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Cerebrospinal Fluid

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The analysis of cerebrospinal fluid (CSF) has been described as the central nervous system equivalent of the complete blood count (Jamison and Lumsden, 1988), and the analogy is a good one. A CSF analysis provides a general index of neurologic health and often provides evidence of the presence of disease. Similar to a complete blood count, CSF analysis has reasonable sensitivity but low specificity. The possible alterations of CSF are relatively limited compared to the varieties of neurologic diseases that exist (particularly if the analysis is restricted to total cell counts and total protein determination). Additionally, the type and degree of CSF abnormality seems to be related as much to the location of disease as to the cause or the severity of...
the lesion; meningeal and paraventricular diseases generally produce greater abnormalities than deep parenchymal diseases. Previous therapy may affect the type, degree, and duration of CSF abnormalities as well. The CSF abnormalities identified upon analysis are also dependent on the CSF collection site with respect to lesion location (Thomson et al., 1990, 1989). Lastly, the CSF of animals with neurological disease is not always abnormal (Tipold, 1995). Only occasionally does CSF analysis provide a specific diagnosis (Kjeldsberg and Knight, 1993)—for example, if infectious agents (bacteria or fungi) or neoplastic cells are observed. In most situations, the chief utility of CSF analysis is to assist in the diagnostic process by excluding the likelihood of certain disease processes being present. As is the case with all tests of relatively low specificity, examination of CSF is most useful when the results are correlated with the history, clinical findings, imaging studies, and ancillary laboratory studies. As stated by Fankhauser (1962), "It is futile to make a diagnosis based solely on the CSF findings and particularly on single alterations of it. Only the entire picture of all findings linked with the other clinical symptoms is of value in reaching a diagnosis."

I. FUNCTIONS OF CEREBROSPINAL FLUID

Cerebrospinal fluid has four major functions: (1) physical support of neural structures, (2) excretion and "sink" action, (3) intracerebral transport, and (4) control of the chemical environment of the central nervous system. Cerebrospinal fluid provides a "water jacket" physical support and buoyancy. When suspended in CSF, a 1500-g brain weighs only about 50 g. The CSF is also protective because its volume changes reciprocally with changes in the volume of intracranial contents, particularly blood. Thus, the CSF protects the brain from changes in arterial and central venous pressure associated with posture, respiration, and exertion. Acute or chronic pathological changes in intracranial contents can also be accommodated, to a point, by changes in the CSF volume (Fishman, 1992; Rosenberg, 1990; Milhorat, 1987).

Excretory function is provided by the direct transfer of brain metabolites into the CSF. This capacity is particularly important because the brain lacks a lymphatic system. The lymphatic function of the CSF is also manifested in the removal of large proteins, and even cells such as bacteria or blood cells, by bulk CSF absorption (see Section II.D, CSF Absorption). The "sink" action of the CSF arises from the restricted access of watersoluble substances to the CSF and the low concentration of these solutes in the CSF. Therefore, solutes entering the brain, as well as those synthesized by the brain, diffuse freely from the brain interstitial fluid into the CSF. Removal may then occur by bulk CSF absorption or, in some cases, by transport across the choroid plexus into the capillaries (Fishman, 1992; Rosenberg, 1990; Milhorat, 1987; Davson and Segal, 1996).

Because CSF bathes and irrigates the brain, including those regions known to participate in endocrine functions, CSF may serve as a vehicle for intracerebral transport of biologically active substances. For example, hormone-releasing factors, formed in the hypothalamus and discharged into the CSF of the third ventricle, may be carried in the CSF to their effective sites in the median eminence. The CSF may also be the vehicle for intracerebral transport of opiates and other neuroactive substances (Fishman, 1992; Milhorat, 1987).

An essential function of CSF is the provision and maintenance of an appropriate chemical environment for neural tissue. Anatomically, the interstitial fluid of the central nervous system and the CSF are in continuity (see Section II.A, Anatomy of Brain–Fluid Interfaces); therefore, the chemical composition of the CSF reflects and affects the cellular environment. The composition of the CSF (and the interstitial fluid) is controlled by cells forming the interfaces, or barriers, between the "body" and the neural tissue. These semipermeable interfaces, the blood–brain barrier, the blood–CSF barrier, and the CSF–brain barrier, control the production and absorption of CSF and provide a fluid environment that is relatively stable despite changes in the composition of blood (Fishman, 1992; Milhorat, 1987; Davson and Segal, 1996).

II. CSF FORMATION, CIRCULATION, AND ABSORPTION

The brain (and the spinal cord) as an organ is isolated in many ways from the body and the systemic circulation. This isolation is accomplished anatomically by several interfaces between brain tissue and systemic fluids (Table 27.1). At these interfaces, sele

| Interface          | Cell type            | Junction type |
|--------------------|----------------------|---------------|
| Blood–brain        | Brain capillary endothelium | Tight junction |
| Blood–CSF          | Choroid plexus epithelium | Apical tight junction |
| Blood–CSF          | Arachnoid cells      | Tight junction |
| CSF–blood          | Arachnoid villi     | Valve         |
| CSF–brain          | Ependyma             | Gap junction  |
|                    | Pia mater            | Gap junction  |

* Modified from Rosenberg (1990).
tive carriers and ion pumps transport electrolytes and essential nutrients and thereby control the brain's microenvironment. A substantial portion of this control is achieved through the formation, circulation, and absorption of CSF at these brain-fluid interfaces (Rosenberg, 1990; Davson and Segal, 1996).

A. Anatomy of Brain–Fluid Interfaces

1. The Blood–Brain Barrier

The important blood–brain (and blood–spinal cord) interface is formed by the endothelial cells of the intraparenchymal capillaries. In most areas of the brain and spinal cord, the capillary endothelium differs from that of other body tissues in the following ways: (1) the absence of fenestrae, (2) the presence of tight junctions between adjacent cells, (3) a lower number of pinocytic pits and vesicles, (4) a higher number of mitochondria, and (5) closely applied, perivascular, astrocytic foot processes. These features result in the capillary endothelium being a selective barrier—the blood–brain barrier—that regulates the entry, and probably the exit, of biologically important substances and aids in the maintenance of a precise, stable environment for the neural tissues (Rosenberg, 1990; Milhorat, 1987; Davson and Segal, 1996).

2. The Blood–CSF Barrier

One part of the blood–CSF interface is formed by the epithelial cells of the circumventricular organs. The circumventricular organs, which include the four choroid plexuses, the median eminence, the neural lobe of the hypophysis, and other specialized areas, border the brain ventricles and are involved with specific secretory activities that appear to require a direct contact with plasma. The capillaries within these organs are fenestrated, similar to capillaries in other organs of the body. Overlying each of the organs are specialized epithelial cells joined by intercellular tight junctions at their apical (ventricular) borders. These epithelial cells also are characterized by an abundance of intracellular organelles and lysosomes. These organelles are probably an important aspect of the barrier and secretory functions of these cells (Rosenberg, 1990; Milhorat, 1987). The choroid plexuses are the major source of CSF. They are formed by evaginations of the ependyma and the pial blood vessels into the ventricles, and they consist of a single row of cuboidal, specialized epithelial cells thrown into villi around a core of blood vessels and connective tissue. The apical (ventricular) surface of the epithelial cells has a brush border of microvilli with occasional cilia. The basal and lateral cell surfaces have numerous infoldings. Overall, the structure of these cells resembles that of other epithelia specialized for fluid transport, such as proximal renal tubular epithelium (Rosenberg, 1990; Milhorat, 1987; Davson and Segal, 1996). Autonomic nerve termina have also been identified in the choroid plexus, but their function is unclear (Fishman, 1992; Nilsson et al., 1992).

The second part of the blood–CSF interface formed by the arachnoid membrane at the arachnoid villi. These villi are microscopic evaginations of the arachnoid membrane into the lumen of the dural sinuses. The barrier function of these arachnoid cells demonstrated by their tight junctions. Their transport function is indicated by giant intracellular vacuoles some of which have both basal and apical opening and pinocytotic vesicles. The sinus surface of a villus is covered by sinus endothelium (Rosenberg, 1990; Milhorat, 1987). Endothelium-lined channels may link directly with the subarachnoid space (Davson and Segal, 1996; Bell, 1995). Arachnoid villi are not limited to intracranial venous sinuses, but also are present in the spinal nerve roots penetrating into the spinal vein (Milhorat, 1987; Bell, 1995).

3. The CSF–Brain Interface

The extensive CSF–brain (and spinal cord) interface consists of the ependyma within the cavities of the central nervous system and the pia mater covering the central nervous system. These two layers are each composed of a single layer of cells joined by gap junctions. The ependyma and the pia mater are not important permeability barriers; the CSF (ventricular and subarachnoid) and the brain interstitial fluid are directly continuous (Milhorat, 1987; Davson and Segal, 1996).

B. CSF Formation

1. Choroidal and Extrachoroidal Formation

Cerebrospinal fluid is formed principally by the choroid plexus, with a smaller amount being formed extrachoroidally (Milhorat, 1987; Davson and Segal, 1996). Choroidal formation involves two processes that occur in series: first, filtration across the choroidal capillary wall, and second, secretion by the choroidal epithelium. Within the choroid plexus, hydrostatic pressure of the choroidal capillaries initiates the transfer of water and ions to the interstitial fluid and then to the choroidal epithelium. Water and ions are then transferred into the ventricles by either (1) intracellular movement across the epithelial membranes or (2) intercellular movement across the apical tight junctions between epithelial cells. Both of these processes are probably dependent upon ion pumps. Secretion of CSF results from the active transport of sodium which is dependent on the membrane-bound, sodium-
potassium activated ATPase present at the apical (ventricular) surface of the choroidal epithelium (Rosenberg, 1990; Davson and Segal, 1996). The presence of autonomic nerve terminals in the choroid plexus suggests a neural control of CSF secretion. However, the functional role of this innervation in normal and pathologic conditions is unknown (Fishman, 1992; Nilsson et al., 1992).

Spurred primarily by clinical evidence that excision of the choroid plexus did not benefit human patients with hydrocephalus, experimental evidence now supports the existence of an extrachoroidal source of CSF. The diffusion of brain interstitial fluid across the ependyma or pia mater is the apparent source of this extrachoroidal CSF component. Formation of the interstitial fluid is thought to occur by active transport processes (secretion) at the cerebral capillaries, but an alternative theory proposes passive permeability of the capillary endothelium and active transport by the surrounding astrocytes (Rosenberg, 1990; Milhorat, 1987). The relative contributions of choroidal and extrachoroidal sources to CSF in normal and pathologic conditions are not certain. Some investigators report the choroid plexus to be the major if not the sole source of CSF, whereas others conclude that at least one-third of newly formed CSF is extrachoroidal (Milhorat, 1987; Davson and Segal, 1996).

2. Rate of CSF Formation

Regardless of the amount of extrachoroidal formation, the rate of CSF formation is closely correlated to the weight of the choroid plexus and varies among species (Table 27.2) (Cserr, 1971; Welch, 1975). Increases and decreases in formation rate have been achieved experimentally, but the general tendency is for the formation rate to remain relatively constant. The formation rate directly parallels the rate of sodium exchange, which is linked to the bicarbonate ion. The enzyme carbonic anhydrase plays an important role because it provides the bicarbonate. Inhibition of carbonic anhydrase slows (but does not abolish) sodium, bicarbonate, and chloride flow, resulting in a reduction of CSF secretion (Maren, 1992). Several drugs and conditions inhibit CSF production (Table 27.3), but their clinical utility is limited either by their time frame of action or by their toxicity (Rosenberg, 1990; Pollay 1992; Davson and Segal, 1996).

Moderate variations in intracranial pressure probably do not affect CSF formation. However, studies of chronically hydrocephalic animals have shown a reduction of CSF formation with increasing intraventricular pressure. The secretion process may also be affected by chronically increased intracranial pressure (Fishman, 1992).

C. CSF Circulation

Cerebrospinal fluid flows in bulk from sites of production to sites of absorption. Fluid formed in the lateral ventricles flows through the paired interventricular foramina (foramina of Monro) into the third ventricle, then through the mesencephalic aqueduct

| TABLE 27.2 Rate of CSF Formation in Various Species |
|-----------------------------------------------|
| Species | Rate (µl/min) |
|-----------------|-------------|
| Mouse           | 0.325       |
| Rat             | 2.1–5.4     |
| Guinea pig      | 3.5         |
| Rabbit          | 10          |
| Cat             | 20–22       |
| Dog             | 47–66       |
| Sheep           | 118         |
| Goat            | 164         |
| Calf            | 290         |
| Monkey          | 28.6–41     |
| Human           | 350–370     |

| TABLE 27.3 Factors Influencing CSF Formation |
|---------------------------------------------|
| Effect | Substance or condition | Site of action |
|--------|------------------------|----------------|
| Increase | Cholera toxin | cAMP |
| Decrease | Phenylephrine | Cholinergic pathways |
|         | Acetazolamide, furosemide | Carbonic anhydrase |
|         | Atrial natriuretic hormone | cGMP |
|         | Diazepam analog | Choroidal benzodiazepine receptor |
|         | Dopamine D1 receptor agonist | Choroidal dopamine receptor |
|         | Hyperosmolarity | Choroidal capillaries |
|         | Hyperthermia | Metabolism (decreased) |
|         | Noradrenaline | cAMP/Choroidal Na+/K+-ATPase |
|         | Omeprazole | H+-K+-ATPase? |
|         | Ouabain | Na+/K+-ATPase |
|         | Serotonin receptor agonist | Choroidal serotonin receptor |
|         | Steroids | Choroidal Na+/K+-ATPase |
|         | Vasopressin | Choroidal vasopressin (V1) receptor |

a Estimated by ventriculo-cisternal perfusion. Modified from Davson and Segal (1996).

b Calhoun et al. (1967).

c Davson and Segal (1996).
(aqueduct of Sylvius) into the fourth ventricle. The majority of CSF exits from the fourth ventricle into the subarachnoid space; a small amount may enter the central canal of the spinal cord. In people, CSF enters the subarachnoid space through the lateral apertures (foramina of Luschka) and the median aperture (foramen of Magendie) of the fourth ventricle. Animals below the anthropoid apes do not have a median aperture (Fletcher, 1993; Fankhauser, 1962). Cerebrospinal fluid has also been shown to flow from the spinal subarachnoid space into the spinal perivascular spaces, across the interstitial space, then into the central canal (Stoodley et al., 1996). Mechanisms for propelling the CSF along its route probably include (1) the continuous outpouring of newly formed ventricular fluid, (2) the ciliary action of the ventricular ependyma, (3) respiratory and vascular pulsations, and (4) the pressure gradient across the arachnoid villi (Milhorat, 1987).

D. CSF Absorption

Absorption of CSF occurs by bulk absorption of the fluid and by absorption or exchange of individual constituents of the fluid, that is, ions, proteins, and drugs. Bulk absorption occurs directly into the venous system and depends primarily upon the CSF hydrostatic pressure; as the pressure rises, the absorption rate increases (Davson and Segal, 1996). If intracranial pressure falls below a critical point, bulk absorption decreases, a homeostatic response to stabilize the intracranial pressure and the CSF volume. The primary site of bulk absorption, at least in people, is the arachnoid villi that project into the dural sinuses. Two other routes are through lymphatic channels in the dura and through the perineural sheaths of cranial nerves (particularly the olfactory nerve) and spinal nerves. Perineural absorption may be through arachnoid villi projecting into perineural veins, lymphatics, or connective tissue (Milhorat, 1987; Davson and Segal, 1996). The importance of these various absorption routes varies with the species (Bell, 1995).

Absorption through the arachnoid villi occurs transcellularly through microperiventricular vesicles and giant intracellular vesicles, but may also occur through endothelium-lined, intercellular clefts. The mechanisms appear to vary among species (Bell, 1995). Absorption is unidirectional from the CSF into the venous blood—the villi act like one-way valves. The basis for the valve-like mechanism appears to be transport by the giant vesicles (see Section II.A.2). Particles ranging in size from colloidal gold (0.2 μm) to erythrocytes (7.5 μm) can be transported across the villi. In disease conditions, accumulations of larger particles (e.g., protein molecules, erythrocytes, leukocytes) within the villi may impair absorption, leading to hydrocephalus (Fishman, 1992; Milhorat, 1987). The choroid plexus also has an absorptive function, acting on specific substances in the CSF rather than by bulk fluid absorption. A variety of compounds are actively transported from the CSF, in a fashion reminiscent of the proximal renal tubule. Solute may also be cleared from the CSF by diffusion into adjacent brain cells or capillaries (Fishman, 1992; Milhorat, 1987).

III. CELLULAR COMPOSITION OF NORMAL CSF

A. Total Erythrocyte and Nucleated Cell Count

Cerebrospinal fluid normally does not contain erythrocytes (Chrisman, 1992; Rand et al., 1990b; Cook and DeNicola, 1988; Wilson and Stevens, 1977). Most commonly, the presence of erythrocytes in a CSF sample is iatrogenic, due to inadvertent trauma associated with the needle placement. However, CSF erythrocytes may also originate from pathologic hemorrhage. The normal nucleated-cell counts of CSF in domestic animals are given in Table 27.4. The most widely accepted reference ranges for the numbers of leukocytes in the CSF of dogs and cats are 0–5 cells/μl (deLahunta, 1983; Oliver and Lorenz, 1993) to 0–8 cells/μl (Duncan et al., 1994). However, these ranges are a little too broad in our experience and the most comprehensive series in the literature confirm this. Jamison and Lumsden

| TABLE 27.4 Total White Blood Cell Count of Normal CSF in Domestic Animals |
|-----------------------------|----------------------------|----------------|----------------|
| Species | Collection site | N | Cells/μl | Reference |
| Dog | C | 50 | 0–2 | Jamison (1988) |
| Dog | C, L | 31 | 0–4 | Bailey (1985) |
| Cat | C | 33 | 0–2 | Rand (1990) |
| Horse | Pooled C and L | 44 | 0–6 | Mayhew (1977) |
| Horse | C | 14 | 0–5 | Furr (1994) |
| Cow | L | 16 | 0.85–3.52 | Welles (1992) |
| Llama | L | 17 | 0–3 | Welles (1994) |
| Sheep | NS | 17 | 0–5 | Fankhauser (1962) |
| Goat | NS | 17 | 0–4 | Brewer (1983) |
| Pig | NS | 17 | 0–7 | Fankhauser (1962) |

\[ C = \text{cerebellomedullary cistern. L = lumbar subarachnoid space.} \]
\[ N = \text{number of animals.} \]
\[ \text{Range.} \]
\[ \text{95% confidence interval.} \]
\[ \text{NS, not stated.} \]
(1988) examined 50 clinically and histopathologically normal dogs and derived cerebellomedullary CSF reference limits of 0–2 cells/μL. In fact, all except one of these dogs had counts of 0–1 cells/μL (personal communication). Bailey and Higgins (1985) examined 31 dogs that were clinically and histopathologically normal. For cerebellomedullary CSF, the mean was 1.45 cells/μL, with the 95% confidence intervals being 1.04–1.86 and the observed range being 0–4 cells/μL. Of 31 dogs, 26 had counts between 0 and 2 cells/μL. They also found that lumbar CSF had significantly lower counts with a mean of 0.55 cells/μL, a 95% confidence interval of 0.22–0.88 and an observed range of 0–2 cells/μL, although 30/31 dogs had counts of 0–2 cells/μL. We currently feel that a normal count for cerebellomedullary CSF in dogs is 0–2 cells/μL, with 3 cells/μL being in the gray zone and 4 cells/μL being abnormal. Rand et al., (1990b), derived reference limits for cerebellomedullary CSF from 33 cats that were clinically and histopathologically normal. The samples did not have blood contamination. The mean ± 1 SD for the white blood cell count was 0.1 ± 0.4 with an observed range of 0–2 cells/μL of 33 cats, 30 had counts of 0 cells/μL. Three cells or more per microliter is therefore considered abnormal in cerebellomedullary fluid of cats.

