Cerebrospinal Fluid (22-Aug-2003)

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Introduction
The presence of cerebrospinal fluid (CSF) within the cavities of the brain was already known to the ancients. Probably, the first report of the existence of CSF was performed in the 17th century B.C., and Hippocrates described the occurrence of fluid in brain cavities in the 4th century B.C.; however, it was thought to be pathological. Galen described the ventricular cavities in the 2nd century A.D. For a long time these cavities were thought to be filled with "vital spirit". Only in the 16th century A.D. did Vesalius again discover a watery humor. Systematic studies of this fluid started later and in 1825 Magendie performed the first tap of the cisterna magna in animals. The examination of CSF was introduced by Quincke 1891, and in 1901 cytological techniques were established by Widal and others [1].

Cerebrospinal Fluid and the Ventricular System

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Cerebrospinal Fluid and the Ventricular System

Anatomy
Cerebrospinal fluid is mostly located in the ventricular system and the subarachnoid space. The ventricular system develops from the neural tube and includes the lateral ventricles, third ventricle, the mesencephalic aqueduct and the fourth ventricle, which continues into the central canal of the spinal cord. The cranial cavity is a closed space and requires a continuous adjustment of the intracranial pressure. In the cavity the volumes responsible for this pressure are the brain parenchyma, the CSF and the blood [1,2].

In part the CSF is produced by the choroid plexus which includes the choroidal epithelium, blood vessels and interstitial connective tissue. This plexus is formed as a result of the invagination of the ependyma into the ventricular cavities by blood vessels of the pia mater. The epithelium of the plexus is continued by the ependymal lining of the ventricles. The choroid plexus has an extensive blood supply that reflects its active metabolic activity. In addition, there exists a nerve supply, an extensive perivascular autonomic innervation derived in part from the sympathetic and the vagal nuclei. There is much evidence for adrenergic, cholinergic and peptidergic innervation. The choroidal epithelium is composed of a single row of epithelial cells, arranged in villi around a core of blood vessels and connective tissue. Numerous infoldings and microvilli provide a structure which resembles other epithelia noted for fluid transport. A membranous barrier to the movement of macromolecules are the tight junctions, which join adjacent choroidal epithelial cells. Tight junctions also characterize brain endothelial cells and the cells of the arachnoid membrane. The electrical resistance found on the apical surface of the choroidal epithelium has also been attributed to these tight junctions [1].
**Brain capillaries** have a special morphology in comparison to capillaries of other organs. Endothelial cells in brain capillaries are joined by tight junctions, which results in a continuous layer of cells separating blood from extracellular fluid of the brain. Also, the surface of the vessels have high electrical resistance, which is thought to be an additional function of the tight junctions. Since these junctions serve as a barrier to the movement of ions and molecules the transport is performed by endothelial cells. In addition brain capillaries are surrounded by a basement membrane, approximately 25% the width of endothelial cells [1]. The function of this membrane is to maintain the integrity of the capillary tube under adverse conditions such as osmotic changes or increased hydrostatic pressure from sudden elevations in the blood flow. The capillaries are surrounded by different cell types, pericytes, perivascular macrophages and astrocytic end-feet. The cerebral ventricles are lined with a layer of ependymal cells followed usually by a subependymal layer of glial fibers and glial cells. At this location an exchange between ventricular fluid and the adjacent subependymal extracellular fluid of the brain occurs.

The brain and the spinal cord are surrounded by the **leptomeninges**. They are divided into the arachnoid mater and the inner layer, the pia mater. The two membranes contain the subarachnoid space filled with the extraventricular CSF. The leptomeninges contain few capillaries. The pia mater forms the outer surface of the perivascular space through "invagination" into the nervous system and tissue derived from the arachnoidea contributes to the inner wall of this space. Filaments of spinal and cranial nerves are surrounded in a similar way by a reticular (endoneurial) sheath derived from the pia. The pia mater is also involved in the formation of the ventricles (e.g., roof of the 3rd ventricle and in part of the 4th ventricle). The perivascular space of Virchow-Robin extends from the subarachnoid space to a variable depth within the brain [2].

The **extracellular interstitial fluid** of the brain is thought to have a volume in the range of 15 to 20%. The space is greater in gray matter than in white matter, the former having a higher water content than the latter.

