for performing CSF cell counts, owing to high background counts and poor reproducibility of low counts.

The Advia 120 Hematology System (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.) has received Food and Drug Administration (FDA) approval for addition of a CSF assay. The instrument performs WBC counts on all samples, RBC counts on samples with less than 1500 cells/μL, and differential counts for neutrophils, lymphocytes, and monocytes.

Calculation of CSF Cell Counts

The standard Neubauer calculation formula used for blood cell counts is also applied to CSF cell counts to determine the number of cells per microliter.

$$\frac{\text{Number of cells counted} \times \text{dilution}}{\text{Number of squares counted} \times \text{volume of 1 square}} = \text{cells}/\mu\text{L}$$

This formula can be used for both diluted and undiluted specimens and offers flexibility in the number and size of the squares counted. Many varied calculations are available, including condensations of the formula to provide single factors by which to multiply the cell count. Keep in mind that the purpose of any calculation is to convert the number of cells counted in a specific amount of fluid to the number of cells that would be present in 1 μL of fluid. Therefore, a factor can be used only when the dilution and counting area are specific for that factor.

The methodology presented in this chapter eliminates the need to correct for the volume counted by counting the four large corner squares (0.4 μL) and the large center square (0.1 μL) on each side of the counting chamber.[7]

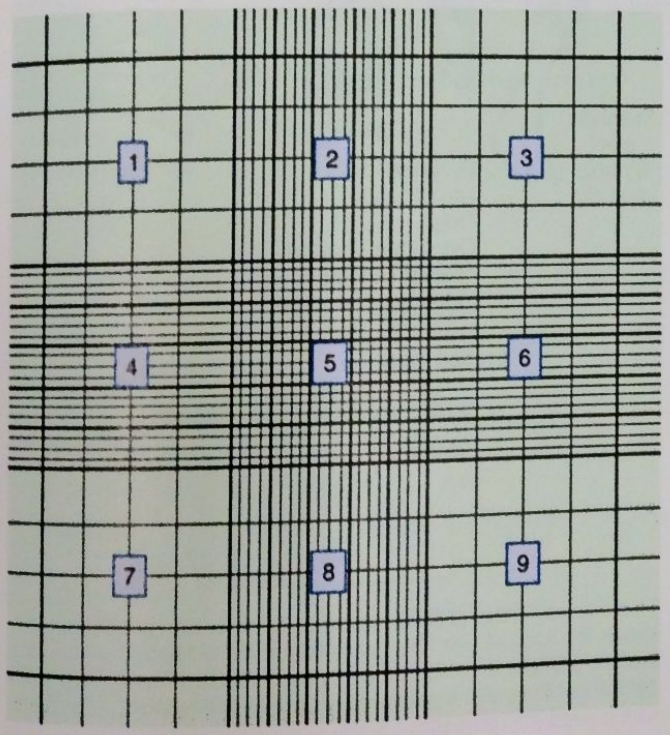


Figure 10–4 Neubauer counting chamber depicting the nine large square counting areas.

Example

$$\text{Number of cells counted} \times \text{dilution} \times \frac{1\ \mu\text{L}}{1\ \mu\text{L}\ (0.1 \times 10)\ \text{(volume counted)}} = \text{cells}/\mu\text{L}$$

Total Cell Count

Clear specimens may be counted undiluted, provided no overlapping of cells is seen during the microscopic examination. When dilutions are required, calibrated automatic pipettes, not mouth pipetting, are used. Dilutions for total cell counts are made with normal saline, mixed by inversion, and loaded into the hemocytometer with a Pasteur pipette. Cells are counted in the four corner squares and the center square on both sides of the hemocytometer. As shown in the preceding example, the number of cells counted multiplied by the dilution factor equals the number of cells per microliter.

WBC Count

Lysis of RBCs must be obtained prior to performing the WBC count on either diluted or undiluted specimens. Specimens requiring dilution can be diluted in the manner described previously, substituting 3% glacial acetic acid to lyse the RBCs. Addition of methylene blue to the diluting fluid stains the WBCs, providing better differentiation between neutrophils and mononuclear cells.

To prepare a clear specimen that does not require dilution for counting, place four drops of mixed specimen in a clean tube. Rinse a Pasteur pipette with 3% glacial acetic acid, draining thoroughly, and draw the four drops of CSF into the rinsed pipette. Allow the pipette to sit for 1 minute, mix the solution in the pipette, discard the first drop, and load the hemocytometer. As in the total cell count, WBCs are counted in the four corner squares, and the center square on both sides of the hemocytometer and the number is multiplied by the dilution factor to obtain the number of WBCs per microliter. If a different number of squares is counted, the standard Neubauer formula should be used to obtain the number of cells per microliter.

Corrections for Contamination

Calculations are possible to correct for WBCs and protein artificially introduced into the CSF as the result of a traumatic tap. Determination of the CSF RBC count and the blood RBC and WBC counts is necessary to perform the correction. By determining the ratio of WBCs to RBCs in the peripheral blood and comparing this ratio with the number of contaminating RBCs, the number of artificially added WBCs can be calculated using the following formula:

$$\text{WBC (added)} = \frac{\text{WBC (blood)} \times \text{RBC (CSF)}}{\text{RBC (blood)}}$$

An approximate CSF WBC count can then be obtained by subtracting the "added" WBCs from the actual count. When peripheral blood RBC and WBC counts are in the normal range, many laboratories choose to simply subtract 1 WBC for every 700 RBCs present in the CSF. Studies have shown a high percentage of error in the correction of fluids containing a large number of RBCs, indicating correction may be of little value under these circumstances.[8]

Quality Control of Cerebrospinal Fluid and Other Body Fluid Cell Counts

Liquid commercial controls for spinal fluid RBC and WBC counts are available from several manufacturers. They can be purchased at two levels of concentration. In-house controls can also be prepared.

On a biweekly basis, all diluents should be checked for contamination by examination in a counting chamber under 4 × magnification. Contaminated diluents should be discarded and new solutions prepared.

On a monthly basis, the speed of the cytocentrifuge should be checked with a tachometer, and the timing should be checked with a stopwatch.

If nondisposable counting chambers are used, they must be soaked in a bactericidal solution for at least 15 minutes and then thoroughly rinsed with water and cleaned with isopropyl alcohol.

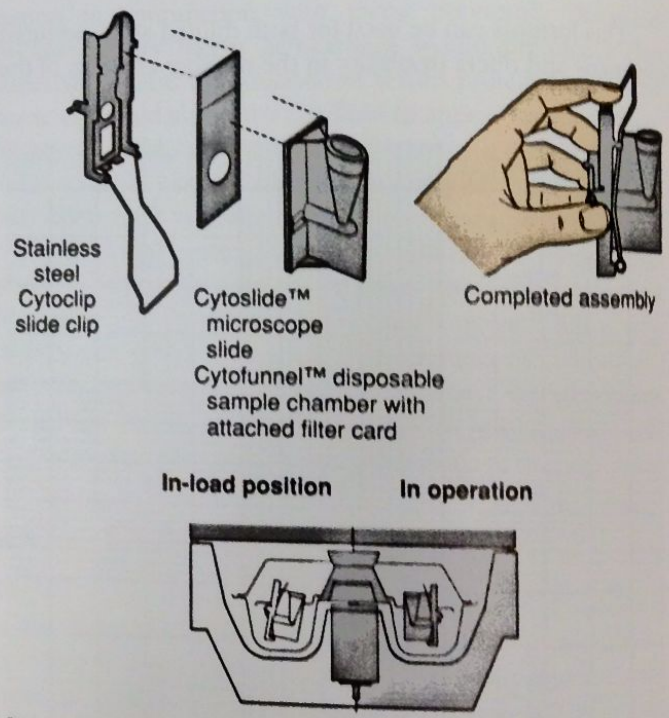
Differential Count on a Cerebrospinal Fluid Specimen

Identifying the type or types of cells present in the CSF is a valuable diagnostic aid. The differential count should be performed on a stained smear and not from the cells in the counting chamber. Poor visualization of the cells as they appear in the counting chamber has led to the laboratory practice of reporting only the percentage of mononuclear and polynuclear cells present, and this can result in the overlooking of abnormal cells with considerable diagnostic importance. To ensure that the maximum number of cells are available for examination, the specimen should be concentrated prior to the preparation of the smear.

Methods available for specimen concentration include sedimentation, filtration, centrifugation, and cytocentrifugation. Sedimentation and filtration are not routinely used in the clinical laboratory, although they do produce less cellular distortion. Most laboratories that do not have a cytocentrifuge concentrate specimens with routine centrifugation. The specimen is centrifuged for 5 to 10 minutes, supernatant fluid is removed and saved for additional tests, and slides made from the suspended sediment are allowed to air dry and are stained with Wright's stain. When the differential count is performed, 100 cells should be counted, classified, and reported in terms of percentage. If the cell count is low and finding 100 cells is not possible, report only the numbers of the cell types seen.