B. Differential Cell Count

1. Leukocytes

Excellent morphologic descriptions of the cell types normally found in the CSF of domestic animals can be found elsewhere (Rand et al., 1990b; Cook and DeNicola, 1988; Jamison and Lumsden, 1988). Normal CSF consists of varying proportions of small lymphocytes and monocytes. The proportions are species and age dependent (Kjeldsberg and Knight, 1993). In dogs, monocytic type cells predominate (Jamison and Lumsden, 1988), although there is significant individual variation. In cats (Rand et al., 1990b), monocytoid cells also predominate, with a mean of 87%, whereas small lymphocytes have a mean of 9%. This same trend is observed in horses, with 73.6% monocytes and 26.2% lymphocytes (Furr and Bender, 1994). However, small lymphocytes predominate in cattle (Welles et al., 1992) and llamas (Welles et al., 1994). In the human literature prior to 1975, any neutrophils present at all in the CSF were thought to be indicative of disease (Kjeldsberg and Knight, 1993). However, with the advent of techniques for concentrating CSF specimens, such as the cytocentrifuge, it became clear that a very small number of neutrophils may be found in normal human CSF (Fishman, 1992; Kjeldsberg and Knight, 1993). Similar observations have been made in many veterinary species, and rare neutrophils are a common and normal finding in the CSF of all domestic species. Eosinophils are not present in normal CSF, although a single cell is occasionally seen on cytocentrifuge slides of fluid with normal total cell counts. Large foamy macrophages or phagocytes are not seen in normal CSF (Fishman, 1992; Christopher et al., 1988) and their presence is nonspecific evidence of an inflammatory disorder. Plasma cells are not seen in normal CSF (Fishman, 1992; Pelc et al., 1981; Kjeldsberg and Knight 1993). Their presence is indicative of underlying inflammatory disease. In people, plasma cells are seen particularly in acute viral disease and various chronic inflammatory conditions, including tuberculosis meningitis, syphilis, multiple sclerosis, and the Guillain Barré syndrome (Pelc et al., 1981; Kjeldsberg and Knight, 1993). In animals, plasma cells have been observed in various conditions, including distemper (Vandeveld and Spano, 1977), other viral meningitis (Vandeveld and Spano, 1977), rabies (Green et al., 1992), granulomatous meningoencephalomyelitis (Baley and Higgins, 1986a; Vandeveld and Spano, 1977) neoplasia, and abscessation (personal observations). Therefore, although they are abnormal, no specificity is associated with their presence in CSF. Similarly, reactive lymphocytes are not found in normal CSF, but their presence has no specificity. They can be seen in active or resolving infectious disease, immunemediated diseases, and neoplasia (Cook and DeNicola, 1988).

2. Other Cells

Cells other than leukocytes can be seen in both normal and abnormal CSF. Cells lining the leptomeningeal choroid plexus cells, and ependymal cells can be seen as single cells, or, more often, as small papillary clusters or sheets. Cytologically, choroid plexus cells and ependymal cells are indistinguishable (Cook and DeNicola, 1988; Kjeldsberg and Knight, 1993). The majority of lining cells seen in normal CSF are thought to be choroid plexus cells (Kjeldsberg and Knight, 1993). Excellent descriptions and photographs of these cell types can be found elsewhere (Rand et al., 1990b; Cook and DeNicola, 1988; Kjeldsberg and Knight, 1993). Choroid plexus cells are occasionally seen in CSF sampled by lumbar puncture, likely due to the spinal needle nicking the intervertebral disc (Bigner and Johnston, 1981). Squamous cells can be observed in CSF and may be due to skin contamination or an underlying pathologic process such as epidermoid cysts (Kormey and Gogacz, 1982) or metastatic carcinomas. Bone-marrow cells (immature hematopoietic precursors) have been
described in the CSF of people (Kjeldsberg and Knight, 1993) and dogs (Christopher, 1992). In people, bone-marrow cells in the CSF are usually associated with lumbar puncture, usually in infants or in patients with vertebral bone abnormalities that create difficulties during the sampling process. The cells are present because of sampling from the vertebral body or articular process. Christopher (1992) observed hematopoietic cells in the lumbar CSF of two dogs and speculated that it was due either to marrow penetration or to dural extramedullary hematopoiesis (Christopher, 1992). Extramedullary hematopoiesis has been observed in the choroid plexus of five dogs that did not have underlying hematologic abnormalities or the presence of extramedullary hematopoiesis elsewhere (Bienzle et al., 1995). Although the CSF was normal in these dogs and hematopoietic cells were not observed, this site could provide another potential source for the presence of these cells in CSF. Metastatic myeloid leukemia could conceivably produce similar findings, but peripheral blood and marrow examination would clarify the origin of the cells in question. Neurons, astrocytes, glial cells, and neutral tissue have been observed in the CSF of people (Bigner and Johnston, 1981) and also in cerebellomedullary cisternal samples associated with traumatic taps in animals (personal observation).

IV. BIOCHEMICAL CONSTITUENTS OF NORMAL CSF

Because CSF is a product of plasma filtration and membrane secretion, its composition is different from that of plasma. In general, CSF is a clear, colorless, nearly acellular, low-protein fluid. Various ions, enzymes, and other substances are also found in normal CSF. In health, the CSF composition is maintained relatively constant by the various membrane interfaces, although some fluctuations occur with fluctuations in plasma composition. The chemical composition of the CSF of various animal species is summarized in Tables 27.5 through 27.8. These values should serve only as a guide; normal values must be established for individual laboratories.

A. Ontogeny of CSF

In people and animals, differences in CSF appearance and composition exist between neonates and adults. Human neonatal CSF is usually xanthochromic, probably because of a greater protein and bilirubin content than adult CSF. Glucose content is also increased, more closely approximating the blood glucose level. Many of these differences, such as protein content, are attributed to immaturity of the blood–brain barrier. Immaturity of the blood–brain barrier may be due to an increased number of fenestrae in the brain capillaries or inadequate closure of their endothelia tight junctions. Other factors that may contribute to age differences in CSF composition are the integrity of the blood–CSF barrier, the rate of CSF secretion and efficiency of absorption, the volume of the extracellular space of the brain, and the lipid solubility of the substances (Fishman, 1992; Davson and Segal, 1996). Protein also decreases with age in foals and puppies (Fur and Bender, 1994; Rossdale et al., 1982; Meeks et al., 1994). In contrast, two studies of calves found that CSF protein increased with age (St. Jean et al., 1995 Binkhorst, 1982). Foals also had xanthochromia and a higher CSF glucose and creatine kinase levels than adults (Fur and Bender, 1994; Rossdale et al., 1982). The WBC count decreased with age in puppies and calves (Meeks et al., 1994; Binkhorst, 1982). Studies done in prenatal, neonatal, and adult laboratory animals (including rats, rabbits, pigs, sheep, cats, dogs, and monkeys) and people have shown that, in general, the CSF/plasma concentration ratios (RCSF) of Na+, Mg2+, and Cl– increase with age. The RCSF of K+, HCO3–, and urea decrease. In some instances, however (e.g., Cl– and K+), changing plasma levels of these substances contribute to the change in the RCSF. The RCSF of total protein, as well as those of the individual proteins, decreases with age. The decreasing concentration of proteins in the CSF compared to plasma protein is an indication of the maturation of the blood–brain and blood–CSF barriers. In rats, the RCSF of amino acids also falls quickly with age, although large individual variations exist. Taurine, for example, has a higher level in the adult than the newborn. This fact, as well as the species-specific transport of some proteins (e.g., albumin) into the CSF indicates a special mechanism of transport based on factors other than molecular weight (Davson and Segal, 1996).

B. Proteins in the CSF

Proteins identified in the CSF are given in Tables 27.9 and 27.10. In general, the concentration of a CSF protein is inversely related to its molecular weight. If the blood–brain barrier is normal, serum proteins with a molecular weight greater than 160,000 Da are largely excluded. However, Felgenhauer (1974) reported CSF:serum protein distribution ratios to be better correlated with the hydrodynamic radii than with the molecular weight of the proteins. Almost all the proteins normally present in CSF are derived from the serum. The exceptions are transthyretin (prealbumin,
| Constituent          | Tipold et al. (1994) | Sorjonen et al. (1987) | Bailey and Higgins (1985) | Bichsel et al. (1984b) | Sorjonen et al. (1981) | Krakowka et al. (1981) | Coles (1980) | Bleich et al. (1964) | Fankhauser (1962) |
|----------------------|----------------------|------------------------|---------------------------|------------------------|------------------------|------------------------|--------------|---------------------|------------------|
| Methods              |                      |                        |                           |                        |                        |                        |              |                     |                   |
| #RBC/μl              | NS                   | ≤10                    | <1500                     | 0                      | ≤10                    | 0                      | NS           | NS                  | NS               |
| Necropsy             | NS                   | Yes                    | Yes                       | NS                     | Yes                    | NS                     | NS           | NS                  | NS               |
| Total protein (mg/dl)|                      |                        |                           |                        |                        |                        |              |                     |                   |
|                      |                      |                        |                           |                        |                        |                        |              |                     |                   |
|                      |                      | 29.9 ± 1.57            | 27.6 ± 1.1SE              |                        |                        |                        |              |                     |                   |
|                      |                      | (23–38.5)              | (15.5–42)                 |                        |                        |                        |              |                     |                   |
| Lumbar Method        |                      |                        |                           |                        |                        |                        |              |                     |                   |
|                      |                      |                        |                           |                        |                        |                        |              |                     |                   |
|                      |                      | 27.5 ± 1.06            | 27 ± 1.06                 |                        |                        |                        |              |                     |                   |
|                      |                      | (11–55)                | (23–35)                   |                        |                        |                        |              |                     |                   |
| Albumin (mg/dl)      |                      | 37 ± 4.29%             | 17.1 ± 6.7                | 12.43 ± 0.96*          | 10.28 ± 0.8SE          | 27                      |              |                     |                   |
|                      |                      | (31–44%)               | (7.5–27.6)                | (10.5–17.4)            | (5.8–18.9)             | (16.5–37.5)             |              |                     |                   |
| Albumin quotient     |                      | 0.22 ± 0.05            |                           |                        |                        |                        |              |                     |                   |
|                      |                      | (0.17–0.3)             |                           |                        |                        |                        |              |                     |                   |
| Globulin (mg/dl)     |                      |                        |                           | 17.45 ± 0.83           |                        |                        |              |                     |                   |
|                      |                      |                        |                           | (14.0–21.1)            |                        |                        |              |                     |                   |
| IgG (mg/dl)          |                      | 0.85 ± 0.14            | 4.68 ± 0.68               | 1.16 ± 0.1SE           |                        |                        |              |                     |                   |
|                      |                      | (0.71–1.09)            | (2.5–8.5)                 |                        |                        |                        |              |                     |                   |
| IgG index            | 0.7 ± 0.3            |                        | 0.38 ± 0.24               |                        |                        |                        |              |                     |                   |
|                      | (0.2–1.3)            |                        | (0.15–0.9)                |                        |                        |                        |              |                     |                   |
| IgM (μg/ml)          | 1.7                  |                        |                            | 0                      | 0                      |                        |              |                     |                   |
|                      | (0–5.8)              |                        |                            |                        |                        |                        |              |                     |                   |
| IgA (μg/ml)          | 0.08 (0–0.2)         |                        |                            | 0                      | 0                      |                        |              |                     |                   |
| Test                      | Value                        | Reference Range       |
|---------------------------|------------------------------|-----------------------|
| Alanine transferase       | 13.7 ± 1.35SE                | (2–32)                |
| (Reitman–Frankel units)   |                              |                       |
| Aspartate transferase     | 20.1 ± 1.64SE                | (9–46)                |
| (Reitman–Frankel units)   |                              |                       |
| Creatine kinase (SU)      | <1                           |                       |
| Bicarbonate (meq/liter)   | 23.5 ± 0.19SE                | 6.56 (5.13–7.40)      |
| Calcium (mg/dl)           |                              |                       |
| Chloride (meq/liter)      | 130 ± 0.5SE                  | 808 (761–883) mg/dl   |
|                           |                              | 667 (602–783) mg/dl   |
| Magnesium (mg/dl)         | 3.09                         | (2.58–3.81)           |
| Phosphorus (mg/dl)        | 3.09                         | (2.58–3.81)           |
| Potassium (meq/liter)     | 3.3 ± 0.04SE                 | 674 (61–116)          |
| Sodium (meq/liter)        | 153 ± 0.5SE                  |                       |
| Glucose (mg/dl)           | 74                           |                       |
| pH                        | 10–11                        |                       |
| Urea (mg/dl)              | 1.005                        | (1.003–1.012)         |
| Specific gravity          |                              |                       |

* Mean ± 1 SD, observed range in parentheses, unless otherwise noted. Values are for cerebellomedullary fluid unless otherwise noted. NS, not stated.

* By electrophoresis.

* By radial immunodiffusion.


| Methods | Rand et al. (1990a) | Hochwald et al. (1969) | Ames (1964) | Fankhauser (1962) |
|---------|--------------------|------------------------|-------------|-------------------|
| # RBC/μl | <30 | NS | NS | NS |
| Necropsy | Yes | No | | |
| Total protein (mg/dl) | | | | |
| Cerebellomedullary | 18 ± 7<sup>a</sup> | 27.0 ± 8.8 | <20 | |
| Reference range | 6–36 | | | |
| Lumbar | | 44.0 ± 1.7 | | |
| Method | Ponceau S | Biuret | NS | |
| Albumin (mg/dl) | | | | |
| Cerebellomedullary | 6.5 ± 2.1 | | | |
| Lumbar | 10.1 ± 2.9 | | | |
| Gamma globulin (mg/dl ± SD) | | | | |
| Cerebellomedullary | | | | |
| Lumbar | 1.2 ± 0.27 | | | |
| IgG (mg/dl) | 1.4 ± 1.7 | | | |
| Reference range | 0–5.3 | | | |
| IgG–total protein index | 0.321 ± 0.210 | (0.086–1.297) | | |
| Aspartate transferase (U/liter) | 17 ± 7 | | | |
| Reference range | 0–34 | | | |
| Creatine kinase (U/liter) | 47 ± 51<sup>b</sup> | | | |
| Reference range | 2–236 | | | |
| Lactate dehydrogenase (U/liter) | 12 ± 5<sup>b</sup> | | | |
| Reference range | 0–24 | | | |
| Calcium (meq/kg H₂O ± SE) | | 1.50 ± 0.06 | 5.2 mg/dl | |
| Chloride (meq/kg H₂O ± SE) | | 144 ± 2 | 900 mg/dl | |
| Magnesium (meq/kg H₂O ± SE) | | 1.33 ± 0.02 | | |
| Potassium (meq/kg H₂O ± SE) | | 2.69 ± 0.09 | | |
| Sodium (meq/kg H₂O ± SE) | | 158 ± 4 | | |
| Glucose (mg/dl) | 4.1 ± 1.3 mmol/l | | 85 | |
| Reference range | 1.0–7.2 mmol/l | | | |
| pH | | | Slightly alkaline | |

<sup>a</sup> Mean ± 1 SD, observed range in parentheses, unless otherwise noted. Values are for cerebellomedullary fluid unless otherwise noted.

<sup>b</sup> Not stated.

and transferrin, which are also synthesized by the choroidal plexus, and beta and gamma trace proteins, tau protein (tau fraction, modified transferrin), glial fibrillary acidic protein, and myelin basic protein, which appear to be synthesized intrathecally (Thompson, 1988).

1. Albumin

With electrophoretic techniques, protein in the CSF can be separated into prealbumin, albumin, and alpha, beta, and gamma globulins. The major protein in CSF is albumin, which is synthesized only in the liver. The limited entry of albumin into the CSF is dependent upon the blood–brain/CSF barrier to macromolecules.

When total CSF protein is increased, the albumin concentration is increased disproportionately. This phenomenon illustrates the role of molecular size in determining the distribution of serum proteins into the CSF (Felgenhauer, 1974).

2. Alpha and Beta Globulins

Immunoelectrophoresis can separate the alpha and beta globulins into several proteins (Table 27.9). The origin of tau protein (beta<sub>2</sub> transferrin) is uncertain. This protein may be modified serum transferrin (beta<sub>2</sub> transferrin) or it may be a unique protein, “tau protein,” in the CSF (Fishman, 1992). In veterinary and human medicine, no correlation has been made between the levels of these proteins and other diseases.
3. Gamma Globulins

Because of the changes found in association with multiple sclerosis and other inflammatory diseases, the gamma globulins have received a great deal of attention. Electrophoretic techniques define the gamma globulins as a heterogeneous group of proteins with migrations at similar rates (see Table 27.9). The gamma globulin fraction contains immunoglobulins. Immunologic assays identify three major immunoglobulins in normal CSF—\( \text{IgG} \), \( \text{IgA} \), and \( \text{IgM} \). Minute amounts of other immunoglobulins have also been detected in normal CSF (Fishman, 1992; Kjeldsberg and Knight, 1993).

a. \( \text{IgG} \)

The major immunoglobulin in normal CSF is \( \text{IgG} \), which normally originates from the serum. An increased level of CSF gamma globulin has been reported in a number of inflammatory central nervous system disorders. In disease conditions, gamma globulin may enter the CSF through dysfunctional blood–brain/CSF barriers, or may be synthesized intrathecally by cells that have migrated into the brain or CSF and are participating in the disease process (Fishman, 1992; Kjeldsberg and Knight, 1993).

b. \( \text{IgM} \) and \( \text{IgA} \)

Cerebrospinal fluid \( \text{IgM} \) and \( \text{IgA} \) also originate normally from the serum. However, in certain diseases, particularly inflammatory diseases, these immunoglobulins are produced within the central nervous system as well (Fishman, 1992; Kjeldsberg and Knight, 1993). \( \text{IgM} \) is ontogenetically and phylogenetically the most primitive immunoglobulin and is therefore detected at an earlier stage of the general immune response of the body. \( \text{IgM} \) is also the first immunoglobulin to return to normal when the offending antigen disappears. The characteristics of \( \text{IgM} \) and \( \text{IgA} \) participation in the intrathecal immune response still need to be resolved, however (Tipold et al., 1994; Felgenhauer, 1982).

4. Other Proteins

Many other proteins have been identified in CSF, including myelin basic protein, S-100 protein, C-reactive protein, interferon, embryonic proteins, fibronectin, and glial fibrillary acidic protein. In general, the CSF concentrations of these proteins may be altered by a number of neurologic disease processes. The utility of assay of these proteins in clinical veterinary or human medicine has yet to be established (Fishman, 1992; Kjeldsberg and Knight, 1993).

C. Glucose

The CSF glucose is derived solely from the plasma by facilitated diffusion. The concentration of CSF glucose depends upon the blood glucose concentration, the rate of glucose transport into the CSF, and the metabolic rate of the central nervous system. The normal CSF glucose level is about 60–80% of the blood glucose concentration, reflecting at least in part the high metabolic rate of the central nervous system. Equilibration with plasma glucose requires about 1 to 2 hours; thus, ideally, plasma glucose should be determined about 1 hour prior to CSF aspiration and analysis. In people, a glucose gradient exists along the neuraxis; the concentration decreases from ventricular to lumbar fluid (Fishman, 1992; Kjeldsberg and Knight, 1993).

D. Enzymes

Numerous enzymes have been assayed in the CSF of animals (see Tables 27.5 through 27.8) (Rand et al., 1990a; Wilson, 1977; Jackson et al., 1996) and people (Banik and Hogan, 1983). These enzymes have three possible sources: (1) blood, (2) neural tissue or neural tumors, or (3) cells within the CSF (Fishman, 1992; Kjeldsberg and Knight, 1993). In probably every instance, the blood enzyme levels are higher than the CSF levels. Unfortunately, many studies of CSF levels in disease fail to report the concurrent blood level and a measure of blood–brain/CSF barrier integrity. However, studies of CSF creatine kinase (CK) in dogs and horses did not find a relationship between WBC counts, serum CK, or CSF total protein and CSF CK (Furr and Tyler, 1990; Jackson et al., 1996). Regarding correlation of CSF RBC with CSF CK, one study reported a significant correlation (Indrieri et al., 1980), whereas another study did not find a statistical association between the two parameters (Jackson et al., 1996). To date, none of the enzyme assays has been shown to be sufficiently sensitive or specific to warrant its routine use in clinical practice (Rand et al., 1994b; Fishman, 1992; Kjeldsberg and Knight, 1993; Jackson et al., 1996; Indrieri et al., 1980).