The **dura mater** is a thick and inelastic membrane which encompasses the brain, spinal cord and lumbar sac. The meningeal layer is the protective envelope of the brain. The falx, the tentorium and the diaphragma of the sella are formed by reduplication of the inner meningeal layer of the dura. The dura has an extensive lymphatic supply and a parasympathetic and sympathetic innervation system. The dura is outside the blood-brain barrier.

A connection exists between the scala tympani and the subarachnoid space by the cochlear aqueduct. The pressure in the three compartments of the cochlea shows changes parallel to those recorded in the CSF in several animal species. However, the actual rate of flow between the CSF and the perilymph via the cochlear aqueduct is not exactly known. It is possible that the endolymph duct may be important in the spread of infection between the inner ear and the meninges [1].

**Physiology**

**Secretion**

CSF is produced by a number of sites. Beside the secretion of CSF by the choroid plexus the fluid derives also directly from the brain by the ependymal lining of the ventricular system and the pia-glial membrane and from blood vessels in the pia-arachnoid. The rate of CSF formation in various species varies between 0.2 and 0.5 ml/minute/gm choroid plexus. In the dog the formation rate is approximately 0.047 ml/minute (dependent on the size of the animal), in the cat 0.017 ml/minute, in the rat 0.002 ml/minute and in man 0.35 ml/minute [1-3]. CSF is produced by ultrafiltration from blood plasma and by active transport mechanisms. Hydrostatic pressure in the capillaries initiates the transfer of water and ions to the interstitium and then to the choroidal epithelium. The further transport occurs across tight apical junctions and through cells. Both transmembranal transfers are probably dependent upon ion pumps and are directly related to sodium transport, which depends upon the membrane-bound enzyme sodium-potassium activated ATPase present at the apical surface as well as at the intercellular clefts. A further important enzyme is carbonic anhydrase. The sodium content of plasma and CSF is about the same; however, CSF has an excess of chloride and magnesium and a deficit of potassium and bicarbonate. A further difference is seen in the water content. Plasma is 93% water, CSF 99% [1]. The fact that the concentration ratios of the major ions in CSF are different from those in a protein-free filtrate indicates that the composition of the CSF depends upon secretory processes. Further specific mechanisms such as facilitated diffusion into the CSF exist for the membrane transport of vitamins, nucleosides, purines, glucose and amino acids essential for brain development and metabolism, whereas toxic metabolites are cleared from CSF to plasma. It was shown that the rate of CSF production is independent of moderate variation in the level of intraventricular pressure of short duration. However, in studies with chronically hydrocephalic animals a reduction in CSF formation is observed with increasing pressure. In contrast, acute changes in blood osmolality alter the CSF production, but it is suspected that the choroid plexus will adapt to chronic osmotic derangement, so that any changes in function would be transient. In addition the choroid plexus serves as an "ectopic renal tubular epithelium" in clearing the CSF from toxic metabolites.

**Absorption**

The CSF circulates from the ventricular system to the subarachnoid space. Along the spinal cord and the central canal in animals a caudal flow, as well as a circulating one exists. In humans besides this caudal flow the CSF has the tendency to
flow also cranially. The cranial cavity is a closed space and in the equilibrium state the rate of absorption of CSF equals its rate of formation. The arachnoid villi are the major place for CSF absorption [2,3]. The mechanism for the bulk flow reabsorption into the venous system depends upon the hydrostatic pressure within the subarachnoidal space. Other sites are the choroid plexus, diffusion into brain and capillaries, veins and lymphatics placed around spinal nerve roots.

Function
The CSF has several functions, such as physical support, excretory function, intracerebral transport and control of the chemical environment of the central nervous system (CNS). The CSF helps in the protection of the brain from acute blood pressure changes and, therefore, in the regulation of intracranial pressure. Since the CSF is considered to be an intracerebral transport medium, the fluid is also useful for clinical research.

Composition
As already described, the CSF is a watery solution containing ions and different substances to serve as an intracerebral transport medium for nutrients, neuroendocrine substances and neurotransmitters. Despite the different composition in relation to plasma, the osmolality of the two fluids remains the same (289 mOsm/L) [1]. In comparison to plasma, glucose is slightly diminished in the CSF (about 80%) and there is much less protein, which is mostly albumin. In the normal dog the protein content is less than 25 mg/dl. In addition to these substances a few leukocytes are seen in the CSF, since the CNS is constantly screened by the immune system. In the CSF of normal dogs, 0 - 3 cells/µl, mostly lymphocytes, are counted.