Cytocentrifugation

A diagramatic view of the principle of cytocentrifugation is shown in Figure 10-5. Fluid is added to the conical chamber, and as the specimen is centrifuged, cells present in the fluid are forced into a monolayer within a 6-mm diameter circle on the slide. Fluid is absorbed by the filter paper blotter, producing a more concentrated area of cells. As little as 0.1 mL of CSF combined with one drop of 30% albumin produces an adequate cell yield when processed with the cytocentrifuge. Addition of albumin increases the cell yield and decreases the cellular distortion frequently seen on cytocentrifuged specimens. Positively charged coated slides to attract cells (Shandon, Inc, Pittsburgh, Pa.) are also available. Cellular distortion may include cytoplasmic vacuoles, nuclear clefting, prominent nucleoli, indistinct nuclear and cytoplasmic borders and cellular clumping that resembles malignancy. Cells from both the center and periphery of the slide should be examined because cellular characteristics may vary between areas of the slide.



This cutaway drawing illustrates, at left, the in-load position which shows the sample chamber assembly in a tilted-back position, so that the sample is not absorbed by the filter card. During spinning, centrifugal force tilts the assembly upright and forces the sample to flow toward the microscope slide.

Figure 10–5 Cytospin 3 cytocentrifuge specimen processing assembly (Courtesy of Shandon, Inc., Pittsburgh, Pa.).

Table 10–2 Cytocentrifuge Recovery Chart[7]

Number of WBCs Counted in Chamber	Number of Cells Counted on Cytocentrifuge Slide
0	0–40
1–5	20–100
6–10	60–150
11–20	150–250
20	250

A daily control slide for bacteria should also be prepared using 0.2 mL saline and two drops of the 30% albumin currently being used. The slide is stained and examined if bacteria are seen on a patient's slide.

In Table 10–2, a cytocentrifuge recovery chart is provided for comparison with chamber counts. The chamber count should be repeated if too many cells are seen on the slide, and a new slide should be prepared if not enough cells are seen on the slide.[7]

Cerebrospinal Fluid Cellular Constituents

The cells found in normal CSF are primarily lymphocytes and monocytes (Figs. 10-6 and 10-7). Adults usually have a predominance of lymphocytes to monocytes (70:30), whereas the ratio is essentially reversed in children.[5] Improved concentration methods are also showing occasional neutrophils in normal CSF.[9] The presence of increased numbers of these normal cells (termed ***pleocytosis***) is considered abnormal, as is the finding of immature leukocytes, eosinophils, plasma cells, macrophages, increased tissue cells, and malignant cells.

When pleocytosis involving neutrophils, lymphocytes, or monocytes is present, the CSF differential count is most frequently associated with its role in providing diagnostic information about the type of microorganism that is causing an infection of the meninges (meningitis). A high CSF WBC count of which the majority of the cells are neutrophils is considered indicative of bacterial meningitis. Likewise, a moderately elevated CSF WBC count with a high percentage of lymphocytes and monocytes suggests meningitis of viral, tubercular, fungal, or parasitic origin.

As seen in Table 10–3, many pathologic conditions other than meningitis can be associated with the finding of abnor-

Table 10–3 Predominant Cells Seen in Cerebrospinal Fluid

Type of Cell	Major Clinical Significance	Microscopic Findings
Lymphocytes	Normal Viral, tubercular, and fungal meningitis Multiple sclerosis	All stages of development may be found
Neutrophils	Bacterial meningitis Early cases of viral, tubercular, and fungal meningitis Cerebral hemorrhage	Granules may be less prominent than in blood Cells disintegrate rapidly
Monocytes	Normal Viral, tubercular, and fungal meningitis Multiple sclerosis	Found mixed with lymphocytes
Macrophages	RBCs in spinal fluid Contrast media	May contain phagocytized RBCs appearing as empty vacuoles or ghost cells, hemosiderin granules and hematoidin crystals
Blast forms	Acute leukemia	Lymphoblasts, myeloblasts, or monoblasts
Lymphoma cells	Disseminated lymphomas	Resemble lymphocytes with cleft nuclei
Plasma cells	Multiple sclerosis Lymphocyte reactions	Traditional and classic forms seen
Ependymal, choroidal, and spindle-shaped cells	Diagnostic procedures	Seen in clusters with distinct nuclei and distinct cell walls
Malignant cells	Metastatic carcinomas Primary central nervous system carcinoma	Seen in clusters with fusing of cell borders and nuclei

mal cells in the CSF. Therefore, because laboratory personnel become so accustomed to finding neutrophils, lymphocytes, and monocytes, they should be careful not to overlook other types of cells. Cell forms differing from those found in blood include macrophages, choroid plexus and ependymal cells, spindle-shaped cells, and malignant cells.

Neutrophils

In additon to bacterial meningitis, increased neutrophils also are seen in the early stages (1 to 2 days) of viral, fungal, tubercular, and parasitic meningitis. Neutrophils may also contain cytoplasmic vacuoles following cytocentrifugation (Fig. 10-8). Granules are also lost more rapidly in CSF. Neutrophils associated with bacterial meningitis may contain phagocytized bacteria (Figs. 10-9 and 10-10). Although of little clinical significance, neutrophils may be increased following central nervous system (CNS) hemorrhage, repeated lumbar punctures, and injection of medications or radiographic dye.

Neutrophils with ***pyknotic*** nuclei indicate degenerating cells. They may resemble nucleated red blood cells (NRBCs) but usually have multiple nuclei. When a single nucleus is present they can appear similar to NRBCs (Fig. 10-11). NRBCs are seen as a result of bone marrow contamination during the spinal tap (Figs. 10-12 and 10-13). This is found in approximately 1% of specimens.[10] Capillary structures and endothelial cells may be seen following a traumatic tap (Fig. 10-14).

Lymphocytes and Monocytes

A mixture of lymphocytes and monocytes is common in cases of viral, tubercular, and fungal meningitis. Reactive lymphocytes containing increased dark blue cytoplasm and clumped chromatin are frequently present during viral infections in conjunction with normal cells (Fig. 10-15). Increased lymphocytes are seen in cases of both asymptomatic HIV infection and AIDS. A moderately elevated WBC count (less than 50 WBCs/μL) with increased normal and reactive lymphocytes and plasma cells may be indicative of multiple sclerosis or other degenerative neurologic disorders.[11]

Eosinophils

Increased eosinophils are seen in the CSF in association with parasitic infections, fungal infections (primarily *Coccidioides immitis*), and introduction of foreign material, including medications and shunts into the CNS (Fig. 10-16).

Macrophages

The purpose of macrophages in the CSF is to remove cellular debris and foreign objects such as RBCs. Macrophages appear within 2 to 4 hours after RBCs enter the CSF and are frequently seen following repeated taps. They tend to have more cytoplasm than monocytes in the peripheral blood (PB) (Fig. 10-17).

The finding of increased macrophages is indicative of a previous hemorrhage (Fig. 10-18). Further degradation of the phagocytized RBCs results in the appearance of dark blue or black iron-containing hemosiderin granules (Figs. 10-19 through 10-22). Yellow ***hematoidin*** crystals represent further degeneration. They are iron-free, consisting of hemoglobin and unconjugated bilirubin (Figs. 10-23 and 10-24).

Nonpathologically Significant Cells

These cells are most frequently seen following diagnostic procedures such as pneumoencephalography and in fluid obtained from ventricular taps or during neurosurgery. The cells often appear in clusters and can be distinguished from malignant cells by their uniform appearance.

Choroidal cells are from the epithelial lining of the choroid plexus. They are seen singularly and in clumps. Nucleoli are usually absent and nuclei have a uniform appearance (Fig. 10-25).

Ependymal cells are from the lining of the ventricles and neural canal. They have less defined cell membranes and are frequently seen in clusters. Nucleoli are often present (Fig. 10-26).

Spindle-shaped cells represent lining cells from the arachnoid. They are ususally seen in clusters and may be seen with systemic malignancies (Fig. 10-27).

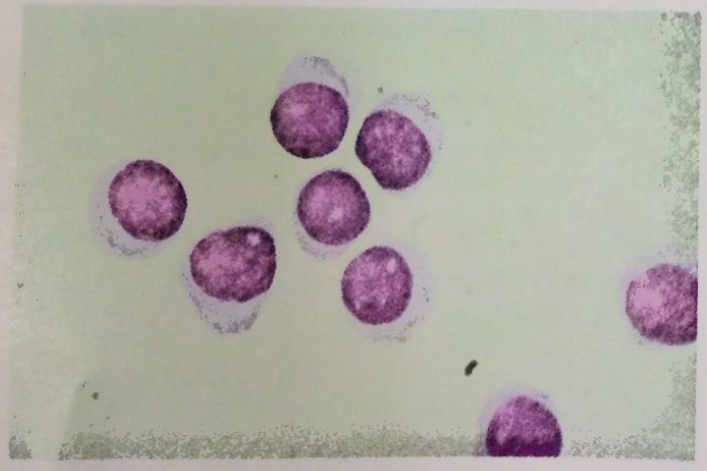


Figure 10–6 Normal lymphocytes. Some cytocentrifuge distortion of cytoplasm (×1000).