E. Neurotransmitters

Because they are produced by neurons, neurotransmitters and their metabolites have been extensively studied in people for their potential use as markers
| Constituent                  | Rossdale et al. (1982) | Andrews et al. (1994) | Andrews et al. (1990a) | Andrews et al. (1990b) | Rossdale et al. (1982) | Mayhew et al. (1977)$^b$ | Fankhauser (1962) |
|-----------------------------|------------------------|-----------------------|------------------------|------------------------|------------------------|--------------------------|---------------------|
| Age                         | <40 hr$^c$             | ≤10 days              | 4–9 years              | NS                     | Adult                  | 0.75–15 years            | Adult?              |
| Methods                     |                        |                       |                        |                        |                        |                          |                     |
| # RBC/μl                    | NS                     | <2000                 | <600                   | NS                     | NS                     | 195.15 ± 511.96          | NS                  |
| Necropy                     | NS                     | No                    | 2 of 12                | NS                     | NS                     | No                       | NS                  |
| Total protein (mg/dl)       |                        |                       |                        |                        |                        |                          |                     |
| Cerebellomedullary          | 138 ± 50 (70–210)      | 82.8 ± 19.2 (56.7–115) | 87.0 ± 17.0 (59–118)   | 105 ± 38 (40–170)      | 37.23 ± 28.4$^d$        | 47.58                   |                     |
| Lumbosacral                 | 83.6 ± 16.1 (60.5–116) | 93 ± 16               | (65–124)               | 37.23 ± 28.4$^d$       | 5–100$^e$              | (28.75–71.75)           |                     |
| Method                      | Biuret                 | Coomassie             | Coomassie              | Biuret                 | TCA                    | NS                       |                     |
| Albumin (mg/dl ± SD)        |                        |                       |                        |                        |                        |                          |                     |
| Cerebellomedullary          | 52.0 ± 8.6 (34–64)     | 35.8 ± 9.7 (24–51)    |                        |                        |                        |                          | 38.64               |
| Lumbosacral                 | 53.8 ± 15.7 (30–92)    | 37.8 ± 11.2 (24–56)   |                        |                        |                        |                          | (22.62–67.94)       |
| Albumin quotient (±SD)      |                        |                       |                        |                        |                        |                          |                     |
| Cerebellomedullary          | 1.86 ± 0.29 (1.55–2.33)| 1.4 ± 0.4 (1–2.1)     |                        |                        |                        |                          |                     |
| Lumbosacral                 | 1.85 ± 0.51 (1.07 ± 2.88)| 1.5 ± 0.4 (1–2.4)    |                        |                        |                        |                          |                     |
| Globulin (mg/dl)            |                        |                       |                        |                        |                        |                          | 9.34                |
| IgG (mg/dl ± SD)            |                        |                       |                        |                        |                        |                          | (3.37–18.37)        |
| Cerebellomedullary          | 10.2 ± 5.5 (3–22)      | 5.6 ± 1.4 (3–8)       |                        |                        |                        |                          |                     |
| Lumbosacral                 | 9.9 ± 5.7 (3–22.5)     | 6.0 ± 2.1 (3–10)      |                        |                        |                        |                          |                     |
| IgG index (±SD)             |                        |                       |                        |                        |                        |                          |                     |
| Cerebellomedullary          | 0.519 ± 0.284 (0.0095–0.942) | 0.19 ± 0.046 (0.12–0.27) |                        |                        |                        |                          |                     |
| Lumbosacral                 | 0.482 ± 0.27 (0.091–2.089) | 0.194 ± 0.05 (0.12–0.26) |                        |                        |                        |                          |                     |
| Alkaline phosphatase (IU)   |                        |                       |                        |                        |                        |                          | 0.83 ± 0.95          |
| Aspartate transferase (IU)  |                        |                       |                        |                        |                        |                          | 0–8$^f$             |
| Cerebellomedullary          | 16.6 ± 7.6 (6–26)      | 4–16$^f$              | 18.27 ± 10.8 (7.5–30)  |                        |                        |                          | 30.74 ± 6.31 SFU     |
| Lumbosacral                 |                        | 0–16$^f$              |                        |                        |                        |                          | 15–50$^e$           |
| Test                                | Normal Range        | Reference Range          |
|-------------------------------------|---------------------|--------------------------|
| Creatine kinase (IU)                |                     |                          |
| Cerebellomedullary                  | 15.2 ± 9.2          | 5.78 ± 3.7               |
| Lumbosacral                         | 0–8'                | 0–8'                     |
| (4–33)                              | (3.2–11)            |                          |
| γ-Glutamyl transferase (IU)         | 1.5 ± 1.5           | 2.45 ± 1.9               |
| (0.9–2.3)                           | (0.8–4.2)           |                          |
| Lactate dehydrogenase (IU)         | 23.2 ± 10.7         | 27.7 ± 8.0               |
| (10–40)                             | (12–34)             | 1.54 ± 1.75              |
| Calcium (mg/dl)                     |                     |                          |
|                                     | 4.18 ± 0.87         | 6.26                     |
|                                     | 2.5–6.0'            | (5.55–6.98)              |
| Chloride (meq/liter)                | 109 ± 3.4           | 103.3 ± 13.5             |
| (104–113)                           | (92–116)            | 109.22 ± 6.90            |
| Magnesium (mg/dl)                   |                     |                          |
|                                     | 1.98                |                          |
| (1.06–2.95)                         |                     |                          |
| Phosphorus (mg/dl)                  |                     |                          |
|                                     | 0.83–0.20           | 1.44                     |
|                                     | 0.5–1.5'            | (0.87–2.20)              |
| Potassium (meq/liter)               | 3.6 ± 2.1           | 2.9 ± 0.6                |
| (1.3 ± 4.6)                         | (1.9 ± 3.9)         | 2.95 ± 0.05              |
| Sodium (meq/liter)                  | 142.6 ± 2.8         | 143.9 ± 2.6              |
| (139–147)                           | (139–147)           | 144.58 ± 1.86            |
| Cholesterol (mg/dl)                 |                     |                          |
|                                     | 4.76 ± 5.72         | 0.36–0.55                |
| Glucose (mg/dl)                     |                     |                          |
| Cerebellomedullary                  | 35–70% of blood glucose<sup>a</sup> | 48.0 ± 9.92 |
| Lumbosacral                         | 35–70% of blood glucose<sup>a</sup> | 30–70' |
| Lactic acid (mg/dl)                 |                     |                          |
| Cerebellomedullary                  | 1.92 ± 0.12         | 55.13 ± 8.22             |
| Lumbosacral                         | 2.3 ± 0.21          | 40–75'                   |
| pH                                  |                     |                          |
| Specific gravity                    | 7.13–7.36           |                          |
| Urea nitrogen (mg/dl)               | 1.003–1.005<sup>c</sup> | 1.004–1.008 | 11.82 ± 3.26 |
|                                     |                      | 0–20'                    |

<sup>a</sup> Mean ± 1 SD, observed range in parentheses, unless otherwise noted. Values are for cerebellomedullary fluid unless otherwise noted. NS, not stated; LS, lumbar subarachnoid space CSF; CM, cerebellomedullary cistern CSF.

<sup>b</sup> Except where noted, values are for pooled cerebellomedullary and lumbosacral fluid.

<sup>c</sup> Spontaneously delivered.

<sup>d</sup> Total protein for ponies—60.48 ± 20.45, reference range 20–105 (significantly different from that for horses).

<sup>e</sup> Reference range.
TABLE 27.8 Biochemical Constituents of CSF of the Cow, Sheep, Goat, Pig, and Llama

| Constituent                  | Cow       | Sheep     | Goat       | Pig        | Llama     |
|-----------------------------|-----------|-----------|------------|------------|-----------|
|                             | (Welles et al., 1992) | (Altman and Dittmer, 1974) | (Altman and Dittmer, 1974) | (Altman and Dittmer, 1974) | (Welles et al., 1994) |
| **Methods**                 |           |           |            |            |           |
| # RBC/μl                    | (5–1930)  | NS        | NS         | NS         | (0–1360)  |
| Necropsy                    | No        | NS        | NS         | NS         | No        |
| Total protein (mg/dl)       |           |           |            |            |           |
| Cerebellomedullary          |           |           |            |            |           |
| Lumbosacral                 |           |           |            |            |           |
|                             | 39.16 ± 3.39 | (8–70)    | 12         | (24–29)    | 43.1 ± 9.0 | (31.2–66.8) |
| Method                      |           |           |            |            |           |
| Coomassie brilliant blue    |           |           |            |            |           |
| Albumin (mg/dl)             |           |           |            |            |           |
| Cerebellomedullary          |           |           |            |            |           |
| Lumbosacral                 |           |           |            |            |           |
|                             | 15.75 ± 1.53% | (8.21–28.71) | (17–24)    | 17.9 ± 4.45 | (11.8–27.1) |
| Albumin quotient            |           |           |            |            |           |
| Globulin (mg/dl)            |           |           |            |            |           |
|                             | 6.4 ± 2.50 | (3.4–13.8) |           |            |           |
| Gamma globulin (mg/dl)      |           |           |            |            |           |
|                             | 4.94 ± 0.44% | (2.46–8.85) |           |            |           |
| IgG (mg/dl)                 |           |           |            |            |           |
|                             | 4.6 ± 4.69 | (0.0–15.0) |           |            |           |
|                             | 13 ± 5.6  | (7–24)    |           |            |           |
| Creatine kinase (U/liter)   |           |           |            |            |           |
|                             | 11.44 ± 3.43 | (2–48)    |           |            |           |
| Lactate dehydrogenase (U/liter) | 13.94 ± 1.318 | (2–25) |           |            |           |
| Calcium (mg/dl)             | 5.6 ± 0.3 | (2–25)    |           |            |           |
| Chloride (meq/liter)        | 832 mg/dl | (750–868) | 681 mg/dl |            |           |
| Magnesium (mg/dl)           | 1.99 ± 0.03 meq/liter | (1.8–2.1) | 2.88       |            |           |
| Potassium (meq/liter)       | 2.96 ± 0.03 | (2.7–3.2) | 3.19 ± 0.10 | (2.9–3.3) |            |
| Sodium (meq/liter)          | 140 ± 0.78 | (132–142) | 154 ± 5.8  | (134–160)  |            |
| Glucose (mg/dl)             | 42.88 ± 0.99 | (37–51) | 71         | (45–87)    | 69.3 ± 7.35 | (59–86) |
| pH                          | 7.35      | (7.3–7.4) |           |            |           |

ᵃ Mean ± 1 SD, observed range in parentheses, unless otherwise noted. NS, not stated.
ᵇ Lumbosacral fluid. Mean ± SEM.
ᶜ Cerebellomedullary fluid.
ᵈ Lumbosacral fluid.

of neuronal activity and neurologic and psychiatric disease (Davis, 1990). The concentrations of several neurotransmitters (e.g., γ-aminobutyric acid, dopamine) and their metabolites (e.g., 5-hydroxyindolacetic acid, homovanillic acid, and dihydroxyphenylacetic acid) have been measured in the CSF from various sites in dogs, sheep, goats, cattle, and horses (Vaughn et al., 1989b, 1988a; Sisk et al., 1990; Loscher and Schwartz: Porsche, 1986; Ruckebusch and Costes, 1988; Bardo and Ruckebusch, 1984; Ruckebusch and Sutra, 198; Faull et al., 1982). Some metabolite concentrations have a gradient along the neuraxis (Vaughn and Smyt...
TABLE 27.9  Cerebrospinal Fluid Proteins Identified by Electrophoresis (Top Row) and Immunoelectrophoresis (Underlying Columns) a

| Protein Fraction | Dog (Sorjonen, 1987) | Cat (Rand et al., 1990) | Horse (Kristensen and Firth, 1977) |
|------------------|----------------------|------------------------|-----------------------------------|
| Prealbumin       | 37 ± 4.29            | 11 ± 15                | 2.0 ± 0.9                         |
|                  | (31–44)              | (1–53)                 |                                   |
| Albumin          | 28 ± 5.27            | 21 ± 11                | 43.4 ± 6.8                        |
|                  | (24–31)              | (0–48)                 |                                   |
| Alpha alpha      | 25 ± 5.31            | 57 ± 15                | 5.3 ± 1.3                         |
|                  | (19–30)              | (37–91)                |                                   |
| Alpha beta       |                      |                       | 3.3 ± 0.8                         |
| Alpha g            |                      |                       | 6.4 ± 1.8                         |
| Beta globulin     | 7.75 ± 1.84          | 12 ± 7                 | 17.0 ± 3.2                        |
|                  | (6–9)                | (0–29)                 | 7.8 ± 2.3                         |

a Mean ± SD percentage of total CSF protein; range in parentheses.
CSF for dogs, cats, and horses are given in Tables 27.5, 27.6, and 27.7, respectively.) The concentration of the albumin and globulin fractions also increases from ventricular to lumbar fluid. The increased protein content may be the result of a greater permeability of the spinal blood–CSF barrier than the ventricular barrier to albumin (Fishman, 1992), additions of protein from adjacent nervous tissue (e.g., IgG from lymphocytes located in or near the CSF pathway (Weisner and Bernhardt, 1978)), progressive equilibration of CSF with plasma through the capillary walls (Weisner and Bernhardt, 1978), and low flow rates of lumbar CSF (Davson and Segal, 1996).

A study of healthy dogs also identified a small but significant gradient for the CSF WBCs; lumbar fluid contained significantly fewer cells than cerebellomedullary fluid (Bailey and Higgins, 1985). Another study did not find a difference in WBC counts between fluids from the two sites (Vaughn et al., 1988b). However, 4 of the 10 dogs in this study had CSF total WBC counts >3/μl, and none of the dogs were necropsied to verify their health. Therefore, some of these dogs may have had subclinical neurologic disease, disguising a small cellular gradient. The small number of WBCs in normal fluid may make a cellular gradient more of a theoretical issue than a practical issue, however. If a cellular gradient exists, it may be due to fewer cells entering the lumbar CSF than the cerebellomedullary CSF, a greater rate of cell lysis in the lumbar CSF, a greater migration rate of WBCs from lumbar CSF back into the blood, or loss of WBCs that entered the CSF rostrally and lysed as CSF circulated to the caudal subarachnoid space.

A gradient has also been reported for CSF neurotransmitter metabolites in the dog (Vaughn et al., 1988b) and the horse (Vaughn and Smyth, 1989). In each species, the neurotransmitter metabolite content of cerebellomedullary CSF was greater than that of lumbar subarachnoid CSF. This gradient probably reflects the major source of the neurotransmitter (brain) and transport of the metabolite from the CSF into the blood along the spinal axis (Vaughn et al., 1988b) and will not be covered here except for the authors’ preferred technique of collection from the cerebellomedullary cistern of dogs and cats (see later discussion). Considerations that apply regardless of species are sterility, use of a spinal needle, and collection from animals with increased intracranial pressure. In order to prevent iatrogenic central nervous system infection sterility during the collection procedure is vital. A generous area around the puncture site should be clipped and surgically prepared. Preparation of too small an area can lead to contamination if any difficulty in palpating landmarks or entering the subarachnoid space is encountered. Additionally, the use of a fenestrated drape is highly recommended. Spinal puncture is contraindicated in an area of severe pyodermia/furunculosis, cellulitis, or epidural abscess. A needle with a stylet (spinal needle) should be used to prevent implantation of a plug of epidermis in the subarachnoid space which not only could lead to infection but could also seed an epidermoid tumor. Replacement of the stylet upon withdrawal is controversial, either preventing causing entrapment and severance or dislocation of nerve root filaments (Fishman, 1992). Aspiration of CSF from animals with increased intracranial pressure may result in brain herniation. Appropriate anesthetic agents, hyperventilation, and mannitol may decrease the probability of herniation. Use of the smallest gauge needle possible may also help prevent herniation by decreasing CSF leakage through the puncture hole in the meninges. Only the minimal amount of CSF necessary to perform the desired tests should be withdrawn. Brain herniation can occur following lumbar taps as well as cerebellomedullary cistern taps.

2. Collection Site

The choice of collection site is influenced by the species and breed of animal, the location of the neurologic lesion, and anesthetic considerations. The site of some animals may make lumbar subarachnoid puncture difficult if not impossible. However, cerebellomedullary puncture usually can be accomplished even in very large or obese animals. Because of differences in anatomy, the type or breed influences the exact site for lumbar puncture in the dog; L3–4 or L4–5 is recommended for large-breed, nonchondrodystrophic dogs (e.g., German shepherd dogs), whereas L4–5 or L5–6 is recommended for small, chondrodystrophic dogs (e.g., dachshunds) (Morgan et al., 1987). The puncture site chosen should be as close to the lesion as possible without penetrating the lesion, or the site should be caudal to the lesion. In animals with spinal disease, cerebellomedullary fluid is abnormal more frequently with cervical disease than it is with thorac...
lumbar disease, but overall lumbar fluid is abnormal more often than cerebellomedullary fluid. With intracranial disease, CSF from both sites is usually abnormal, perhaps because both sites are caudal to the lesion (Thomson et al., 1990; Scott, 1992; Thomson et al., 1989). Occasionally, CSF is collected from both sites. Although the order of collection, cerebellomedullary or lumbar CSF collected first, appears not to influence the analytical results significantly (Bailey and Higgins, 1985), aspiration from the relatively small lumbar subarachnoid space is easier if the CSF pressure has not just been lowered by cerebellomedullary CSF collection. Cerebellomedullary puncture should be done under general anesthesia. In most instances, lumbar puncture can be done with sedation and local anesthesia. Therefore, if general anesthesia is contraindicated, a lumbar puncture should be done.

3. CSF Collection from the Cerebellomedullary Cistern

The authors' preferred technique for CSF collection from the cerebellomedullary cistern is to utilize the palpable bony landmarks that are the closest to the puncture site. These structures are the vertebral arch of C1 and the external occipital protuberance. After anesthetic induction and intubation, the animal is placed in lateral recumbancy and padding is placed under the neck to align the dorsal cervical and cranial midline parallel to the tabletop. The assistant is instructed to "tuck in the animal's chin" and push the external occipital protuberance toward the operator. This procedure flexes the atlanto-occipital joint and maximizes the space between the occipital bone and C1. Asking the assistant to simply flex the neck seems to produce flexion of the midcervical area more than the atlanto-occipital area. The external occipital protuberance, the C2 spinous process, and the C1 vertebral arch are palpated. The last structure is located by rolling a fingertip off the cranial edge of the C2 spinous process and palpating firmly, feeling for a transverse bony ridge (the C1 vertebral arch). The C1 vertebral arch can usually be palpated, and if so, the puncture is made on the midline just in front of the fingertip palpating the vertebral arch. If C1 is not palpable, the distance between the cranial edge of the C2 spinous process and the occipital protuberance is noted, and the puncture is made on the midline about one-third of that distance cranial to the cranial edge of the C2 spinous process. In rare cases, neither C1 nor C2 can be palpated. In this situation, the lateral edge of each C1 transverse process is palpated and a triangle from each edge to the occipital protuberance is constructed visually. The puncture is made on the midline in the center of that triangle.

B. Physical Examination: Clarity, Color, and Viscosity

After collection, the CSF is examined visually and the clarity, color, and viscosity are recorded. Normal CSF is clear and colorless and has essentially the same viscosity as water. For accurate assessment, the CSF can be compared to the same amount of distilled water in the same type of container. The containers can be held against a white, typewritten page to judge color and clarity, and gently shaken to assess viscosity. If the CSF appears abnormal, the color and clarity of the supernatant after centrifugation should be noted.