Acquisition (techniques of CSF Tap)
In clinics the CSF is analyzed for its cellular and chemical constituents. CSF is obtained by cisternal puncture in lateral recumbency during general anesthesia. After surgical preparation the puncture of the cerebellomedullary cistern is performed between the occipital bone and the atlas using 22 gauge, 1.5-inch spinal needles with a stylet. For the procedure, the head is held at a right angle to the vertebral column. Avoid excessive flexion of the head since it might occlude the airway. The animal has to be observed for adequate ventilation during the whole procedure. The landmark is a triangle composed from the occipital protuberance and the wings of the atlas. The puncture follows in the middle of this triangle. In animals which are not heavily muscled the space between the occipital bone and the arch of the atlas can be palpated. The needle is carefully inserted, the puncture of the dura mater and atlantooccipital membrane is felt, sometimes a slight muscle twitching occurs, when the needle is in the right place. When the stylet is removed, CSF flow is observed, which is the only reliable sign of a successful puncture. The CSF is collected in a sterile tube. The pressure can be measured using a spinal manometer [4,5], but the additional information is minimal. Therefore, most examiners do not recommend the additional manipulation. The amount of CSF, which can be obtained per animal, is 1 ml / 5 kg. Mostly, 1 - 2 ml are taken and sufficient for the most important examinations. If only a few drops can be obtained, the measurement of the protein content and the cell count is feasible with only 100 - 200 µl CSF. By puncture of radicular vessels blood contamination might occur. Another technique to obtain a CSF sample is the lumbar puncture (L5-L6). CSF can be taken in most cats with this procedure, although in dogs the technique is more difficult. Sometimes only a few drops are obtained and blood contamination occurs more frequently than with the suboccipital puncture. In addition, in normal CSF the number of cells counted after lumbar puncture is somewhat higher.

Cerebrospinal fluid pressure in the subarachnoid space can be measured after the puncture. Values of both the cervical and lumbar spines are affected by changes in body position. Pressure is reduced by inclining the body position to 30, 60 and 90 degrees [6].

Normal and Pathological Findings
Color
The CSF is a watery, clear and colorless fluid. Every change from this waterlike appearance is abnormal. The CSF is cloudy when a high amount of cells are present (> 500/µl). A high protein content can enhance this turbidity and produces a more viscous fluid. Red color is indicative of hemorrhage, in most cases caused by puncture of radicular vessels. If the CSF clears after centrifugation, the coloration was caused by a traumatic tap. However, a red or yellow staining after centrifugation is indicative for a pathologic hemorrhage, since the erythrocytes degenerate easily in the CSF. Xanthochromia, a yellow color of the CSF, is typical for subarachnoid hemorrhage in the absence of hyperbilirubinemia. It is caused by an accumulation of blood pigments and occurs several hours after the hemorrhagic insult (bleeding after trauma, in arteritis cases or other severe CNS inflammation, as well as in vascular or bleeding disorders).

Cell Counts
The cerebrospinal fluid and its cells are a valuable source of information for diagnostics and research since they reflect, at least in part, the immune response occurring in the CNS. The examination of CSF cells is especially useful in cases in which
an ongoing inflammatory reaction is suspected (inflammatory/infectious diseases of the CNS, tumors). The total number of cells is determined by use of a cell counting chamber: mostly the Fuchs-Rosenthal chamber is used. The counting should be performed within about 30 minutes after CSF collection, since the cells degrade rapidly in a fluid with low protein content. In particular, granulocytes are very sensitive and are lysed up to 40% after 2 hours at room temperature. Refrigerating helps to minimize the degeneration [7]. In the CSF of normal dogs and cats 0 - 3 cells/µl are counted, mostly mononuclear cells (lymphocytes and monocytes). It should be reminded that a normal cell count of 3 leukocytes/µl CSF is equivalent to 3000 white blood cells/ml. Slight contamination with erythrocytes because of a traumatic puncture does not severely effect the counting results. Approximately one white blood cell is subtracted from the CSF leukocyte count for every 500 - 700 erythrocytes present.