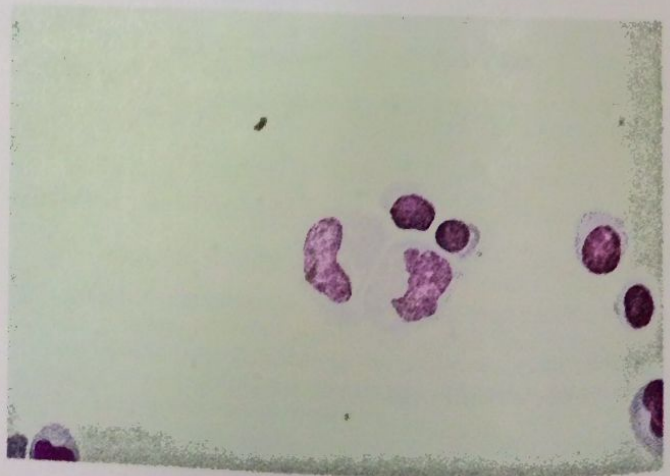


Figure 10–7 Normal lymphocytes and monocytes (×500).

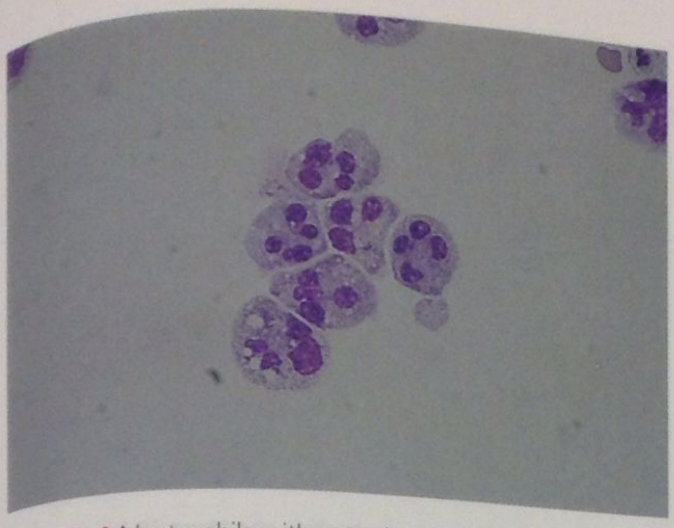


Figure 10–8 Neutrophils with cytoplasmic vacuoles resulting from cytocentrifugation (×500).

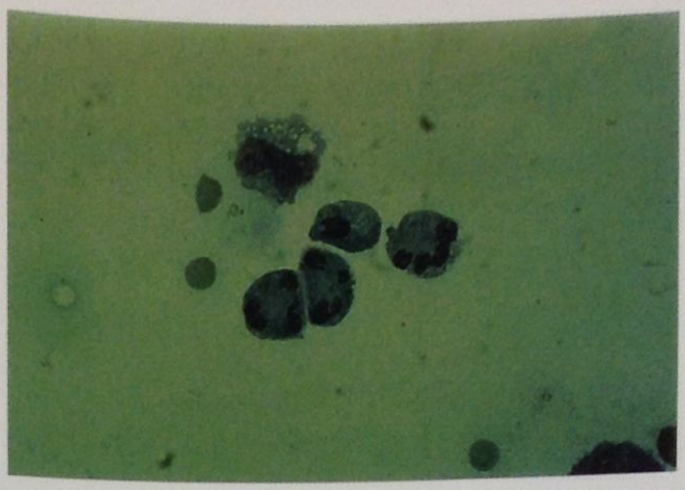


Figure 10–11 Neutrophils with pyknotic nuclei. Notice the cell with a single nucleus in the center (×1000).

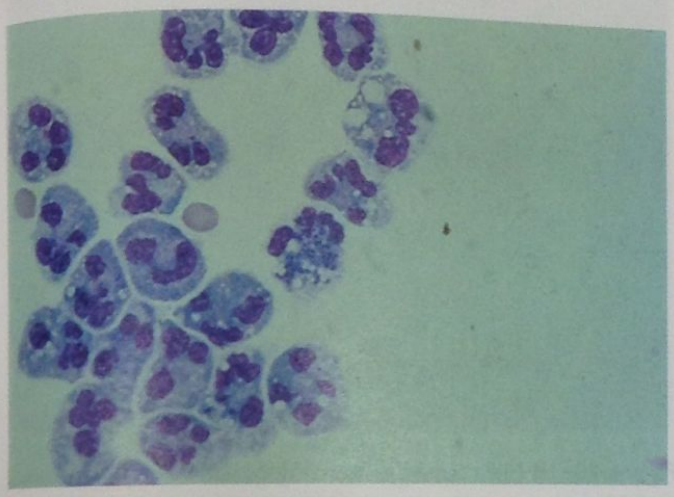


Figure 10–9 Neutrophils with intracellular bacteria (×1000).

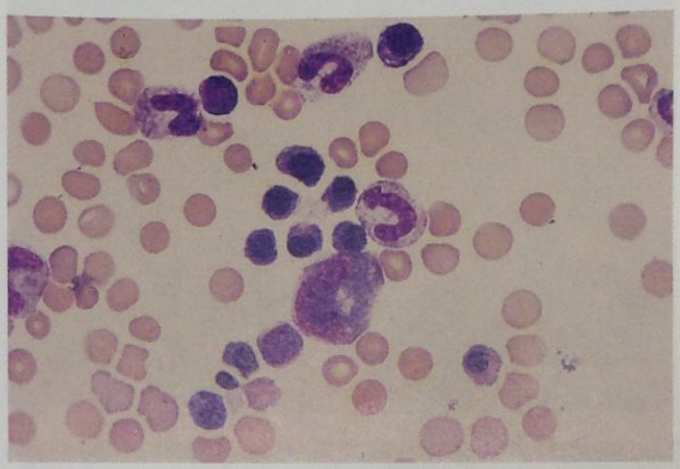


Figure 10–12 Nucleated RBCs seen with bone marrow contamination (×1000).

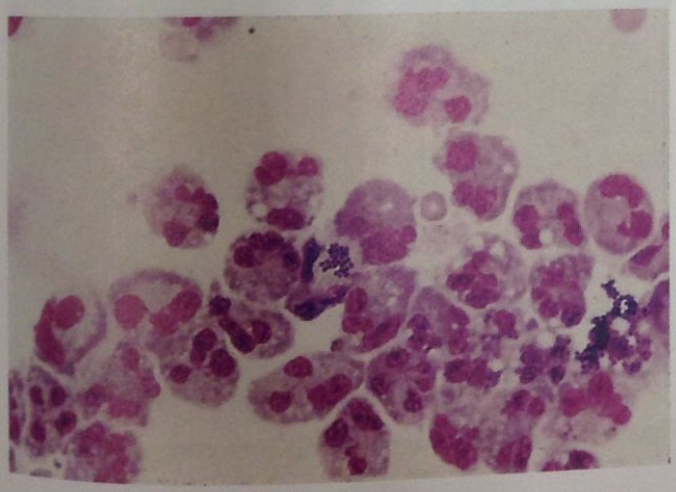


Figure 10–10 Neutrophils with intracellular and extracellular bacteria (×1000).

Figure 10–13 Bone marrow contamination (×1000). Notice the immature RBCs and granulocytes.

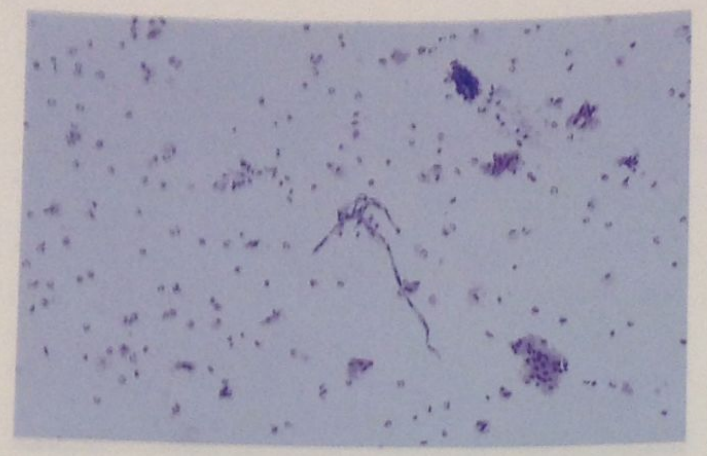


Figure 10–14 Capillary and tissue fragments from a traumatic tap (×100).

