Cerebrospinal Fluid

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I. INTRODUCTION

The analysis of cerebrospinal fluid (CSF) has been described as the central nervous system (CNS) equivalent of the complete blood count (Jamison and Lumsden, 1988), and the analogy is appropriate. CSF analysis is a general index of neurological 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 neurological 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 seem to be related as much to the location of disease as to the cause or the severity of lesion; meningeal and paraventricular diseases generally produce greater abnormalities than deep parenchymal diseases. Previous therapy may affect the type, degree, and duration of CSF (Jamison and Lumsden, 1988) abnormalities as well. The CSF abnormalities identified are also dependent on the CSF collection site with respect to lesion location

(Thomson *et al.*, 1989, 1990). Lastly, the CSF of animals with neurological disease is not always abnormal (Tipold *et al.*, 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."

II. 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" of physical support and buoyancy. When suspended in CSF, a 1500-gm brain weighs only about 50 gm. 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; Milhorat, 1987; Rosenberg, 1990).

The direct transfer of brain metabolites into the CSF provides excretory function. 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 cells, such as bacteria or blood cells, by bulk CSF absorption (see Section II.D). The "sink" action of the CSF arises from the restricted access of water-soluble 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 (Davson and Segal, 1996; Fishman, 1992; Milhorat, 1987; Rosenberg, 1990).

Because CSF bathes and irrigates the brain, including those regions known to participate in endocrine functions, the suggestion has been made that 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 (Davson and Segal, 1996; Fishman, 1992; Milhorat, 1987; Rosenberg, 1990).

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); 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 (Davson and Segal, 1996; Fishman, 1992; Milhorat, 1987; Rosenberg, 1990).

III. 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 26-1). At these interfaces, selective 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 (Davson and Segal, 1996; Fishman, 1992; Milhorat, 1987; Rosenberg, 1990).

A. Anatomy of Brain-Fluid Interfaces

1. 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,

TABLE 26-1 Composition of the Brain–Fluid Interfaces

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

Modified from Rosenberg (1990).

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 pinocytotic 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 (Davson and Segal, 1996; Fishman, 1992; Milhorat, 1987; Rosenberg, 1990).

2. Blood-CSF Barrier

The epithelial cells of the circumventricular organs form one part of the blood-CSF interface. 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 (Davson and Segal, 1996; Fishman, 1992; Milhorat, 1987; Rosenberg, 1990). 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 other epithelia specialized for fluid transport, such as proximal renal tubular epithelium (Davson and Segal, 1996; Fishman, 1992; Milhorat, 1987; Rosenberg, 1990). Autonomic nerve terminals 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 is 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 is demonstrated by their tight junctions. Their transport function is indicated by giant intracellular vacuoles, some of which have both basal and apical openings, and pinocytotic vesicles. The sinus surface of a villus is covered by sinus endothelium (Milhorat, 1987; Rosenberg, 1990). Endothelium-lined channels may link directly with the subarachnoid space (Bell, 1995; Davson and Segal, 1996).

Arachnoid villi are not limited to intracranial venous sinuses but also are present at the spinal nerve roots penetrating into the spinal veins (Bell, 1995; Milhorat, 1987).

3. 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 (Davson and Segal, 1996; Milhorat, 1987).

B. CSF Formation

1. Choroidal and Extrachoroidal Formation

Cerebrospinal fluid is formed principally by the choroid plexuses, with a smaller amount formed extrachoroidally (Davson and Segal, 1996; Milhorat, 1987). Choroidal formation involves two processes that occur in series; first is filtration across the choroidal capillary wall, and second is 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 either by (1) intracellular movement across the epithelial membranes, or (2) intercellular movement across the apical tight junctions between epithelial cells. Both of these processes probably depend on 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 (Davson and Segal, 1996; Rosenberg, 1990). 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 pathological 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 (Milhorat, 1987; Rosenberg, 1990). The relative contributions of choroidal and extrachoroidal sources to CSF in normal and pathological conditions are not certain. Some investigators report the choroid plexus to be the major if not the sole source of CSF; whereas others conclude

TABLE 26-2 Rate of CSF Formation in Various Species[a]

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[b]	290
Monkey	28.6–41
Human being	350–370

Modified from Davson and Segal (1996).
[a] *Estimated by ventriculo-cisternal perfusion.*
[b] *Calhoun* et al. (1967).

TABLE 26-3 Factors Influencing CSF Formation

Effect	Substance or Condition	Site of Action
Increase	Cholera toxin	cAMP
	Phenylephrine[b]	Cholinergic pathways
Decrease	Acetazolamide, furosemide	Carbonic anhydrase
	Atrial natriuretic hormone	cGMP
	Diazepam analogue[c]	Choroidal benzodiazepine receptor
	Dopamine D_1 receptor agonist	Choroidal dopamine receptor
	Hyperosmolarity	Choroidal capillaries
	Hypothermia	Metabolism (decreased)
	Noradrenaline[a]	cAMP/choroidal Na^+-K^+ ATPase
	Omeprazole[b]	H^+-K^+ ATPase?
	Ouabain	Na^+-K^+ ATPase
	Serotonin receptor agonist	Choroidal serotonin receptor
	Steroids[b]	Choroidal Na^+-K^+ ATPase
	Vasopressin	Choroidal vasopressin (V_1) receptor

Modified from Fishman (1992).
[a] *Nilsson* et al. (1992).
[b] *Davson and Segal (1996).*

that at least one-third of newly formed CSF is extrachoroidal (Davson and Segal, 1996; Milhorat, 1987).

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 26-2) (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 26-3), but their clinical utility is limited either by their time frame of action or toxicity (Davson and Segal, 1996; Pollay, 1992; Rosenberg, 1990).

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 (foramen of Monro) into the third ventricle, then through the mesencephalic aqueduct (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 (Fankhauser, 1962; Fletcher, 1993). 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 (i.e., ions, proteins, and drugs). Bulk absorption occurs directly into the venous system and depends primarily

TABLE 26-4 Total White Blood Cell Count of Normal CSF in Domestic Animals

Species	Collection Site[a]	N[b]	Cells/ul[c]	Reference
Dog	C	50	0–2	Jamison and Lumsden, 1988
Dog	C, L	31	0–4	Bailey and Higgins, 1985
Cat	C	33	0–2	Rand *et al.*, 1990*b*
Horse	Pooled C & L	44	0–6	Mayhew, 1977
Horse	C	14	0–5	Furr and Bender, 1994
Cow	L	16	0.85–3.52[d]	Welles *et al.*, 1992
Llama	L	17	0–3	Welles *et al.*, 1994
Sheep	L	NS	0–5	Fankhauser, 1962
Goat	NS	NS	0–4	Brewer, 1983
Pig	NS	NS	0–7	Fankhauser, 1962
Ferret	C	42	0–8	Platt *et al.*, 2004
Holsten calf, 8 weeks old	C	10	0–10	St. Jean *et al.*, 1995

[a] C = *cerebellomedullary cistern.* L = *lumbar subarachnoid space.*
[b] N = *number of animals.*
[c] *Range.*
[d] *95% confidence interval.*
NS = *not stated.*

on 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 nerves) and spinal nerves. Perineural absorption may be through arachnoid villi projecting into perineural veins, lymphatics, or connective tissue (Davson and Segal, 1996; Milhorat, 1987). The importance of these various absorption routes varies with the species (Bell, 1995).

Absorption through the arachnoid villi occurs transcellularly through micropinocytotic vesicles and giant intracellular vesicles, but it 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 size 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. Solutes may also be cleared from the CSF by diffusion into adjacent brain cells or capillaries (Fishman, 1992; Milhorat, 1987).

IV. CELLULAR COMPOSITION OF NORMAL CSF

A. Total Erythrocyte and Nucleated Cell Count

Cerebrospinal fluid normally does not contain erythrocytes (Chrisman, 1992; Cook and DeNicola, 1988; Rand *et al.*, 1990b; Wilson and Stevens, 1977). Erythrocytes in a CSF sample are most commonly iatrogenic, because of trauma associated with the needle placement. However, CSF erythrocytes may also originate from pathological hemorrhage. The normal nucleated cell count of CSF in domestic animals is in Table 26-4. The most widely accepted reference ranges for the numbers of leukocytes in the CSF of dogs and cats is 0 to 5 cells/µL (de Lahunta, 1983; Oliver and Lorenz, 1993) to 0 to 8 cells/µL (Duncan, 1994). However, these ranges are too broad in our experience and other studies confirm this (Jamison and Lumsden, 1988). Jamison examined 50 clinically and histopathologically normal dogs and derived cerebellomedullary CSF reference limits of 0 to 2 cells/µL (Jamison and Lumsden, 1988). In fact,

all except one of these dogs had counts of 0 to 1 cells/μL (personal communication). Bailey and Higgins examined 31 dogs that were clinically and histopathologically normal. For cerebellomedullary CSF, the mean nucleated cell count was 1.45 cells/μL with the 95% confidence intervals 1.04 to 1.86, and the observed range 0 to 4 cells/μL. Twenty-six of 31 dogs had counts between 0 to 2 cells/μL. They also found that lumbar CSF had a significantly lower nucleated cell count, with a mean of 0.55 cells/μL, a 95% confidence interval of 0.22 to 0.88 and an observed range of 0 to 4 cells/μL, although 30/31 dogs had counts of 0 to 2 cells/μL (Bailey and Higgins, 1985). In our opinion, a normal nucleated count for cerebellomedullary CSF in dogs is 0 to 2 cells/μL, with 3 cells/μL being questionably abnormal and 4 cells/μL definitely abnormal.

Rand and colleagues (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 to 2 cells/μL. Thirty of 33 cats had counts of 0 cells/μL. Three cells or more per microliter is therefore abnormal in feline cerebellomedullary CSF.

B. Differential Cell Count

1. Leukocytes

Excellent morphological descriptions of the cell types normally found in the CSF of domestic animals can be found elsewhere (Cook and DeNicola, 1988; Jamison and Lumsden, 1988; Rand *et al.*, 1990b). 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 individual variation. In cats (Jamison and Lumsden, 1988; Rand *et al.,* 1990b), (unreactive) macrophages 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 (macrophages) 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 literature published before 1975 that focused on humans, any neutrophils present 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 cytocentrifugation, 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 may be a 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 in animals with normal total nucleated cell counts. Large foamy activated macrophages or phagocytes are not seen in normal CSF (Christopher *et al.*, 1988; Fishman, 1992), and their presence is nonspecific evidence of an inflammatory disorder. Plasma cells are not seen in normal CSF (Fishman, 1992; Kjeldsberg and Knight, 1993; Pelc *et al.*, 1981). Their presence indicates underlying inflammatory disease. In people, plasma cells are seen particularly in acute viral disease and various chronic inflammatory conditions including tuberculous meningitis, syphilis, multiple sclerosis, and the Guillain-Barré syndrome (Kjeldsberg and Knight, 1993; Pelc *et al.*, 1981). In animals, plasma cells have been observed in various conditions including distemper (Vandevelde and Spano, 1977), other viral meningitis (Bichsel *et al.*, 1984a; Vandevelde and Spano, 1977), rabies (Green *et al.*, 1992), granulomatous meningoencephalomyelitis (Bailey and Higgins, 1986a; Vandevelde and Spano, 1977), neoplasia, and abscesses (W. Vernau, 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, immune mediated 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 leptomeninges, 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; Garma-Avina, 2004; Kjeldsberg and Knight, 1993). The majority of lining cells seen in normal CSF is choroid plexus cells (Kjeldsberg and Knight, 1993). Excellent descriptions and illustrations of these cell types can be found elsewhere (Cook and DeNicola, 1988; Garma-Avina, 2004; Kjeldsberg and Knight, 1993; Rand *et al.*, 1990b). Chondrocytes are occasionally observed in CSF sampled by lumbar puncture, likely resulting from the spinal needle puncturing the intervertebral disk (Bigner and Jonston, 1981). Squamous cells can be observed in CSF and may be due to skin contamination or an underlying pathological process such as epidermoid cysts (Kornegay and Gorgacz, 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 bone marrow. 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 was observed in the choroid plexus of five dogs that did not have underlying hematological 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 neural tissue may be observed in the CSF of people (Bigner and Jonston, 1981) and also in cerebellomedullary cisternal samples associated with traumatic CSF taps in animals (Fallin *et al.*, 1996). White matter in CSF is more common in lumbar versus cisternal CSF samples in dogs, most likely because of the sampling method,[1] although the presence of underlying malacia is another potential cause (Mesher *et al.*, 1996). White matter in the CSF is not correlated with a negative prognosis when compared to dogs without white matter in the CSF.[1]

V. BIOCHEMICAL CONSTITUENTS OF NORMAL CSF

Because CSF is a product of plasma filtration and membrane secretion, its composition is different from 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 26-5 through 26-8. 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 (e.g., 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 endothelial 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

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[1] Zabolotzky, S., Vernau, W., Vernau, K. M., *et al.*, manuscript in preparation.

of the extracellular space of the brain, and the lipid-solubility of the substances (Davson and Segal, 1996; Fishman, 1992). Protein also decreases with age in foals and puppies (Furr and Bender, 1994; Meeks *et al.*, 1994; Rossdale *et al.*, 1982). In contrast, two studies of calves found that CSF protein increased with age (Binkhorst, 1982; St. Jean *et al.*, 1995). Foals also had xanthochromia and a higher CSF glucose and creatine kinase level than adults (Furr and Bender, 1994; Rossdale *et al.*, 1982). The white blood count (WBC) decreased with age in puppies and calves (Binkhorst, 1982; Meeks *et al.*, 1994).

