A COMPARISON OF RAPID STAINING TECHNIQUES FOR THE CYTOLOGICAL EVALUATION OF CANINE INTRACRANIAL NEOPLASMS

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Abstract

Intracranial neoplasms represent a significant proportion of all tumours in dogs and cats. While many treatment modalities have been examined in order to treat them, efforts to evaluate success are hampered due to the relatively infrequent attempts at definitive antemortem diagnosis. In humans, stereotactic needle biopsy has become a common method of providing a definitive diagnosis of brain tumours with safety and reliability, and cytological techniques have been developed to aid intraoperative diagnosis from biopsy specimens. Stereotactic techniques have recently become available for use in dogs and cats, but little information has been published in the veterinary literature evaluating the normal cytological appearance of the brain or comparing different cytological techniques for intraoperative diagnosis.

In order to evaluate the normal cytological appearance of the canine brain, smears were prepared from samples taken from a normal brain and stained with toluidine blue, following which the cytological appearance of different regions of the brain were described. Samples were also taken from a variety of lesions at post mortem from 10 dogs and 1 cat to evaluate 3 preparation techniques (touch impression, medium pressure impression and smear preparation) and 4 stains (Diff-Quik, May-Grunwald-Giemsa, toluidine blue and Zynostain).

This study provides a useful guide to the normal cytological appearance of the canine brain, which was found to show similarities with the normal cytological appearance of the human brain. The preparation techniques and stains examined provided good diagnostic accuracy, with smear preparation and Diff-Quick stains showing slightly greater accuracy than other techniques. The greatest difficulty was encountered with

diagnosis of inflammatory lesions, due to the potential for confusion with other pathological processes. The most important factors for diagnosis appeared to be the use of the smear preparation and the Diff-Quick stain, high overall slide quality and familiarity of the cytologist with the stain used. While further work needs to be performed to validate the stains and preparation methods that have shown most promising results, it is to be hoped that diagnostic accuracy will improve with increased familiarity with the stains and preparation methods examined.

Declaration

I, Sam Nicholas Long, do hereby declare that the work carried out in this thesis is original, was carried out by either myself, Fenella Long, Pamela Johnston or with due acknowledgement, and has not been presented for the award of a degree at any other university.

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INTRODUCTION

Part I: Background

Epidemiology

Intracranial neoplasms represent a substantial proportion of all neoplasms found in dogs and cats. Of the domestic species, most examples are seen in the dog. While considerable literature on their occurrence exists, the systematic study of central nervous system (CNS) tumours has been undertaken by only a few authors, and comprehensive data on their incidence is available only for the dog. In this species, the incidence of intracranial neoplasia would appear to be greater than in humans, with a reported incidence of 14.5 tumours per 100,000 dogs compared to 3-4 per 100,000 people (Summers *et al.*, 1995; Braund, 1994; Morrison, 1998). Other figures suggest an incidence of CNS tumours found in 1% - 3% of all canine necropsies, including one report that found 2.83% of 6,175 dogs having intracranial tumours at post-mortem examination (McGrath, 1960). One report examining tumours in young dogs indicated that the 3 most common sites for neoplasia were the haematopoietic system, the brain and the skin, in decreasing order (Keller, Madewell, 1992).

The majority of intracranial tumours seen in dogs and cats are of primary CNS origin. The spectrum of primary brain tumours reported is broad and is similar to that described in humans (see Table 1, page 3).

Table 1 : Type and location of canine brain tumours

Tissue of Origin	Tumour Type	Predilection Site
Neuron	Ganglioneuroma	Variable: cerebellum, cranial nerve roots, eye, cervical ganglion
	Ganglioneuroblastoma	
	Neuroblastoma	
Neuroepithelium	Ependymoma	Third and lateral ventricles
	Neuroepithelioma	Meninges, TL spinal cord
	Choroid plexus papilloma	Fourth ventricle
Neuroglia	Astrocytoma	Piriform area, convexity of cerebral hemispheres, thalamus, hypothalamus
	Oligodendroglioma	Cerebral hemispheres, periventricular white matter
	Glioblastoma	As for astrocytoma
	Spongioblastoma	Variable, ependymal surfaces: cerebellum, optic nerve tracts
	Medulloblastoma	Cerebellum
	Gliomas (unclassified)	Periventricular area, esp cerebral hemispheres
Peripheral nerves and nerve sheaths	Schwannoma	Peripheral nerves
	Neurofibroma	
	Neurinoma	
	Neurofibrosarcoma	
Meninges, vessels and other mesenchymal structures	Meningiomas	Convexities of cerebral hemispheres, floor of vault, cerebellopontine angle, falx cerebri
	Angioblastoma	Variable
	Sarcoma	Variable
Pineal gland, pituitary gland and craniopharyngeal duct	Pinealoma	Pineal body
	Pituitary adenoma	Pituitary gland
	Craniopharyngioma	Hypophyseal/infundibular areas
Heterotopic tissues (malformation tumours)	Epidermoid	Fourth ventricle, cerebellopontine angle
	Dermoid	Variable
	Teratoma	Variable
	Germ Cell Tumours	Base of brain above sella turcica
Metastatic tumours	Mammary gland adenocarcinoma, pulmonary carcinoma, prostatic carcinoma, chemodectoma, malignant melanoma, lymphosarcoma, salivary gland adenocarcinoma, haemangiosarcoma etc	Variable
Primary tumours from surrounding tissues	Osteosarcoma, lipoma, chondrosarcoma, fibrosarcoma, nasal adenocarcinoma, haemangiosarcoma, multiple myeloma, calcifying aponeurotic fibromatosis, epidermoid cyst	Variable