C. Cytological Analysis

1. General Techniques

Collection of CSF in a plastic or silicon-coated glass tube is preferred because monocytes will adhere to glass and can activate in the process (Fishman, 1992). This can result in erroneous cell counts and also alter morphology. In practical terms, this is of little consequence in those specimens that are rapidly processed but becomes more significant as the delay between collection and processing increases. A complete cytologic examination includes both a total cell count and a differential, as well as thorough morphologic assessment. A differential and thorough morphologic assessment should be performed routinely, even on those samples that have cell counts within normal limits. In our experience, very low cell counts alone cannot be used as an indicator of normality. In one study that utilized cytocentrifugation, nearly 25% of canine CSF samples with cell counts in the normal range had abnormalities in cell type or morphology (Christophe et al., 1988). Abnormalities included the presence of phagocytic macrophages, increased percentage of neutrophils in the differential, and the presence of reactive lymphocytes and plasma cells. Malignant cells have been observed in samples with normal counts (Bichse et al., 1984b; Grevel and Machus, 1990). This certainly emphasizes the importance of cytologic assessment, especially in specimens with normal cell counts. In general terms, CSF samples should be processed as soon as possible after collection, as cells degenerate relatively quickly in CSF (Chrisman, 1992; Fishman 1992; Steele et al., 1986; Kjeldsberg and Knight, 1993). This is because of the marginal hypotonicity of the fluid and very low protein content (in nonpathological specimens). Proteins and lipids tend to have
membrane-stabilizing effect (Steele et al., 1986). A multitude of veterinary references state that processing must be performed within 30 minutes of collection (Thomson et al., 1990; Oliver and Lorenz, 1993; Cook and DeNicola, 1988; Chrisman, 1983) and this necessitates analysis in the individual veterinary hospital or practice setting. However, there is a complete lack of controlled veterinary studies to confirm this assertion, although, as stated previously, the sooner the better, in general terms. There have been several human studies performed. Steele found that there were significant differences in rates of lysis that were dependent on cell type. At room temperature, neutrophil counts decreased most rapidly and were significantly reduced at 1 hour and 2 hours, 68 ± 10% (SEM) and 50 ± 12% of original values, respectively. Values were not significantly reduced at half an hour. Lymphocyte and monocyte numbers were not significantly altered until 3 hours (69 ± 7% and 66 ± 7%, respectively). However, refrigeration at 4°C markedly reduces the rate of lysis, with only 15% decrease in the total white blood cell numbers at 2 hours (Kjeldsberg and Knight, 1993). Another study (Stokes et al., 1975) reported the following rates of disappearance for white blood cells at room temperature: 4 hours—10% lysis, 18 hours—70% lysis, 36 hours—100%. Because there is differential sensitivity of cell types, both cell counts and the differential are altered with time. The authors have observed this phenomenon. When differential counts are compared between slides made immediately and those made via cytocentrifugation 3–6 hours later, there is a marked decrease in the percentage of neutrophils (personal observation). This would tend to confirm the preceding data. Therefore, the recommendation that analysis be performed within 30 minutes is reasonable, but it is predicated on the conditions that the sample is exposed to. Refrigeration obviously slows lysis, likely long enough for transport to reference laboratories in some instances. Addition of protein to the sample helps preserve cells and also expedites transport of samples to more remote facilities. There was excellent correlation between the total numbers of cells on the slides and the differential between sediment slides processed immediately and those preserved with fetal bovine serum (200 µl of CSF and 200 µl of fetal bovine serum) and cytocentrifuged 2–4 hours later (Rand et al., 1990b). We have observed excellent morphology on slides made from CSF as long as 12 hours postcollection, when the sample was immediately mixed with approximately 20% by volume of autologous, well-centrifuged serum and transported on an ice pack. However, the effects on cell count and differential are unknown under these conditions. This is perhaps an option for practitioners in remote locations without the capability or expertise to process CSF. The duration of cell preservation under different conditions requires further study.

2. Total Leukocyte and Erythrocyte Counts

Electronic cell or particle counters are not sensitiv enough to be used for enumeration of cells in CSF. The level of background counts with these counters is frequently in excess of the counts present in the majority of CSF samples that are analyzed. Therefore, counts are performed with the use of a standard hemacytometer chamber with a Neubauer ruling (Cook and DeNicola, 1988; Jamison and Lumsden, 1988; Brobst and Bryan, 1989). The chamber is charged with undilute fluid. Ideally, the cells should be allowed to settle for 10 minutes in a humidified environment. This allows all the cells to be visible in the same plane of focus. The cells in the 9 largest squares on both sides of the chamber are counted (18 squares in total) and the result multiplied by 0.55 to obtain the number of cells per microliter. Numerous references recommend counting the cells in 9 large squares and multiplying by 1.1 to determine the count per microliter. To the untrained observer, unstained leukocytes and erythrocytes may be difficult to differentiate. Leukocytes are larger and the presence of nuclei gives them a more granular appearance than erythrocytes. With experience, nuclear morphology can often be appreciated (Cook and DeNicola, 1988). The cytoplasmic border is usually slightly irregular. In contrast, erythrocytes are usually smaller, smooth, and refractile, although they may become crenated upon standing (Jamison and Lumsden, 1988). A number of techniques have been developed to lyse erythrocytes and stain leukocytes in an effort to expedite counting. Technical details can be found in numerous sources (Chrisman, 1992; Cook, 1985; Brobst and Bryan, 1989), but there are several major disadvantages associated with these methods (Jamison and Lumsden, 1988), and we do not recommend them.

3. Cytologic Examination

A wide variety of methods to facilitate cytologic examination of CSF have been reported and compared (Grevel, 1991; Roszel, 1972; Kölml, 1977; Sörnäs, 1996; Barrett and King, 1976; Steinberg and Vandeveld, 1974; Ducos et al., 1979; Hansen et al., 1974; Woodruif, 1973; Jamison and Lumsden, 1988). There is controversy as to which method is optimal, and all have the strengths and weaknesses. Methods include simple centrifugation, sedimentation and variations thereof, membrane filtration, and cytocentrifugation (Jamison and Lumsden, 1988). The reader is referred to the references for specific methodological details. In th
great majority of cases, simple centrifugation produces slides that are completely unsatisfactory for cytologic examination. Membrane filtration techniques have the chief advantage of excellent cellular recovery with yields approaching 90–100% (Barrett and King, 1976). However, the methodology is laborious and time consuming, the morphology relatively poor, many cells are partly hidden in the filter substance, which itself stains variably, and the technique requires specialized, nonroutine staining techniques that the great majority of veterinary cytologists have no experience or expertise in interpreting. For these reasons, they are not recommended. Cytocentrifugation (Hansen et al., 1974; Woodruff, 1973) has become the method of choice in both human (Fishman, 1992; Kjeldsberg and Knight, 1993) and veterinary medicine (Christopher et al., 1993; Jamison and Lumsden, 1988). The technique is rapid and simple and provides slides with good cytologic detail. The technique is enhanced by the addition of protein to the CSF sample prior to centrifugation. This helps preserve cell morphology. Conditions of cytocentrifugation vary from laboratory to laboratory. We personally prefer the method described by Rand (Rand et al., 1990b). The disadvantages of cytocentrifugation include the expense of the instrument and the relatively low cell yield. In one comparative study (Barrett and King, 1976), the following cell yields were determined: Millipore filtration 81 ± 3 % (SEM), Nucleopore filtration 69 ± 3 %, and cytocentrifugation 11 ± 1 %. The Sornas method of centrifugation results in a cell yield, after staining, of approximately 20% (Sörnäs, 1967). The sedimentation technique of Sayk, modified by Kölmel (Grevel, 1991; Grevel and Machus, 1990; Kölmel, 1977) results in a yield of approximately 30% (Kölmel, 1977), although this can be increased to almost 90% if a membrane filter is substituted for direct sedimentation onto a slide. We have some experience with the Kölmel apparatus and technique and have found the cell morphology to be at least as good as that in cytocentrifugation, with an apparently superior cell yield, although this would need to be confirmed with controlled comparative studies. Therefore, most studies would suggest that sedimentation techniques result in greater cell yields than cytocentrifugation, although there is a least one study that found the yield of cytocentrifugation to be marginally higher than that of sedimentation (Ducos et al., 1979). Standard Romanowsky stains are recommended for staining of slides. They provide good cellular detail on air-dried CSF preparations and are familiar to most observers. These stains include the Wright’s and Wright–Giemsa staining methods, as well as a variety of rapid staining methods including Diff-quik and Camco-quik (Jamison and Lumsden, 1988).

4. Immunocytochemistry

There are very few reports in the veterinary literature documenting the use of immunocytochemistry for the assessment of CSF. In people, the value of cytologic diagnosis of CSF can be improved if morphologic studies are appropriately supplemented by immunocytochemistry (Kjeldsberg and Knight, 1993). Immunophenotypic studies of cytocentrifuge slides are useful in the differential diagnosis of leukemia, malignant lymphoma, primary brain tumors, and metastatic tumors (Bigner and Johnston, 1981; Bigner, 1992; Kjeldsberg and Knight, 1993). Panels of monoclonal antibodies are often used, with the greatest limitation being the volume and the cellularity of the specimen that is available for the marker studies. Undifferentiated tumor panels frequently include leukocyte common antigen and cytokeratin antibodies. These can be very helpful in distinguishing single carcinoma cells from lymphocytes or monocytes (Bigner and Johnston, 1981; Bigner, 1992; Kjeldsberg and Knight, 1993). Glial fibrillary acidic protein has proven to be helpful in distinguishing a glial origin, but there are currently no specific markers to distinguish primary brain tumors. Immunocytochemistry can be used also to characterize the lymphocyte subpopulations present in CSF. Of the lymphocytes found in normal human CSF, 75–95% are T cells, with a mean of approximately 85% (Kjeldsberg and Knight, 1993). Within the population of T cells, T helper cells predominate and account for up to 88% of T cells. Alterations of these percentages have been shown to have significant associations with disease in people (Kjeldsberg and Knight, 1993). Similar studies are lacking in domestic animals but would potentially be quite useful. In people, most central nervous system lymphomas are B cell in origin, and immunocytochemistry can be used to document monoclonality (Bigner and Johnston, 1981; Bigner, 1992). This is strong evidence (but not definitive proof) of malignancy. The B-cell origin can also be confirmed. For patients with T-cell lymphomas, marker studies can be more difficult to interpret as T cells predominate in normal and inflammatory CSF (Kjeldsberg and Knight, 1993). If there is systemic involvement, then comparison with the peripheral phenotype is useful to confirm presence in the CSF. Immunocytochemistry has also been utilized to detect infectious agents such as cytomegalovirus in human patients (Kjeldsberg and Knight, 1993).

5. Polymerase Chain Reaction

Polymerase chain reaction (PCR) technology represents a powerful adjunct to routine cytologic assessment and has the potential to revolutionize CSF analysis by dramatically increasing both the sensitivity and
specificity of diagnosis. Because PCR exponentially increases in vitro the number of original DNA copies to a final number dependent on the number of cycles programmed, it is uniquely suited to the low volumes and small cell numbers frequently found in CSF samples. In people, one of the most powerful and useful applications of PCR methodology has been its use to confirm malignancy and detect minimal residual disease in lymphomatous meningitis (Rhodes et al., 1996). This is accomplished via detection of clonal immunoglobulin or T-cell receptor gene rearrangements and the detection of clone-specific rearrangements, respectively. However, the exquisite sensitivity may result in false positive results due either to contamination or to very low initial numbers of cells producing an artifactual clonal band. Other applications include detection of a wide variety of infectious agents, including toxoplasma, borrelia, TB, and herpes simplex (Christen et al., 1995; Lin et al., 1995; Novati et al., 1994; Guffond et al., 1994). In the great majority of these studies, PCR has resulted in a more rapid diagnosis with superior sensitivity and specificity when compared to those of standard culture and serologic diagnostic techniques. In veterinary medicine, PCR has been used to detect several infectious agents in CSF samples, including sarcocystis neurona, toxoplasma, and listeria (Fenger, 1994; Peters et al., 1995; Stiles et al., 1996). The same advantages appear to apply in these instances, although some agents such as listeria may not gain access to the meningoventricular system, resulting in negative results when applied to CSF of confirmed positive cases (Peters et al., 1995). Further developmental work in conjunction with prospective studies will be required before the true utility of PCR-based CSF diagnostics can be accurately assessed in domestic animals.

D. Protein Analysis

1. Measurement of CSF Total Protein

An increase in the concentration of CSF total protein was recognized as an indicator of neurologic disease soon after the introduction of lumbar puncture in human medicine. A number of tests were developed to assess qualitative changes in CSF protein, such as Lange's colloidal gold test, the Nonne-Appelt test, the Pandy test, and others. These qualitative tests largely have been replaced by quantitative methods. Several techniques have been developed for the quantitative measurement of CSF total protein, including turbidimetric methods, biuret procedures, and Lowry's method. The accuracy of these methods in many clinical laboratories is no better than ±5% (Fishman, 1992). Total CSF protein values are reported in numerous articles and vary noticeably with the technique and the laboratory performing the assay. Therefore, clinicians must learn the normal values for their own laboratories.

2. CSF Protein Fractionation

A number of techniques for fractionation of CSF proteins have been developed. These include electrophoresis using paper or cellulose acetate, agar, agarose polyacrylamide, and starch gels. Immunoelctrophoresis, electroimmunodiffusion, radioimmunoassay, and isoelectric focusing are more recent techniques (Fishman, 1992; Kjeldsberg and Knight, 1993). Because of the normally low protein content, most of these methods require concentration of the CSF, which can create technical artifacts in the measured protein content. Techniques that do not require CSF concentration, such as electroimmunodiffusion, are therefore advantageous (Fishman, 1992).

3. Albumin and the CSF/Serum Albumin Index

Because albumin is synthesized only extrathecally increased CSF albumin indicates damage to the blood-brain/CSF barriers, intrathecal hemorrhage, or a traumatic CSF tap. In these conditions, albumin will leak into the CSF in general proportion to its serum concentration. Therefore, in the absence of intrathecal hemorrhage (pathologic or iatrogenic), the ratio of CSF albumin to serum albumin can be used as an indicator of barrier dysfunction (Tibbling et al., 1977; Link and Tibbling, 1977). This ratio is called the albumin index (also known as albumin quota or albumin quotient) and is calculated as follows (Kjeldsberg and Knight, 1993):

\[
\text{Albumin index} = \frac{\text{CSF albumin (mg/dl)}}{\text{serum albumin (g/dl)}}
\]

Values above the normal range indicate increased barrier permeability. The use of this index is potentially limited, however, because the large variability of CSF albumin in normal animals (at least in dogs and horse Andrews et al., 1994, 1990a; Bichsel et al., 1984b; Kowka et al., 1981) yields a large variability in the values for this index (Davson and Segal, 1996). In people the albumin index is age dependent, being highest in newborns and adults over 40 years of age (Kjeldsberg and Knight, 1993).

4. Quantitative Measurement of Immunoglobulins

a. IgG and the IgG/Albumin Index

The identification of intrathecal production of immunoglobulin is helpful in the diagnosis of neurologic disease. Immunoglobulin G is the dominant CSF in
munoglobulin. However, the IgG content of CSF is not a particularly useful measurement by itself, because the IgG present in CSF may be of serum origin (via a dysfunctional blood–brain/CSF barrier, intrathecal hemorrhage, or traumatic puncture) or intrathecally produced (as in various neural diseases). Varied opinions exist regarding the best way to calculate the contribution of IgG from each source (Trotter and Rust, 1989; Thompson, 1988). To determine the probable origin of CSF IgG, it can be related mathematically to a protein of purely extrathecal origin. Because albumin is synthesized entirely extrathecally, it is the preferred comparison protein and is the most widely used (Fishman, 1992). Transferrin and alpha2 macroglobulin have also been recommended because of their extrathecal origin (Schliep and Felgenhauer, 1974).

The simplest formula for correction of the CSF IgG level for extrathecal “contamination” and thereby demonstration of intrathecal IgG synthesis is the IgG/albumin index (Tibbling et al., 1977; Link and Tibbling, 1977). This index is calculated using the CSF and serum concentrations of albumin and IgG as follows (Kjeldsberg and Knight, 1993):

\[
\text{IgG index} = \frac{\text{CSF IgG (mg/dl)}}{\text{serum IgG (g/dl)}} \times \frac{\text{CSF albumin (mg/dl)}}{\text{serum albumin (g/dl)}}.
\]

The denominator of this index (CSF albumin/serum albumin) is the albumin index. Because albumin is synthesized only extrathecally, the albumin index assesses the amount of albumin crossing the blood–brain/CSF barriers, and therefore is a measure of barrier integrity. Blood contamination of the CSF with as little as 0.2% serum (equivalent to about 5000–10000 RBC/μl) by a traumatic puncture falsely elevates the IgG index in people (Peter and Tourtelotte, 1986). Also, the IgG index loses reliability when CSF protein levels are less than 25 mg/dl or greater than 150 mg/dl (Boerman et al., 1991).

An additional problem with the IgG index is its basic premise that the selectivity of the protein transfer at the blood–CSF barrier is independent of the actual permeability condition. This concept has been shown to be incorrect, and the IgG index, as well as the IgA and IgM indices, vary in a nonlinear fashion with progressive impairment of the barrier (Reiber and Felgenhauer, 1987). Therefore, Reiber and Felgenhauer (1987) developed a formula to calculate the intrathecally synthesized fractions of IgG, IgM, and IgA in the CSF.

b. IgM and IgA Indices

As with IgG, CSF IgM and IgA may be of serum origin or intrathecally produced. Indices for IgM and IgA can be calculated in the same fashion as those for IgG (Fryden et al., 1978). However, because of high variability in normal IgM and IgA levels and the biological variation of these large molecules, the application of the same formula for IgM and IgA indices as used for the IgG index may only provide rough estimates (Tipold et al., 1994; Reiber and Felgenhauer, 1987).

5. Qualitative Immunoglobulin Assays

Qualitative assays of CSF immunoglobulins include agarose-gel electrophoresis, acrylamide immunoelectrophoresis, isoelectric focusing, and immunofixation. These tests separate the proteins into “bands” and provide information regarding the CSF protein composition. Although abnormal band patterns are not specific for a particular disease, they do indicate pathology and may indicate a type of disease. Abnormal band patterns may be detected even in patients with a normal IgG index. Thus, both quantitative and qualitative immunoglobulin assays are useful in the assessment of central nervous system disorders, particularly immunologic or inflammatory diseases (Fishman, 1992; Kjeldsberg and Knight, 1993).

E. Antibody/Antigen Tests

A variety of CSF antibody and antigen tests are now available for viruses, fungi, rickettsia, protozoa, and other organisms (Greene, 1990). For antibody titers, two samples taken 2 weeks apart should be assayed. Because of interrun variability, the samples should be assayed at the same time in the same analytical run. Interpretation of CSF antibody titers must take into account the possibility of transudation of serum antibodies through a defective blood–brain/CSF barrier. Serum antibodies could be present because of disease, previous exposure to antigen, or vaccination. Ideally, the CSF/serum albumin index and IgG index are also determined (see Sections V.D.3 and 4) to identify blood–CSF barrier dysfunction and intrathecal production of immunoglobulin. Intrathecal production of antigen-specific antibody (specific Ig) can be determined with an antibody index in the same way as intrathecal IgG production is detected with the IgG index. The formula is (Reiber and Lange, 1991)

\[
\text{Antibody index} = \frac{\text{CSF specific Ig}}{\text{serum specific Ig}} \times \frac{\text{CSF total Ig}}{\text{serum total Ig}}.
\]

A modification of this formula accounting for large local synthesis of polyclonal IgG in the central nervous system may be necessary (Reiber and Lange, 1991).
antibody index > 1 suggests intrathecal production of the specific antibody (Reiber and Lange, 1991; Munana et al., 1995). Antibody indices have been calculated in human patients with a variety of diseases (Reiber and Lange, 1991). The diagnostic reliability of these indices and application to clinical veterinary medicine needs further study.

Antigen detection tests include immunoelectrophoretic techniques, agglutination tests, and ELISA for bacterial antigens and latex agglutination for cryptococcal antigens. The recently developed PCR procedures detect specific antigen DNA in CSF and are highly sensitive, specific, and rapid (see Section V.C.5). The ELISA and PCR procedures have much promise for the diagnosis of neural infections (Kjeldsberg and Knight, 1993).

**F. Microbial Tests**

The Gram stain, the Ziehl–Neelson acid-fast stain, and both aerobic and anaerobic cultures of CSF are time-honored methods for diagnosis of bacterial central nervous system infections. Bacteriological tests must be performed as soon as possible after CSF acquisition because some bacteria undergo rapid autolysis in the test tube. Additional tests such as the acridine orange stain for bacteria and tests for microbial antigens by counterimmuno electrophoresis or agglutination techniques may also be useful (Fishman, 1992).