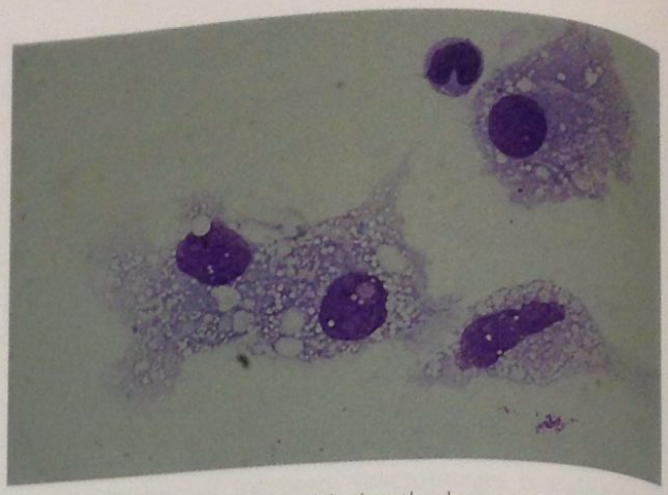


Figure 10–17 Macrophages. Notice the large amount of cytoplasm and vacuoles (×500).

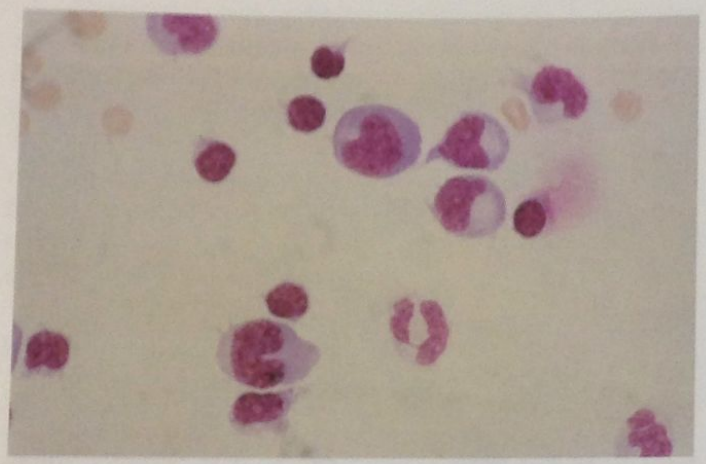


Figure 10–15 Broad spectrum of lymphocytes and monocytes in viral meningitis (×1000).

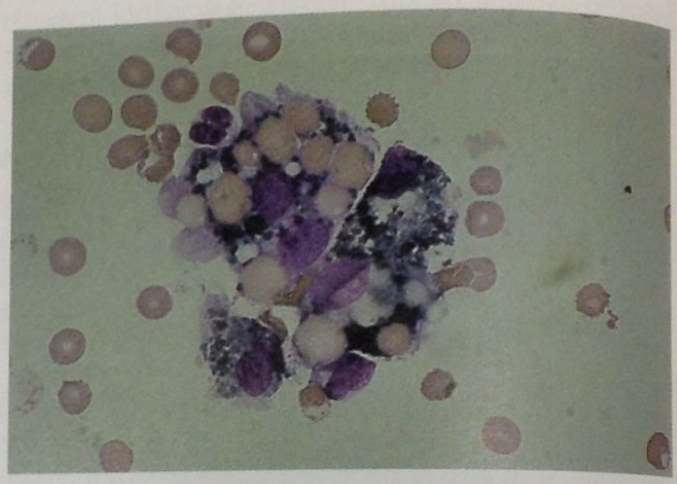


Figure 10–18 Macrophages showing erythrophagocytosis (×500).

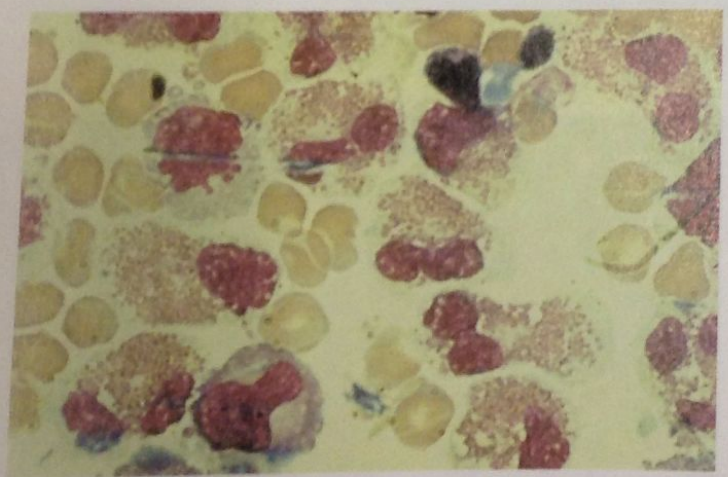


Figure 10–16 Eosinophils (×1000). Notice cytocentrifuge distortion.

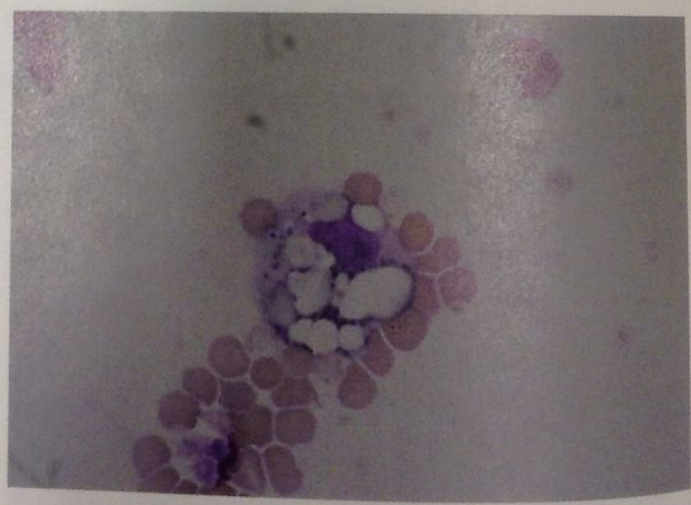


Figure 10–19 Macrophage with RBC remnants (×500).

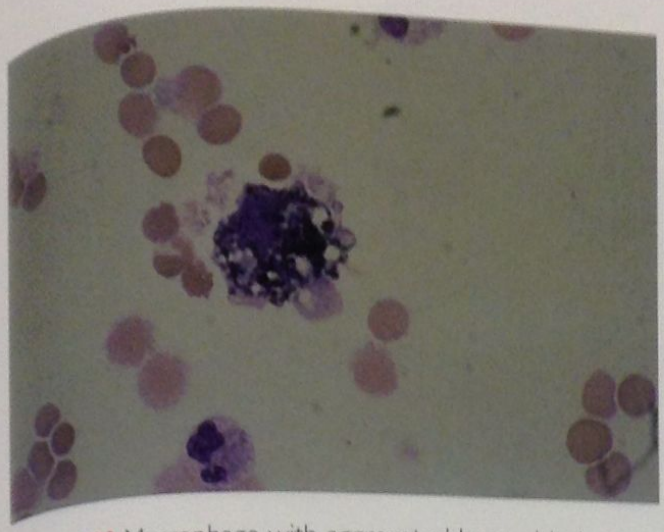


Figure 10–20 Macrophage with aggregated hemosiderin granules (×500).

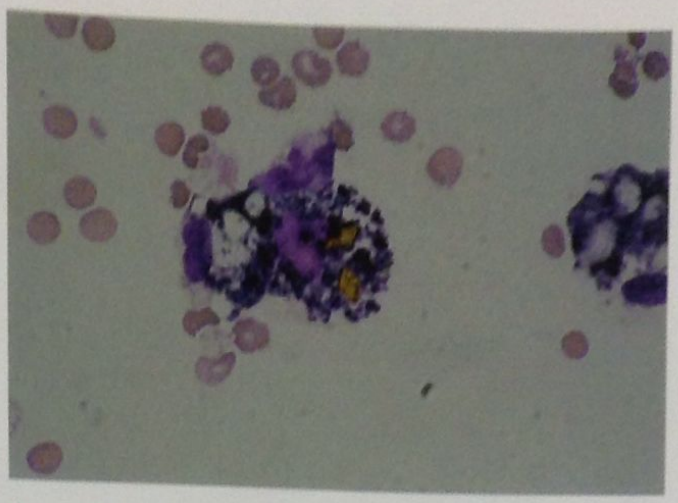


Figure 10–23 Macrophage containing hemosiderin and hematoidin crystals (×500).