In cases with severe anemia or leukocytosis the following formula may be used to correct the leukocyte count in the CSF:

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

W = actual or calculated leukocyte content of CSF;
WBCF and WBCB = counted white blood cells in the CSF and blood;
RBCF and RBCB are the numbers of erythrocytes/µl in CSF and blood [1].

In the author’s experience these calculations are probably unnecessary and do not improve the clinical diagnosis in that they do not influence the degree of pleocytosis.

In case of a pleocytosis, an increased cell count, which is graded as mild (5 - 50 cells), moderate (50 - 200 cells) or marked (> 200 cells), a differential cell count is performed as soon as possible. Enumeration and identification of the cells helps to narrow the clinical differential diagnosis [8,9]. For closer evaluation of the CSF cells cytopsin preparations are commonly used [10]. The cytocentrifuge accumulates all the cells in a volume of 0.5 to 1.0 ml of CSF. In case of a marked pleocytosis, 200 µl is sufficient for a differential cell count. Thus, cytocentrifugation can prepare about 300 times more cells for evaluation than observed in the counting chamber. A sedimentation chamber can also give nice results. To prevent rapid cell degeneration during the time of the laboratory procedure and to obtain good cytopsin preparations protein has to be added to the CSF sample (about 1/3 of a 10% bovine serum albumin solution and 2/3 of CSF). CSF samples with a high protein content are centrifuged without addition of albumin. A darkly stained background complicates the evaluation of the cells. After staining (DiffQuick, Papanicolaou) the percentage of lymphocytes, plasma cells, monocytes, macrophages, neutrophils and eosinophils is counted, the cells are evaluated by their size and appearance and mitosis or tumor cells are searched for [10]. Pleocytosis with predominantly lymphocytes and plasma cells is found in viral infections and during the chronic phase of steroid responsive meningitis-arteritis (SRMA) [11], in granulomatous meningoencephalomyelitis (GME) and in breed-specific necrotizing encephalitis. A predominantly neutrophilic pleocytosis is characteristic for bacterial infections and the acute stage of SRMA [11]. A mixed cell population is frequently seen in protozoal diseases, FIP, in chronic bacterial infections, in necrotic lesions and in GME [12]. Eosinophils are found in the rare eosinophilic encephalitis of unknown origin, in protozoal, parasitic and mycotic infections, but also occasionally in GME, FIP and certain tumor types (e.g., histiocytosis) [13]. Tumors mostly have an unspecific differential cell count. In lymphosarcoma large uniform lymphoid cells are characteristic and mixed with a "normal" lymphocyte population. In meningioma a neutrophilic pleocytosis might occur. The differential cell count is only helpful in combination with the clinical examination, signalment, history and further testing (blood, imaging etc.) to establish a clinical diagnosis. Because of this limitation, the evaluation of changes in lymphocyte subpopulations of CSF cells in neurologic diseases might be helpful to further characterize a disease in vivo. Therefore, normal control data for the dog were established using flow cytometry and immunocytochemistry [14,15]. It could be shown that lymphocyte populations in CSF differ from peripheral blood in a few subsets. A relatively high degree of individual variation was found, not only in dogs of different breeds and ages, but also in an inbred Beagle population. These large individual variations suggest that only repeated paired CSF-blood samples taken during the course of the neurologic disease within the same individual would provide meaningful results. CD3+ and CD4+ T-cells were significantly lower in normal CSF. Of great interest is the fact that T-cells, characterized by double staining CD3/CD45RA are present in variable numbers in normal CSF. In other species they are known to be naive or resting T-cells. CD4/CD45RA positive cells seem to be an important subpopulation of these CD45RA positive T-cells underlining the fact that the CNS is constantly observed and screened by the immune system. However, systematic flow cytometry analysis of CSF is feasible in larger animals such as dogs [14]. Occasionally bone marrow contamination of CSF might occur after lumbar puncture [16].