**G. Blood Contamination**

Erythrocytes may be present in CSF samples because of subarachnoid hemorrhage or, more commonly, because of traumatic puncture. Blood contamination due to traumatic puncture is a common problem during CSF collection and, depending on its degree, can interfere with cytologic interpretation. Blood contamination is more likely to occur with lumbar puncture as opposed to cerebellomedullary cisternal puncture (Thomson et al., 1990; Bailey and Higgins, 1985; Oliver and Lorenz, 1993). Blood contamination is a source of leukocytes and hence can affect both the count and the differential. In one study of CSF in cats (Rand et al., 1990b), the total leukocyte count, the neutrophil percentage, and the eosinophil percentage were positively correlated with the CSF erythrocyte count once this count exceeded 500 erythrocytes per microliter. However, there was no significant increase in total white blood cell count or alteration in the differential percentages with up to 500 RBC/μl of CSF. Numerous correction factors have been used to correct leukocyte counts for the effect of blood contamination and include the following: In people, 1 white blood cell per 700 red blood cells is subtracted from the total white blood cell count (Fishman, 1992); in dogs, 1 white blood cell per 500 red blood cells is subtracted from the total count (Bailey and Higgins, 1985), and in cats, a maximum of 1 white blood cell per 100 red blood cells is subtracted (Rand et al., 1990b). A more accurate formula takes into account the actual white blood cell and red blood cell counts of the patient and hence compensates for any significant alterations in these counts (Fishman, 1992).

\[
W = \text{WBC}_{F} - \frac{\text{WBC}_{B} \times \text{RBC}_{F}}{\text{RBC}_{B}},
\]

where \(W\) is the white blood cell count of the fluid before blood was added, that is, the corrected count; \(\text{WBC}_{F}\) is the total white blood cell count in the blood fluid; \(\text{WBC}_{B}\) is the white blood cell count in the peripher al blood per microliter; and \(\text{RBC}_{F}\) and \(\text{RBC}_{B}\) are the numbers of red blood cells per microliter in the CSF and blood, respectively. Despite all of these elaboration corrections, our own experience is that many thousands of red blood cells in contaminated samples of CSF will frequently be observed without any accompanying white blood cells, suggesting that these correction factors may not be valid. This empirical observation has been made by others (deLahunta, 1983). The lack of validity has been proven by several studies (Wilson and Stevens, 1977; Novak, 1984). Wilson and Stevens (1977) concluded that blood contamination appeared to have little effect on white blood cell number and that the preceding correction formula was unreliable. They found 91 samples from both normal and diseased animals where there were numerous red blood cells but no white blood cells. Some of the red blood cell counts exceeded 15,000 RBC/μl, but white blood cells were still absent. Novak (1984) concluded that the standard computations frequently overcor rected white blood cell counts in blood-contaminated CSF and the magnitude of the overcorrection may obscure disease in some instances—in eight infants with significant blood contamination but proven bacterial meningitis, correction computations normalized but overcorrected the white blood cell counts. The mechanism of this overcorrection could not be defined, but it is clear that the presence of low numbers of neutrophils should not be immediately discounted when red cells are concurrently found (Christopher et al., 1988).

A study of feline CSF (Rand et al., 1990a) also found that values for CSF total protein, lactate dehydrogenase, creatine kinase, IgG ratio, and γ-globulin percentage were affected by blood contamination. The CSF total protein value of blood-contaminated CSF may be corrected using the formula for white blood cell...
correction given earlier but substituting the total protein levels of the bloody CSF and the serum for the corresponding white blood cell counts (Kjeldsberg and Knight, 1993). In people, bloody contamination of CSF with as little as 0.2% serum (equivalent to about 5000–10000 RBC/ml) elevates the IgG index (Fishman, 1992).

VI. GENERAL CHARACTERISTICS OF CSF IN DISEASE

A. Physical Characteristics: Clarity, Color, and Viscosity

Normal CSF is clear and colorless and has the consistency of water. In pathological conditions the clarity, color, and/or consistency may change.

1. Clarity

Cloudy or turbid CSF is usually due to pleocytosis; about 200 WBC/µL or 400 RBC/µL will produce a visible change. With these low levels of cellularity the CSF may appear opalescent or slightly hazy. Microorganisms, epidural fat, or myelographic contrast agent may also produce hazy or turbid CSF.

2. Color

Although the term xanthochromia means yellow color, it has often been used to describe pink CSF as well. The color of CSF is most usefully described as (1) pink or orange, (2) yellow, or (3) brown. These colors correspond to the major pigments derived from red cells: oxyhemoglobin, bilirubin, and methemoglobin. Oxyhemoglobin is red in color, but after dilution in the CSF it appears pink or orange. Oxyhemoglobin is released from lysed red cells and may be detected in the CSF supernatant about 2 hours after red cells enter the CSF. The level of oxyhemoglobin reaches its peak about 36 hours later and disappears over the next 4–10 days. Bilirubin is yellow in color. Bilirubin is the derivative of hemoglobin and is considered to be formed in macrophages and other leptomeningeal cells that degrade the hemoglobin from lysed red cells. Bilirubin is detected about 10 hours after red cells enter the CSF, reaches a maximum at about 48 hours, and may persist for 2 to 4 weeks. Bilirubin is also the major pigment responsible for the abnormal color of CSF with a high protein content. Methemoglobin in CSF is dark yellow-brown. Methemoglobin is an oxidation product of hemoglobin characteristically found in encapsulated subdural hematomas and in old, loculated intracerebral hemorrhages (Fishman, 1992; Kjeldsberg and Knight, 1993). Occasionally blackish CSF is encountered in animals with melanin-producing tumors in the nervous system.

Causes of a CSF color change other than red-cell contamination include icterus due to liver disease or hemolytic disease, markedly increased CSF total protein level, and drug effects. Both free and conjugated bilirubin may be present in the CSF, although the amount of bilirubin in the CSF does not correlate well with the degree of hyperbilirubinemia. If the CSF protein level is increased, the color change will be greater because of increased amounts of the albumin-bound bilirubin. High CSF protein content alone can impart a yellow color to the CSF (Fishman, 1992; Kjeldsberg and Knight, 1993). The drug rifampin is well known to impart an orange-red color to body fluids. Rifampin is 90% bound to protein, and hypoproteinemia may result in the staining of CSF (Fishman, 1992).

3. Viscosity

Increased viscosity is usually due to a very high CSF protein content, particularly fibrinogen. If pleocytosis is present, a surface pellicle or a clot may form. In this situation, collection of the CSF in a heparinized or EDTA tube may be necessary to obtain an accurate cell count. Cryptococcosis may increase CSF viscosity because of the polysaccharide capsule of the yeast. Epidural fat or nucleus pulposus in the CSF may also increase viscosity, or it may result in globules within the fluid (Fishman, 1992; Kjeldsberg and Knight, 1993).

B. Cytology

An increase in the cellularity of CSF is termed pleocytosis. In general terms, the degree of pleocytosis is dependent upon several factors, including the nature of the inciting cause and the severity and location of the lesion with respect to the subarachnoid space or ventricular system (Cook and DeNicola, 1988). It should also be emphasized that normal CSF analysis does not exclude the presence of disease (Fishman, 1992; Kjeldsberg and Knight, 1993). This is especially true with deep parenchymal lesions that do not communicate with the leptomeninges, and hence the subarachnoid space or the ependymal surfaces. In these cases, despite the presence of neurologic disease that is often severe, the lesion may not affect the CSF cellularity (Cook and DeNicola, 1988). Abnormal CSF findings always indicate the presence of pathologic abnormality.

1. Neutrophilia

A marked pleocytosis with neutrophil predominance generally suggests either bacterial meningitis (Kornegay et al., 1996; Kjeldsberg and Knight, 1993)
or suppurative, nonseptic (corticosteroid-responsive) meningitis (Tipold and Jaggy, 1994; Meric, 1988, 1992a). Total leukocyte counts in excess of 2000 cells per microliter are frequently encountered in these diseases and may even exceed 10,000 cells per microliter (Meric, 1992a). Observation of bacteria or a positive culture confirms septic meningitis. In our experience, bacteria are more commonly observed in the CSF of large animals afflicted with septic meningitis than in dogs or cats with septic meningitis. Neutrophil nuclear morphology is often used as a criterion for determining the likelihood of sepsis, with nuclear degenerative changes or karyolysis being interpreted as evidence of bacterial disease. However, the neutrophils in confirmed cases of septic meningitis in dogs and cats are frequently well preserved, especially if there has been prior therapy. Therefore, absence of bacteria or degenerative nuclear changes in neutrophils cannot be used to exclude unequivocally a diagnosis of septic meningitis, although it does make it less likely. In people, acute viral meningoencephalitis can initially produce a neutrophilic pleocytosis (Fishman, 1992; Converse et al., 1973; Kjeldsberg and Knight, 1993) that may persist from a few hours to several days prior to the development of the more typical mononuclear reaction. A similar phenomenon has been documented in animals (Green, 1993). Occasionally, distemper virus infection can cause massive encephalomalacia (Vandebelde and Spano, 1977), resulting in a neutrophilic pleocytosis in contrast to the moderate mononuclear pleocytosis that is more typical. Central nervous system neoplasia may result in a neutrophil predominance in the CSF, especially if there is significant necrosis and inflammation associated with the tumor. Moderate to marked pleocytosis with neutrophil predominance is a common finding in canine meningioma (Bailey and Higgins, 1986b). Canine intervertebral disc disease is predominately characterized by a mild mononuclear pleocytosis (Thomson et al., 1989). However, acute severe disease may result in counts greater than 50 cells per microliter with more than 50% neutrophils (Thomson et al., 1989). This finding is a reflection of acute inflammation that may be exacerbated by myelomalacia in some instances. The authors have seen a similar phenomenon associated with fibrocartilaginous thromboembolism in dogs. A neutrophilic pleocytosis of varying severity often occurs following myelography with iodinated contrast agents (Widmer et al., 1992; Johnson et al., 1985; Carakostas et al., 1983). These changes usually peak at 24 hours post myelogram (see Section VII for further details). Similarly, a neutrophilic pleocytosis has been observed postictally in people. We have occasionally observed similar findings in dogs (see Section VII).

2. **Lymphocytosis**

Alterations in both numbers and morphology of lymphocytes (see Section III.B) in the CSF occur in a variety of diseases. Central nervous system viral infections often result in a predominantly lymphocytic pleocytosis, which has been documented in dogs (Vandebelde and Spano, 1977), cats (Rand et al., 1994a; Dow et al., 1990), horses (Green et al., 1992; Hamir et al., 1992), sheep and goats (Brewer, 1983) and numerous other species. In people, CSF lymphocytosis has been observed in bacterial meningitis following antibiotic therapy (Fishman, 1992; Cargill, 1975; Converse et al., 1973; Kjeldsberg and Knight, 1993). This is a good example of how therapy and chronicity can alter the CSF findings. A similar finding has been reported in the dog (Tipold and Jaggy, 1994; Sarfaty et al., 1986) and the calf (Green and Smith, 1992).

3. **Eosinophilia**

Eosinophils are not present in normal, uncontaminated (by blood) CSF, although single eosinophils are occasionally seen on cytocentrifuge slides from animals with CSF that has normal nucleated counts and protein content. Although the presence of eosinophil in CSF is abnormal and is evidence of underlying disease, no diagnostic specificity is associated with their presence, as they can be found in a variety of disease (Bosch and Oehmichen, 1978). Additionally, CSF eosinophilia and peripheral blood eosinophilia do not necessarily occur together, and if they do, no positive correlation exists between the magnitude of peripheral blood eosinophilia and the severity of the CSF eosinophilia (Smith-Maxie et al., 1989; Bosch and Oehmichen, 1978). In one case series of eight dogs with eosinophilic meningoencephalitis, five of eight had concurrent peripheral eosinophilia, but no correlation was present between the peripheral and CSF eosinophil counts. The two dogs with the highest CSF eosinophil counts had peripheral eosinophil counts within normal reference limits. In people, central nervous system invasion by parasites, especially *Angiostrongylus cantonensis*, is the most frequent cause of eosinophilic pleocytosis, and in many of these cases eosinophils predominate in the differential cell count (Bosch and Oehmichen, 1979; Kuberski, 1979). CSF eosinophilia can also occur in association with bacterial, fungal, and viral infections and hence can be seen concurrently with suppurative granulomatous, and lymphocytic inflammatory processes of the central nervous system (Smith-Maxie et al., 1989; Jamison and Lumsden, 1988). However, many of these cases, eosinophils represent less than 5% of the total cell count in CSF (Smith-Maxie et al., 1989; Bosch and Oehmichen, 1978). Other documents
causes in people include neurosyphilis, tuberculosis, ricketsial disease, foreign-body reactions to shunt tubes, intrathecal penicillin or contrast agents, hyper-eosinophilic syndrome, multiple sclerosis, lymphoma, Hodgkin's disease, leukemia, melanoma, disseminated glioblastoma, idiopathic disorders, and systemic allergic reactions (Fishman, 1992; Smith-Maxie et al., 1989; Kuberski, 1979; Kjeldsberg and Knight, 1993). In animals, CSF pleocytosis that consists predominantly or almost exclusively of eosinophils is rare. We have personally seen CSF eosinophilia in association with idiopathic or steroid responsive eosinophilic meningoencephalitis (Smith-Maxie et al., 1989) and canine neural angiostrongylosis (Mason, 1989). Pleocytosis with eosinophil predominance has also been described in central nervous system cryptococcosis (Vandevelde and Spano, 1977), although this finding is not common in our experience. Other documented causes of CSF eosinophilia (though not predominance) in animals include bacterial encephalitis, distemper, rabies, toxoplasmosis, neosporosis, cuterebral encephalitis, central nervous system nematodiasis, protothecosis, granulomatous meningoencephalomyelitis, lymphoma, astrocytoma, and cerebral infarction (Chrisman, 1992; Lester, 1992; Darien et al., 1988; Vandevelde and Spano, 1977; Jamison and Lumsden, 1988).

4. Neoplastic Cells

The observation of neoplastic cells in CSF samples from animals with central nervous system neoplasia is rare in our experience. Very few veterinary studies have investigated the incidence of positive CSF cytology in confirmed cases of central nervous system neoplasia. In one study involving 77 histopathologically confirmed cases of primary central nervous system neoplasia in dogs, neoplastic cells were not observed in a single case (Bailey and Higgins, 1986b). However, in this study, cytologic assessment was performed only on those cases with an elevated cell count and these only accounted for 41.3% of cases. As stated previously, cases have been reported in the veterinary literature in which tumor cells were observed in the CSF but the CSF cell counts were within normal limits (Grevel et al., 1992; Bichsel et al., 1984b; Grevel and Machus, 1990). Additionally, in the study assessing primary brain tumors in 77 dogs (Bailey and Higgins, 1986b), CSF differentials and cytology were performed on cytospin slides. The cell yield with cytospin smears is very low, approximating 10% (Barrett and King, 1976) in some studies, and this may partly explain the failure to observe neoplastic cells in this study. Other veterinary studies utilizing different techniques report a higher incidence of neoplastic cell observation in the CSF from confirmed cases of central nervous system neoplasia.

In two studies utilizing a Kölmel sedimentation apparatus, tumor cells were seen in the CSF of five of eight cases (Grevel and Machus, 1990) and four of nine cases (Grevel et al., 1992). In the former study, two of the five cytologically positive cases had normal cell counts. In another study utilizing a membrane filter technique (Bichsel et al., 1984b), three of nine cases with central nervous system neoplasia had CSF samples in which neoplastic cells were observed. Both of the techniques utilized in these studies result in a higher cell yield than cytocentrifugation, which may be partly responsible for the increased incidence of neoplastic cell observation in the CSF in these studies.

A much larger number of studies assess the incidence of CSF neoplastic cells in people with central nervous system neoplasia. Overall sensitivities that are frequently quoted are 70% for central nervous system leukemia, 20-60% for metastatic meningeal carcinoma and approximately 30% for primary central nervous system tumors (Kjeldsberg and Knight, 1993), regardless of the technique utilized. The detection rate of malignant cells in the CSF is significantly improved by the collection of multiple samples (Olson et al., 1974). These figures are supported by one study utilizing cytocentrifugation in 117 cases of histopathologically confirmed central nervous system neoplasia (Glass et al., 1979). Overall, 26% (31/117) were positive. However, if only those cases with leptomeningeal involvement were considered, the incidence increased to 59%. Conversely, of 66 cases in which the tumor did not reach the leptomeninges, only a single case was positive. In another study, only 13.9% of all gliomas had a positive CSF cytology (Balhuizen et al., 1978). This low incidence is likely due to the fact that the great majority of gliomas do not extend into the subarachnoid space (Balhuizen et al., 1978). As a result of these studies, the following generalizations are frequently made in human medicine: (1) A positive CSF cytology is a reliable indicator of central nervous system malignancy and almost always reflects a leptomeningeal tumor (or one involving the ventricular system), and (2) a negative cytology does not exclude the presence of an intracerebral tumor, particularly a deep parenchymal mass that does not breach the pia or the ventricular system. Controlled studies are required in veterinary medicine to determine the incidence of positive CSF cytology in confirmed cases of the different types of central nervous system neoplasia and also to compare the sensitivities of different preparative methods. These studies may be hampered by the general lack of experience at identifying cells derived from central nervous system neoplasms. Tumor cells can be erroneously identified as normal ependymal or choroid...
plexus cells. Solitary tumor cells from metastatic carcinomas can be mistaken for lymphocytes or monocytes (Kjeldsberg and Knight, 1993). The need for the preceding types of studies has been somewhat decreased by the advent of routine access to advanced imaging techniques.

C. Protein

1. Changes in CSF Total Protein Content

An increase in the total protein content of CSF is the single most useful alteration in the chemical composition of the fluid (Fishman, 1992). However, this alteration accompanies many diseases and is therefore nonspecific. Increased total protein may be caused by (1) increased permeability of the blood–brain/spinal cord/CSF barriers allowing passage of serum proteins into the CSF, (2) intrathecal globulin production, and (3) interruption of CSF flow and/or absorption. Particular emphasis has been put on CSF flow rate as a major factor in CSF protein content (Reiber, 1994). In many diseases, two or all three of these mechanisms are at work. In complete spinal subarachnoid space blockage (e.g., by a compressive lesion or arachnoiditis), CSF withdrawn caudal to the block may clot when aspirated. This phenomenon is called Froin’s syndrome and results from very high CSF protein levels caused by the defective flow and absorption and blood–spinal cord barrier breakdown (Fishman, 1992; Kjeldsberg and Knight, 1993).

Decreased total protein is much less common. Theoretically, low levels of CSF protein could result from decreased entry of protein into the CSF or increased removal. No evidence exists to support the first mechanism. Increased removal can occur, however, if intracranial pressure is increased while the barriers to serum protein remain normal. In this situation, bulk flow absorption of CSF is increased, while entrance of protein into the CSF remains normal. Protein content of fluid collected from the lumbosacral site could be decreased if large volumes are removed, or if ongoing leakage of CSF from the lumbar area is occurring. In these situations, lumbosacral CSF is replaced more quickly than normal by ventricular CSF, which has a lower protein content than lumbosacral CSF (Fishman, 1992; Kjeldsberg and Knight, 1993). Low CSF protein has also occurred in people with hyperthyroidism, leukemia, or water intoxication (Fishman, 1992; Kjeldsberg and Knight, 1993).

2. Albuminocytologic Dissociation

In many disease processes, the CSF cell count and CSF total protein increase in rough parallel. In some disorders, though, the cell count remains normal, or nearly so, whereas the total protein is notably increased, a phenomenon termed albuminocytologic dissociation. Some degenerative disorders, ischemia/infarction, immune-mediated diseases (e.g., polyradiculoneuritis), tumors, and neural compression produce albuminocytologic dissociation (Laterre, 1996).

3. Increased CSF Albumin and Albumin Index

Elevation of CSF albumin (which originates in the serum), and consequently an increased albumin index, is indicative of dysfunction of the blood–brain/spinal cord/CSF barriers or contamination of the CSF by blood (from intrathecal hemorrhage or traumatic spinal tap). Barrier damage occurs in most types of neurologic disorders, including inflammatory diseases, neoplasia, trauma, compression, and occasionally metabolic diseases (Sorjonen, 1987; Bichsel et al., 1984b; Sorjonen et al., 1991; Krakowka et al., 1981).

4. Increased CSF IgG and IgG Index

The CSF IgG can be increased by transudation of protein across damaged blood–brain/CSF barriers, by intrathecal hemorrhage (pathologic or iatrogenic), or by intrathecal IgG synthesis. An elevated CSF IgG content and increased IgG index, indicating intrathecal IgG synthesis, is typical for infectious inflammatory diseases (Tipold et al., 1994, 1993b). In contrast, animals with noninflammatory diseases usually have normal IgG indices (Tipold et al., 1993b). In a few animals with noninfectious disorders, mild intrathecal IgG synthesis occurs, reflecting the presence of inflammatory infiltrates around the lesion (Tipold et al., 1993b). Therefore the IgG index is often useful for distinguishing between inflammatory and noninflammatory lesions which is not always possible on the basis of CSF cell counts alone (Bichsel et al., 1984b). In one study (Tipold et al., 1993b), 7 of 66 dogs with noninflammatory lesions had no pleocytosis but had an elevated IgG index; in contrast, 17 of 32 dogs with noninflammatory disease had pleocytic CSF and a normal IgG index. The authors of this study consider an IgG index ≥2.8 as proof of intrathecal synthesis allowing a diagnosis of meningocerephalomyelitis, and an IgG index between 1.2 and 2.8 as suggestive of inflammatory disease. In a few dogs with intense inflammatory lesions and intrathecal IgG production, the IgG index may not be elevated because of marked IgG transudation against which the local IgG synthesis is undetectable (Bichsel et al. 1984b). Traumatic puncture and red blood cell contamination of the CSF can artifactually increase the IgG index. Additionally, the normal IgG index of cerebellomedullary fluid and that of lumbar fluid are likely...
to be different because of the different protein concentrations of these fluids.