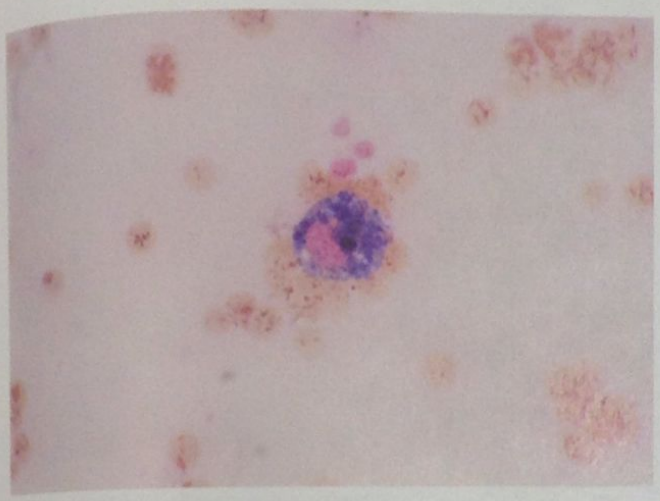


Figure 10–21 Macrophage containing hemosiderin stained with Prussian blue (×250).

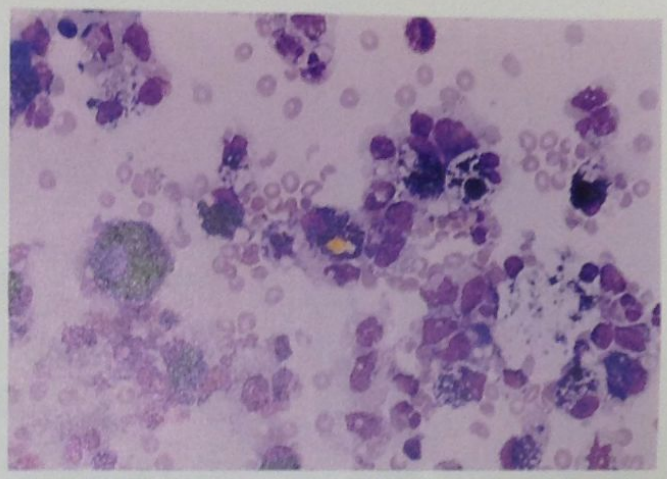


Figure 10–24 Macrophages with hemosiderin and hematoidin (×250). Notice the bright yellow color.

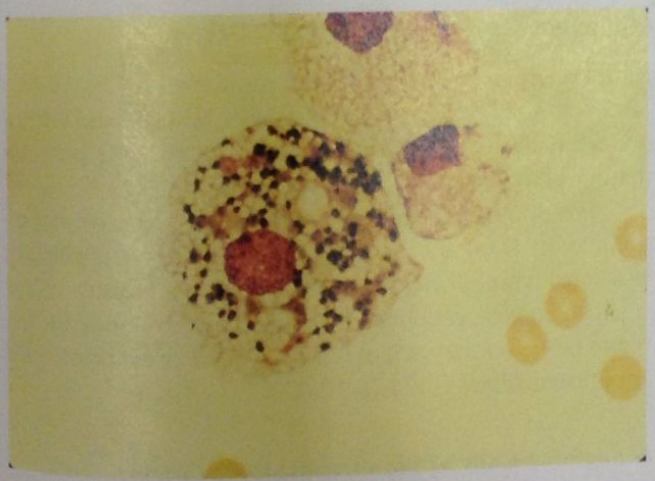


Figure 10–22 Macrophage with coarse hemosiderin granules (×500).

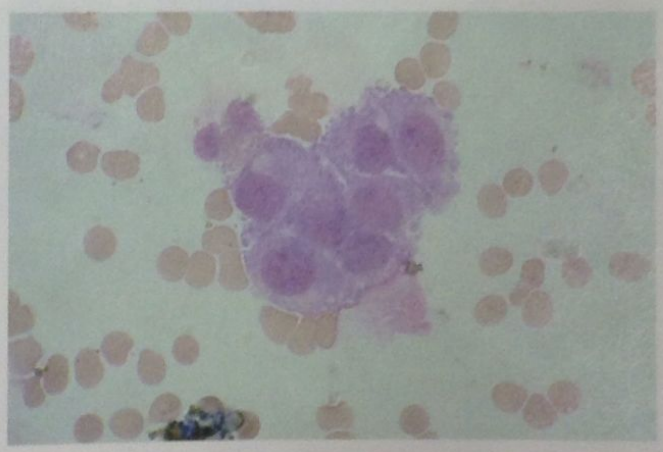


Figure 10–25 Choroidal cells showing distinct cell borders and nuclear uniformity (×500).

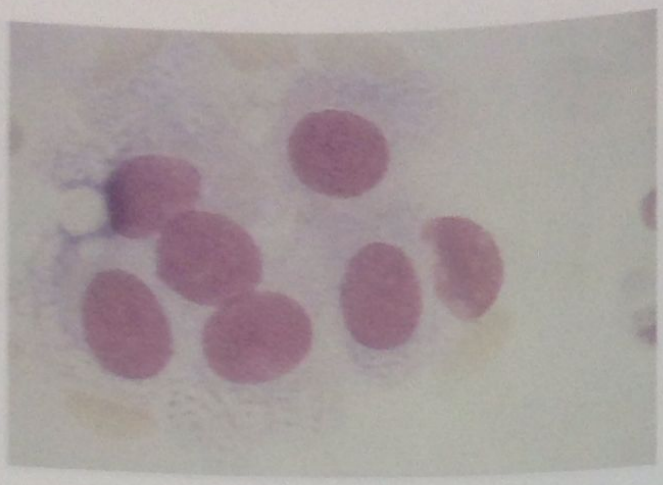


Figure 10–26 Ependymal cells. Notice the nucleoli and less distinct cell borders (×1000).

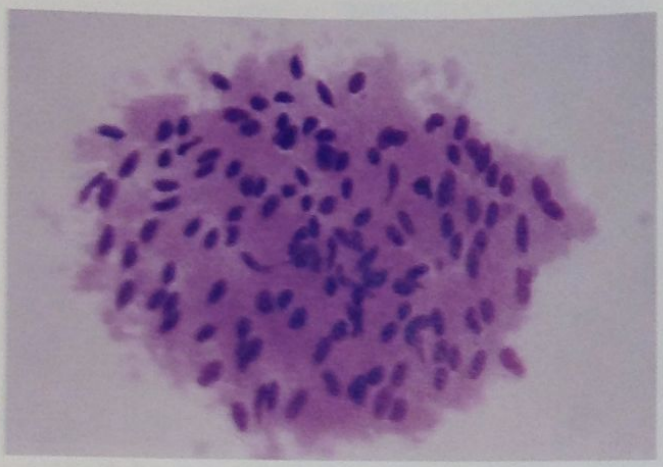


Figure 10–27 Cluster of spindle-shaped cells (×500).

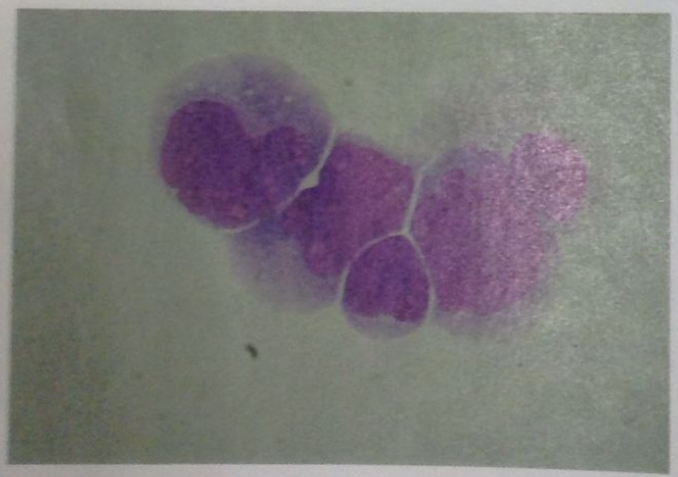


Figure 10–30 Monoblasts and two lymphocytes (×1000). Notice the prominent nucleoli.

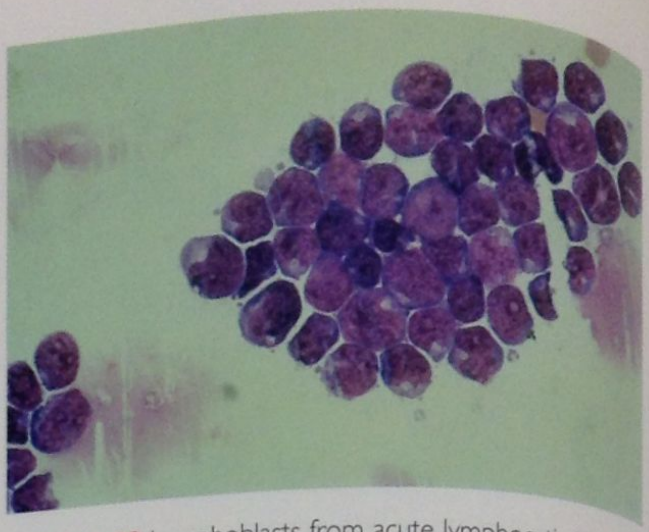


Figure 10–28 Lymphoblasts from acute lymphocytic leukemia (×500).