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 (R_{CSF}) of Na^+, Mg^{2+}, and Cl^- increase with age. The R_{CSF} of K^+, HCO_3^-, and urea decrease. In some instances, however (e.g., Cl^- and K^+), changing plasma levels of these substances contribute to the change in the R_{CSF}. The R_{CSF} of total protein, as well as 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 R_{CSF} 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 Cerebrospinal Fluid

Proteins identified in the CSF are given in Tables 26-9 and 26-10. 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 daltons are largely excluded. However, Felgenhaur (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) and transferrin, which are also synthesized by the choroid 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 on the blood-brain/CSF

TABLE 26-5 Biochemical Constituents of CSF of the Dog[a]

Constituent	Tipold et al., 1994	Sorjonen, 1987	Bailey and Higgins, 1985	Bichsel et al., 1984b	Sorjonen et al., 1991	Krakowka et al., 1981	Coles, 1980	Bleich, 1964	Fankhauser, 1962
Methods									
# RBC/μl	NS	≤10	<1500	0	≤10	0	NS	NS	NS
Necropsy	NS	Yes	Yes	NS	NS	Yes	NS	NS	NS
Total protein (mg/dl)									
Cerebellomedullary		27 ± 4.2 (23–35)	13.97 ± 4.54 (3–23)		29.9 ± 1.57 (23–38.5)	27.6 ± 1.1SE (15.5–42)			27.5 (11–55)
Lumbar			28.68 ± 5.52 (18–44)						
Method		Coomassie brilliant blue	Coomassie brilliant blue		Micro-Lowry	Coomassie brilliant blue			NS
Albumin (mg/dl)		37 ± 4.29% (31–44%)		17.1 ± 6.7 (7.5–27.6)	12.43 ± 0.96[b] (10.5–17.4) 11.27 ± 1.0[c] (7.8–19.0)	10.28 ± 0.8SE (5.8–18.9)			27 (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)				9.0 (5.5–16.5)
IgG (mg/dl)				0.85 ± 0.14 (0.71–1.09)	4.68 ± 0.68 (2.5–8.5)	1.16 ± 0.1SE			
IgG index	0.7 ± 0.3 (0.2–1.3)			0.38 ± 0.24 (0.15–0.9)					
IgM (μg/ml)	1.7 (0–5.8)				0	0			
IgA (μg/ml)	0.08(0–0.2)				0	0			

Constituent	Value
Alanine transferase (Reitman-Frankel units)	13.7 ± 1.35SE (2–32)
Aspartate transferase (Reitman-Frankel units)	20.1 ± 1.64SE (9–46)
Creatine kinase (SU)	<1
Bicarbonate (mEq/L)	23.5 ± 0.19SE
Calcium (mg/dl)	6.56 (5.13–7.40)
Chloride (mEq/L)	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 (2.82–3.47)
Potassium (mEq/L)	3.3 ± 0.04SE
Sodium (mEq/L)	153 ± 0.5SE
Glucose (mg/dl)	74 (61–116)
pH	7.36
Urea (mg/dl)	10–11
Specific gravity	1.005 (1.003–1.012)

[a] *Mean ± 1 SD, observed range in parentheses, unless otherwise noted. Values are for cerebellomedullary fluid unless otherwise noted.*
[b] *By electrophoresis.*
[c] *By radial immunodiffusion.*
NS = *not stated.*

TABLE 26-6 Biochemical Constituents of CSF of the Cat[a]

Constituent	Rand *et al.*,1990b	Hochwald *et al.*,1969	Ames, 1964	Fankhauser, 1962
Methods				
# RBC/μl	<30	NS		NS
Necropsy	Yes	No		NS
Total protein (mg/dl)				
Cerebellomedullary	18 ± 7[b]	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		
γ-Globulin (mg/dl $\pm$ SD)				
Cerebellomedullary		1.2 ± 0.27		
Lumbar		1.6 ± 0.30		
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/L)	17 ± 7			
Reference range	0–34			
Creatine kinase (U/L)	47 ± 51[b]			
Reference range	2–236			
Lactate dehydrogenase (U/L)	12 ± 5[b]			
Reference range	0–24			
Calcium (mEq/kg $H_2O \pm$ SE)			1.50 ± 0.06	5.2 mg/dl
Chloride (mEq/kg $H_2O \pm$ SE)			144 ± 2	900 mg/dl
Magnesium (mEq/kg $H_2O \pm$ SE))			1.33 ± 0.02	
Potassium (mEq/kg $H_2O \pm$ SE)			2.69 ± 0.09	
Sodium (mEq/kg $H_2O \pm$ SE)			158 ± 4	
Glucose (mg/dl)	74.54 ± 23.6			85
Reference range	18.2–130.9			
pH				Slightly alkaline

[a] *Mean$\pm$1 SD, observed range in parentheses, unless otherwise noted. Values are for cerebellomedullary fluid unless otherwise noted.*
[b] *Significantly correlated with CSF RBC count.*
NS = *not stated.*

barrier to macromolecules. When total CSF protein increases, the albumin concentration increases 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 26.9). The origin of tau protein (beta$_2$ transferrin) is uncertain. This protein may be modified serum transferrin (beta$_1$ 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 changes in the concentrations of these globulins and specific neurological disease (Fishman, 1992; Sorjonen *et al.,* 1991). Thus, their measurement has limited clinical use at this time.

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 26.9). The gamma globulin fraction contains immunoglobulins. Immunological assays identify three major immunoglobulins

TABLE 26-7 Biochemical Constituents of CSF of Foals and Horses[a]

Constituent	Rossdale et al., 1982	Andrews et al., 1994	Andrews et al., 1990a	Andrews et al., 1990b	Rossdale et al., 1982	Mayhew, 1977[b]	Fankhauser, 1962
Age	<40 hrs[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
Necropsy	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] 5–100[e]–0.46 ± 13.7 (LS-CM difference)	47.58 (28.75–71.75)
Lumbosacral		83.6 ± 16.1 (60.5–116)	93 ± 16 (65–124)				
Method	Biuret	Coomassie brilliant blue	Coomassie brilliant blue		Biuret	TCA	NS
Albumin (mg/dl ± SD)							
Cerebellomedullary		52.0 ± 8.6 (34–64)	35.8 ± 9.7 (24–51)				38.64 (22.62–67.94)
Lumbosacral		53.8 ± 15.7 (30–92)	37.8 ± 11.2 (24–56)				
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(3.37–18.37)
IgG (mg/dl±SD)							
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 (0.095–0.942)	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 0–8[e]	
Aspartate transferase (IU)							
Cerebellomedullary	16.6 ± 7.6 (6–26)			4–16[e]	18.27 ± 10.8 (7.5–30)	30.74 ± 6.31 SFU 15–50[e]	
Lumbosacral				0–16[e]			

(*continued*)

TABLE 26-7 Continued

Constituent	Rossdale et al., 1982	Andrews et al., 1994	Andrews et al., 1990a	Andrews et al., 1990b	Rossdale et al., 1982	Mayhew, 1977[b]	Fankhauser, 1962
Creatine kinase (IU)							
Cerebellomedullary	15.2 ± 9.2 (4–33)			0–8[e]	5.78 ± 3.7 (3.2–11)	1.08 ± 3.13 0–8[e]	
Lumbosacral				0–8[e]			
γ glutamyl transferase (IU)	1.5 ± 1.5 (0.9–2.3)				2.45 ± 1.9 (0.8–4.2)		
Lactate dehydrogenase (IU)	23.2–10.7 (10–40)			0–8[e]	27.7 ± 8.0 (12–34)	1.54 ± 1.75 0–8[e]	
Calcium (mg/dl)						4.18 ± 0.87 2.5–6.0[e]	6.26 (5.55–6.98)
Chloride (mEq/L)	109 ± 3.4 (104–113)				103.3 ± 13.5 (92–116)	109.22 ± 6.90 95–123[e]	737 mg/dl (690–792)
Magnesium (mg/dl)							1.98 (1.06–2.95)
Phosphorus (mg/dl)						0.83 ± 0.20 0.5–1.5[e]	1.44 (0.87–2.20)
Potassium (mEq/L)	3.6 ± 2.1 (1.3–4.6)				2.9 ± 0.6 (1.9–3.9)	2.95 ± 0.05 2.5–3.5[e]	12.66 mg/dl (10.65–14.20)
Sodium (mEq/L)	142.6 ± 2.8 (139–147)				143.9 ± 2.6 (139–147)	144.58 ± 1.86 140–150[e]	
Cholesterol (mg/dl)						4.76 ± 5.7 20–20[e]	0.36–0.55
Glucose (mg/dl)							
Cerebellomedullary				35–70% of blood glucose[e]		48.0 ± 9.92 30–70[e]	57.2 (40–78)
Lumbosacral				35–70% of blood glucose[e]		55.13 ± 8.22 40–75[e]	
Lactic acid (mg/dl)							
Cerebellomedullary				1.92 ± 0.12			
Lumbosacral				2.3 ± 0.21			
Specific gravity				1.003–1.005[e]			1.004–1.008
pH							7.13–7.36
Urea nitrogen (mg/dl)						11.82 ± 3.26 0–20[e]	

LS = *lumbar subarachnoid space* CSF. CM = *cerebellomedullary cistern* CSF.

[a] *Mean ± 1 SD, observed range in parentheses, unless otherwise noted. Values are for cerebellomedullary fluid unless otherwise noted.*
[b] *Except where noted, values are for pooled cerebellomedullary and lumbosacral fluid.*
[c] *Spontaneously delivered.*
[d] *Total protein for ponies – 60.48±20.45, reference range 20–105 (significantly different from horses).*
[e] *Reference range.*
NS = *not stated.*

TABLE 26-8 Biochemical Constituents of CSF of the Cow, Sheep, Goat, Pig, and Llama[a]

Constituent	Cow Welles *et al.*, 1992[b]	Sheep Altman and Dittmer, 1974[c]	Goat Altman and Dittmer, 1974[c]	Pig Altman and Dittmer, 1974[c]	Llama Welles *et al.*, 1994[d]
Methods					
# RBC/µl	(5–1,930)	NS	NS	NS	(0–1,360)
Necropsy	No	NS	NS	NS	No
Total protein (mg/dl)					
Cerebellomedullary		(8–70)	12	(24–29)	
Lumbosacral	39.16 ± 3.39 (23.4–66.3)				43.1 ± 9.0 (31.2–66.8)
Method	Coomassie brilliant blue	NS	NS	NS	Coomassie brilliant blue
Albumin (mg/dl)					
Cerebellomedullary				(17–24)	
Lumbosacral	15.75 ± 1.53% (8.21–28.71)				17.9 ± 4.45 (11.8–27.1)
Albumin quotient					0523 ± 0.114 (0.38–0.75)
Globulin (mg/dl)				(5–10)	
γ-Globulin (mg/dl)	4.84 ± 0.44% (2.46–8.85)				6.4 ± 2.50 (3.4–13.8)
IgG (mg/dl)	9.49 ± 1.03i (4.88–16.57)				
Creatine kinase (U/L)	11.44 ± 3.43 (2–48)				4.6 ± 4,69 (0.0–15.0)
Lactate dehydrogenase (U/L)	13.94 ± 1.318 (2–25)				13 ± 5.6 (7–24)
Calcium (mg/dl)		5.6 ± 0.3			
Chloride (mEq/L)		832 mg/dl (750–868)	681 mg/dl		134 ± 6.5 (116–143)
Magnesium (mg/dl)	1.99 ± 0.03 mEq/L (1.8–2.1)	2.88			
Potassium (mEq/L)	2.96 ± 0.03 (2.7–3.2)				3.19 ± 0.10 (2.9–3.3)
Sodium (mEq/L)	140 ± 0.78 (132–142)				154 ± 5.8 (134–160)
Glucose (mg/dl)	42.88 ± 0.99 (37–51)	(48–109)	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.*
[b] *Lumbosacral fluid. Mean ± SEM.*
[c] *Cerebellomedullary fluid.*
[d] *Lumbosacral fluid.*1
NS = *not stated.*

TABLE 26-9 Cerebrospinal Fluid Proteins Identified by Electrophoresis (Top Row) and Immunoelectrophoresis (Underlying Columns)

Transthyretin (Prealbumin)	Albumin	Alpha$_1$ Globulin	Alpha$_2$ Globulin	Beta Globulin	Gamma Globulin
Transthyretin	Albumin	Alpha$_1$ antitrypsin	Alpha$_2$ macroglobulin	Beta lipoprotein	IgG
		Alpha$_1$ lipoprotein	Alpha$_2$ lipoprotein	Transferrin	IgA
		Alpha$_1$ glycoprotein	Haptoglobulin	Tau protein (modified transferrin)	IgM
			Ceruloplasmin	Plasminogen	
			Erythropoietin	Complement	IgD
				Hemopexin	IgF
				Beta-trace	Gamma-trace

Modified from Fishman (1992).