5. Classification of Disease Based on Albumin Index and IgG Index

Alterations of the albumin index and the IgG index can be grouped into three pathogenetic categories: (1) blood–brain/CSF barrier disturbance (increased albumin index), (2) intrathecal IgG synthesis (increased IgG index), and (3) barrier disturbance combined with intrathecal IgG production (both indices increased). These categories correlate somewhat with certain types of disease. Barrier disturbance may be seen in degenerative, inflammatory, metabolic, space-occupying, vascular, and traumatic conditions (Sorjonen, 1987; Bichsel et al., 1984b; Sorjonen et al., 1991). Intrathecal IgG synthesis is typical of inflammatory conditions (Tipold et al., 1994), but also occurs in noninfectious disorders that have secondary inflammation, such as some tumors (Tipold et al., 1993b; Bichsel et al., 1984b). Barrier disturbance coupled with intrathecal IgG production is typical of infectious–inflammatory diseases (Bichsel et al., 1984b).

6. Increased CSF IgM, IgA, and IgM and IgA Indices

The immunoglobulins IgM and IgA may be increased in the CSF of animals with inflammatory neurologic disease. A study of 69 dogs with inflammatory disease detected IgM elevations in 16 dogs and IgA elevations in 40 (Tipold et al., 1994). An increased CSF IgM index is considered by some investigators to be a good indication of recent or persistent immunological stimulation in people (Sharief and Thompson, 1989). In contrast, Felgenhauer (1982) reported IgM was present through all stages of human herpes and bacterial meningitis, and increased and decreased with IgG. Perhaps a transition from IgM to IgG production does not occur in the central nervous system (Tipold et al., 1994); or perhaps, in the presence of a normal or near-normal blood-CSF barrier, IgM accumulates in the CSF (Felgenhauer, 1982). In people with Borrelia infection, CSF IgM is persistently produced and the IgM index is a better indicator of this disease than is the IgG index (Fishman, 1992). Further studies need to be done in animals to determine the specificity of the various immunoglobulin alterations.

7. Electrophoretic Patterns of CSF Protein in Disease

Abnormalities in the CSF electrophoretic pattern can suggest categories of disease (Sorjonen, 1987; Sorjonen et al., 1991). In one study, dogs with inflammatory diseases had one of three patterns: (1) little or no blood–brain barrier disturbance (as determined by CSF albumin concentration and the albumin quota) with decreased gamma globulin, (2) mild blood–brain barrier disturbance with markedly increased gamma globulin, and (3) moderate or marked blood–brain barrier disturbance with increased gamma globulin. Dogs with intervertebral disc protrusion or cervical spondylomyelopathy had a pattern of normal barrier function or severe barrier disturbance with decreased alpha globulin. Dogs with brain neoplasia had marked barrier disturbance and normal or mildly increased alpha and beta globulins (Sorjonen et al., 1991).

In the gamma globulin region, three patterns of protein bands can occur—monoclonal, oligoclonal, and polyclonal. Oligoclonal bands are associated with disease and are seen in a high percentage of people with multiple sclerosis or encephalitis. These bands, readily identifiable against the low background of normal polyclonal IgG in the CSF, are thought to represent the products of a limited number of plasma-cell clones. Oligoclonal bands unique to CSF (i.e., not present in serum) indicate intrathecal synthesis of immunoglobulin and may be more sensitive than the IgG index in detecting this synthesis. People with multiple sclerosis may have a normal IgG index yet have CSF oligoclonal banding; thus, the demonstration of these bands is considered by some to be the single most useful test in the diagnosis of multiple sclerosis (Kjeldsberg and Knight, 1993). Oligoclonal bands are also seen in patients with inflammatory diseases and in some patients with neoplasia (Fishman, 1992). Occasionally, a single (monoclonal) band is identified in the CSF electrophoretic pattern of people. Monoclonal bands have been seen in neurologically normal people as well as patients with neurologic disease (Kjeldsberg and Knight, 1993).

8. Other CSF Proteins

Numerous attempts have been made to correlate specific CSF proteins, particularly “brain-specific” proteins, with specific diseases. Proteins such as C-reactive protein, interferon, myelin basic protein, and S-100 are increased in the CSF associated with neurologic disease, but these increases are found in many heterogeneous conditions. This nonspecificity limits the clinical usefulness of these protein assays. However, the measurement of some of these proteins is thought to be useful as a screening procedure for neurologic disease or as an indication of prognosis (Fishman, 1992; Kjeldsberg and Knight, 1993; Lowenthal et al., 1984). The clinical usefulness of these assays needs further investigation.
9. Plasma Proteins in the CSF

Alterations in plasma proteins may be reflected in the CSF. For example, the serum protein monoclonal gammopathy of multiple myeloma may be evident in the CSF. Bence-Jones proteins are also readily seen in the CSF. The high-molecular-weight paraproteins do not cross the normal blood–brain barrier, however. Serum protein electrophoresis is indicated in patients with elevated CSF globulins to clarify the source of the globulins (Fishman, 1992).

D. Antibody Titters

The CSF antibody titer can be measured for a number of diseases (Green et al., 1993; Greene, 1990). Interpretation of the results is confounded by the need to differentiate among titers caused by vaccination, exposure to the antigen without development of the disease, and actual disease. Interpretation of CSF antibody titers could be aided by an accurate vaccination history, comparison of CSF and serum titers, assessment of blood–brain/CSF barrier function and intrathecal immunoglobulin production by determination of albumin and immunoglobulin indices, determination of CSF IgM levels, and analysis of acute and convalescent samples (Chrisman, 1992; Green et al., 1993).

E. Glucose

Increased CSF glucose usually reflects hyperglycemia. Decreased CSF glucose occurs with several disorders of the nervous system, particularly acute, bacterial, fungal, amebic, or tuberculous meningitis. In people, low CSF glucose is also characteristic of diffuse carcinomatous meningitis, meningeal cysticercosis or trichinosis, and syphilitic meningitis. The major factors responsible for low CSF glucose levels are inhibition of the entry of glucose due to alteration of membrane glucose transport and increased anaerobic glycolysis by neural tissue. As noted earlier, hyperglycemia elevates the CSF glucose, which may mask a decreased CSF level. Therefore, calculation of a CSF/serum glucose ratio has been recommended to identify pathologically low CSF glucose level (Kjeldsberg and Knight, 1993). A low CSF glucose level in the absence of hypoglycemia indicates a diffuse, meningeal disorder, rather than focal disease (Fishman, 1992). Decreased CSF glucose classically has been associated with bacterial meningitis, but many human patients with bacterial meningitis have normal CSF glucose levels. Therefore, the recommendation has been made that CSF glucose need be measured only if the opening CSF pressure, cell count, cytospin differential, and protein are inconclusive (Hayward et al., 1987).

F. Enzymes

Numerous enzymes have been assayed in the CSF of animals (Rand et al., 1994b; Furr and Tyler, 1990; Wilson, 1977; Jackson et al., 1996). Of these, creatine kinase has received the most attention and opinions of its usefulness are somewhat conflicting. Although Furr and Tyler (1990) confirmed previous observations that CSF creatine kinase activity was elevated in several neurologic diseases, they concluded that the greater frequency of elevation in the CSF of horses with protozoal myelitis vs horses with cervical compressive myelopathy indicated this enzyme assay was useful in differentiating these two diseases. This conclusion was disputed by Jackson et al. (1996), who did not find the sensitivity or specificity of creatine kinase measurement sufficient for diagnosis of a specific disease. Jackson et al. (1996) also concluded that contamination of the CSF sample with epidural fat or dura mater may contribute to previously unexplained elevations in CSF creatine kinase activity. Their conclusion regarding the enzyme’s lack of sensitivity and specificity reflects the current situation with all of the enzymes in CSF studies to date—none have sufficient specificity to warrant their routine use as diagnostic tests (Rand et al., 1994b; Fishman, 1992; Kjeldsberg and Knight, 1993; Jackson et al., 1996; Indrieri et al., 1980). The site of CSF collection with respect to the location of the lesion may be responsible for some of the lack of diagnostic significance in CSF enzyme analysis. Cerebellomedullary fluid may be less affected than lumbar fluid in animal with spinal disease (Indrieri et al., 1980). Measurement of enzyme isomers may increase the specificity (Kjeldsberg and Knight, 1993).

G. Other Constituents

1. Interferon

Interferon is increased in the CSF in a large percentage of people with viral encephalitis–meningitis. This finding is not specific, however, as increases are also found in patients with bacterial meningitis (Glimakos et al., 1994) or multiple sclerosis, and occasionally in patients with noninflammatory neurologic disease (Brooks et al., 1983). In an experimental study of canine distemper, interferon appeared to be a valid marker for persistence of the virus in the central nervous system (Tsai et al., 1982). Studies on the clinical application of CSF interferon assay would be interesting.

2. Neurotransmitters

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter, whose dysfunction has been suggested to play a role in experimental (Griffi
et al., 1991) and clinical seizure disorders. A study of epileptic dogs found the average CSF concentration of GABA to be significantly reduced, a situation similar to that in people (Loscher and Schwartz-Porsche, 1986). Increased CSF levels of the biogenic amine neurotransmitter metabolites homovanillic acid and 5-hydroxyindoleacetic acid were found in 2 of 10 collies experimentally given invermectin (Vaughn et al., 1989a). Both of these collies had severe neurologic deficits. Neurotransmitter metabolite concentrations were also elevated in the CSF of goats demonstrating neurologic abnormalities after experimental boron toxinosis (Sisk et al., 1990). Significant differences in neurotransmitter concentrations were also found between the CSF of normal dogs and that of narcoleptic dogs (Faull et al., 1982). The use of neurotransmitter assays in clinical diagnosis needs more investigation.

3. Quinolinic Acid

Quinolinic acid is a neuroexcitotoxic metabolite of L-tryptophan and an agonist of N-methyl-D-aspartate receptors. Increased levels have been found in people with a variety of neurological diseases, including AIDS (Heyes et al., 1992), and in macaques infected with simian immunodeficiency virus (Smith, 1995). Quinolinic acid levels may be elevated in CSF of animals with inflammatory nervous system disease. Therefore, it may be useful as a marker of inflammation and perhaps also as an indicator of prognosis (Smith, 1995).

4. Lactic Acid

In human medicine, the measurement of CSF lactic acid has been advocated in differentiating bacterial from viral meningitis. The concentration of CSF lactic acid is elevated in conditions causing severe or global brain ischemia and anaerobic glycolysis. Therefore, many diseases may elevate CSF lactic acid, and the overlapping CSF lactate levels limit the value of CSF lactate assay (Fishman, 1992; Kjeldsberg and Knight, 1993). A study of CSF lactic acid levels in horses with neurologic disease found elevated lactate levels in several types of central nervous system diseases (Green and Green, 1990). Therefore, as with people, increased CSF lactic acid in the horse appears to be a nonspecific indicator of central nervous system disease. Interestingly, in the horses studied, elevated lactic acid was the only CSF abnormality associated with brain abscess (Green and Green, 1990).

5. 3-OH Butyrate

The measurement of serum 3-OH butyrate concentration is useful in the feeding management of pregnant ewes and in the diagnosis of pregnancy toxemia. Following death, however, rapid autolytic change renders serum biochemical analysis useless. Scott et al. (1995) compared the 3-OH butyrate concentrations of serum collected antemortem and aqueous humor and CSF collected within 6 hours of death. Their results indicated that either fluid was suitable for postmortem determination of 3-OH butyrate levels, and that such data could be extrapolated to indicate antemortem serum 3-OH butyrate concentration and the possibility that pregnancy toxemia contributed to the death of the animal.

VII. CHARACTERISTICS OF CSF ASSOCIATED WITH SPECIFIC DISEASES

A. Degenerative Disorders

This group of disorders includes a variety of diseases such as the inherited, breed-specific polyradiculoneuropathies, myelopathies, and encephalopathies; motor neuron diseases; and cerebellar abiotrophies. The storage diseases can also be included in this group. The inclusion of canine degenerative myelopathy is arguable, awaiting further clarification of its pathogenesis. The CSF in animals with degenerative disorders is characteristically normal, reflecting the lack of inflammation in the disease process (Braund, 1994; Oliver and Lorenz, 1993). A mild to moderate increase of CSF total protein may occur in several of these disorders, however. Increased total protein is also found in people with motor neuron disease, Parkinson's disease, and various hereditary neuropathies and myelopathies. The mechanism of the protein increase is unknown. Electrophoretic studies of CSF associated with some human neurodegenerative disorders have shown a transudative pattern. Intrathecal immunoglobulin production has also been found in people with motor neuron disease (Fishman, 1992). In storage disease such as globoid-cell leukodystrophy, accumulations of metabolic material may be seen in CSF cells (Roszel 1972).

1. Canine Degenerative Myelopathy

Although the CSF of dogs with degenerative myelopathy may be normal, a mild elevation of the white blood cell count is present occasionally (Bichsel et al., 1984b). More common is a normal cell count coupled with a mild to moderate elevation of total protein (approximately 40–70 mg/dl). This albuminocytologic dissociation may support the theory that this disorder is an immune-mediated disease (Waxman et al., 1980). However, the common existence of concurrent chronic, spinal cord compression by Type II disc pro
trusion in these dogs complicates the situation, because chronic cord compression may also produce an increase in total protein. The elevated total protein in canine degenerative myelopathy is probably due to increased CSF albumin (Bichsel et al., 1984b). The CSF IgG index is usually normal (Tipold et al., 1993b; Bichsel et al., 1984b), indicating a lack of intrathecal IgG production.

2. Degenerative Myeloencephalopathy of Llamas

A degenerative myeloencephalopathy has been identified in two adult llamas. Lesions consist of bilateral white-matter degeneration in all spinal-cord segments, and degenerate neurons in the brain-stem nuclei or degeneration of brain-stem white-matter tracts. Inflammation is not evident. Lumbosacral CSF from both animals was normal (Morin et al., 1994).

3. Equine Motor Neuron Disease

The CSF of horses with this disorder is either normal or has albuminocytologic dissociation (Divers et al., 1994; Cummings et al., 1990). In a study of 28 cases (Divers et al., 1994), 9 of 26 horses had elevated CSF protein. The albumin quotient was abnormal in only 2 of 19 horses. The IgG index was increased in 8 of 16 horses. The abnormalities in total protein and IgG index did not appear to be associated with the duration or severity of clinical signs. The increased protein and IgG index in these horses suggest that intrathecal production occurs. Blood–brain barrier damage and intrathecal IgG production also occur in people with motor neuron disease (Apostolski et al., 1991).

B. Idiopathic Disorders

1. Granulomatous Meningoencephalomyelitis

The CSF associated with granulomatous meningoencephalomyelitis (GME) is usually abnormal. The fluid may be clear or hazy and is generally colorless. The total white blood cell count is moderately to markedly elevated, as is the total protein. The white blood cell differential is variable, but typically lymphocytes predominate, with monocytes/macrophages and neutrophils making up the remainder in about equal percentages (Bailey and Higgins, 1986a; Braund, 1994; Tipold, 1995; Thomas and Eger, 1989; Sarfaty et al., 1986). A 15–30% neutrophilic component is suggestive of GME, but the white blood cell differential can range from 95% neutrophils (Sorjonen, 1990) to 100% mononuclear cells. Plasma cells, cells undergoing mitosis, and large, mononuclear cells with abundant foamy cytoplasm are occasionally present (Bailey and Higgins, 1986a; Braund, 1994). Lumbar fluid is also abnormal, although it generally has fewer cells and less protein than cerebellomedullary fluid (Bailey and Higgins, 1986a). Electrophoresis of CSF suggests that blood–brain barrier dysfunction is present in the acute stage of disease; intrathecal IgG production with resolution of the barrier dysfunction occurs in chronic disease (Sorjonen, 1990). The albumin quota is elevated (Sorjonen, 1987), and the IgG index is usually elevated (Tipold et al., 1994, 1993b; Bichsel et al., 1984b). If barrier dysfunction is severe, with marked transudation of protein, the IgG index may be normal because the amount of intrathecally produced IgG is small in comparison to the amount of transudated serum IgG (Fishman, 1992; Bichsel et al., 1984b).

2. Necrotizing Encephalitis of Pug Dogs, Maltese Dogs, and Yorkshire Terriers

A necrotizing encephalitis of unknown cause has been identified in Pug Dogs (deLahunta, 1983; Cordy and Holliday, 1989), Maltese dogs (Stalis et al., 1995), and Yorkshire terriers (Tipold et al., 1993a). The lesions are similar in each breed, although the distribution of lesions in the Pug and Maltese dogs (large, diffuse, cerebral) is different than in the Yorkshire terriers (well-defined multifocal, brain stem). The CSF associated with the Pug and Maltese-dog diseases has a moderate to marked, predominantly lymphocytic white blood cell count (although one Maltese had 62% neutrophils) and moderate to marked elevation in total protein (Stalis et al., 1995; deLahunta, 1983; Cordy and Holliday, 1989; Bradley, 1991). The CSF of the Yorkshire terriers has mild to moderate increases of white blood cell and protein, with a predominantly mononuclear differential count (Tipold, 1995; Tipold et al., 1993a). Seizures are a consistent clinical sign for the Pugs and the Maltese dogs, but not the Yorkshire terriers. The relationship of the seizures to the more severe CSF abnormalities of the Pug and Maltese dogs compared to those of the Yorkshire terriers is interesting to consider.

C. Immune-Mediated Diseases

1. Acute Idiopathic Polyradiculoneuritis/Coonhound Paralysis

Acute idiopathic polyradiculoneuritis is one of the most common canine polynuropathies, and coonhound paralysis is the most common form. The disorder resembles Guillain–Barré syndrome of people. In affected dogs, the classical CSF abnormality is albuminocytologic dissociation without pleocytosis. The abnormality is more obvious in lumbosacral CSF than
in cerebellomedullary CSF (Cuddon, 1990; Cummings et al., 1982). The CSF IgG level and IgG index may also be increased, indicating intrathecal immunoglobulin production (Tipold et al., 1993b; Cuddon, 1990).

2. Equine Cauda Equina Neuritis

This disease is thought to be an autoimmune polyneuritis. The CSF of affected horses may be xanthochromic and typically has a prominent, usually lymphocytic pleocytosis (at least in the chronic stage) and moderately elevated protein. The CSF can also be normal (Mayhew, 1989; Yvorchuk, 1992).

3. Steroid-Responsive Meningitis/Arteritis

Steroid-responsive meningitis/arteritis is a common, supplicative meningitis of dogs. The CSF has a marked, often extreme, neutrophilic pleocytosis and moderately to markedly increased protein. Occasionally, a single sample collected early in the disease is normal (deLahunta, 1983; Tipold, 1995; Meric, 1988). The IgG index is typically elevated (Tipold et al., 1994, 1993b), and IgM and IgA levels are often elevated as well (Tipold et al., 1995, 1994). Microbial cultures are negative. In protracted or inadequately treated cases, the pleocytosis is mild to moderate with a mixed population or even a mononuclear cell predominance; the protein level may be normal or slightly elevated. The CSF may even be normal (Tipold and Jaggy, 1994). A polyarteritis/vasculitis reported in beagles, Bernese mountain dogs, German short-haired pointers, and sporadically in other breeds (Meric, 1988) has similar CSF abnormalities and pathologic changes and may be the same disease as steroid-responsive meningitis/arteritis (Tipold and Jaggy, 1994).