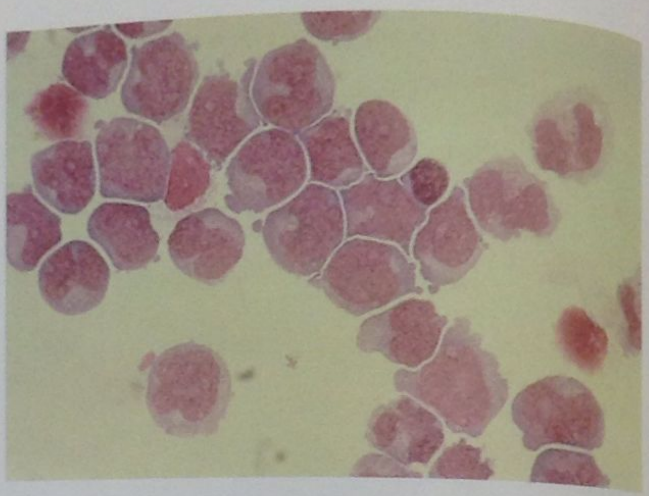


Figure 10–29 Myeloblasts from acute myelocytic leukemia (×500).

Malignant Cells of Hematologic Origin

Lymphoblasts, myeloblasts, and monoblasts (Figs 10-28 to 10-30) in the CSF are frequently seen as a serious complication of acute leukemias. Nucleoli are often more prominent than in blood smears.

Lymphoma cells are also seen in the CSF indicating dissemination from the lymphoid tissue. They resemble large and small lymphocytes and usually appear in clusters of large, small, or mixed cells based on the classification of the lymphoma. Nuclei may appear cleaved, and prominent nucleoli are present (Figs. 10-31 to 10-33).

Malignant Cells of Nonhematologic Origin

Metastatic carcinoma cells of nonhematologic origin are primarily from lung, breast, renal, and gastrointestinal malig-

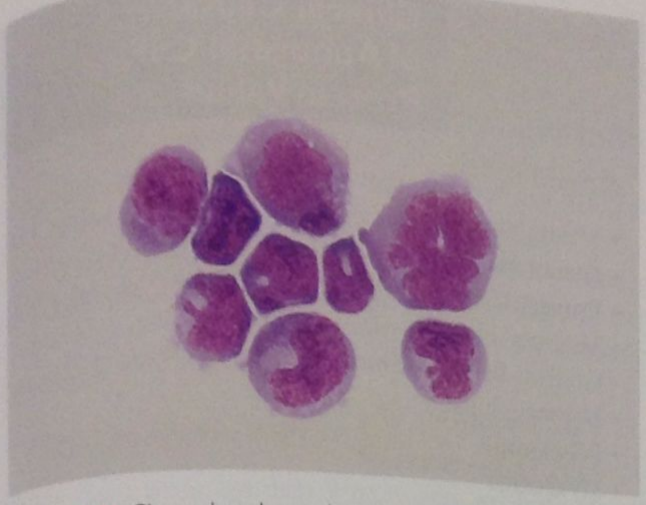


Figure 10–31 Cleaved and noncleaved lymphoma cells (×1000).

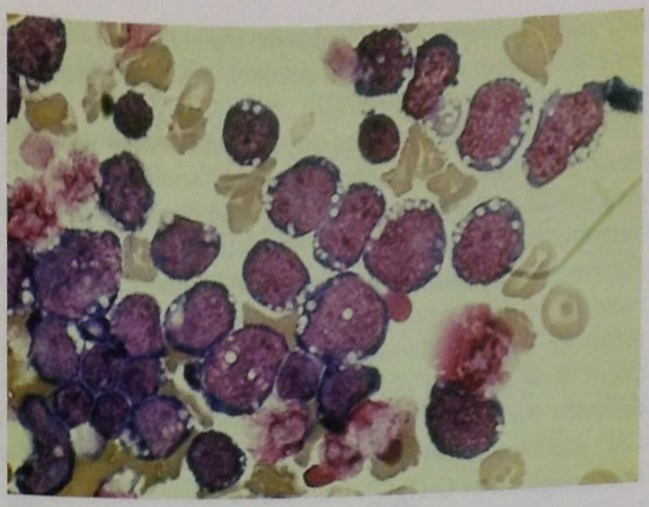


Figure 10–33 Burkitt lymphoma. Notice characteristic vacuoles (×500).

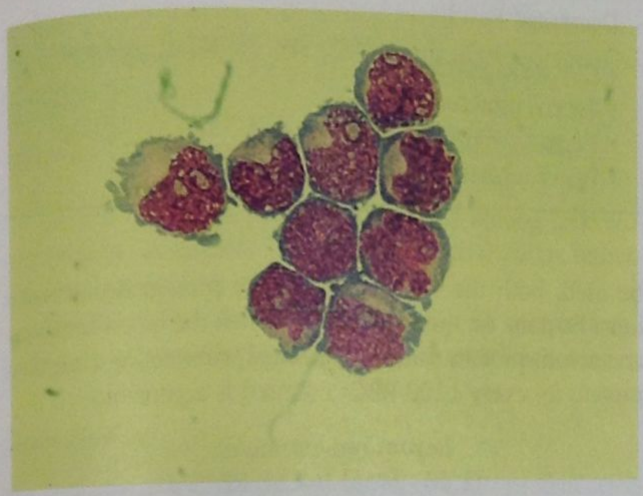


Figure 10–32 Lymphoma cells with nucleoli (×500).

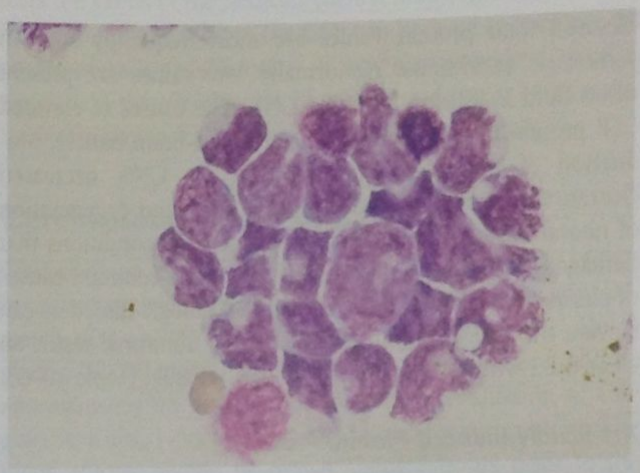


Figure 10–34 Medulloblastoma (×1000). Notice cellular clustering, nuclear irregularities, and rosette formation.

nancies. Cells from primary CNS tumors include *astrocytomas*, *retinoblastomas*, and *medulloblastomas* (Fig. 10-34). They usually appear in clusters and must be distinguished from normal clusters of ependymal, choroid plexus, lymphoma, and leukemia cells. Fusing of cell walls and nuclear irregularities and hyperchromatic nucleoli are seen in clusters of malignant cells. Slides containing abnormal cells must be referred to pathology.

Chemistry Tests

Because CSF is formed by filtration of the plasma, one would expect to find the same low-molecular-weight chemicals in the CSF that are found in the plasma. This is essentially true; however, because the filtration process is selective and the chemical composition is controlled by the blood-brain barrier, normal values for CSF chemicals are not the same as the plasma values. Abnormal values result from alterations in the permeability of the blood-brain barrier or increased production or metabolism by the neural cells in response to a pathologic condition. They seldom have the same diagnostic significance as plasma abnormalities. The clinically important CSF chemicals are few; under certain conditions it may be necessary to measure a larger variety. Many CSF metabolites are currently under investigation to determine their possible diagnostic significance.

Cerebrospinal Protein

The most frequently performed chemical test on CSF is the protein determination. Normal CSF contains a very small amount of protein. Normal values for total CSF protein are usually listed as 15 to 45 mg/dL, but are somewhat method dependent, and higher values are found in infants

and older persons.[12] This value is reported in milligrams per deciliter and not grams per deciliter, as are plasma protein concentrations.