TABLE 26-10 Protein Content of Cerebellomedullary Cisternal CSF of Healthy Dogs, Cats, and Horses as Identified by Electrophoresis[a]

Protein fraction	Dog Sorjonen, 1987	Cat Rand *et al.,* 1990*a*	Horse, Kristensen and Firth, 1977
Prealbumin			2.0 ± 0.9
Albumin	37 ± 4.29 (31–44)	11 ± 15 (1–53)	43.4 ± 6.8
Alpha globulin	28 ± 5.27 (24–31)	21 ± 11 (0–48)	
Alpha$_1$			5.3 ± 1.3
Alpha$_{2a}$			3.3 ± 0.8
Alpha$_{2bc}$			6.4 ± 1.8
Beta globulin	25 ± 5.31 (19–30)	57 ± 15 (37–91)	
Beta$_1$			17.0 ± 3.2
Beta$_2$			7.8 ± 2.3
Gamma globulin	7.75 ± 1.84 (6–9)	12 ± 7 (0–29)	14.8 ± 3.3

[a] *Mean* ± SD *percentage of total CSF protein; range in parentheses.*

in normal CSF: IgG, IgM, and IgA. Minute amounts of other immunoglobulins have also been detected in normal CSF (Fishman, 1992; Kjeldsberg and Knight, 1993).

a. IgG

The major immunoglobulin in normal CSF is IgG, which normally originates from the serum. An increased level of CSF gamma globulin is 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 it 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. IgM and IgA

Cerebrospinal fluid IgM and 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). 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. IgM is also the first immunoglobulin to return to normal when the offending antigen disappears. The characteristics of IgM and IgA participation in the intrathecal immune response still need to be resolved, however (Felgenhauer, 1982; Tipold and Jaggy, 1994).

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 neurological disease processes. The utility of assaying these proteins in clinical veterinary or human medicine has yet to be established (Fishman, 1992; Kjeldsberg and Knight, 1993).

C. Glucose

CSF glucose is derived solely from the plasma by facilitated diffusion. The concentration of CSF glucose depends on 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% to 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 2h; thus, ideally, plasma glucose should be determined about 1h before 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). In people, a CSF:serum glucose ratio less than 0.4 to 0.5 is abnormal and associated with bacterial and fungal infections, as well as metastasis to the leptomeninges (Deisenhammer et al., 2006). CSF glucose and serum CSF: glucose ratio is not routinely used in veterinary medicine, possibly because of the lack of specificity and availability of more specific tests in most instances.

D. Enzymes

Numerous enzymes have been assayed in the CSF of animals (see Tables 26.5 to 26.8) (Jackson et al., 1996; Lobert et al., 2003; Rand et al., 1990a; Wilson, 1977) and people (Banik, 1983). These enzymes have three possible sources: (1) blood, (2) neural tissue or neural tumors, and (3) cells within the CSF (Fishman, 1992; Kjeldsberg and Knight, 1993). The blood enzyme levels are usually 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 red blood count (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). CSF lactate is independent of blood glucose, and it may be measured in people and animals (Deisenhammer et al., 2006; Lobert et al., 2003). In people, blood:CSF lactate ratio may be elevated with mitochondrial disease and correlates inversely with the blood:CSF glucose ratio. To date, none of the enzyme assays is sufficiently sensitive or specific to warrant routine use in clinical practice (Fishman, 1992; Indrieri et al., 1980; Jackson et al., 1996; Kjeldsberg and Knight, 1993; Rand et al., 1994a).

E. Neurotransmitters

Because they are produced by neurons, neurotransmitters and their metabolites have been extensively studied in people for their potential use as markers of neuronal activity and neurological and psychiatric disease (Davis, 1990). The concentrations of several neurotransmitters (e.g., γ-aminobutyric acid [GABA], glutamate, aspartate and 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 (Bardon and Ruckebusch, 1984; Ellenberger et al., 2004; Faull et al., 1982; Holt et al., 2002; Loscher and Schwartz-Porsche, 1986; Podell and Hadjiconstantinou, 1997; Ruckebusch and Costes, 1988; Ruckebusch and Sutra, 1984; Sisk et al., 1990; Vaughn et al., 1988a, 1989). Some metabolite concentrations have a gradient along the neuraxis (Ruckebusch and Costes, 1988; Ruckebusch and Sutra, 1984; Vaughn et al., 1988b; Vaughn and Smyth, 1989), and some are age-related (Ruckebusch and Costes, 1988; Smyth et al., 1994; Vaughn and Smyth, 1989).

Despite intense interest, more research is needed to verify the clinical utility of assay of these substances in the CSF (Fishman, 1992; Kjeldsberg and Knight, 1993).

F. Other CSF Constituents

Many other substances have been measured in CSF in experimental and clinical situations. These include electrolytes, gases, organic and amino acids, ammonia, urea, creatinine, prostaglandins, cytokines, and hormones. Assay of these substances is not particularly helpful in the diagnosis of neurological disease in people because the substances are not generally associated with specific disease (Fishman, 1992; Kjeldsberg and Knight, 1993). Some substances, such as S-100B, a calcium-binding protein, have been used as a marker protein in

people with brain injury, but its utility in veterinary medicine is unproven (Shimada *et al.*, 2005). The usefulness of these substances in veterinary medicine has yet to be established.

G. Concentration Gradient along the Neuraxis

In cats (Hochwald *et al.*, 1969), dogs (Bailey and Higgins, 1985; Vaughn *et al.*, 1988b), horses (Andrews *et al.*, 1990a; Vaughn and Smyth, 1989), rhesus macaques (Smith and Lackner, 1993), and people (Davson and Segal, 1996; Fishman, 1992), the total protein concentration increases along the neuraxis from rostral to caudal. For example, in people the total protein concentration of ventricular, cerebellomedullary cistern, and lumbar subarachnoid fluid is about 26, 32, and 42 mg/dl, respectively (Weisner and Bernhardt, 1978). Total protein, albumin, and globulin content of cerebellomedullary cistern and lumbar subarachnoid CSF for dogs, cats, and horses is given in Tables 26.5, 26.6, and 26.7, 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 of 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 $>3/\mu$l, and none of the dogs was necropsied to verify its health. Therefore, some of these dogs may have had subclinical neurological 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.*, 1988a).

VI. CSF COLLECTION AND ANALYTICAL TECHNIQUES

A. Collection

1. *General Techniques*

Specific details about the collection of CSF from the various species are covered in many excellent articles and textbooks (Boogerd and Peters, 1986; Brewer, 1983, 1987, de Lahunta, 1983; Fowler, 1989; Holbrook and White, 1992; Kornegay, 1981; Mayhew, 1989) and will not be covered here except for the authors' preferred technique for collection from the cerebellomedullary cistern of dogs and cats (discussed later). Considerations that apply regardless of species are sterility, use of a specialized spinal needle, and collection from animals with increased intracranial pressure. To prevent iatrogenic central nervous system infection, sterility during the collection procedure is essential. 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 site is encountered. Additionally, the use of a fenestrated drape is highly recommended. Spinal puncture is contraindicated in an area of severe pyoderma/furunculosis or cellulitis. A needle with a stylet (spinal needle) should be used to prevent implantation of a plug of epidermis in the subarachnoid space that not only could lead to infection but also could seed an epidermoid tumor. Replacement of the stylet upon withdrawal is controversial, either preventing or causing entrapment and severance or dislocation of nerve root filaments (Fishman, 1992). Collection of CSF from animals with increased intracranial pressure may result in brain herniation. Appropriate anesthetic agents, hyperventilation, and mannitol (to treat intracranial hypertension) 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*

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.

The choice of collection site is influenced by the species and breed of animal, the location of the neurological lesion, and anesthetic considerations. The size of some animals may make lumbar subarachnoid puncture difficult, if not impossible. However, cerebellomedullary puncture usually can be accomplished even in large or obese animals. Because of differences in anatomy, the type or breed influences the exact site for lumbar puncture in the dog; L4-5 is

recommended for large breed, nonchondrodystrophic dogs (e.g., German shepherd dogs), whereas L5 to 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 thoracolumbar 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 (Scott, 1992; Thomson *et al.*, 1989, 1990). Occasionally, CSF is collected from both sites. Although the order of collection (cerebellomedullary or lumbar CSF collected first) appears not to influence significantly the analytical results (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.

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 right lateral recumbency, 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 (flex the neck) and push the external occipital protuberance toward the operator. This procedure flexes the atlantooccipital 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 atlantooccipital area.

The clinician faces the dorsal aspect of the patient's neck, kneeling on a pad. The external occipital protuberance, the C2 spinous process, and the C1 vertebral arch are palpated. The latter 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. The needle should be advanced slowly

and the stylet removed regularly. A "pop" may be palpated when the dura mater is punctured with the needle. The clinician should hold the spinal needle with one hand (to hold it steady) and remove the stylet with the other hand. The CSF should be allowed to drip out of the spinal needle into a tube. A volume of at least 0.5 mLs should be collected for a full CSF analysis (partial analysis may be done with smaller volumes). Larger volumes may be collected for other tests such as culture and sensitivity, polymerase chain reaction for infectious agents, antigen/antibody testing, immunophenotyping, and clonality assessment. To collect CSF for culture and sensitivity testing, aspirate CSF directly from the spinal needle hub using a needle and syringe.

B. Physical Examination: Clarity, Color, and Viscosity

After collection, the CSF is examined visually and the color, clarity, 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 it becomes important as the delay between collection and processing increases. A complete cytological examination includes both a total and differential cell count, as well as thorough morphological assessment. A differential and thorough morphological assessment should be done 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 utilizing cytocentrifugation, about 25% of canine CSF samples with cell counts in the normal range had abnormalities in cell type or morphology (Christopher *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 nucleated cell counts (Bichsel *et al.*, 1984b; Grevel and Machus, 1990). CSF samples should be processed as soon as possible after collection. Cells degenerate quickly in CSF (Chrisman, 1992; Fishman, 1992; Fry

et al., 2006; Kjeldsberg and Knight, 1993; Steele *et al.*, 1986), likely secondary to the CSF hypotonicity and very low protein content (in nonpathological specimens). Proteins and lipids tend to have a membrane stabilizing effect (Fry *et al.*, 2006; Steele *et al.*, 1986). A multitude of veterinary references state that CSF must be processed within 30 min of collection (Chrisman, 1983; Cook and DeNicola, 1988; Oliver and Lorenz, 1993; Thomson *et al.*, 1990). However, these references do not cite scientific data to support this statement. One study systematically evaluated the effects of time, initial composition and stabilizing agents on the results of abnormal (TNCC $\geq$ 5 cells/ul) canine CSF evaluation (Fry *et al.*, 2006). Statistically significant changes ($p = <0.05$) in the total nucleated cell count were not noted at any time point (0, 2 h, 4 h, 8 h, 12 h, 24 h, and 48 h) in unaltered (refrigerated) CSF, CSF with added fetal calf serum, or CSF with added hetastarch. However, differential cell percentages deteriorated in a time dependent fashion and macrophages were the most labile cell type in this study with their differential percentage being significantly decreased by 2 h. Concurrently, the percentage of unrecognizable cells was significantly increased at 2 h. At 12 and 24 h, the percentages of lymphocytes and neutrophils, respectively, were significantly decreased. Samples with a higher protein concentration ($\geq$50 mg/dl) were less susceptible to deterioration than those with a lower protein concentration ($<$50 mg,dl). The addition of fetal calf serum or hetastarch improved the stability of the CSF. Ultimately, the authors supported the contention that CSF should be analyzed as soon as possible post collection but that delays of 4 to 8 h were unlikely to alter the overall clinical interpretation (Fry *et al.*, 2006). If the protein concentration is $>$50 mg/dL, the analysis may be delayed up to 12 h without altering the overall clinical interpretation as the mean percentage of unrecognizable cells was only 6% at this time point (versus 33% in samples with protein concentration $<$50 mg/dl) (Fry *et al.*, 2006). Several reports recommend altering CSF processing when it is not analyzed within 1 h of collection (Bienzle *et al.*, 2000; Fry *et al.*, 2006). If there is a delay in processing, CSF samples should be divided into two aliquots. The unaltered aliquot should be submitted for TNCC and protein concentration. The second aliquot should be treated by the addition of either 20% fetal calf serum or 10% autologous serum, and the differential cell counts and morphology should be assessed on the second altered aliquot.

In a study of feline CSF, there was excellent correlation between the total numbers of cells on the slides and the differential cell count 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 to 4 h later (Rand *et al.*, 1990b).