(Braund 1994)

The most common types of primary tumours affecting the brains of dogs are meningiomas and glial tumours (astrocytomas and oligodendrogliomas) (Morrison, 1998). Pituitary tumours, choroid plexus papillomas and ependymomas are also commonly described. Meningiomas are reported to comprise between 30 and 39% of all intracranial neoplasms (Braund, Ribas, 1986; Turrel *et al.*, 1986; Heidner *et al.*, 1991). Of the neuroectodermal brain tumours, astrocytomas are probably the most common, representing 55% of one report of 215 cases of neuroglial tumours (Braund, 1994). Of the remaining cases, oligodendrogliomas comprised 28%, glioblastoma multiforme approximately 12%, choroid papillomas approximately 12% and ependymomas 2%. Metastasis of primary brain tumours to non-neural tissue is rare, although it has been reported (Schulman *et al.*, 1992; Dugan *et al.*, 1993; Helman *et al.*, 1980). However, a number of tumours may spread within the CNS along CSF pathways, notably medulloblastoma and choroid plexus papilloma (LeCouteur *et al.*, 1981), although this is more properly referred to as implantation rather than metastasis (Johnson, 1990).

Tumours of the nervous system are most commonly reported in mature and older dogs, with one study reporting a peak incidence at 9 year of age (Heidner *et al.*, 1991), although there are sporadic reports of brain tumours in dogs less than 1 year of age (Keller, Madewell, 1992). There would appear to be no gender predisposition, but there is an increased incidence of brain tumours in brachycephalic breeds such as boxers, English bulldogs and Boston terriers, especially of glial tumours (Braund, 1994). Meningiomas are more commonly reported in mesocephalic and dolichocephalic breeds such as golden retrievers, Dobermans and collies (Gavin *et al.*, 1995).

In cats the most common type of primary CNS tumour is the meningioma, making up 9.8% of all nonhaemopoietic tumours and 56% of CNS tumours found at necropsy (Morrison, 1998). However, while the vast majority of meningiomas in dogs are solitary, multiple primary meningiomas have been reported in up to 40% of cats (Nafe, 1990a), and in young cats with mucopolysacharidosis type I (Haskins, McGrath, 1983). The high incidence of virus–associated lymphoreticular tumours is likely to affect the proportion of brain tumours found in this species (Summers *et al.*, 1995).

The incidence of metastatic brain neoplasms in animals is unknown, but it is likely that it is underestimated, as the cranial vault is uncommonly evaluated during routine post mortem examination in cats and dogs. In the human field, the incidence of silent brain metastases suggests that most epidemiological studies underestimate their occurrence, currently reported to comprise between 30% and 50% of all CNS neoplasms (Johnson, 1990). Up to 20% of people with malignant tumours have brain metastases at death, but many of these patients die of systemic disease before these metastases become symptomatic. Metastases often become apparent after neoplastic involvement of the lung, as 15% of cardiac output returning from the lung goes to the brain.

Classification

Intracranial neoplasms can occur as either primary neoplasms, secondary neoplasms or as metastases of extracranial neoplasms. Primary brain tumours can arise from cells of mesodermal, ectodermal or neuroectodermal origin. The majority of these arise from cells of mesodermal or neuroectodermal origin, which include nerve cells, glia, neuroepithelium, meninges, lymphoid tissue, nerve sheaths, blood vessels, pineal and

neurohypohyseal tissue, and germ cells (see Table 1, page 3). Secondary tumours arise from invasion of the brain by tumours arising in adjacent non-neural structures. Examples include tumours originating from the nasal and frontal sinus, cranial nerves, the pituitary gland and surrounding calvarial bone (Moore *et al.*, 1991). Metastatic tumours most commonly seen in the dog include haemangiosarcoma and carcinomas of the mammary gland, prostate and bronchus (Moore, Taylor, 1988; Sackman *et al.*, 1989; Nafe, 1990b; Mori *et al.*, 1991). Lymphoma may arise as either a primary or metastatic neoplasm, although the majority of cases seen in animals arise as part of a multicentric process with the primary focus outside the CNS.