D. Infectious Diseases

The variety of CSF abnormalities associated with infectious disease reflects the variety of infectious diseases affecting the central nervous system. If the infection causes inflammation, the total white blood cell count and protein usually will be elevated, but the degree and type of abnormality depend on the infectious agent, the immune status of the animal, the location of the infectious process (e.g., surface-related vs parenchymal), the duration of the infection, and previous treatment. The general rules of inflammation due to infection apply, that is, bacterial infections result in suppurative inflammation, whereas viral infections result in nonsuppurative inflammation. Several important exceptions exist, however.

1. Bacterial Diseases

The CSF associated with aerobic or anaerobic bacterial infections of the central nervous system may be clear, hazy or turbid (depending on the cell count), and colorless or amber with moderate to marked elevations of total white blood cell count and total protein. Because of the elevated protein, the CSF may clot or foam when shaken. The white blood cell differential count has a characteristically high percentage of neutrophils (>75%), which may be degenerate (Rand et al., 1994a; Green and Smith, 1992; Scott, 1995; Foreman and Santschi, 1989; Santschi and Foreman, 1989; Dow et al., 1988; Tipold, 1995; Kornegay, 1981; Meric, 1988; Baum, 1994). The protein is composed of albumin that has crossed the diseased blood–brain/CSF barrier and immunoglobulin produced intrathecaclly therefore, the IgG index is usually elevated (Tipold et al., 1994, 1993b). The IgM and IgA levels may be normal or increased (Tipold et al., 1994). The CSF of animals with chronic or treated bacterial infections may be nonsuppurative with mild to moderate elevations of total white blood cell count and total protein (Green and Smith, 1992). Occasionally, extracellular or intracellular bacteria may be seen on a Gram stain (Green and Smith, 1992; Foreman and Santschi, 1989; Kornegay, 1981). Because some bacteria undergo rapid autolysis in the test tube, bacterial culture of these infections is often unrewarding. Nonetheless, culture should be attempted. Polymerase chain reaction techniques may be used to detect the presence of bacterial DNA (Peters et al., 1995).

a. Listeriosis

The CSF of cattle with meningoencephalitis caused by Listeria monocytogenes typically has mild to moderate elevations in total white blood cell count and total protein, with the white cells being mostly mononuclear cells, in spite of the fact that it is a bacterial infection (Rebhun and deLahunta, 1982). These mild changes probably reflect the characteristic lesions of this disease, which are mononuclear vascular cuffing and parenchymal microabscesses. The disease in sheep may produce a CSF similar to that of infected cattle (Scarratt, 1987). However, two studies reported ovine CSF to have moderate to marked elevations in white blood cell count and protein, with a neutrophilic pleocytosis (53 to 100% neutrophils) (Scott, 1993, 1992). Perhaps the mononuclear CSF reported in cattle reflects a more chronic stage or resolution of the disease (Green and Smith, 1992; Kjeldsberg and Knight, 1993). A study of bacterial culture and polymerase chain reaction (PCR) for the detection of L. monocytogenes in the CSF of 14 infected ruminants yielded no positive cultures and
only one positive PCR. The authors concluded that *L. monocytogenes* only occasionally gains access to the meningoventricular system in the course of the disease, and that reliable, in vivo diagnosis of listeric encephalitis generally cannot be based on the detection of the organism in the CSF (Peters et al., 1995).

b. Neuroborreliosis (Lyme Disease)

Although neuroborreliosis caused by the Lyme disease spirochete, *Borrelia burgdorferi*, has been suspected in dogs (Mandel et al., 1993; Feder et al., 1991) and horses (Hahn et al., 1996; Burgess and Mattison, 1987), the actual incidence in animals is unknown, largely because of diagnostic difficulties. These difficulties arise from a delay or repression of seroconversion after infection; the high number of seropositive, clinically normal animals; the persistence of infection and sero-reactivity; and difficulty in culturing the organism from tissue or fluid samples (Appel et al., 1993; Madigan, 1993; Parker and White, 1992; Levy et al., 1993). The CSF associated with neuroborreliosis in animals has not been characterized. In people, CSF abnormalities are related to the stage of the disease. When present, typical abnormalities are a mononuclear pleocytosis (T lymphocytes, plasma cells, and IgM-positive B cells (Sindern and Malin, 1995)) with a moderately elevated total protein and normal or decreased CSF glucose (Fishman, 1992). Persistent CSF oligoclonal bands and intrathecal synthesis of IgG, IgM, and IgA occur (Henriksson et al., 1986). Diagnosis is enhanced by the determination of intrathecal synthesis of specific *B. burgdorferi* antibodies (Kaiser and Lucking, 1993), but cross reactivity is a problem (Fishman, 1992). *Borrelia burgdorferi* antibodies have also been detected in the CSF of dogs (Mandel et al., 1993; Feder et al., 1991). PCR techniques for CSF have been recently developed, but the diagnostic success rate is variable (Lebech, 1994). The CSF of a horse was reported PCR positive for *B. burgdorferi* (Hahn et al., 1996).

c. Rickettsial Diseases

Rickettsial diseases such as ehrlichiosis, usually caused by *Ehrlichia canis*, and rocky mountain spotted fever, caused by *Rickettsia rickettsii*, sporadically involve the central nervous system of animals. In dogs with neural ehrlichiosis, the CSF resembles that of viral diseases, that is, the white blood cell count and protein may be normal or slightly to moderately elevated with a predominantly mononuclear pleocytosis (Maretzki et al., 1994; Greene et al., 1985; Buoro et al., 1990; Mein-koth et al., 1989; Firneisz et al., 1990). The albumin quotient is reported to be elevated (Sorjonen et al., 1991). Occasionally, *Ehrlichia morulae* may be observed in CSF mononuclear cells or neutrophils (Maretzki et al., 1994). The few reports of CSF associated with Rocky Mountain spotted fever suggest a difference from ehrlichiosis in that the CSF pleocytosis of Rocky Mountain spotted fever may be predominantly neutrophilic, particularly early in the disease (Breitschwerdt, 1995 Breitschwerdt et al., 1985; Greene et al., 1985; Rutger et al., 1985). A predominantly neutrophilic pleocytosis has also been reported in dogs experimentally infected with *R. rickettsii* (Breitschwerdt et al., 1990). In this same study, IgG or IgM antibodies were not detected in the CSF of experimentally infected dogs, but were detected in the CSF of one naturally infected dog that also had a high serum titer (Breitschwerdt et al., 1990).

d. Thromboembolic Meningoencephalitis

In cattle, *Hemophilus somnus* causes bacteremia and thromboembolism, with some preference for neuraxis tissue. The vascular lesion results in multifocal hemorrhages. Consequently, the CSF is characteristically yelow with a high red blood cell count (not iatrogenic in origin), and moderately to markedly increased white blood cell count (predominantly neutrophilic) and protein (George, 1996; Mayhew, 1989; Ames, 1987; Littl and Sorensen, 1969). The bacterium can be cultured only occasionally from CSF, and more easily from septicemic animals (Little, 1986; Nayer et al., 1977).

2. Viral Diseases

The CSF abnormalities associated with viral diseases is characterized by nonsuppurative inflammatory changes. The total white blood cell count and total protein are generally mildly to moderately elevated. The white cell population may be mixed with a majority of mononuclear cells or may be entirely mononuclear cells. Occasionally, neutrophils predominate, particularly in the early stages of disease or in certain diseases (see later discussion). The IgG index is commonly elevated (Tipold et al., 1993b; Bichsel et al., 1984b). The IgA and IgM levels may also be elevated. The CSF of viral infections may also be normal, particularly if the meninges or epithyma are not involved (Rand et al., 1994a; Tipold et al. 1994; Fishman, 1992; Fankhauser, 1962; Tipold, 1995).

a. Canine Distemper

The CSF abnormalities associated with canine distemper vary strikingly with the stage of the disease. Dogs with acute, demyelinating, noninflammatory distemper encephalitis may have normal or near-norm CSF (mild elevations of total cell count and total protein) (Tipold, 1995; Johnson et al., 1988). Protein elevation is most likely due to blood–brain barrier dysfunction (Bichsel et al., 1984b). The IgG index may also
normal or occasionally mildly elevated, which correlates with the histological findings of multifocal demyelination with very little or no infiltration of inflammatory cells (Tipold et al., 1994, 1993b; Bichsel et al., 1984b; Vandevelde et al., 1986; Johnson et al., 1988). The acute form of nervous canine distemper is an exception to the usual association of an elevated IgG index with infectious neurologic diseases because infiltration with inflammatory cells occurs only in the chronic stage of distemper encephalitis (Vandevelde et al., 1986). The CSF IgM and IgA content is also usually normal (Tipold et al., 1994; Johnson et al., 1988). The CSF of subacute/chronic, inflammatory distemper usually has a moderately elevated total white blood cell count, primarily mononuclear, and moderately elevated protein (Bichsel et al., 1984b; Tipold, 1995). The IgG index is typically elevated (Bichsel et al., 1984b; Vandevelde et al., 1986) and IgA levels are commonly increased. Interestingly, IgM levels are increased more often in the chronic stage than in the dogs with acute, noninflammatory distemper (Tipold et al., 1994, 1993b). The IgM and IgA are presumably of intrathecal origin (Tipold et al., 1994), although blood–brain barrier dysfunction is also present in some dogs and therefore protein could be of serum origin (Sorjonen, 1987; Bichsel et al., 1984b; Sorjonen et al., 1991). Occasionally the CSF is normal or has only mild changes in cell count or total protein content (Tipold et al., 1994, 1993b; Bichsel et al., 1984b; Sorjonen et al., 1991; Tipold, 1995; Vandevelde et al., 1986). Antimyelin antibody and antiviral antibody have also been identified in the CSF of inflammatory distemper (Vandevelde et al., 1986). Canine distemper virus antibody is normally absent from CSF; when present it is diagnostic of infection. False-positive results can occur, however, if the CSF is contaminated by serum distemper virus antibody by either iatrogenic or pathologic blood–brain barrier disturbance. The CSF of delayed-onset canine distemper (also known as old-dog encephalitis) has an elevated protein and nonsuppurative, inflammatory cytology. The IgG index is elevated, and much of the CSF IgG is virus specific, suggesting an intrathecal anti-viral immune response. The IgM and IgA concentrations are normal (Johnson et al., 1988).

### b. Equine Herpesvirus Myeloencephalitis

With its predilection for endothelial cells, the equine herpesvirus 1 (EHV-1) may cause vasculitis and perivascular hemorrhage in the brain and spinal cord. As a result, the CSF is often xanthochromic. The total white blood cell count may be normal while the total protein level is moderately to markedly elevated (albuminocytologic dissociation). The CSF/serum albumin ratio is increased (Klingeborn et al., 1983). In some cases the total protein is normal, perhaps because the CSF is analyzed very early in the course of the disease before the protein level has risen, or late in the disease after the level has subsided (Kohn and Fenner, 1987). Antibodies to the virus may be identified in the CSF (Blythe et al., 1985; Klingeborn et al., 1983; Jackson et al., 1977) Antiviral CSF antibodies are not present routinely in neurologically normal horses, horses vaccinated with modified live EHV-1, or horses with other neurologic diseases (Blythe et al., 1985). However, because of destruction of the blood–brain barrier, serum antiviral antibodies may pass into the CSF and confound the interpretation of the CSF titers (Blythe et al., 1985; Klingeborn et al., 1983; Kohn and Fenner, 1987; Jackson et al., 1977). Determining the CSF IgG index may help to assess the relevance of a positive CSF EHV-1 titer.

### c. Feline Infectious Peritonitis

The feline infectious peritonitis (FIP) coronavirus may cause a multifocal, pyogranulomatous meningoencephalitis choroid plexitis, and ependymitis characterized by perivascular granulomas around small blood vessels. The CSF associated with these lesions consistently has a moderate to marked elevation of white blood cells and protein. In one study, the CSF of cats with FIP was distinctive compared to that of cats with other inflammatory central nervous system diseases in having greater than 200 mg/dl total protein (Rand et al., 1994a). Despite the virally nature of the disease, the white cell population is dominated by neutrophils, commonly greater than 70% (Rand et al., 1994a; Kline et al., 1994; Baroni and Heinold, 1995). Prolonged glucocorticosteroid therapy may result in a normal CSF in rare instances; the authors have observed this on at least one occasion.

### d. Feline Immunodeficiency Virus

The CSF associated with feline immunodeficiency virus (FIV) neurologic disease typically has a mild primarily lymphocytic, pleocytosis (Dow et al., 1990; Phillips et al., 1994). In experimentally infected cats, the pleocytosis appears related to the duration, and perhaps route, of infection, as well as to the age of the cat. In one study, pleocytosis appeared within 2–8 weeks of inoculation of adult cats, then disappeared by 20 weeks (Dow et al., 1990). In a study of kittens, the total and differential cell counts were normal at 5 and 12–16 months post inoculation (Podell et al., 1993). The total protein content is typically normal, although the albumin quotient and IgG index may be elevated (Dow et al., 1990; Podell et al., 1993). Antibodies to the virus may be detected in the CSF, and their presence in CSF that has not been contaminated by peripherally blood is presumptive evidence of FIV neural infection.
(Dow et al., 1990; Phillips et al., 1994). In experimentally inoculated cats, FIV antibodies developed in the CSF 4–8 weeks after the appearance of CSF pleocytosis (Dow et al., 1990). The virus can be recovered from the CSF of most cats that have intrathecal antibodies (Dow et al., 1990; Phillips et al., 1994). In the immunodeficient, chronic stage of FIV infection, the effect of possible opportunistic neural infections on CSF must be considered.

e. Rabies

Because rabies is an overwhelmingly fatal, zoonotic disease, there is a paucity of information regarding its CSF abnormalities. In people, the total white blood cell count is normal or has a mild, lymphocytic pleocytosis, and total protein is mildly increased. Occasionally, the pleocytosis is marked (Fishman, 1992). The CSF of animals with rabies may be normal or abnormal. Typical abnormalities include a mild to moderate mononuclear pleocytosis and mild to moderate elevations in total protein. The white cells may be predominantly lymphocytes, with macrophages, neutrophils, and occasionally plasma cells and eosinophils (Braund, 1994; Green et al., 1992; Hanlon et al., 1989; Coles, 1980; Hamir et al., 1992). A neutrophilic pleocytosis reported for one horse was thought to reflect an early stage of the disease (Green, 1993). Xanthochromia was detected in the CSF of 3 of 5 horses in one study (Green et al., 1992), perhaps due to antemortem head trauma. The CSF IgM titer increases in 2–3 weeks or more after the onset of clinical rabies (Murphy et al., 1980). Because of this delay, a negative titer result does not eliminate rabies infection as a possibility (Greene and Dreesen, 1990). Infective virus may be isolated from the CSF before clinical signs of the disease appear, and neutralizing antibodies in the CSF may not be identified until after clinical signs develop (Fekadu and Shaddock, 1984). Because of the human health hazard, CSF collection should be avoided if rabies is suspected.

3. Fungal Diseases

Fungal infection of nervous tissue is relatively uncommon, although Cryptococcus neoformans has a predilection for the central nervous system. The CSF associated with neural cryptococcosis is quite variable. The total white blood cell count can be near normal or markedly increased. The white blood cell differential count is typically mixed with a majority of neutrophils (Berthelin et al., 1994; Steckel et al., 1982). However, mononuclear CSF has been reported (deLahunta, 1983; Berthelin et al., 1994), as has eosinophilic fluid (deLahunta, 1983; Vandeveld and Spano, 1977). The total protein is typically elevated, although sometimes only marginally so. The albumin quotient and IgG index are mildly to markedly elevated (Sorjonen et al., 1991). Cryptococcal organisms are commonly seen in the CSF (93% in one report/review) (Berthelin et al., 1994) and cultures are often, but not invariably, positive. Latex agglutination for cryptococcal antigen in the CSF may also be positive, but experience with this test with CSF is limited (Berthelin et al., 1994).

The literature contains only a few reports of the CSF abnormalities associated with neural aspergillosis, blastomycosis, coccidioidomycosis, or histoplasmosis. Again, the CSF abnormalities are variable; a mixed pleocytosis and elevated protein is typical (Vandeveld and Spano, 1977; Mullaney et al., 1983; Coates, 1995; Gelatt et al., 1991; Nafe et al., 1983; Schaer et al., 1983; Kormegay, 1981). In a reported case of aspergillosis of the brain of a dog, the CSF had a normal total nucleated cell count (differential count was not done) and a normal protein (Parker and Cunningham, 1971).

4. Prion Disorders

The transmissible spongiform encephalopathies are a group of neurodegenerative diseases of people and animals caused by prions (proteinaceous infectious particles). The diseases in this group include kuru and Creutzfeldt–Jakob disease of people, bovine spongiform encephalopathy, scrapie of sheep and goats, transmissible mink encephalopathy, and spongiform encephalopathies in deer, captive ungulates, and domestic cats (Schreuder, 1994a, b). The CSF associated with the spongiform encephalopathies in animals has normal cytology, protein content, and electrophoretic pattern. Thus, these diseases, although apparently infectious, do not appear to damage the blood–brain barrier or elicit an immune response in the central nervous system (Scott et al., 1990; Lowenthal and Karcher, 1994; Millson et al., 1960; Strain et al., 1984).

E. Ischemic Disorders

In general, neural ischemia causes blood–brain/CSF barrier dysfunction resulting in increased CSF protein. If infarction occurs, the tissue destruction and cellular response may result in CSF pleocytosis. With extensive particularly acute, infarction the pleocytosis may be substantially neutrophilic (Fishman, 1992). In animals CSF abnormalities have been reported for fibrocartilaginous embolism and cerebral ischemia/infarction.

1. Fibrocartilaginous Embolism

The characteristics of CSF associated with fibrocartilaginous embolism have been reported for dogs and horses. About one-third of the reported canine cases have normal CSF, about one-third have pleocytosis usually mononuclear, and increased protein, and abou
1. Intracranial Intra-arachnoid Cysts

Intracranial intra-arachnoid cysts have been identified in six dogs: two Lhasa apsos, two pugs, one shih tzu, and one German short-haired pointer (Vernau et al., 1996). Analysis of cerebellomedullary CSF was performed in three of the dogs. In two, the CSF was normal (clear, colorless, <2 WBC/µl, <25 mg/dl total protein). The CSF of the third dog had a moderate, lymphocytic pleocytosis and moderately increased total protein, suggesting a nonsuppurative, inflammatory process. Necropsy of this dog identified a nonsuppurative, necrotizing meningoencephalitis unrelated to its cyst (Vernau et al., 1996).

2. Cerebral Ischemia/Infarction

Cerebral infarction (ischemic encephalopathy) has been reported primarily in cats, but also in a few dogs. In cats within the first week of onset, the white blood cell count is normal or mildly elevated with a mixed, predominantly mononuclear, pleocytosis, and protein is mildly to markedly elevated (Rand et al., 1994b; deLahunta, 1983). Dogs with cerebral infarction have similar CSF characteristics (Bichsel et al., 1984b; deLahunta, 1983; Vandevelde and Spano, 1977; Joseph et al., 1988), although two dogs in one report had a mixed, but predominantly neutrophilic, pleocytosis and normal protein (Vandevelde and Spano, 1977). The neutrophilic pleocytosis reflected the acute encephalomalacia noted on histopathologic examination (Vandevelde and Spano, 1977). In one dog of another report, the CSF albumin and IgG index were normal (Bichsel et al., 1984b). Another dog with a deep, parenchymal, hemorrhagic infarct in the basal nuclear region had normal CSF (Norton, 1992).

F. Malformations of Neural Structures

Although reports with CSF analysis are relatively few, the CSF of animals with neural malformations is generally normal (Rand et al., 1994b; Vandevelde and Spano, 1977; Milner et al., 1996; Meric, 1992b; Shell et al., 1988; Greene et al., 1976; Wilson et al., 1979). However, if the malformation interferes with CSF circulation or absorption, abnormalities in protein and even cell count may be present. The CSF may also be altered by secondary or additional unrelated processes (Rishniw et al., 1994). For example, intraventricular hemorrhage can occur in hydrocephalic animals, producing xanthochromic CSF with an increased white blood cell count and protein content.