There have also been several human studies performed on the effects of time and temperature on CSF (Kjeldsberg and Knight, 1993; Steele *et al.*, 1986; Stokes *et al.*, 1975). Interestingly, in these studies, and in contrast to the above study assessing canine CSF, neutrophils and not large mononuclear cells were the most labile cell type. Refrigeration at 4°C markedly reduced the rate of lysis of all cell types in the human CSF studies. Therefore, the recommendation that analysis be performed within 30 min is reasonable, but it is predicated by 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 therefore attenuates the temporal effects involved in transport of samples to more remote facilities.

2. Total Leukocyte and Erythrocyte Counts

Electronic cell or particle counters are typically not sensitive 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, cells are usually counted with a standard hemacytometer chamber with Neubauer ruling (Brobst, 1989; Cook and DeNicola, 1988; Jamison and Lumsden, 1988). The chamber is charged with undiluted fluid. Ideally, the cells are allowed to settle for 10 min in a humidified environment. This allows all the cells to be visible in the same plane of focus. The cells in the nine 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. Alternatively, the cells in nine large squares are counted and the number multiplied 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). Differentiating nucleated cells and erythrocytes in a hemacytometer chamber can be expedited by staining with New Methylene Blue before counting (Fry *et al.*, 2006). This latter technique can be used without significant dilutional effects.

A laser based cell counter and dedicated software are used to count and differentiate cells in human CSF (Aune *et al.*, 2004; Mahieu *et al.*, 2004). This technique has the advantage of markedly superior precision and accuracy. The same methodology has been used to assess canine CSF (Ruotsalo *et al.*, 2005). Although there was good correlation between the leukocyte and erythrocyte concentrations when compared with standard hemacytometer methods, the current software algorithms were not suitable for determining an accurate differential count in canine CSF. Additionally, it is likely that the cost and logistics of this methodology will preclude routine use in veterinary medicine.

3. Cytological Examination

There are a variety of methods to facilitate cytological examination of CSF (Barrett and King, 1976; Ducos *et al.*, 1979; Grevel, 1991; Hansen *et al.*, 1974; Jamison and Lumsden, 1988; Kolmel, 1977; Roszel, 1972; Sornas, 1967; Steinberg and Vandevelde, 1974; Woodruff, 1973). There is controversy as to which method is optimal, and all have their strengths and weaknesses. Methods include simple centrifugation, sedimentation, and variations thereof; membrane filtration; and cytocentrifugation (Jamison and Lumsden, 1988). Consult these references for specific methodological details. Simple centrifugation usually produces slides that are unsatisfactory for cytological examination. The chief advantage of membrane filtration techniques is excellent cellular recovery with yields approaching 90% to 100% (Barrett and King, 1976). However, the methodology is laborious and time consuming, the cellular 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 most veterinary clinical pathologists do not have experience or expertise in interpreting. For these reasons, they are not recommended. Cytocentrifugation (Hansen *et al.*, 1974; Woodruff, 1973) is the method of choice in both human (Fishman, 1992; Kjeldsberg and Knight, 1993) and veterinary medicine (Christopher *et al.*, 1988; Jamison and Lumsden, 1988). It is rapid, simple, and produces slides with good cytological detail. The technique is enhanced by the addition of protein to the CSF sample before centrifugation, which helps to preserve cell morphology. Conditions of cytocentrifugation vary from laboratory to laboratory. We prefer the method described by Rand and colleagues (Rand *et al.*, 1990b). The disadvantages of cytocentrifugation are 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 $81 \pm 3\%$ (SEM), nucleopore filtration $69 \pm 3\%$, and cytocentrifugation $11 \pm 1\%$. The Sornas method of centrifugation results in a cell yield, after staining, of approximately 20% (Sornas, 1967). The sedimentation technique of Sayk, modified by Kölmel (Grevel, 1991; Grevel and Machus, 1990; Kolmel, 1977), results in a yield of approximately 30% (Kolmel, 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 cytocentrifugation with an apparently superior cell yield, although this needs to be confirmed with controlled comparative studies. Therefore, most studies suggest that sedimentation techniques result in greater cell yields than does cytocentrifugation. However, there is at least one study that found the yield of cytocentrifugation to be marginally higher than 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

In people, the value of cytological diagnosis of CSF can be improved if morphological studies are appropriately supplemented by immunocytochemistry (Kjeldsberg and Knight, 1993). Immunophenotypic studies of cytocentrifuge slides are useful in the differential diagnosis of leukemia, lymphosarcoma, primary brain tumors, and metastatic tumors (Bigner, 1992; Bigner and Jonston, 1981; Jorda *et al.*, 1998; Kjeldsberg and Knight, 1993; Tosaka *et al.*, 2001). Few veterinary studies document CSF immunocytochemistry. In dogs, immunophenotyping is useful in the diagnosis of lymphoma and infiltrative leukemia[2] and histiocytic sarcoma, both postmortem (Zimmerman *et al.*, 2006) and antemortem.[3]

Panels of monoclonal antibodies are typically used for the immunocytochemical assessment of CSF. The greatest limitation is therefore the volume and cellularity of the specimen available for the marker studies. Undifferentiated tumor panels frequently include leukocyte common antigen and cytokeratin antibodies. These can be helpful in distinguishing single carcinoma cells from lymphocytes or monocytes (Bigner, 1992; Bigner and Jonston, 1981; 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. Seventy-five to 95% of the lymphocytes found in normal human CSF 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 assessing CSF lymphocyte subset alterations in disease are lacking in domestic animals but may be useful. Lymphocyte subset distribution has been assessed in the brains of dogs with different types of diseases (Tipold *et al.*, 1999). T cells predominated in viral encephalitides, whereas B cells predominated in bacterial and protozoal diseases and in steroid responsive meningitis-arteritis (Tipold *et al.*, 1999). However, it has not been determined if similar changes are reflected in the CSF. Lymphocyte subset distribution has been assessed in normal dogs and horses (Duque *et al.*, 2002; Furr *et al.*, 2001; Tipold *et al.*, 1998) Similar to people, T lymphocytes predominate in canine CSF but are present as a lower percentage than in people, accounting for approximately 50% to 60% of lymphocytes (Duque *et al.*, 2002; Tipold *et al.*, 1998). However, there appears to be

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[2]Vernau, W., unpublished observations.
[3]Tzipory, Vernau, Moore, in preparation.

much individual variation that may complicate use of this type of assessment clinically (Tipold *et al.*, 1998). Horses appear to be more similar to people, with T cells accounting for approximately 80% of lymphocytes in the CSF (Furr *et al.*, 2001). However, in contrast to people, CD8+ T cells in normal equine CSF may constitute a greater subset of T cells, accounting for approximately 30% of CSF T cells in one study (Furr *et al.*, 2001). In people, most central nervous system lymphomas are B cell in origin; immunocytochemistry assessing immunoglobulin light chain expression can be used to document monoclonality (Bigner, 1992; Bigner and Jonston, 1981). This is strong evidence (but not definitive proof) of malignancy. This assessment cannot be made in most domestic animals because of the marked light chain skewing that exists normally in these species (Arun *et al.*, 1996: Butler, 1998, #387). The B cell origin of the lymphocytes can also be confirmed with demonstration of immunoglobulin light chain expression. 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 and mycobacterium tuberculosis in human patients (Stark *et al.*, 1993; Sumi *et al.*, 2002) and distemper virus in dogs (Abate *et al.*, 1998).

5. *Polymerase Chain Reaction*

Polymerase chain reaction (PCR) technology is a powerful adjunct to routine cytological assessment of CSF that may increase 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 useful applications of PCR methodology is the confirmation of malignancy and detection of 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 because of either contamination or very low initial numbers of cells producing an artifactual clonal band. Tumor specific quantitative reverse transcriptase PCR (qRT-PCR) has been used for the sensitive detection of (neoplastic) neuroblastoma cells in the CSF of a human patient (Rosanda *et al.*, 2006). Other applications in people include detection of a wide variety of infectious agents, such as toxoplasma, borrelia, tuberculosis, human immunodeficiency virus, rabies virus, herpes simplex virus, and various amebas (Christen *et al.*, 1995; Guffond *et al.*, 1994; Lin *et al.*, 1995; Novati *et al.*, 1994; Qvarnstrom *et al.*, 2006). In the majority of these studies, PCR results in a

more rapid diagnosis with superior sensitivity and specificity when compared to standard culture and serological diagnostic techniques (Deisenhammer *et al.*, 2006).

In veterinary medicine, PCR is used to detect several infectious agents in CSF samples, such as bacteria (*listeria monocytogenes* and *Streptococcus equi*), protozoa (*sarcocystis neurona*, *toxoplasma gondii*, *neospora caninum*), and viruses (canine distemper virus, West Nile virus, and equine herpesvirus-1) (Amude *et al.*, 2006a, 2006b; Cannon *et al.*, 2006; Fenger, 1994; Finno *et al.*, 2006; Frisk *et al.*, 1999; Goehring *et al.*, 2006; Kim *et al.*, 2006; Peters *et al.*, 1995; Schatzberg *et al.*, 2003; Stiles *et al.*, 1996). Some agents such as listeria, encysted neospora or toxoplasma bradyzoites in the CNS parenchyma may not gain access to the meningoventricular system. This may result in negative CSF PCR results in confirmed positive cases (Peters *et al.*, 1995). A combination of diagnostic information (clinical information, CSF assessment, serology, PCR, biopsy, and immunohistochemistry) is the most practical way to make a clinical diagnosis, rather than the use of a single test result, such as PCR (Schatzberg *et al.*, 2003). PCR assays for the detection of clonal immunoglobulin or T-cell receptor gene rearrangements in dogs and cats have now been developed (Burnett *et al.*, 2003; Moore *et al.*, 2005; Vernau and Moore, 1999; Werner *et al.*, 2005). These assays have been used for the confirmation of malignancy in the CSF of dogs with suspected CNS lymphoma (W. Vernau, unpublished data). Recently, qRT-PCR was used to assess the cytokine profiles present in the CSF of horses with different neurological disorders (Pusterla *et al.*, 2006b). Some differences were noted between the different types of diseases but significant overlap of values also occurred. Further developmental work in conjunction with additional 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 neurological 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 have largely been replaced by quantitative methods. Urinary dipsticks have been used to determine CSF protein concentration, but false negative and false positive test results occur using this methodology, which preclude recommendation for routine use (Behr *et al.*, 2003; Jacobs *et al.*, 1990). Techniques for quantitative measurement of CSF total protein include turbidimetric methods, biuret procedures, and Lowry's method. The accuracy of these methods in many clinical laboratories is no better than ±5% (Fishman, 1992). Dye binding microprotein assays such as Coomassie Brilliant Blue, Ponceau

S red, and Pyrogallol red (Marshall and Williams, 2000; Pesce and Strande, 1973) are more accurate and are now the methods of choice for measurement of CSF protein concentration. Total CSF protein values are reported in numerous articles and vary noticeably with the methodology and the laboratory performing the assay. Therefore, clinicians must use laboratory-specific normal values when assessing CSF protein concentration.

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. Immunoelectrophoresis, electroimmunodiffusion, radioimmunoassay, isoelectric focusing, and high-resolution protein electrophoresis are more recent techniques (Behr *et al.*, 2006; 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 (pathological or iatrogenic), the ratio of CSF albumin to serum albumin can be used as an indicator of barrier dysfunction (Link and Tibbling, 1977; Tibbling *et al.*, 1977). This ratio is also called the albumin index (a.k.a. albumin quota, 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 horses) (Andrews *et al.*, 1990a, 1994; Bichsel *et al.*, 1984b; Krakowka *et al.*, 1981) results in 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 lowest in childhood. The albumin index increases with age (Deisenhammer *et al.*, 2006).

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 neurological disease.

Immunoglobulin G is the dominant CSF immunoglobulin. 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 (Thompson, 1988; Trotter, 1989). 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 alpha$_2$ 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" (Zimmerman *et al.*, 2006), and thereby demonstration of intrathecal IgG synthesis, is the IgG/albumin index (Link and Tibbling, 1977; Tibbling *et al.*, 1977). This index is calculated using the CSF and serum concentrations of albumin and IgG as follows (Kjeldsberg and Knight, 1993):

$$\text{IgG Index} = \frac{\dfrac{\text{CSF IgG (mg/dl)}}{\text{serum IgG (g/dl)}}}{\dfrac{\text{CSF albumin (mg/dl)}}{\text{serum albumin (g/dl)}}}$$

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 to 10,000 RBC/μl) by a traumatic puncture falsely elevates the IgG index in people (Peter and Tourtellotte, 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 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 (Reiber and Felgenhauer, 1987; Tipold *et al.*, 1994).

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 in both people and animals, particularly immunological or inflammatory diseases (Bichsel *et al.*, 1984b; Deisenhammer *et al.*, 2006; Fishman, 1992; Kjeldsberg and Knight, 1993).