Glucose
The CSF glucose is dependent upon blood glucose level and the rate of metabolism in the CNS. There are two mechanisms
responsible for the entry of glucose into the CSF: carrier mediated diffusion (glucose transporter protein) and simple diffusion. A variable time is required before CSF glucose level reaches an equilibrium with blood glucose (2 - 4 hours) [1]. In general the CSF glucose level is a complex function of the blood glucose level during the previous 4 hours. CSF levels are usually 60 - 80% of blood levels. CSF and blood glucose levels should be obtained simultaneously. An increase in the CSF glucose level is not diagnostically important and reflects hyperglycemia within 4 hours prior to the puncture. Decreased CSF glucose levels were associated with bacterial and fungal meningitis, in which microorganisms and polymorphonuclear leukocytes utilize glucose. However, in the dog, bacterial meningitis is a rare finding in comparison to steroid responsive meningitis-arteritis. In the later disease extremely high numbers of neutrophils may be found (up to 2000 cells/µl). In this disease low glucose levels are measured despite the fact that no infectious agent has been found to date. Neutrophils are supposed to have an increased glycolysis, particularly when these cells are active (phagocytosis, production of oxygen radicals etc.). Low glucose levels were also reported in diffuse meningeal neoplasia and are therefore not considered anymore to be of specific diagnostic value. In summary, low CSF glucose levels in the absence of hypoglycemia indicate the presence of a diffuse meningeal disorder.

**Protein**

In contrast to glucose levels, the measurement of protein levels in the CSF is important in the quest to obtain a differential diagnosis. Most proteins normally present in the CSF are derived from blood. In the dog and cat, normal CSF protein levels after suboccipital puncture are usually less than 25 mg/dl and might be somewhat higher in lumbar puncture samples. The kinetics of protein exchanges were studied with labeled albumin. Following intravenous injection, it needed 20 hours for albumin to reach equilibrium levels in the CSF of dogs. Protein entry depends chiefly upon pinocytosis across capillary endothelial cells, but also upon the isoelectric point. It is considered that more processes are involved than restricted filtration. The exit rate of protein from the CSF to blood is about 200 times the entry rate and normally is performed by passage across the arachnoid villi into the venous blood, presumably by macrovesicular transport. Qualitative measurement of the protein level is performed with the Pandy solution, a 10% carboic acid solution, which precipitates globulin. Normal CSF samples do not show any turbidity after being added to the Pandy solution. Depending on the protein level a recognized turbidity is specified as 1+ to 4+ positivity. The biuret method, normally used for quantification, is not sensitive enough to measure CSF protein levels. Several methods for quantitative measurement of CSF protein level were adapted for use in animals, such as turbidometric methods using trichloracetic acid, benzethonium chloride in an alkaline environment or nephelometry. An increased protein content serves as a nonspecific indicator of CNS disease and may be caused by a damaged blood-brain barrier or an increased local IgG production within the CNS. Elevated protein levels are therefore found in inflammatory/infectious, toxic/metabolic, vascular and neoplastic diseases.

**IgG-Index**

Measuring the IgG-index, which is a calculated quotient using IgG and albumin content of cerebrospinal fluid and serum to detect intrathecal IgG-synthesis, can distinguish inflammatory/infectious diseases of the CNS from other disorders [17].

\[
\frac{\text{IgG}_{\text{CSF}}}{\text{IgG}_{\text{Serum}}} \quad \frac{\text{Albumin}_{\text{CSF}}}{\text{Albumin}_{\text{Serum}}}
\]

In most dogs with infectious/inflammatory diseases, with the exception of the acute form of nervous canine distemper, an elevation in the IgG-index can be determined. Tumors of the CNS, where pleocytosis can be detected, have an IgG-index within the normal range with an exception of lymphoid tumors and meningiomas with secondary cellular infiltration. The demonstration of intrathecal immunoglobulin synthesis resulting from infiltration of IgG-producing lymphocytes inside brain and spinal cord is a specific indication for the presence of inflammation in the CNS. Intrathecal IgG-synthesis can be shown by comparing the amount of immunoglobulin in the CSF with that in serum using albumin as a reference protein. Albumin can cross the blood-brain barrier, but in contrast to IgG, cannot be produced in the CNS itself. Several methods have been used for quantitation of these proteins in animal CSF, such as rocket immunoelectrophoresis, single radial immunodiffusion, agarose electrophoresis, ELISA and laser nephelometry.