Classification of brain tumours in animals has traditionally employed criteria used for human tumours, and most commonly follows the grading system established by the World Health Organisation for prognostic purposes (see Appendix I, page 98). However, not all of these tumours have been reported in animals, and some authors question the value of such a grading system in animals given the comparatively infrequent attempts at antemortem biopsy and definitive therapy (Summers *et al.*, 1995; Johnson, 1990). Classification is primarily based on the histological and cytological determination of the cell of origin, the degree of differentiation, pathological behaviour, topographic pattern and secondary changes within and surrounding the tumour (Braund, 1994; LeCouteur, 1999). Classification may also be facilitated by the use of immunocytochemical studies, although substantial differences exist between staining characteristics of human and animal brain tumours, limiting their usefulness (Summers *et al.*, 1995).

Assessment of the malignant potential of a brain tumour can be made on cytological and biological basis, and the difference between these should be emphasised. Cytological malignancy can be defined as the morphological assessment of anaplasia, while biological malignancy can be defined as the likelihood that a tumour will kill its host. While the majority of cytologically malignant tumours are also biologically malignant, many cytologically benign tumours are biologically malignant. A good example is the canine meningioma, which, although cytologically benign, is often locally invasive into the Virchow-Robin spaces. In contrast, the majority of feline meningiomas are well defined with a clear demarcation between normal brain and tumour tissue, and appear to grow more slowly than canine meningiomas (LeCouteur, 1999).

In addition to pathological classification, the classification of brain tumours along anatomic grounds can prove useful for diagnostic purposes. The osseous tentorium is commonly used as a reference point in describing the location of tumours within the cranial vault. Tumours arising in the brainstem and cerebellum may be described as caudotentorial or caudal fossa tumours, while those occurring in the cerebral hemispheres or diencephalon are often referred to as rostrotentorial or rostral fossa tumours. Additionally, brain tumours may be classified as being either intra-axial or extra-axial in origin. Intra-axial tumours arise from within the neural axis and include glial tumours, metastatic tumours and tumours of the choroid plexus and ependyma. Extra-axial tumours arise from outside the neural axis and include meningeal tumours, pituitary tumours and secondary tumours that invade the brain by local extension. The imaging characteristics of these two categories differ and may be useful in providing a diagnosis (Gavin *et al.*, 1995).

Aetiology

While it is beyond the scope of this work to review all aspects of research into the oncogenesis of brain tumours, a brief discussion of some relevant factors may be useful. Currently, there exists no single unifying theory to explain the development of nervous system neoplasms or even all glial tumours. In humans the age and site predilection of different tumour types is well characterised (see Appendix II, page 102 and Figure 1, page 9), suggesting factors involved in tumour development specific to various regions of the brain. In dogs, as in man, the consistent location of some neuroectodermal tumours in particular areas of the brain also suggests that oncogenesis is not a random process. For example, the germinal subependymal plate contains discrete populations of cells with active and persistent mitotic activity and is a zone associated with an increased incidence of gliomas (Summers *et al.*, 1995).