E. Antibody/Antigen Tests

A variety of CSF antibody and antigen tests are available for viruses, fungi, rickettsia, protozoa, parasites, and other organisms (Berthelin *et al.*, 1994a; Duarte *et al.*, 2006; Dubey, 1990b; Jacobs and Medleau, 1998; Lunn *et al.*, 2003; Madhusudana *et al.*, 2004; Porter *et al.*, 2004; Rossano *et al.*, 2003). For antibody titers, two samples taken 2 weeks apart should be assayed. Because of inter-run 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 passage 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 V.D.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 fashion as intrathecal IgG production is detected with the IgG index. The formula is (Reiber and Lange, 1991):

$$\text{Antibody Index} = \frac{\dfrac{\text{CSF specific Ig}}{\text{serum specific Ig}}}{\dfrac{\text{CSF total Ig}}{\text{serum total Ig}}}$$

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). An antibody index >1 suggests intrathecal production of the specific antibody (Munana *et al.*, 1995; Reiber and Lange, 1991). 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 need further study.

Antigen detection tests include immunoelectrophoretic techniques, agglutination tests, and enzyme-linked immunosorbent assay (ELISA) for bacterial antigens and latex agglutination for cryptococcal antigens. The polymerase chain reaction (PCR) procedures detect the presence of specific antigen DNA (or RNA) in CSF and can be highly sensitive, specific, and rapid (see Section V.C.5).

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, tests for microbial antigens by counterimmunoelectrophoresis or agglutination techniques, and the G test for the broad spectrum detection of fungi (tests for (1,3)-β-D-glucan in most fungal cell walls) (Stevens, 2002) may also be useful (Fishman, 1992). PCR may be used to detect microbes in CSF (Finno *et al.*, 2006; Peters *et al.*, 1995; Stevens, 2002).

G. Blood Contamination

Erythrocytes may be present in CSF samples because of subarachnoid hemorrhage or, more commonly, because of traumatic puncture. Blood contamination resulting from traumatic puncture is a common problem during CSF collection and, depending on its degree, can interfere with cytological interpretation. Blood contamination is more likely to occur with lumbar puncture as opposed to cerebellomedullary cisternal puncture (Bailey and Higgins, 1985; Oliver and Lorenz, 1993; Thomson *et al.*, 1990). Blood contamination is a source of leukocytes and hence can affect both the leukocyte count and the differential. In one study of CSF analysis 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); in cats, a maximum of

one 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 cells counts of the patient and hence compensates for any significant alterations in these counts (Fishman, 1992):

$$W = WBC_F - \frac{WBC_B \times RBC_F}{RBC_B}$$

where W is the white blood cell count of the fluid before blood was added (i.e., the corrected count), WBC_F is the total white blood cell count in the bloody fluid, WBC_B is the white blood cell count in the peripheral blood per microliter, and RBC_F and RBC_B are the numbers of red blood cells per microliter in the CSF and blood, respectively. Despite all of these elaborate 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 (de Lahunta, 1983). This lack of validity has been proven by several studies (Novak, 1984; Wilson and Stevens, 1977). In one article, blood contamination appeared to have little effect on white blood cell numbers, and the above correction formula was considered unreliable. The authors evaluated 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 (Wilson and Stevens, 1977). In another article, the authors concluded that the standard computations frequently overcorrect white blood cell counts in blood contaminated CSF, and the magnitude of the overcorrection may obscure disease in some instances—in eight infants with marked blood contamination but proven bacterial meningitis, correction computations normalized or overcorrected the white blood cell counts (Novak, 1984). The mechanism of this overcorrection was not 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 previously 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 to 10,000 RBC/ml) elevates the IgG index (Fishman, 1992).

VII. 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, 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 2h after red cells enter the CSF. The level of oxyhemoglobin reaches its peak about 36h later and disappears over the next 4 to 10 days. Bilirubin is yellow in color. Bilirubin is derived from hemoglobin and is formed by macrophages and other leptomeningeal cells that degrade the hemoglobin from lysed red blood cells. Bilirubin is detected about 10h after red cells enter the CSF, reaches a maximum at about 48h, 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 a reduction product of hemoglobin characteristically found in encapsulated subdural hematomas and in old, loculated intracerebral hemorrhages (Fishman, 1992; Kjeldsberg and Knight, 1993). Occasionally the CSF may be black tinged CSF in animals with melanin-producing tumors in the nervous system.

Causes of a CSF color change other than red cell contamination include icterus resulting from 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 imparts an

orange-red color to body fluids. Rifampin is 90% bound to protein; hypoproteinemia may result in rifampin staining of CSF in patients receiving this drug (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 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 depends on 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). A 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 neurological 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 pathology.

1. Neutrophilia

A marked pleocytosis with neutrophil predominance suggests either bacterial meningitis (Kjeldsberg and Knight, 1993; Kornegay et al., 1978) or suppurative, nonseptic (corticosteroid responsive) meningitis (Meric, 1988, 1992a; Tipold and Jaggy, 1994). 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 criteria for determining the likelihood of sepsis with nuclear degenerative changes or karyolysis 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 unequivocally exclude a diagnosis of septic meningitis, although it does make it less likely. In people, acute viral meningoencephalitis initially causes a neutrophilic pleocytosis (Converse et al., 1973; Fishman, 1992; Kjeldsberg

and Knight, 1993) that may persist from a few hours to several days before the development of the more typical mononuclear reaction. A similar phenomenon is documented in animals (Green et al., 1993). Occasionally, distemper virus infection causes massive encephalomalacia (Vandevelde and Spano, 1977) resulting in a neutrophilic pleocytosis, in contrast to the more typical moderate mononuclear pleocytosis. 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 may be noted in dogs with meningioma (Bailey and Higgins, 1986b). However, in another study of dogs with meningioma, about 30% of dogs had a normal CSF analysis (Dickinson et al., 2006). In this study, a significant association between meningiomas in the caudal portion of the cranial fossa and an elevated CSF nucleated cell count was found; but only 19% of the dogs had an elevated total nucleated white cell count with a predominance of neutrophils (Dickinson et al., 2006).

Canine intervertebral disk disease is associated with variable alterations in CSF that depend on factors such as disease severity and chronicity (Thomson et al., 1989). Acute severe disease may result in counts greater than 50 cells per microliter with more than 50% neutrophils (Thomson et al., 1989). This finding may be a reflection of acute inflammation secondary to trauma that may be exacerbated by myelomalacia in some instances. The authors have seen a similar phenomenon associated with fibrocartilaginous embolic myelopathy in dogs. A neutrophilic pleocytosis of varying severity often occurs following myelography with iodinated contrast agents (Carakostas et al., 1983; Johnson et al., 1985; Widmer et al., 1992). These changes usually peak at 24 h postmyelogram (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, documented in dogs (Vandevelde and Spano, 1977), cats (Dow et al., 1990; Rand et al., 1994b), horses (Green et al., 1992; Hamir et al., 1992; Wamsley et al., 2002), sheep, goats (Brewer, 1983), and numerous other species. In people, CSF lymphocytosis has been observed in bacterial meningitis following antibiotic therapy (Cargill, 1975; Converse et al., 1973; Fishman, 1992; Kjeldsberg and Knight, 1993), indicating that therapy and chronicity can alter the CSF findings. A similar finding has been reported in dogs (Sarfaty et al., 1986; Tipold and Jaggy, 1994) and calves (Green and Smith, 1992). We have noted that dogs with chronic or acute on chronic type I intervertebral disk disease have a pleocytosis that is more commonly lymphocytic than neutrophilic (Windsor et al., 2007). The CSF

findings with granulomatous meningoencephalitis (GME) are somewhat variable, but a marked lymphocytic pleocytosis is common (Bailey and Higgins, 1986a; Thomas and Eger, 1989). Similarly, dogs with necrotizing meningoencephalitis (small breed dogs including pug dogs, Yorkshire terriers, and Maltese terriers) frequently have a marked lymphocytic pleocytosis (Cordy and Holliday, 1989; Stalis *et al.*, 1995; Tipold *et al.*, 1993a).

3. E*osinophilia*

Eosinophils are not present in normal, uncontaminated (by blood) CSF. Single eosinophils are occasionally noted on cytocentrifuge slides from animals with normal CSF (normal nucleated counts and protein concentration). Although the presence of eosinophils in CSF is abnormal and evidence of underlying disease, no diagnostic specificity is associated with their presence in human CSF, as they can be found in a variety of diseases (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 (Bosch and Oehmichen, 1978; Smith-Maxie *et al.*, 1989). 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; in many of these cases eosinophils predominate in the CSF differential cell count (Bosch and Oehmichen, 1978; Kuberski, 1979). A marked eosinophilic pleocytosis is also reported in dogs with neural angiostrongylosis (Lunn *et al.*, 2003; Mason, 1989). 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 (Jamison and Lumsden, 1988; Smith-Maxie *et al.*, 1989). However, in many of these cases, eosinophils represent less than 5% of the total cell count in CSF (Bosch and Oehmichen, 1978; Smith-Maxie *et al.*, 1989). Other documented causes in people include neurosyphilis, tuberculosis, rickettsial disease, foreign body reactions to shunt tubes, intrathecal penicillin or contrast agents, hypereosinophilic syndrome, multiple sclerosis, lymphoma, Hodgkin's disease, leukemia, melanoma, disseminated glioblastoma, idiopathic, and systemic allergic reactions (Bell *et al.*, 2006; Fishman, 1992; Kjeldsberg and Knight, 1993; Kuberski, 1979; Smith-Maxie *et al.*, 1989). In animals, CSF pleocytosis that consists predominantly, or almost exclusively, of eosinophils is rare. We have personally seen CSF eosinophilia with marked eosinophil predominance in association with idiopathic or steroid

responsive eosinophilic meningoencephalitis (Smith-Maxie *et al.*, 1989), canine neural angiostrongylosis (Mason, 1989), and histopathologically confirmed canine CNS neosporosis.[4] Golden retriever dogs and rottweilers may be predisposed to idiopathic or steroid responsive eosinophilic meningoencephalitis (Bennett *et al.*, 1997; Smith-Maxie *et al.*, 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 necessarily predominance) in animals include bacterial encephalitis, distemper, rabies, toxoplasmosis, neosporosis, cuterebral encephalitis, central nervous system nematodiasis and cestodiasis, prototheccosis, granulomatous meningoencephalomyelitis, lymphoma, astrocytoma, cerebral infarction, canine neural angiostrongylosis, and salt poisoning (Chrisman, 1992; Darien *et al.*, 1988; Jamison and Lumsden, 1988; Lester, 1992; Mac Donald *et al.*, 1976; Mason, 1989; Oruc and Uslu, 2006; Smith, 1957; Tyler *et al.*, 1980; Vandevelde and Spano, 1977).

4. N*eoplastic Cells*

Lymphoma has been diagnosed on the basis of CSF assessment in both small and large animals (Lane *et al.*, 1994; Pusterla *et al.*, 2006a; Vandevelde and Spano, 1977). However, the observation of neoplastic cells in CSF samples from animals with central nervous system neoplasia other than lymphoma is uncommon in our experience. Few veterinary studies have investigated the prevalence of positive CSF cytology in animals with confirmed 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 any sample (Bailey and Higgins, 1986b). However, in this study, cytological assessment was done only on those samples with an elevated cell count and these only accounted for 41.3% of cases. Tumor cells have been observed in the CSF when the CSF cell counts were within normal limits (Grevel and Machus, 1990; Grevel *et al.*, 1992). Additionally, in the study assessing primary brain tumors in 77 dogs (Bailey and Higgins, 1986b), CSF differential and cytology were done on cytospin samples. The cell yield with cytospin slide preparation is low, approximating 10% (Barrett and King, 1976) in some studies, and this may partly explain the failure to observe neoplastic cells in the above study. Other veterinary studies utilizing different techniques report a higher prevalence of neoplastic cells 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 in five of eight dogs (Grevel and Machus, 1990) and four of nine dogs (Grevel *et al.*, 1992). In the former study, two of the five cytologically

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[4]W. Vernau, unpublished data.

positive cases had normal cell counts. The Kölmel technique results in a higher cell yield than cytocentrifugation, which may be partly responsible for the increased incidence of neoplastic cell observation in the CSF. Despite the low yield of cytospin slides, the presence of neoplastic cells in cytospin CSF slides from animals with CNS tumors other than lymphoma has been reported in cats with intracranial oligodendroglioma, dogs with CNS histiocytic sarcoma, and dogs with choroid plexus carcinoma (Dickinson *et al.*, 2000; Zimmerman *et al.*, 2006).[3,5]

A large number of studies assess the prevalence of neoplastic cells in the CSF of people with central nervous system neoplasia. Overall sensitivities that are frequently quoted are 70% for CNS leukemia, 20% to 60% for metastatic meningeal carcinoma, and approximately 30% for primary CNS tumors (Kjeldsberg and Knight, 1993), regardless of the technique utilized. The detection rate of malignant cells in the CSF is 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 people with leptomeningeal involvement were considered, the prevalence increased to 59%. Conversely, of 66 cases in which the tumor did not reach the leptomeninges, only a single sample was positive. In another study, only 13.9% of all gliomas had a positive CSF cytology (Balhuizen *et al.*, 1978). This low prevalence is likely because the majority of gliomas does 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 prevalence of positive CSF cytology in confirmed cases of different types of central nervous system neoplasias, 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 above type of study has been somewhat decreased by the advent of more routine access to advanced imaging and biopsy techniques (Koblik *et al.*, 1999; Vernau *et al.*, 2001).