In general, the CSF contains protein fractions similar to those found in serum; however, as can be seen in Table 10–4, the ratio of CSF proteins to serum proteins varies among the fractions. As in serum, albumin makes up the majority of CSF protein. But in contrast to serum, prealbumin is the second most prevalent fraction in CSF. The alpha globulins include primarily haptoglobin and ceruloplasmin. Transferrin is the major beta globulin present; also, a separate carbohydrate-deficient transferrin fraction, referred to as "tau," is seen in CSF and not in serum. CSF gamma globulin is primarily immunoglobulin G (IgG), with only a small amount of IgA. Immunoglobulin M (IgM), fibrinogen, and beta lipoprotein are not found in normal CSF.[13]

Clinical Significance of Elevated Protein Values

Elevated total protein values are most frequently seen in pathologic conditions. Abnormally low values are present when fluid is leaking from the CNS. The causes of elevated CSF protein include damage to the blood-brain barrier, production of immunoglobulins within the CNS, decreased clearance of normal protein from the fluid, and degeneration of neural tissue. Meningitis and hemorrhage conditions that damage the blood-brain barrier are the most common causes of elevated CSF protein. Many other neurologic disorders can elevate the CSF protein, and finding an abnormal result on clear fluid with a low cell count is not unusual (Table 10–5).

Artificially Induced Plasma Proteins

Plasma protein can be artificially introduced into a specimen by a traumatic tap in the same manner as blood cells. A correction calculation similar to that used in cell counts is available for protein measurements; however, if the correction is to be used, both the cell count and the protein determination must be done on the same tube.[6] When the blood hematocrit and serum protein values are normal, subtracting 1 mg/dL of protein for every 1200 RBCs counted is acceptable.

$$\text{mg/dL protein added} = \frac{[\text{serum protein mg/dL} \times (1.00 - \text{Hct})] \times \text{CSF RBCs}/\mu\text{L}}{\text{(plasma volume)}\ \text{blood RBCs}/\mu\text{L}}$$

Table 10–4 Cerebrospinal Fluid and Serum Protein Correlations

	Fluid (mg/dL)	Cerebrospinal (mg/dL)	Serum Ratio
Prealbumin	1.7	23.8	14
Albumin	15.5	3600	236
Ceruloplasmin	0.1	36.6	366
Transferrin	1.4	204	142
Immunoglobulin G	1.2	987	802
Immunoglobulin A	0.13	175	1346

Adapted from Fishman, RA: Cerebrospinal fluid in diseases of the nervous system, 2nd ed, WB Saunders, Philadelphia, 1992.

Table 10–5 Clinical Causes of Abnormal CSF Protein Values

Elevated Results

- Meningitis
- Hemorrhage
- Primary CNS tumors
- Multiple sclerosis
- Guillain-Barré syndrome
- Neurosyphilis
- Polyneuritis
- Myxedema
- Cushing disease
- Connective tissue disease
- Polyneuritis
- Diabetes
- Uremia

Decreased Results

- CSF leakage/trauma
- Recent puncture
- Rapid CSF production
- Water intoxication

Methodology

The two most routinely used techniques for measuring total CSF protein use the principles of turbidity production or dye-binding ability. The turbidity method has been adapted to automated instrumentation in the form of nephelometry. Methods for the measurement of CSF protein are available for most automated chemistry analyzers.

Protein Fractions

Routine CSF protein procedures are designed to measure total protein concentration. However, diagnosis of neurologic disorders associated with abnormal CSF protein often requires measurement of the individual protein fractions. Protein that appears in the CSF as a result of damage to the integrity of the blood-brain barrier contains fractions proportional to those in plasma, with albumin present in the highest concentration. Diseases, including multiple sclerosis, that stimulate the immunocompetent cells in the CNS show a higher proportion of IgG.

To accurately determine whether IgG is increased because it is being produced within the CNS or is elevated as the result of a defect in the blood-brain barrier, comparisons between serum and CSF levels of albumin and IgG must be made. Methods include the CSF/serum albumin index to evaluate the integrity of the blood-brain barrier and the CSF IgG index to measure IgG synthesis within the CNS.

The CSF/serum albumin index is calculated after determining the concentration of CSF albumin in milligrams per deciliter and the serum concentration in grams per deciliter. The formula used is as follows:

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

An index value less than 9 represents an intact blood-brain barrier. The index increases relative to the amount of damage to the barrier.

Calculation of an IgG index, which is actually a comparison of the CSF/serum albumin index with the CSF/ serum IgG index, compensates for any IgG entering the CSF via the blood-brain barrier.[14] It is performed by dividing the CSF/serum IgG index by the CSF/serum albumin index as follows:

$$\text{IgG index} = \frac{\text{CSF IgG (mg/dL)/serum IgG (g/dL)}}{\text{CSF albumin (mg/dL)/serum albumin (g/dL)}}$$

Normal IgG index values vary slightly among laboratories; however, in general, values greater than 0.70 are indicative of IgG production within the CNS.

Techniques for the measurement of CSF albumin and globulin have been adapted to automated instrumentation.

Electrophoresis

The primary purpose for performing CSF protein electrophoresis is for the detection of *oligoclonal bands* representing inflammation within the CNS. The bands are located in the gamma region of the protein electrophoresis, indicating immunoglobulin production. To ensure that the oligoclonal bands are present as the result of neurologic inflammation, simultaneous serum electrophoresis must be performed. Disorders such as leukemia, lymphoma, and viral infections may produce serum banding, which can appear in the CSF as a result of blood-brain barrier leakage or traumatic introduction of blood into the CSF specimen. Banding representing both systemic and neurologic involvement is seen in the serum and CSF with HIV infection.[15]

The presence of two or more oligoclonal bands in the CSF that are not present in the serum can be a valuable tool in the diagnosis of multiple sclerosis, particularly when accompanied by an increased IgG index. Other neurologic disorders including encephalitis, neurosyphilis, *Guillain-Barré* syndrome, and neoplastic disorders also produce oligoclonal banding that may not be present in the serum. Therefore, the presence of oligoclonal banding must be considered in conjunction with clinical symptoms. Oligoclonal banding remains positive during remission of multiple sclerosis, but disappears in other disorders.[11]

Low protein levels in the CSF make concentration of the fluid prior to performing electrophoresis essential for most electrophoretic techniques. Agarose gel electrophoresis followed by Coomassie brilliant blue staining is most frequently performed in the clinical laboratory. Better resolution can be obtained using immunofixation electrophoresis **(IFE)** and isoelectric focusing **(IEF)** followed by silver staining. Specimen concentration is not required by the more sensitive IEF procedure.

Electrophoresis is also the method of choice when determining if a fluid is actually CSF. Identification can be made based on the appearance of the previously mentioned extra isoform of transferrin, tau, that is found only in CSF.[16]

Myelin Basic Protein

The presence of myelin basic protein **(MBP)** in the CSF is indicative of recent destruction of the myelin sheath that protects the axons of the neurons *(demyelination)*. Measurement of the amount of MBP in the CSF can be used to monitor the course of multiple sclerosis.[17] It may also provide a valuable measure of the effectiveness of current and future treatments. Immunoassay techniques are used for measurement.[18]

Cerebrospinal Fluid Glucose

Glucose enters the CSF by selective transport across the blood-brain barrier, which results in a normal value that is approximately 60% to 70% that of the plasma glucose. If the plasma glucose is 100 mg/dL, then a normal CSF glucose would be approximately 65 mg/dL. For an accurate evaluation of CSF glucose, a blood glucose test must be run for comparison. The blood glucose should be drawn about 2 hours prior to the spinal tap to allow time for equilibration between the blood and fluid. CSF glucose is analyzed using the same procedures employed for blood glucose. Specimens should be tested immediately because glycolysis occurs rapidly in the CSF.

The diagnostic significance of CSF glucose is confined to the finding of values that are decreased in relation to plasma values. Elevated CSF glucose values are always a result of plasma elevations. Low CSF glucose values can be of considerable diagnostic value in determining the causative agents in meningitis. The finding of a markedly decreased CSF glucose accompanied by an increased WBC count and a large percentage of neutrophils is indicative of bacterial meningitis. If the WBCs are lymphocytes instead of neutrophils, tubercular meningitis is suspected. Likewise, if a normal CSF glucose value is found with an increased number of lymphocytes, the diagnosis would favor viral meningitis. Classic laboratory patterns such as those just described may not be found in all cases of meningitis, but they can be helpful when they are present.

Decreased CSF glucose values are caused primarily by alterations in the mechanisms of glucose transport across the blood-brain barrier and by increased use of glucose by the brain cells. The common tendency to associate the decreased glucose totally with its use by microorganisms and leukocytes

cannot account for the variations in glucose concentrations seen in different types of meningitis and the decreased levels seen in other disorders producing damage to the CNS.[19]

Cerebrospinal Fluid Lactate

The determination of CSF lactate levels can be a valuable aid in the diagnosis and management of meningitis cases. In bacterial, tubercular, and fungal meningitis, the elevation of CSF lactate to levels greater than 25 mg/dL occurs much more consistently than does the depression of glucose and provides more reliable information when the initial diagnosis is difficult. Levels greater than 35 mg/dL are frequently seen with bacterial meningitis, whereas in viral meningitis, lactate levels remain lower than 25 mg/dL. CSF lactate levels remain elevated during initial treatment but fall rapidly when treatment is successful, thus offering a sensitive method for evaluating the effectiveness of antibiotic therapy.