Another immunoglobulin that can be measured in the CSF by ELISA is IgA [18,19]. A combined elevation of CSF and serum IgA levels is highly indicative for steroid responsive meningitis-arteritis [11]. A single elevation of IgA in the CSF is only indicative of a primary (inflammatory/infectious disease) or secondary immune reaction (e.g., neoplasia). Various electrophoretic techniques were also applied in animals but did not enhance the diagnostic value of the CSF examination [20,21]. Other proteins were measured such as the myelin basic protein [22], S-100 protein and the C-reactive protein to narrow the clinical diagnosis to demyelination or to distinguish bacterial from viral meningoencephalitis [1,23]. However, the measurement of these proteins remains of the oretical interest and is not useful in clinical practice since too many disorders
Antigen Detection
The IgG-index is a valuable tool to diagnose an inflammatory/infectious disease. The differential cell count together with the signalment, history and additional examinations such as blood values can give a hint as to the etiology of this group of diseases. However, to prove the etiology of an inflammatory/infectious disease, antigen detection is necessary. Occasionally bacterial or fungal organisms may be seen by microscopic evaluation, especially cryptococcus neoformans [24]. However, it must be determined if the microorganisms are a contamination of the CSF after the puncture or are the causative agent of a bacterial encephalitis. If bacteria contaminate collecting tubes or slides for cytospins, neutrophils are still able to phagocytose. Therefore intracellular bacteria are not proof of a causative agent for an encephalitis. If bacterial encephalitis is

<table>
<thead>
<tr>
<th>Disease</th>
<th>Protein Content</th>
<th>Cell Count</th>
<th>Dominant Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-inflammatory distemper</td>
<td>Normal - slightly elevated</td>
<td>Normal, mild pleocytosis (rare)</td>
<td>Mononuclear cells</td>
</tr>
<tr>
<td>Inflammatory distemper</td>
<td>Slightly - strongly elevated</td>
<td>Mild - moderate pleocytosis</td>
<td>Mononuclear cells</td>
</tr>
<tr>
<td>Other viral diseases</td>
<td>Slightly - strongly elevated</td>
<td>Mild - moderate pleocytosis</td>
<td>Mononuclear cells</td>
</tr>
<tr>
<td>Bacterial encephalitis</td>
<td>Slightly - strongly elevated</td>
<td>Moderate - marked pleocytosis</td>
<td>Predominantly neutrophils</td>
</tr>
<tr>
<td>Protozoal encephalitis</td>
<td>Slightly - strongly elevated</td>
<td>Moderate pleocytosis</td>
<td>Mixed population, sometimes eosinophils</td>
</tr>
<tr>
<td>Fungal encephalitis</td>
<td>Strongly elevated</td>
<td>Moderate - marked pleocytosis</td>
<td>Mixed population, sometimes eosinophils</td>
</tr>
<tr>
<td>Parasitic infection</td>
<td>Slightly - strongly elevated</td>
<td>Mild - moderate pleocytosis</td>
<td>Mixed population, sometimes eosinophils</td>
</tr>
<tr>
<td>Granulomatous meningoencephalo-myelitis</td>
<td>Slightly - strongly elevated</td>
<td>Moderate - marked pleocytosis</td>
<td>Varying: mononuclear cells, mixed population, occasionally eosinophils</td>
</tr>
<tr>
<td>Steroid-responsive meningitis-arteritis</td>
<td>Slightly - strongly elevated</td>
<td>Marked pleocytosis</td>
<td>Acute: neutrophils; Protracted: mononuclear cells</td>
</tr>
<tr>
<td>Breed specific necrotizing encephalitis</td>
<td>Slightly elevated</td>
<td>Mild - moderate pleocytosis</td>
<td>Mononuclear cells</td>
</tr>
<tr>
<td>Feline Infectious Peritonitis</td>
<td>Strongly elevated</td>
<td>Marked pleocytosis</td>
<td>Mixed population, occasionally eosinophils</td>
</tr>
<tr>
<td>Eosinophilic encephalitis</td>
<td>Slightly - strongly elevated</td>
<td>Mild - moderate pleocytosis</td>
<td>Eosinophils</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>Varying: normal - strongly elevated</td>
<td>Varying: normal - marked pleocytosis</td>
<td>Varying: mononuclear cells, neutrophils (e.g., meningioma), occasionally eosinophils and tumor cells</td>
</tr>
<tr>
<td>Necrotic lesions (different causes)</td>
<td>Normal - slightly elevated</td>
<td>Varying: normal - marked pleocytosis</td>
<td>Mixed population</td>
</tr>
<tr>
<td>Degenerative lesions</td>
<td>Normal - slightly elevated</td>
<td>normal</td>
<td>---</td>
</tr>
</tbody>
</table>
suspected, the CSF should be cultured and growing bacteria have to be classified. In most cases of bacterial meningoencephalomyelitis, microorganisms are either not present in the CSF or only present in low numbers. Culture results can be negative. PCR techniques are evaluated to improve the diagnostic work-up. In cases of suspected viral encephalitis, such as canine distemper encephalitis, virus detection is performed either by staining techniques (e.g., indirect immunofluorescent antibody examination) or by PCR [25]. Specific antibodies can be found in the CSF [26,27]; however, they are not diagnostic [28,2]. Serial serum determinations are necessary for the determination of a causative agent or the evaluation of specific indices [30]. For the latter, no experience exists yet in small animal medicine.