[5]Westworth, D., in preparation.

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 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. In people, 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, whereas entrance of protein into the CSF remains normal. Protein content of fluid collected from the lumbar site could be decreased if large volumes are removed or if ongoing leakage of CSF from the lumbar area is occurring. In these situations, lumbar CSF is replaced more quickly than normal by ventricular CSF, which has a lower protein content than lumbar 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. *Albuminocytological Dissociation*

In many disease processes, the CSF cell count and CSF total protein increase in rough parallel. In some disorders, the cell count remains normal, whereas the total protein is notably increased, a phenomenon termed albuminocytological dissociation. Some degenerative disorders, ischemia/infarction, immune-mediated diseases (e.g., polyradiculoneuritis), tumors, and neural compression produce albuminocytological 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, indicates 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 neurological disorders, including inflammatory diseases, neoplasia, trauma, compression, and occasionally metabolic diseases (Bichsel *et al.,* 1984b; Krakowka *et al.*, 1981; Sorjonen, 1987; Sorjonen *et al.*, 1991).

4. *Increased CSF IgG and IgG Index*

The CSF IgG can be increased by movement of protein across damaged blood-brain/CSF barriers, intrathecal hemorrhage (pathological or iatrogenic), or intrathecal IgG synthesis. An elevated CSF IgG content and increased IgG index, indicating intrathecal IgG synthesis, are typical for infectious inflammatory diseases (Tipold *et al.*, 1993b, 1994). 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 inflammatory lesions had no pleocytosis but had an elevated IgG index; in contrast, 17 of 32 dogs with noninflammatory disease had pleocytotic 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 meningoencephalomyelitis, and an IgG index between 1.3 and 2.8 as suggestive of inflammatory disease. In a few dogs with marked inflammatory lesions and intrathecal IgG production, the IgG index may not be elevated because of marked IgG exudation 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 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 pathogenetical 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 diseases. Barrier disturbance may be seen in degenerative, inflammatory, metabolic, space-occupying, vascular, and traumatic conditions (Bichsel *et al.*, 1984b; Sorjonen, 1987; 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). 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 neurological 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, one study reported that IgM was present through all stages of human herpes and bacterial meningitis, and increased and decreased with IgG (Felgenhauer, 1982). 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). Anti-West Nile Virus (WNV) IgM antibody production appears to occur intrathecally in horses and detection of CSF anti-WNV IgM may be used to differentiate previously vaccinated horses versus infected horses (Porter *et al.*, 2004) Further studies need to be done in animals to determine the sensitivity and specificity of the various immunoglobulin alterations occurring in disease.

7. *Electrophoretic Patterns of CSF Protein in Disease*

Abnormalities in the CSF electrophoretic pattern can suggest categories of diseases (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 disk 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). However, a more recent study using high-resolution agarose electrophoresis was unable to differentiate various categories of neurological disease in dogs using this technique (Behr *et al.*, 2006).

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 in patients with neurological disease (Kjeldsberg and Knight, 1993).

8. *Other* CSF P*roteins*

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 neurological disease, but these increases are found in many heterogeneous conditions. This nonspecificity limits the clinical utility of many of these specific protein assays. However, the measurement of some of these proteins is thought to be useful as a screening procedure for neurological disease or as an indication of prognosis (Fishman, 1992; Kjeldsberg and Knight, 1993; Lowenthal *et al.*, 1984). Immunoassay detection in the CSF of the brain-derived protein 14-3-3 appears to be helpful for the diagnosis of transmissible spongiform encephalopathies in both animals and people (Hsich *et al.*, 1996; Sanchez-Juan *et al.*, 2006). An autoantibody against canine glial fibrillary acidic protein present in astrocytes has been detected in the CSF of two pug dogs affected with necrotizing encephalitis (Uchida *et al.*, 1999). However, it is unknown if the presence of this antibody is a primary or secondary phenomenon.

9. P*lasma* P*roteins in the* CSF

Alterations in plasma proteins may be reflected in the CSF. For example, in people, 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 Titers

The CSF antibody titer can be measured for a number of diseases (Dubey, 1990b; 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; Porter *et al.*, 2004).

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 because of the alteration of membrane glucose transport and increased anaerobic glycolysis by neural tissue. As noted previously, 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 levels (Deisenhammer *et al.*, 2006; Kjeldsberg and Knight, 1993). A CSF/serum glucose ratio less than 0.4 to 0.5 is considered to be pathological in people (Deisenhammer *et al.*, 2006). A low CSF glucose level in the absence of hypoglycemia indicates a diffuse, meningeal disorder, rather than focal disease (Fishman, 1992; Kjeldsberg and Knight, 1993). 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). CSF glucose concentration and CSF/serum glucose ratio are not routinely measured in veterinary medicine, possibly because of the lack of specificity and availability of more specific tests in most instances.

F. Enzymes

Numerous enzymes have been assayed in the CSF of animals (Furr and Tyler, 1990; Jackson *et al.*, 1996; Rand *et al.*, 1994b; Wilson, 1977). Of these, creatine kinase has received the most attention, and opinions of its usefulness are conflicting. Although Furr and Tyler confirmed previous observations that CSF creatine kinase activity was elevated in

several neurological diseases, they concluded that the greater frequency of elevation in the CSF of horses with protozoal myelitis versus horses with cervical compressive myelopathy indicated this enzyme assay was useful in differentiating these two diseases (Furr and Tyler, 1990). 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 this enzyme's lack of sensitivity and specificity reflects the current situation with all of the enzymes in CSF studied to date—none has sufficient specificity to warrant its routine use as diagnostic test (Fishman, 1992; Indrieri *et al.,* 1980; Jackson *et al.*, 1996; Kjeldsberg and Knight, 1993; Rand *et al.*, 1994b). 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 animals with spinal disease (Indrieri *et al.*, 1980). Measurement of enzyme isomers may increase the specificity (Kjeldsberg and Knight, 1993).

To date, none of the enzyme assays are sufficiently sensitive or specific to warrant routine use in clinical practice (Fishman, 1992; Indrieri *et al.*, 1980; Jackson *et al.*, 1996; Kjeldsberg and Knight, 1993; Rand *et al.*, 1994a).

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 (Glimaker *et al.*, 1994) or multiple sclerosis and occasionally in patients with noninflammatory neurological 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).

2. Neurotransmitters

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter, whose dysfunction has been suggested to play a role in experimental (Griffith *et al.*, 1991) and clinical seizure disorders. Conversely, glutamate (GLU) is a major excitatory neurotransmitter in the CNS that plays an important role in the initiation, spread, and maintenance of epileptic activity in people (Meldrum, 1994). Increased extracellular concentrations of glutamate in the CNS may also mediate secondary tissue damage and cell death (Meldrum, 2000). 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). Inhibitory and excitatory neurotransmitters have been assayed in the CSF of epileptic dogs (Ellenberger *et al.*, 2004; Podell and Hadjiconstantinou, 1997). In one study, epileptic dogs were found to have lower levels of CSF GABA and higher levels of CSF glutamate than normal dogs (Podell and Hadjiconstantinou, 1997). In another study, CSF GABA was also found to be decreased in epileptic Labradors, but in contrast to the former report, these dogs had lower levels of CSF glutamate than normal dogs or epileptic non-Labradors (Ellenberger *et al.*, 2004). CSF neurotransmitters have also been assayed in dogs with portosystemic shunts (PSS) and clinical signs of hepatic encephalopathy (Holt *et al.*, 2002). Dogs with PSS had significantly higher levels of glutamine, tryptophan, and 5-hydroxyindoleacetic acid. These alterations may play a role in the neurological abnormalities associated with hepatic encephalopathy (Holt *et al.*, 2002). Because of its potential role in secondary tissue damage, glutamate concentrations in lumbar CSF have been measured in dogs with intervertebral disk herniation and acute and chronic compressive spinal cord lesions (Olby *et al.*, 1999). Dogs with severe, acute thoracolumbar disk herniations have two- to ten-fold increases in their lumbar CSF 12h or more after injury. The severity of the clinical signs appeared to be related to the lumbar CSF glutamate concentration (Olby *et al.*, 1999). Dogs with chronic compressive thoracolumbar lesions have a two-fold elevation of lumbar CSF glutamate concentration. However, focal spinal cord injuries did not alter glutamate concentrations in cisternal CSF (Olby *et al.*, 1999).

Increased CSF levels of the biogenic amine neurotransmitter metabolites homovanillic acid and 5-hydroxyindoleacetic acid were found in 2 of 10 collies experimentally given ivermectin (Vaughn *et al.*, 1989). Both of these collies had severe neurological deficits. Neurotransmitter metabolite concentrations were also elevated in the CSF of goats demonstrating neurological abnormalities after experimental boron toxicosis (Sisk *et al.*, 1990). Significant differences in neurotransmitter concentrations were found between the CSF of normal dogs and narcoleptic dogs (Faull *et al.*, 1982). Hyopocretins are neuropeptides that bind to the G-protein coupled hypocretin receptors Hcrtr 1 and Hcrtr 2. Hypocretins are undetectable in the CSF of sporadic narcoleptic dogs but are normal in familial narcoleptic dogs that have mutations in the hypocretin receptor 2 gene (Ripley *et al.*, 2001).

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 macaques infected with simian immunodeficiency virus (Smith, 1995). Quinolinic acid levels

may be elevated in the CSF of animals with inflammatory nervous system disease. Therefore, they may be useful as a marker of inflammation and perhaps also as an indicator of prognosis (Smith, 1995). Increased CSF quinolinic acid concentrations were also found in dogs with portosystemic shunts (PSS) and signs of hepatic encephalopathy (HE) (Holt *et al.*, 2002); it was speculated in this study that quinolinic acid and other tryptophan metabolites may contribute to the neurological abnormalities present in dogs with PSS and HE.

4. *Lactic Acid*

In people, the measurement of CSF lactic acid has been advocated in differentiating bacterial from viral meningitis. CSF lactate concentration is also increased in CNS fungal infections and leptomeningeal metastatic disease (Deisenhammer *et al.*, 2006). CSF lactate concentration is independent of blood lactate concentration (Deisenhammer *et al.*, 2006). In people, CSF lactate concentration may be elevated in diseases resulting in severe or global brain ischemia and anaerobic glycolysis or mitochondrial disease. It correlates inversely with the CSF: blood glucose ratio. However, because many diseases may elevate CSF lactic acid, the overlapping CSF lactate levels limit the value of CSF lactate assay (Fishman, 1992; Kjeldsberg and Knight, 1993). In a study of dogs with a variety of CNS diseases, blood and CSF pyruvate and lactate levels were measured. Levels of pyruvate were variable. Lactate levels were significantly elevated only in dogs with disk disease over CSF lactate levels in normal dogs (Lobert *et al.*, 2003). A study of CSF lactate levels in horses with neurological 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 6h of death. Their results indicated 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.

VIII. 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 abiatrophies. 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 with 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 diseases such as globoid cell leukodystrophy, mucopolysaccharidosis, and fucosidosis, accumulated metabolic material may be seen in the white blood cells in the CSF (Keller and Lamarre, 1992; Roszel, 1972; Silverstein Dombrowski *et al.*, 2004).

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 to 70 mg/dl). This albuminocytological dissociation may support the theory that this disorder is an immune-mediated disease (Waxman *et al.*, 1980). However, concurrent, chronic, spinal cord compression by type II disk protrusion in many of these dogs complicates the situation, because chronic cord compression may also produce an increase in total protein. The elevated total protein concentration in canine degenerative myelopathy is probably the result of increased CSF albumin (Bichsel *et al.*, 1984b). The CSF IgG index is usually normal (Bichsel *et al.*, 1984b; Tipold *et al.*, 1993b), 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 (Loscher and Schwartz-Porsche, 1986) disorder is either normal or has albuminocytological dissociation (Cummings *et al.*, 1990; Divers *et al.*, 1994; Morin *et al.*, 1994). 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 immunoglobulin production occurs. Blood-brain barrier damage and intrathecal IgG production also occur in people with motor neuron disease (Apostolski *et al.*, 1991).

B. Idiopathic Diseases

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 comprising the remainder in about equal percentages (Bailey and Higgins, 1986a; Braund, 1994; Sarfaty *et al.*, 1986; Thomas and Eger, 1989; Tipold, 1995). A 15% to 30% neutrophilic component suggests 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 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 (Bichsel *et al.*, 1984b; Tipold *et al.*, 1993b, 1994). 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 transduced serum IgG (Bichsel *et al.*, 1984b; Fishman, 1992).