Destruction of tissue within the CNS owing to oxygen deprivation (*hypoxia*) causes the production of increased CSF lactic acid levels. Therefore, elevated CSF lactate is not limited to meningitis and can result from any condition that decreases the flow of oxygen to the tissues. CSF lactate levels are frequently used to monitor severe head injuries. RBCs contain high concentrations of lactate, and falsely elevated results may be obtained on xanthochromic or hemolyzed fluid.[9]

Cerebrospinal Fluid Glutamine

Glutamine is produced from ammonia and α-ketoglutarate by the brain cells. This process serves to remove the toxic metabolic waste product ammonia from the CNS. The normal concentration of glutamine in the CSF is 8 to 18 mg/dL.[20] Elevated levels are found in association with liver disorders that result in increased blood and CSF ammonia. Increased synthesis of glutamine is caused by the excess ammonia that is present in the CNS; therefore, the determination of CSF glutamine provides an indirect test for the presence of excess ammonia in the CSF. Several methods of assaying glutamine are available and are based on the measurement of ammonia liberated from the glutamine. This is preferred over the direct measurement of CSF ammonia because the concentration of glutamine remains more stable than the concentration of volatile ammonia in the collected specimen. The CSF glutamine level also correlates with clinical symptoms much better than does the blood ammonia.[20]

As the concentration of ammonia in the CSF increases, the supply of α-ketoglutarate becomes depleted; glutamine can no longer be produced to remove the toxic ammonia, and coma ensues. Some disturbance of consciousness is almost always seen when glutamine levels are more than 35 mg/dL.[13] Therefore, the CSF glutamine test is a frequently requested procedure for patients with coma of unknown origin. Approximately 75% of children with *Reye syndrome* have elevated CSF glutamine levels.[21]

Microbiology Tests

The role of the microbiology laboratory in the analysis of CSF lies in the identification of the causative agent in meningitis. For positive identification, the microorganism must be recovered from the fluid by growing it on the appropriate culture medium. This can take anywhere from 24 hours in cases of bacterial meningitis to 6 weeks for tubercular meningitis. Consequently, in many instances, the CSF culture is actually a confirmatory rather than a diagnostic procedure. However, the microbiology laboratory does have several methods available to provide information for a preliminary diagnosis. These methods include the Gram stain, acid-fast stain, India ink preparation, and latex agglutination tests. In Table 10–6, the laboratory tests used in the differential diagnosis of meningitis are compared.

Table 10–6 Major Laboratory Results for the Differential Diagnosis of Meningitis

Bacterial	Viral	Tubercular	Fungal
Elevated WBC count	Elevated WBC count	Elevated WBC count	Elevated WBC count
Neutrophils present	Lymphocytes present	Lymphocytes and monocytes present	Lymphocytes and monocytes present
Marked protein elevation	Moderate protein elevation	Moderate to marked protein elevation	Moderate to marked protein elevation
Markedly decreased glucose level	Normal glucose level	Decreased glucose level	Normal to decreased glucose level
Lactate level >35 mg/dL	Normal lactate level	Lactate level>25 mg/dL	Lactate level >25 mg/dL
		Pellicle formation	Positive India ink with *Cryptococcus neoformans*
Positive Gram stain and bacterial antigen tests			Positive immunologic test for *C. neoformans*

Summary of Cerebrospinal Fluid Chemistry Tests

Protein
1. Normal concentration is 15 to 45 mg/dL.
2. Elevated values are most frequently seen in patients with meningitis, hemorrhage, and multiple sclerosis.

Glucose
1. Normal value is 60% to 70% of the plasma concentration.
2. Decreased levels are seen in patients with bacterial, tubercular, and fungal meningitis.

Lactate
1. Levels >35 mg/dL are seen in patients with bacterial meningitis.
2. Levels >25 mg/dL are found in patients with tubercular and fungal meningitis.
3. Lower levels are seen in patients with viral meningitis.

Glutamine
1. Normal concentration is 8 to 18 mg/dL.
2. Levels >35 mg/dL are associated with some disturbance of consciousness.

Gram Stain

The Gram stain is routinely performed on CSF from all suspected cases of meningitis, although its value lies in the detection of bacterial and fungal organisms. All smears and cultures should be performed on concentrated specimens because often only a few organisms are present at the onset of the disease. The CSF should be centrifuged at 1500 g for 15 minutes, and slides and cultures should be prepared from the sediment.[23] Use of the cytocentrifuge provides a highly concentrated specimen for Gram stains. Even when concentrated specimens are used, at least a 10% chance exists that Gram stains and cultures will be negative. Thus, blood cultures should be taken, because the causative organism is often present in both the CSF and the blood.[9] A CSF Gram stain is one of the most difficult slides to interpret because the number of organisms present is usually small, and they can easily be overlooked, resulting in a false-negative report. Also, false-positive reports can occur if precipitated stain or debris is mistaken for microorganisms. Therefore, considerable care should be taken when interpreting a Gram stain. Organisms most frequently encountered include *Streptococcus pneumoniae* (gram-positive cocci), *Haemophilus influenzae* (pleomorphic gram-negative rods), *Escherichia coli* (gram-negative rods), and *Neisseria meningitidis* (gram-negative cocci). The gram-positive cocci, *Streptococcus agalactiae* and the gram-positive rods *Listeria monocytogenes* may be encountered in newborns.

Acid-fast or fluorescent antibody stains are not routinely performed on specimens unless tubercular meningitis is suspected. Considering the length of time required to culture mycobacteria, a positive report from this smear is extremely valuable.

Specimens from possible cases of fungal meningitis are Gram stained and often have an India ink preparation performed on them to detect the presence of thickly encapsulated *Cryptococcus neoformans* (Fig. 10-35). As one of the more frequently occurring complications of AIDS, cryptococcal meningitis is now commonly encountered in the clinical laboratory. Particular attention should be paid to the Gram stain for the classic starburst pattern produced by *Cryptococcus*, as this may be seen more often than a positive India ink (Fig. 10-36).[24]

Latex agglutination tests to detect the presence of *C. neoformans* antigen in serum and CSF provide a more sensitive method than the India ink preparation. However, immunologic testing results should be confirmed by culture and demonstration of the organisms by India ink, because false-positive reactions do occur. Interference by ***rheumatoid factor*** is the most common cause of false-positive reactions. Several commercial kits with pretreatment techniques are available and include incubation with dithiothreitol or pronase and boiling with ethylenediaminetetra-acetic acid.[21,25] An enzyme immunoassay technique has been shown to produce fewer false-positive results.[26]

Latex agglutination and enzyme-linked immunosorbent assay (**ELISA**) methods provide a rapid means for detecting and identifying microorganisms in CSF. Test kits are available to detect *Streptococcus* group B, *H. influenzae* type b, *S. pneumoniae*, *N. meningitidis* A, B, C, Y, W135, and *E. coli K1* antigens. The bacterial antigen test (**BAT**) does not appear to be as sensitive to detection of *N. meningitidis* as it is to the other organisms.[27] The BAT should be used in combination with results from the hematology and clinical chemistry laboratories for diagnosing meningitis.[28] The Gram stain is still the recommended method for detection of organisms.[29]

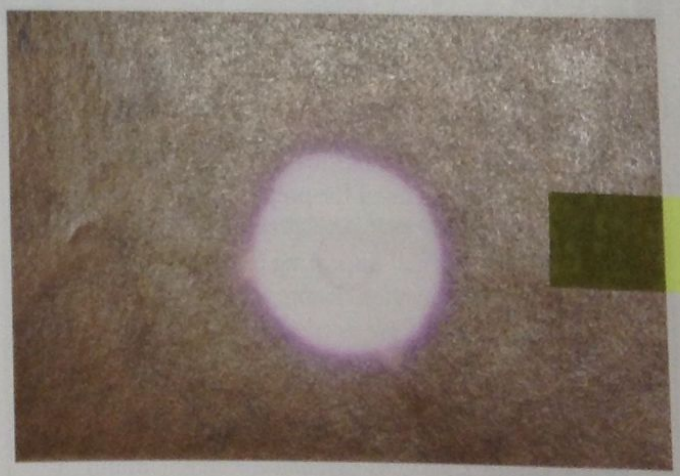


Figure 10–35 India ink preparation of *C. neoformans* (×400). Notice budding yeast form. (Courtesy of Ann K. Fulenwider, Md.)