Enzymes
A wide variety of assays to measure enzymes in serum were also applied to study the CSF but have provided little data with sufficient diagnostic specificity. In veterinary neurology, creatine kinase (CK) and lactate dehydrogenase (LDH) are sometimes measured. However, increased levels are found in a variety of different diseases and are considered to be nonspecific.

Other Metabolites
Numerous metabolites were measured in the CSF, mostly in pathogenesis studies, to improve diagnostic work-up or to improve the prognostic value of the CSF examination. Changes in lactate and pyruvate levels may be indicative for a mitochondrial disease. The lactate/pyruvate ratio reflects the redox state in the brain. The measurement of these metabolites has only been performed in case reports, and values in large numbers of small animals and different diseases are lacking. The concentration of lactic acid in brain is dependent upon its rate of production and independent of blood lactate concentrations. Increased malondialdehyde (MDA) levels are an indicator for lipid peroxidation and was detected in the prefrontal cortex of dogs, but not in the CSF [31]. Gamma-aminobutyric Acid (GABA) is a major inhibitory neurotransmitter in the brain and spinal cord. Low levels of GABA were found in dogs with epilepsy [32-35]. A correlation was found between GABA concentrations in cerebrospinal fluid and seizure excitability. Treatment of dogs with seizures was also monitored by CSF examinations and the measurement of phenobarbital in the CSF [36]. The accumulation of excessive concentrations of glutamate in the extracellular space causes excitotoxic damage. Glutamate is considered to be a mediator of secondary tissue damage and elevated levels are found in several diseases [37,38]. Chronic and acute compressive spinal cord lesions in dogs due to intervertebral disc herniation are associated with elevation in lumbar CSF glutamate concentration [39]. Also biogenic amines were studied in the CSF and are of interest in studies in dogs with behavior abnormalities [40,41]. Changes in normal levels in serotonin, dopamine and norepinephrine are suspected to be important in aggressive dogs or in dogs with compulsive disorders. Low levels of neuropeptides such as orexin and hypocretin can be measured in dogs with sleeping disorders such as narcolepsy [42-44]. Numerous other metabolites were studied, mostly in pathogenesis studies on inflammatory/infectious diseases [45-49]: several cytokines (e.g., interferon gamma, interleukin 8, interleukin 10, transforming growth factor beta) were measured in canine distemper or steroid responsive meningitis-arteritis in comparison to other CNS diseases [50-55]. An elevation of cytokine levels in the CSF is not specific for certain diseases. The CSF of inflammatory/infectious diseases can have chemotactic abilities for neutrophils and/or mononuclear cells [51]. Values for prostaglandins, lipids, neuropeptides, hormones and vitamins are known [1].

Intracranial Pressure (ICP)
The intracranial cavity is closed by bony structures and its contents are fixed in total volume. The cavity is open through the foramen magnum into the spinal subarachnoid space with some degree of elasticity. Three components are important regarding the intracranial pressure: brain, CSF, and blood. Brain tissue may undergo displacement by herniation or by pressure atrophy as observed in hydrocephalus [3]. The volumes of intracranial blood and CSF vary reciprocally as described before, which help maintain intracranial pressure within normal limits. The regulation is performed by autoregulation of cerebrospinal blood flow and CSF absorption, but also by changes in body position [6].

Most dogs have a pressure between 5 and 12 mm Hg under general anesthesia, while slightly lower CSF pressures have been found in cats [4]. Causes for an elevated CSF pressure are space-occupying lesions (e.g., tumors), which cause compression of venous sinuses and therefore prevention of CSF absorption in the arachnoid villi. Elevated CSF pressure also occurs in cerebral edema, usually associated with brain injury, hydrocephalus and inflammatory lesions.

References