1. Intracranial Intra-arachnoid Cysts

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G. Metabolic/Nutritional Disorders

Cerebrospinal fluid analysis is not done commonly in animals with metabolic or nutritional neurologic disorders because most of these disorders are diagnosed from historical and physical findings and laboratory tests of blood and urine. When other procedures are nondiagnostic, or when therapy does not eliminate, or perhaps worsens, the neurologic dysfunction, CSF analysis is indicated to investigate other causes of the neurologic signs. In most cases, routine analysis of CSF associated with metabolic or nutritional disorders does not detect abnormalities (Fishman, 1992; Scott, 1995; Bichsel et al., 1984b; Vandevelde and Spano, 1977). Although brain edema is relatively common with some of these disorders (e.g., hypoxia, hyponatremia, or the osmotic dysequilibrium syndromes of hemodialysis or diabetic ketoacidosis), the edema is usually cytotoxic rather than vasogenic. Therefore, the blood–brain/CSF barrier is usually intact and CSF protein is normal. If edema is severe enough to result in brain ischemia, infarction, or herniation, the blood–brain/CSF barrier becomes dysfunctional, vasogenic edema occurs, and CSF protein rises. If neural necrosis ensues, the white blood cell count may also increase. Even in the (apparent) absence of vasogenic edema, blood–brain barrier leakage may occur, perhaps because of the biochemical effects of the disorder on the barrier cells. Animals and people with uremic or hepatic encephalopathies or hypothyroidism may have increased total protein with a normal IgG index (Fishman, 1992; Bichsel et al., 1984b, 1988). People with diabetic neuropathy may also have increased CSF protein (Fishman, 1992). Animals with severe metabolic encephalopathies often have seizures, and the effect of seizures on the CSF must also be considered (see Section VII.H.2). Disorders in which neural necrosis is a primary feature, such as the polioencephalomalacia of thiamine deficiency, typically have a pleocytosis and increased total protein (Bichsel et al., 1984b; George, 1996; deLahunta, 1983). Specific biochemical analysis of CSF may show abnormalities, such as abnormalities in osmolality or electrolyte content with salt or water intoxication (Mayhew, 1989; Kopcha, 1987), abnormalities in amino acid levels (such as glutamine) with hepatic encephalopathy (Schaeffer...
et al., 1991; Grabner et al., 1991), and elevated citrulline in bovine citrullinemia (Healy et al., 1990).

**H. Miscellaneous Conditions**

1. **Alterations in CSF Following Myelography**

Changes in the composition of CSF following myelography have been reported in people (Fishman, 1992) and animals (Widmer and Blevins, 1991; Burbidge et al., 1989). Most contrast agents are low-grade leptomeningeal irritants, resulting in leptomeningeal inflammation that is reflected in the CSF. By 90 minutes after myelography, the total white blood cell count and total protein can be elevated and the white blood cell differential count altered. The pleocytosis is typically a mixed mononuclear/neutrophilic response, with the proportion of mononuclear cells to neutrophils varying with the contrast agent used and the time interval after myelography. The pleocytosis may resolve within 10 days (Johnson et al., 1985), although individual animals may have a slightly increased total white blood cell count up to 14 days following contrast injection (Spencer et al., 1982; Wood et al., 1985). In contrast, one study of the contrast agents iohexol and iotrolan did not detect any alteration of total white blood cell count in CSF taken between 1 and 14 days following myelography (van Bree et al., 1991). The CSF specific gravity and Pandy test score can also be elevated, presumably partly because of the presence of the contrast media (Widmer et al., 1992). Increased CSF albumin and immunoglobulin levels may be due predominantly to blood–brain/CSF barrier leakage, and may return to normal levels within 5 days (Johnson et al., 1985). In summary, any alteration of CSF within the first week or two following myelography must be assessed cautiously.

2. **Seizures: Interictal and Postictal CSF Characteristics**

Patients with seizures due to progressive intracranial or some extracranial disorders typically have CSF changes reflecting the disorder. In contrast, the interictal CSF of patients with nonprogressive, intracranial disease should be normal. Postictal CSF is often abnormal, however. Pleocytosis of postictal CSF has been well documented in people (Fishman, 1992; Rider et al., 1995; Barry and Hauser, 1994). The white blood cell counts may be up to 80/μl with a neutrophilic component from 5 to 92%. The cell counts are highest at about 24 hours after the seizure. The mechanism of the pleocytosis is obscure (Fishman, 1992). Convulsive seizures, regardless of cause, may also induce a reversible increase in blood–brain/CSF barrier permeability, resulting in a transient elevation of CSF protein. Brain metabolism is also stimulated during the seizure, resulting in an increase in brain lactate production and a decrease in brain pH (Fishman, 1992). However, differentiating the effects of the local (brain) phenomena from the effects of systemic phenomena that occur during seizures (hypertension, acidosis, hypoxia, etc.) is difficult. For example, severe, experimental hyperthermia in dogs (core body temperature >41.2°C) results in increased CSF enzymes, calcium, and chloride, probably due to increased blood–brain/CSF barrier permeability (Deswal and Chohan, 1981). Interpretation of postictal CSF must be done cautiously because of the potential confusion of a postictal, "idiopathic epileptic" condition with a progressive disease that alters the CSF primarily. For children with seizures, the recommendation has been made that CSF with >20 white blood cell/μl or >10 polymorphonuclear cells/μl not be attributed to the seizures (Rider et al., 1995).

**I. Neoplasia**

The CSF associated with neoplastic conditions affecting the central nervous is quite variable, reflecting the variety of tumors, locations, and tissue reactions to the disease. The CSF is usually clear and colorless although xanthochromia may be present if hemorrhage has occurred. The total white blood cell count is often normal, but pleocytosis may occur, particularly with meningiomas and choroid plexus tumors (and occasionally other tumors) (Bailey and Higgins, 1986b; Carillo et al., 1986). Pleocytosis is usually monocellular although meningiomas may have >50% neutrophil (Bailey and Higgins, 1986b). Neural lymphosarcomatous tumors often have a lymphocytic/lymphoblastic pleocytosis (Lane et al., 1994; Williams et al., 1992; Couto et al., 1984), except in cattle, in which the tumor is usually extradural (Sherman, 1987). One report indicates the pleocytotic CSF is associated with a significantly shorter survival time than is normal or albuminocytologic CSF (Heidner et al., 1991). The commonest CS abnormality is increased total protein, with choroid plexus tumors producing the most marked elevation (Rand et al., 1994b; Bailey and Higgins, 1986b; Moor et al., 1994; Sarfaty et al., 1988; Mayhew, 1989; Brew et al., 1992; Waters and Hayden, 1990; Roeder et al., 1990; Kornegay, 1991). Dogs with neural neoplasia, particularly of the meninges or choroid plexus, commonly have blood–brain/spinal cord barrier disturbance and subsequently an increased albumin quotient (Sorjonen, 1987; Bichsel et al., 1984b; Sorjonen et al., 1991; Moore et al., 1994). In one study, this abnormality was most common with choroid plexus tumors and least common with astrocytomas (Moore et al., 1994).
Alpha and beta globulin levels are usually normal; gamma globulins are normal or mildly increased (Sorjonen, 1987; Sorjonen et al., 1991; Moore et al., 1994). The IgG index may be elevated, reflecting the presence of inflammatory infiltrates around the lesion (Tipold et al., 1993b; Bichsel et al., 1984b).

The CSF associated with spinal neoplasia is reported to be normal more often than is the CSF of brain tumors (Fingeroth et al., 1987; Luttgen et al., 1980; Schott et al., 1990). This finding may reflect the fact that most spinal neoplasia is extradural or that most spinal tumors are relatively small at the time of diagnosis. It may also reflect the site of CSF collection—that most of the samples are cerebellomedullary rather than lumbar, although many reports do not state the puncture site. Cerebrospinal fluid collected caudal to the lesion is abnormal more often than is CSF collected cranial to the lesion (Thomson et al., 1990).

J. Parasitic Diseases

1. Equine Protozoal Encephalomyelitis

Equine protozoal encephalomyelitis (EPM), caused by Sarcocystis neurona (S. falcatula (Dame et al., 1995)), is characterized by multifocal areas of mononuclear, perivascular inflammation and necrosis; severe lesions may be hemorrhagic and have neutrophilic infiltration (Mayhew and deLahunta, 1978; Madigan and Higgins, 1987). The CSF may be normal or have mild to moderate mononuclear pleocytosis and increase in total protein (Mayhew, 1989; Reed et al., 1994). Xanthochromia is occasionally present, as well as neutrophils and eosinophils (Mayhew, 1989). The CSF albumin concentration and albumin quotient are reported to be normal and the IgG index elevated, indicating intrathecal IgG production (Andrews and Provenza, 1995). Antibodies to S. neurona can be identified in the CSF by immunoblot analysis (Granstrom, 1993). The test is very sensitive and specific for the diagnosis of EPM; however, its accuracy depends on an intact blood–brain/CSF barrier because many horses have serum antibodies to S. neurona but do not have clinical disease (Andrews and Provenza, 1995; Fenger, 1995). Detection of S. neurona in the CSF by polymerase chain reaction provides definitive evidence of the presence of the parasite in the central nervous system. The results of polymerase chain reaction assay are independent of serum leakage across the blood–brain/CSF barrier (Fenger, 1994).

2. Neosporosis, Toxoplasmosis

Both Neospora and Toxoplasma can invade the central nervous system, causing necrosis, vasculitis and a multifocal, granulomatous meningoencephalomyelitis. Neospora seems to have more of a predilection for the central nervous system than Toxoplasma, particularly in young dogs (Dubey et al., 1989, 1988). The CSF associated with neural protozoal infections generally has a mild to moderate increase in white blood cell count and total protein. Typically, the white blood cell differential count shows a mixed pleocytosis with monocytes, lymphocytes, neutrophils, and eosinophils; in order of decreasing percentage (Rand et al., 1994a; Cuddon et al., 1992; Tipold, 1995; Vandevenelde and Spano, 1977; Kornegay, 1981; Dubey et al., 1990; Averil and deLahunta, 1971; Hass et al., 1989). Occasionally the white blood cell count and protein are normal (Tipold, 1995; Parish et al., 1987). The CSF IgG index was elevated in three of three dogs studied; in two of two dogs, the IgM was normal and the IgA was elevated (Tipold et al., 1994, 1993b). In a study of experimentally infected cats, T. gondii-specific IgG was intrathecal produced, but T. gondii-specific IgM was not detected (Munana et al., 1995). Antiprotozoal antibodies in the CSF may be detected by a variety of methods (Ruehlmann et al., 1995; Cole et al., 1993; Patton et al., 1991). However, the presence of antibodies does not necessarily indicate clinical disease (Munana et al., 1995; Dubey and Lindsay, 1993). Recently, polymerase chain reaction techniques have been developed to identify the protozoa in tissue and fluids, including CSF (Stiles et al., 1996; Parmley et al., 1992; Novati et al., 1994). Occasionally the organisms themselves may be seen in CSF cells (Dubey, 1990; McGlennon et al., 1990).

In considering the CSF abnormalities of toxoplasmosis and neosporosis, two issues must be kept in mind. First, reports of toxoplasmosis prior to 1988 (when Neospora was identified) must be carefully scrutinized because many of these cases were actually neosporosis. Second, because T. gondii is not a primary pathogen, clinical toxoplasmosis is relatively rare and is seen mostly in conjunction with a second disease, particularly canine distemper, which may itself alter the CSF (Dubey et al., 1989).

3. Migratory Parasites

Neural invasion by migratory parasites is relatively common in large and exotic animals, yet rare in dogs and cats. The CSF may reflect the physical trauma and consequent inflammatory response, and in some cases an immune reaction to the parasite tissue. The CSF abnormalities are variable and probably depend to some degree on the specific parasite, as well as on its location and the type of incited response. For example, H. bovis larvae in the cow normally lodge in the lumbar epidural space and their effect on the spinal cord may be primarily compression. The CSF in such a case could
be normal or have only mildly to moderately elevated protein. Parasites that actually invade neural tissue may leave the CSF unchanged or produce CSF with mild to marked pleocytosis and protein elevation, as well as xanthochromia. An eosinophilic pleocytosis is suggestive of parasitism, and typical of some parasites such Parelaphostrongylus (Mason, 1989; George, 1996; Pugh et al., 1995; Baum, 1994). However, eosinophilic pleocytosis is not pathognomonic for parasitism, nor does a lack of eosinophils in the CSF rule out neural parasitism (Lester, 1992; Braund, 1994; George, 1996; de Lahunta, 1983). The development of an ELISA technique using Parelaphostrongylus-specific immunoglobulin to detect parasite antigen in the CSF might be of diagnostic value (Dew et al., 1992).

**K. Toxicity**

Even though neurologic signs may occur, the CSF associated with toxicity is usually normal (e.g., cows with lead poisoning, tetanus, or botulism) (Fankhauser, 1962; Feldman, 1989). Mild elevations of the white blood cell count and protein may occur if the toxin causes breakdown of blood-brain/CSF barrier or neural degeneration or necrosis, such as in some cases of lead poisoning (Fankhauser, 1962; Swarup and Maiti, 1991; Dorman et al., 1990; Dow et al., 1989; George, 1996; Mayhew, 1989; Little and Sorensen, 1969). Lead has been shown to poison capillary endothelial cells selectively (Goldstein et al., 1977), as well as to cause cerebral cortical necrosis (Christian and Tryphonas, 1970). If necrosis is severe, the white blood cell count and the total protein can be markedly increased with a predominance of neutrophils, as with leukoencephalomalacia caused by moldy corn poisoning in horses. Xanthochromia is also a characteristic of moldy corn poisoning, reflecting the perivascular hemorrhages in the central nervous system (McCue, 1989; Masri et al., 1987). With toxicities, biochemical alterations of the CSF may occur more commonly than alterations of CSF cell counts or protein. At the onset of fatal signs of lead poisoning, CSF glucose, urea, creatinine, and creatine kinase levels are increased (Swarup and Maiti, 1991). Neurostimulatory toxins may result in elevated monoamine metabolites in the CSF (Sisk et al., 1990). Ivermectin toxicity producing recumbency in dogs elevates the CSF concentrations of homovanillic acid and 5-hydroxyindoleacetic acid (Vaughn et al., 1989a). Interestingly, copper poisoning in sheep does not produce significant increases in CSF copper, zinc, or iron levels (Gooneratne and Howell, 1979).

**L. Trauma/Compression of Neural Tissue**

The CSF abnormalities associated with trauma and/or compression are variable depending on the rate at which the neural insult developed, the degree of neural damage, the location of the lesion (particularly with respect to the CSF collection site), the elapsed time since the onset of the neural insult, and the maintenance or progression of the insult. With acute trauma the CSF may be pink and hazy or turbid, or actually bloody. After centrifugation, the supernatant can be clear. If hemorrhage occurred more than 48 hours prior to CSF collection, the supernatant may be yellow because of bilirubin. The total red blood cell count may be markedly elevated. The white blood cell count may be mildly to moderately elevated, reflecting either hemorrhage into the subarachnoid space or inflammation instigated by the trauma. Erythrophagocytosis may be present. The pleocytosis is usually a mixed ce population, and a substantial proportion of neutrophils (40–50%) is possible. Total protein may be moderately elevated because of disruption of blood vessels, interruption of CSF flow and absorption, and necrosis (Thomson et al., 1989; Green et al., 1993). Thus the CSF of acute trauma may have a distinct, inflammatory character. With spinal cord trauma/compression, lumbar CSF is more consistently abnormal than cerebellomedullary CSF (Thomson et al., 1990). Th-CSF abnormalities of chronic trauma or sustained compression tend to be milder than the abnormalities of acute damage. The white blood cell count may be normal or mildly elevated with generally a mixed or mononuclear pleocytosis. The cerebellomedullary CSF of horses with cervical stenotic myelopathy is reported to be hypocellular with a reduced number of lymphocytes (Grant et al., 1993). The CSF protein associated with chronic trauma or sustained neural compression may be normal to moderately elevated (Thomson et al., 1989; Mayhew, 1989). The albumin content and the albumin quotient of CSF associated with trauma compression may be normal or increased, the latter reflecting the vascular damage and edema (Sorjonen 1987; Sorjonen et al., 1991; Bichsel et al., 1984b; Andrew and Provenza, 1995). The gamma globulin percent and the IgG index are usually normal. Occasional elevations probably reflect the presence of inflammatory cells in the lesion (Tipold et al., 1993b; Bichsel et al., 1984b; Andrews and Provenza, 1995).

**References**

Altman, P. L., and Dittmer, D. S. (1974). "Biology Data Book," V. III, 2nd Ed., p. 197. Federation of American Societies for Experimental Biology, Bethesda, Maryland.

Ames, A. (1964). J. Neurophysiol. 27, 672.

Ames, T. R. (1987). Vet. Clin. North Am. Food Anim. Pract. 3, 61.

Andrews, F. M., and Provenza, M. (1995). Proc. ACVIM 13, 600.

Andrews, F. M., Maddux, J. M., and Faulk, D. (1990a). Prog. V. Neurol. 1, 197.

Andrews, F. M., Matthews, H. K., and Reed, S. M. (1990b). Veterinary Medicine 85, 1325.
Trotter, J. L., and Rust, R. S. (1989). In "The Cerebrospinal Fluid" (R. M. Herndon and R. A. Brumback, eds.), p. 179. Kluwer Academic Publishers, Boston.

Tsai, S. C., Summers, B. A., and Appel, M. J. G. (1982). Arch. Virol. 72, 257.

van Bree, H., Van Rijssen, B., and Van Ham, L. (1991). Am. J. Vet. Res. 52, 926.

Vandevelde, M., and Spano, J. S. (1977). Am. J. Vet. Res. 38, 1827.

Vandevelde, M., Zurbriggen, A., Steck, A., and Bichsel, P. (1986). J. Neuroimmunol. 11, 41.

Vaughn, D. M., Simpson, S. T., Blagburn, B. L., Whitmer, W. L., Heddens-Mysinger, R., and Hendrix, C. M. (1989a). Vet. Res. Commun. 13, 47.

Vaughn, D. M., Coleman, E., Simpson, S. T., and Satjawatcharaphong, C. (1988a). Am. J. Vet. Res. 49, 1302.

Vaughn, D. M., Coleman, E., Simpson, S. T., Whitmer, B., and Satjawatcharaphong, C. (1988b). Am. J. Vet. Res. 49, 2134.

Vaughn, D. M., Smyth, G. B. (1989). Vet. Res. Commun. 13, 413.

Vaughn, D. M., Smyth, G. B., Whitmer, W. L., and Satjawatcharaphong, C. (1988c). Vet. Res. Commun. 13, 237.

Vernau, K. M., Kortz, G. D., Koblik, P. D., LeCouteur, R. A., Bailey, C. S., and Pedroia, V. (1996). Veterinary Radiology & Ultrasound, in press.

Waters, D. J., and Hayden, D. W. (1990). J. Vet. Intern. Med. 4, 207.

Waxman, F. J., Clemmons, R. M., and Hinrichs, D. J. (1980). J. Immunol. 124, 1216.

Weisner, B., and Bernhardt, W. (1978). J. Neurol. Sci. 37, 205.

Welch, K. (1975). In "Advances in Neurology: Current Reviews" (W. J. Friedlander, ed.), p. 247. Raven Press, New York.

Welles, E. G., Tyler, J. W., Sorjonen, D. C., and Whatley, E. M. (1992). Am. J. Vet. Res. 53, 2050.

Welles, E. G., Pugh, D. G., Wenzel, J. G., and Sorjonen, D. C. (1994). Am. J. Vet. Res. 55, 1075.

Widmer, W. R., and Blevins, W. E. (1991). J. Am. Anim. Hosp. Assoc. 27, 163.

Widmer, W. R., DeNicola, D. B., Blevins, W. E., Cook, J. R., Cantwell, H. D., and Teclaw, R. F. (1992). Am. J. Vet. Res. 53, 396.

Williams, M. A., Welles, E. G., Gailor, R. J., Ewart, S. L., Humburg, J. M., Mullaney, T. P., Stickley, J., Chang, C. D., and Walter, G. L. (1992). Prog. Vet. Neurol. 3, 51.

Wilson, J. W. (1977). J. Am. Vet. Med. Assoc. 171, 200.

Wilson, J. W., and Stevens, J. B. (1977). J. Am. Vet. Med. Assoc. 171, 256.

Wilson, J. W., Kurtz, H. J., Leipold, H. W., and Lees, G. E. (1979). Vet. Pathol. 16, 165.

Wood, A. K., Farrow, B. R. H., and Fairburn, A. J. (1985). Acta Radiol. 26, 767.

Woodruff, K. H. (1973). Am. J. Clin. Pathol. 60, 621.

Yvorochuk, K. (1992). In "Current Therapy in Equine Medicine," 3rd ed. (N. E. Robinson, ed.), p. 569. Saunders, Philadelphia, Pennsylvania.