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

A necrotizing encephalitis (NE) of unknown cause is recognized in pug dogs (Cordy and Holliday, 1989; de Lahunta, 1983), Maltese dogs (Stalis *et al.*, 1995), and Yorkshire terriers (Ducote *et al.*, 1999; Jull *et al.*, 1997; Kuwamura *et al.*, 2002; 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 from that 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, increased white blood cell count (although one Maltese had 62% neutrophils) and moderate to marked elevation in total protein (Bradley, 1991; Cordy and Holliday, 1989; Stalis *et al.*, 1995). The CSF of the Yorkshire terriers has mild to moderate increases in white blood cells and protein, with a predominantly mononuclear differential count (Ducote *et al.*, 1999; Tipold, 1995; Tipold *et al.*, 1993a). Seizures are a consistent clinical sign for the pugs and the Maltese dogs but not the Yorkshire terriers.

An autoantibody against canine astrocytes has been detected in the CSF of dogs with NE (Matsuki *et al.*, 2004; Uchida *et al.*, 1999). This autoantibody, which recognizes glial fibrillary acidic protein, has also been detected in the CSF of dogs with GME and with intracranial tumors (Matsuki *et al.*, 2004); therefore, it is not a specific finding in dogs with NE. It is unknown if the presence of this antibody is a primary or secondary phenomenon. Further research is necessary to determine the clinical utility of the presence of this autoantibody in CSF.

C. Immune-Mediated Diseases

1. Acute Idiopathic Polyradiculoneuritis/Coonhound Paralysis

Acute idiopathic polyradiculoneuritis is one of the most common canine polyneuropathies, and coonhound paralysis is the most common form. The disorder resembles Guillain-Barre syndrome of people. In affected dogs, the classical CSF abnormality is albuminocytological dissociation. The abnormality is more obvious in lumbar 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 (Cuddon, 1990; Tipold *et al.*, 1993b).

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, suppurative 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 (de Lahunta, 1983; Meric, 1988; Tipold et al., 1995). The IgG index is typically elevated (Tipold and Jaggy, 1994; Tipold et al., 1993b), and IgM and IgA levels are often elevated as well (Tipold and Jaggy, 1994; Tipold et al., 1995). 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 pathological changes and may be the same disease as steroid-responsive meningitis/arteritis (Tipold and Jaggy, 1994). Boxer dogs may also be predisposed to this disease (Behr and Cauzinille, 2006).

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 versus parenchymal), the duration of the infection, and previous treatment. The general rules of inflammation resulting from infection apply (i.e., bacterial infections result in suppurative inflammation whereas viral infections result in nonsuppurative inflammation). Several important exceptions exist, however.

1. Bacterial Diseases

In central nervous system aerobic or anaerobic bacterial infections, the CSF 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 concentration. Because of the elevated protein concentration, the CSF may clot or foam when shaken. The white blood cell differential count characteristically has a high percentage of neutrophils (>75%), which may be degenerate (Baum, 1994; Dow et al., 1988; Foreman and Santschi, 1989; Green and Smith, 1992; Kornegay, 1981; Meric, 1988; Rand et al., 1994b; Santschi and Foreman,

1989; Scott, 1995; Sturges et al., 2006; Tipold, 1995). The protein is composed of albumin that has crossed the diseased blood-brain/CSF barrier and immunoglobulin produced intrathecally; therefore, the IgG index is usually elevated (Tipold et al., 1993b, 1994). 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 concentration (Green and Smith, 1992; Sturges et al., 2006). Occasionally extracellular or intracellular bacteria may be seen, either on a routine Wright's stain or a Gram stain (Foreman and Santschi, 1989; Green and Smith, 1992; Kornegay, 1981). Because prior antibiotic therapy is common, and 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 (Finno et al., 2006; Peters et al., 1995).

a. Listeriosis

Despite being a bacterial infection, 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 mostly mononuclear cells (Rebhun and deLahunta, 1982). These mild (to moderate) 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 with moderate to marked elevations in white blood cell count and protein, with a neutrophilic pleocytosis (53% to 100% neutrophils) (Scott, 1992, 1993). The mononuclear CSF reported in cattle likely 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. Direct culture of brain tissue was more frequently positive. 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 (Feder et al., 1991; Mandel et al., 1993) and horses (Burgess and Mattison, 1987; Hahn et al., 1996), the actual incidence in animals is unknown. The diagnostic difficulties arise from a delay or repression of seroconversion

after infection; the high number of seropositive, clinically normal animals; the persistence of infection and seropositivity despite resolution of clinical disease; antibody cross-reactivity; and difficulty in culturing the organism from tissue or fluid samples (Appel *et al.*, 1993; Levy *et al.*, 1993; Madigan, 1993; Parker and White, 1992). 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 (Feder *et al.*, 1991; Mandel *et al.*, 1993). Polymerase chain reaction (PCR) techniques for CSF have been 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. Ehrlichial and Rickettsial Diseases

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 (i.e., the white blood cell count and protein may be normal or slightly to moderately elevated with a predominantly mononuclear pleocytosis) (Buoro *et al.*, 1990; Firneisz *et al.*, 1990; Greene *et al.*, 1985; Maretzki *et al.*, 1994; Meinkoth *et al.*, 1989). 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; Meinkoth *et al.*, 1989). 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; Rutgers *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 they 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 neural tissue. The vascular lesion results in multifocal hemorrhages. Consequently, the CSF is characteristically yellow with a high red blood cell count (not iatrogenic in origin), and moderately to markedly increased white blood cell count (predominantly neutrophils) and protein (Ames, 1987; George, 1996; Little and Sorensen, 1969; Mayhew, 1989). The bacterium can be cultured only occasionally from CSF and more easily from septicemic animals (Little, 1984; Nayar *et al.*, 1977)

2. Viral Diseases

The CSF 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 (discussed later). The IgG index is commonly elevated (Bichsel *et al.*, 1984b; Tipold *et al.*, 1994). The IgA and IgM levels may also be elevated. The CSF of viral infections may also be normal, particularly if the meninges or ependyma is not involved (Fankhauser, 1962; Fishman, 1992; Rand *et al.*, 1994b; Tipold, 1995; Tipold *et al.*, 1994).

a. Canine Distemper

The CSF abnormalities associated with canine distemper (CDV) vary strikingly with the stage of the disease. Dogs with acute, demyelinating, noninflammatory distemper encephalitis may have normal or near normal CSF (mild elevations of total cell count and total protein) (Johnson *et al.*, 1988; Tipold, 1995). Protein elevation is most likely the result of blood-brain barrier dysfunction (Bichsel *et al.*, 1984b). The IgG index may also be normal or occasionally mildly elevated, which correlates with the histological findings of multifocal demyelination with few or no infiltration of inflammatory cells (Bichsel *et al.*, 1984b; Johnson *et al.*, 1988; Tipold *et al.*, 1993b, Vandevelde *et al.*, 1986). The acute form of nervous canine distemper is an exception to the usual association of an elevated IgG index with infectious neurological 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 (Johnson *et al.*, 1988; Tipold *et al.*, 1994). 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 dogs in the chronic stage than in the dogs with acute, noninflammatory distemper (Tipold *et al.*, 1993b, 1994). 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 (Bichsel *et al.*, 1984b; Sorjonen, 1987; Sorjonen *et al.*, 1991). Occasionally the CSF is normal or has only mild changes in cell count or total protein content (Bichsel *et al.*, 1984b; Sorjonen *et al.*, 1991; Tipold, 1995; Tipold *et al.*, 1993b, 1994; 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 pathological blood-brain barrier disturbance. The CSF of delayed-onset canine distemper (a.k.a. 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 antiviral immune response. The IgM and IgA concentrations are normal (Johnson *et al.*, 1988). Occasionally, distemper virus infection causes massive encephalomalacia (Vandevelde and Spano, 1977), resulting in a neutrophilic pleocytosis. Because of the variable presentations and CSF findings associated with CDV infection, definitive antemortem diagnosis can be difficult. Fluorescent antibody testing (IFA) for the detection of viral antigen in conjunctival, tonsillar, and respiratory epithelium has proven useful but only in the acute phases of illness (Greene and Appel, 2006). In subacute and chronic disease, antibody coating of viral antigen may interfere with diagnostic immunofluorescence (Amude *et al.*, 2006a; Andrews *et al.*, 1994; Greene and Appel, 2006). RT-PCR for the detection of viral RNA is likely the most sensitive method for detecting CDV infection; however, sensitivity may be higher in urine than in CSF (Amude *et al.*, 2006a; Frisk *et al.*, 1999; Kim *et al.*, 2006).

b. Equine Herpesvirus Myeloencephalitis

With its predilection for endothelial cells, the equine herpes virus 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, whereas the total protein level is moderately to markedly elevated (albuminocytological 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 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; Jackson *et al.*, 1977; Klingeborn *et al.*, 1983). Antiviral CSF antibodies are not present routinely in neurologically normal horses, horses vaccinated with modified live EHV-1, or horses with other neurological 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; Jackson *et al.*, 1977; Klingeborn *et al.*, 1983; Kohn and Fenner, 1987). 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 meningitis, 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 cell count and protein concentration. 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.*, 1994b). Despite being a viral disease, the white cell population is dominated by neutrophils, commonly greater than 70% (Baroni and Heinold, 1995; Kline *et al.*, 1994; Rand *et al.*, 1994b). 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) neurological 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 the age of the cat. In one study, pleocytosis appeared within 2 to 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 3 and 12 to 16 months postinoculation (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 peripheral 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 to 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; Coles, 1980; Green *et al.*, 1992; Hamir *et al.*, 1992; Hanlon *et al.*, 1989). 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 three of five horses in one study (Green *et al.*, 1992), perhaps because of antemortem head trauma. The CSF IgM titer increases in 2 to 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, 1998). 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.

f. West Nile Virus

West Nile virus (WNV) is a mosquito-borne flavivirus that is endemic in Africa, Europe, and Asia and emerged as a pathogen in the United States in 1999 (Cannon *et al.*, 2006; Davis *et al.*, 2006; Long *et al.*, 2006; Porter *et al.*, 2004). Disease occurs most commonly in birds, horses, and humans and in several other animal species, and rarely in dogs. Clinical signs frequently include fever and neurological disease most often manifest by ataxia, weakness, and muscle tremors (Cannon *et al.*, 2006). Nonsuppurative (lymphoplasmacytic and histiocytic) polioencephalomyelitis is seen histopathologically in the CNS of animals (Cannon *et al.*, 2006; Cantile *et al.*, 2000, 2001; Kiupel *et al.*, 2003; Wunschmann *et al.*, 2005). CSF is usually abnormal in horses (Wamsley *et al.*, 2002). A mild to moderate mononuclear pleocytosis with lymphocyte predominance and mildly increased protein concentration are the most common CSF findings (Wamsley *et al.*, 2002). However, macrophages may predominate, and occasionally only elevated protein concentration is present (albuminocytological dissociation). The albumin quotient is usually normal and the IgG index is elevated in lumbar (but not in cisternal) samples, perhaps reflecting spinal cord involvement, intrathecal IgG production in this location, and the tendency for pelvic limb weakness in many horses with clinical WNV disease (Porter *et al.*, 2004). In one study of horses with neurological disease attributable to WNV infection, all measured CSF parameters were within reference limits in 27% (8/30) of horses (Wamsley *et al.*, 2002). The current gold standard for diagnosis of active WNV infection in horses is the IgM capture ELISA (MAC-ELISA) for the detection of WNV-specific IgM antibodies. This test appears capable of distinguishing infected horses from vaccinated horses (Porter *et al.*, 2004). Additionally, use of the MAC-ELISA in CSF may be slightly more sensitive than application of the same test in serum for horses with WNV infection (Porter *et al.*, 2004).

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.*, 1994b; Steckel *et al.*, 1982). However, mononuclear CSF has been reported (Berthelin *et al.*, 1994b; de Lahunta, 1983), as has eosinophilic fluid (de Lahunta, 1983; Vandevelde 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.*, 1994b), and cultures are often, but not invariably, positive. Latex agglutination for cryptococcal antigen in the CSF may also be positive (Berthelin *et al.*, 1994b; Jacobs and Medleau, 1998).

There are only a few reports of the CSF abnormalities associated with CNS aspergillosis, blastomycosis, coccidioidomycosis, or histoplasmosis. The CSF abnormalities are variable; but a mixed pleocytosis and elevated protein are typical (Coates, 1995; Gelatt *et al.*, 1991; Kornegay, 1981; Mullaney *et al.*, 1983; Nafe *et al.*, 1983; Schaer *et al.*, 1983; Vandevelde and Spano, 1977). In a 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). The tropism of some fungi for CNS white matter might result in failure to access the meninges or ventricular system and hence result in normal CSF.

4. Prion Disorders

The transmissible spongiform encephalopathies (TSEs) 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, 1994b). 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 (Green *et al.*, 2007; Lowenthal and Karcher, 1994;

Millson *et al.*, 1960; Scott *et al.*, 1990; Strain *et al.*, 1984). However, immunoassay detection in the CSF of the brain derived protein 14-3-3 appears to be useful for the diagnosis of TSE in both animals and people (Hsich *et al.*, 1996; Sanchez-Juan *et al.*, 2006).

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 are reported for fibrocartilaginous embolism and cerebral ischemia/infarction.

1. *Fibrocartilaginous Embolism*

The CSF characteristics associated with fibrocartilaginous embolism (FCE) are reported for dogs and horses. About one-third of the reported canine cases have normal CSF, about one-third have a mononuclear pleocytosis and increased protein, and about one-third have albuminocytological dissociation (Bichsel *et al.*, 1984a, 1984b; Cauzinille and Kornegay, 1996; Gandini *et al.*, 2003; Grunenfelder *et al.*, 2005). Similar findings are reported for two horses (Jackson *et al.*, 1995; Taylor *et al.*, 1977). However, we have seen marked neutrophilic pleocytoses associated with (histopathologically confirmed) severe, acute FCE in dogs (unpublished observation). The type of pleocytosis, neutrophilic or mononuclear, probably depends on the size, location, and age of the infarct. The CSF albumin is reported to be normal, and the IgG index either normal or slightly elevated, the latter possibly reflecting the subsequent inflammation around the lesion (Bichsel *et al.*, 1984b; Tipold *et al.*, 1993b). Dogs with normal CSF may have a better prognosis for recovery (Cauzinille and Kornegay, 1996; Gandini *et al.*, 2003).

2. *Cerebral Ischemia/Infarction*

Cerebral infarction (ischemic encephalopathy) is reported primarily in cats, but also in a few dogs. In cats within the first week of onset, the CSF white blood cell count is normal or mildly elevated with a mixed, predominantly mononuclear, pleocytosis, and protein is mildly to markedly elevated (de Lahunta, 1983; Rand *et al.*, 1994a). Dogs with cerebral infarction have similar CSF characteristics (Bichsel *et al.*, 1984b; de Lahunta, 1983; Joseph *et al.*, 1988; Vandevelde and Spano, 1977), 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 histopathological 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 (Greene *et al.*, 1976; Meric, 1992b; Milner *et al.*, 1996; Rand *et al.*, 1994a; Shell *et al.*, 1988; Vandevelde and Spano, 1977; 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*

Although several reports describe intracranial intra-arachnoid cysts (ICIACs) in dogs (Duque *et al.*, 2005; Kitagawa *et al.*, 2003; Saito *et al.*, 2001; Vernau *et al.*, 1997, 2002; Von Kurnatowski *et al.*, 2006), most do not report the CSF findings present with this condition. Several dogs with ICIACs are diagnosed with concurrent inflammatory brain disease (Duque *et al.*, 2005; Kitagawa *et al.*, 2003; Vernau *et al.*, 1997; Von Kurnatowski *et al.*, 2006), and some authors believe that ICIACs may therefore be incidental in some animals. One report describes two dogs with ICIACs with intracystic hemorrhage. One of these dogs had a normal CSF nucleated cell count but had some degenerate red blood cells and a moderate elevation of protein. The other dog had a mild mononuclear pleocytosis, a mild elevation of protein, and there was evidence of erythrocytophagia (Vernau *et al.*, 2002).

2. *Spinal Arachnoid Cysts*

Most dogs with spinal arachnoid cysts have normal CSF analysis (Gnirs *et al.*, 2003; Hashizume, 2000; Jurina and Grevel, 2004; Rylander *et al.*, 2002; Sessums and Ducote, 2006; Skeen *et al.*, 2003). In dogs with a spinal arachnoid cyst that have abnormal CSF, the most common abnormality is an albuminocytological dissociation (Gnirs *et al.*, 2003; Rylander *et al.*, 2002) with protein concentrations that may exceed 100 mg/dl. Less commonly, dogs may have a mild mononuclear pleocytosis and increased protein concentration as high as 216 mg/dl (Rylander *et al.*, 2002).

G. Metabolic/Nutritional Disorders

Cerebrospinal fluid analysis is not done commonly in animals with metabolic or nutritional neurological 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 neurological dysfunction, CSF analysis is indicated to investigate other causes of the neurological signs. In most cases, routine analysis of CSF associated with metabolic or nutritional disorders does not detect abnormalities (Bichsel *et al.*, 1984b; Fishman, 1992; Scott, 1995; 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 (Bichsel *et al.*, 1984b, 1988; Fishman, 1992). People with diabetic neuropathy may also have increased CSF protein (Fishman, 1992). Animals with severe metabolic encephalopathies often suffer seizures and the effect of seizures on the CSF must also be considered (see Section VII.H). 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; de Lahunta, 1983; George, 1996). Specific biochemical analysis of CSF may show abnormalities, such as abnormalities in osmolality or electrolyte content with salt or water intoxication (Kopcha, 1987; Mayhew, 1989), abnormalities in amino acid levels (such as glutamine) with hepatic encephalopathy (Grabner and Goldberg, 1991; Schaeffer *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 (Burbidge *et al.*, 1989; Widmer and Blevins, 1991). Many contrast agents are low-grade leptomeningeal irritants, resulting in leptomeningeal inflammation that is reflected in the CSF. By 90 min 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 resulting from 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 (Barry and Hauser, 1994; Fishman, 1992; Rider *et al.*, 1995). 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 h 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 because of 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 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; Carrillo *et al.*, 1986). Pleocytosis is usually mononuclear, although meningiomas may have > 50% neutrophils (Bailey and Higgins, 1986b). However, in another study of dogs with meningioma, about 30% of dogs had a normal CSF analysis (Dickinson *et al.*, 2006). In this study, a significant association between meningiomas in the caudal portion of the cranial fossa and an elevated CSF nucleated cell count was found; but only 19% of the dogs had an elevated total nucleated white cell count with a predominance of neutrophils (Dickinson *et al.*, 2006). Neural lymphosarcoma often has a lymphocytic/lymphoblastic pleocytosis (Couto and Kallet, 1984; Lane *et al.*, 1994; Williams *et al.*, 1992), except in cattle in which the tumor is usually extradural (Sherman, 1987). One study of brain tumors in dogs found that pleocytotic CSF is associated with a significantly shorter survival time than is normal or albuminocytological CSF (Heidner *et al.*, 1991). The most common CSF abnormality present with CNS neoplasia is increased total protein, with choroid plexus tumors producing the most marked elevations (Bailey and Higgins, 1986b; Brehm *et al.*, 1995; Heidner *et al.*, 1991; Mayhew, 1989; Moore *et al.*, 1994; Rand *et al.*, 1994a; Roeder *et al.*, 1990; Sarfaty *et al.*, 1988; Waters and Hayden, 1990). 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 (Bichsel *et al.*, 1984b; Moore *et al.*, 1994; Sorjonen, 1987; Sorjonen *et al.*, 1991). 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 (Moore *et al.*, 1994; Sorjonen, 1987; Sorjonen *et al.*, 1991). The IgG index may be elevated, reflecting the presence of inflammatory infiltrates around the lesion (Bichsel *et al.*, 1984b; Tipold *et al.*, 1993b).

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). Neoplastic cells can also be observed in CSF, facilitating a definitive diagnosis. Lymphoma has been diagnosed on the basis of CSF assessment in both small and large animals (Lane *et al.*, 1994; Pusterla *et al.*, 2006a; Vandevelde and

Spano, 1977). However, the observation of neoplastic cells in CSF samples from animals with central nervous system neoplasia other than lymphoma is more uncommon in our experience. This may be partly because cytocentrifugation has been the most common technique employed for the cytological assessment of CSF. Although cytospin slides produce excellent morphology, the cell yield is low (Barrett and King, 1976), which may increase the incidence of false negative results in confirmed cases of CNS neoplasia. Additionally, the general lack of experience of veterinary clinical pathologists at identifying cells derived from central nervous system neoplasms may also result in false negative results. Despite these limitations, the presence of neoplastic cells in cytospin CSF slides from animals with CNS tumors other than lymphoma has been reported in cats with intracranial oligodendroglioma, dogs with CNS histiocytic sarcoma, and dogs with choroid plexus carcinoma (Dickinson *et al.*, 2000; Zimmerman *et al.*, 2006)[3,5]

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 (Madigan and Higgins, 1987; Mayhew *et al.,* 1978). The CSF may be normal or have mild to moderate mononuclear pleocytosis and increase in total protein (Mayhew, 1989). 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). IgG 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 infected and previously infected horses have serum antibodies to *S. neurona* but do not have clinical disease (Andrews and Provenza, 1995; Fenger, 1995). Therefore, it may be useful to also measure the albumin quotient and IgG index in CSF that is submitted for *S. neurona* immunoblotting. However, some have questioned the utility of doing this and recommend a CSF RBC count instead, with RBC counts >50 RBCs/μL invalidating interpretation of a positive CSF immunoblot result (Furr *et al.,* 2002). Conversely, a negative CSF result, even when concurrent with RBC counts >50 RBCs/μL, would indicate that EPM is highly unlikely. The development of a Sarcocystis neurona specific IgM capture ELISA, similar to that developed for WNV infection, may further improve EPM clinical diagnostics (Murphy *et al.,* 2006). 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). However, although a powerful and highly specific test, the PCR test for detection of parasite antigen has not been found to be clinically useful because of the high incidence of false negative results (Furr *et al.*, 2002). The reasons for this are unclear but may be due to rarity of the parasite in CSF or rapid destruction of parasite DNA in the CSF environment or both (Furr *et al.*, 2002).

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.*, 1988, 1989). 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 (Averill and DeLahunta, 1971; Cuddon *et al.*, 1992; Dubey, 1990a; Hass *et al.*, 1989; Kornegay, 1981; Rand *et al.*, 1994b; Tipold, 1995; Vandevelde and Spano, 1977). However, we have also seen marked pleocytosis with marked eosinophil predominance in histopathologically confirmed canine CNS neosporosis (W. Vernau, unpublished observation). Occasionally the white blood cell count and protein are normal (Parish *et al.*, 1987; Tipold, 1995). 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.*, 1993b, 1994). In a study of experimentally infected cats, *T. gondii*-specific IgG was intrathecally 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 (Cole *et al.*, 1993; Patton *et al.*, 1991; Ruehlmann *et al.*, 1995). However, the presence of antibodies does not necessarily indicate clinical disease (Dubey and Lindsay, 1993; Munana *et al.*, 1995). Polymerase chain reaction techniques have been developed to identify the protozoa in tissue and fluids, including CSF (Novati *et al.*, 1994; Parmley *et al.*, 1992; Schatzberg *et al.*, 2003; Stiles *et al.*, 1996). Occasionally the organisms themselves may be seen in CSF cells (Dubey, 1990a; Gaitero *et al.*, 2006; McGlennon *et al.*, 1990).

In considering the CSF abnormalities of toxoplasmosis and neosporosis, two issues must be kept in mind. First, reports of toxoplasmosis before 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 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 suggests parasitism and is typical of some parasites such *Parelaphostrongylus* (Baum, 1994; George, 1996; Mason, 1989; Pugh *et al.*, 1995) and *Angiostrongylus cantonensis* (Lunn *et al.*, 2003; Mason, 1989). However, eosinophilic pleocytosis is not pathognomonic for parasitism, nor does a lack of eosinophils in the CSF rule out neural parasitism (Braund, 1994; de Lahunta, 1983; Lester, 1992). The *Parelaphostrongylus*-specific ELISA may be useful to detect parasite antigen in the CSF (Dew *et al.*, 1992). *Angiostrongylus cantonensis*-specific antibodies can be detected in the CSF via ELISA (Lunn *et al.*, 2003).

K. Toxicity

Even though neurological 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 (Dorman *et al.*, 1990; Dow *et al.*, 1989; Fankhauser, 1962; George, 1996; Little and Sorensen, 1969; Mayhew, 1989; Swarup and Maiti, 1991). Lead has been shown to selectively poison capillary endothelial cells (Goldstein *et al.*, 1977), as well as 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 (Masri *et al.*, 1987; McCue, 1989). 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.*, 1989). 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 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 48h before 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 cell population, and a substantial proportion of neutrophils (40% to 50%) is possible; acute severe disease may result in counts greater than 50 cells per microliter with more than 50% neutrophils (Thomson *et al.*, 1989). In another larger study of canine intervertebral disk disease, mixed pleocytoses as high as 428 cells/μL were noted (Windsor *et al.*, 2007). Total protein may be moderately to markedly elevated because of the disruption of blood vessels, interruption of CSF flow and absorption, and necrosis (Green *et al.*, 1993; Thomson *et al.*, 1989). 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). The 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. We have noted that dogs with chronic or acute on chronic type I intervertebral disk disease have a pleocytosis that is more commonly lymphocytic than neutrophilic (Windsor *et al.*, 2007); white blood cell counts in these instances may be as high as 180 cells/μL. 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 (Mayhew, 1989; Thomson *et al.*, 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 (Andrews and Provenza, 1995; Bichsel *et al.*, 1984b; Sorjonen, 1987; Sorjonen *et al.*, 1991). The gamma globulin percentage and the IgG index are usually normal. Occasional elevations probably reflect the presence of inflammatory cells in the lesion (Andrews and Provenza, 1995; Bichsel *et al.*, 1984b; Tipold *et al.*, 1993b).

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