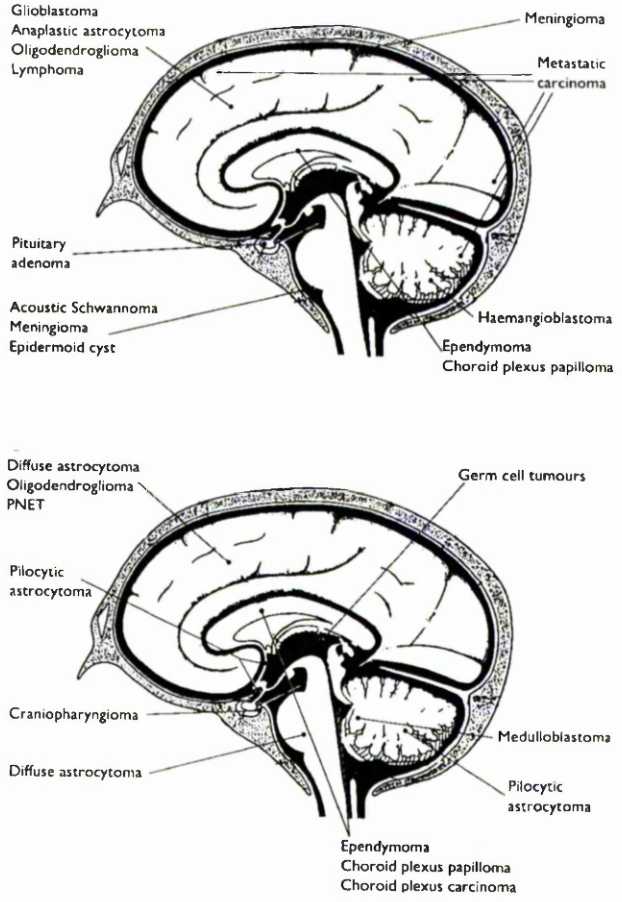

Figure 1: Anatomic distribution of the most common CNS tumours in adults (top) and children (bottom). Adapted from: Moss TH, Nicoll JAR, Ironside JW: Intraoperative diagnosis of CNS tumours. London: Arnold, 1997.

Research into the mechanisms regulating tumour growth has focused on three broad areas: oncogenetic defects within tumours, oncogene expression and growth factor responsiveness of tumours. In humans a number of genetic abnormalities result in a greater incidence of nervous system tumours, perhaps the best characterised of which are the neurofibromatoses. Neurofibromatosis type 2 (NF-2) is characterised by the development of multiple neoplasms of Schwann cell, meningeal and astroglial origin, with more extensive involvement of the CNS. The defect responsible for NF-2 is autosomal-dominant in nature and is associated with the loss of a gene on chromosome 22 (McLendon, Tien, 1998). Interestingly, examination of spontaneous meningiomas in humans has indicated that 43% of human meningiomas have lost portions of at least one copy of chromosome 22, suggesting that chromosome 22 contains a gene that suppresses development of peripheral and central supportive cell neoplasms (Johnson, 1990). However, cytogenetic studies of glial neoplasms have shown that a variety of other genetic defects occur in addition to those affecting chromosome 22 (Johnson, 1990).

Research has also investigated the expression of oncogenes, genes which code for growth factors or their receptors and thus allow more effective competition with other cells for selective growth advantage. Within neoplastic glial cell tumours in humans, growth factors examined have included epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and insulin-like growth factors (IGF), all of which are coded for by separate genes. Progesterone and oestrogen are also thought to play a role in the growth of human meningiomas, and receptors for these sex steroids have been identified in some canine meningeal tumours (Summers *et al.*, 1995).

Although considerable research has been performed into the cellular basis of metastases and their site specificity, the mechanisms involved are poorly understood. While some tumours are not particular about their site of metastasis, others are more selective. Possible factors influencing this include: site-specific growth enhancement by the brain, site-specific adhesion molecules, the absence of growth inhibitors, and the presence of an intracellular matrix readily degradable by tumour emboli (Johnson, 1990). The occurrence and distribution of brain metastases is roughly proportional to the mass of a given brain structure and the volume of its blood supply and they are preferentially distributed in the superficial arterial fields (Johnson, 1990). Unsurprisingly, the majority occur in the cerebrum, most probably reflecting the large relative mass of this portion of the brain, but within this structure preference is given to the border zones between the cerebral artery watersheds. This is likely to reflect lodgement of tumour emboli at the grey-white matter junction, the most distal part of the arterial tree.

Part II: Diagnosis of intracranial neoplasms

Clinical examination

The importance of a detailed neurological examination should not be underestimated in making a diagnosis of intracranial neoplasia. The results of this together with signalment and a detailed history may produce a high index of suspicion of a brain tumour, especially if clinical signs are insidious in onset, the patient is middle-aged or elderly, and the results of neurological examination suggest an asymmetric intracranial lesion. Breed, sex and age of onset have been discussed earlier (see Epidemiology, page 2). Clinical signs seen are related to the location of the tumour, and commonly include seizures, behavioural changes, blindness, circling, pacing, proprioceptive deficits, cranial nerve abnormalities and cerebellar signs (Bagley *et al.*, 1999). Signs may be gradual and slowly progressive or acute and sudden in onset depending on the tumour type and malignancy and the severity of associated pathology (Moore *et al.*, 1996).

Further imaging

Antemortem diagnosis of brain tumours in animals has been limited prior to the availability of advanced imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI). The demonstration of hyperostosis on plain skull radiographs may provide supportive evidence for a diagnosis of meningioma in the cat as it may be associated with a high proportion of meningiomas in this species (Lawson *et al.*, 1984). Plain skull radiographs may also demonstrate the presence of bony destruction with malignant skull tumours of bony origin and nasal tumours with secondary brain involvement. Cerebral angiography may demonstrate disruption of the

arterial circle with some parasellar tumours, but this is not a reliable finding (Brawner, 1993). However, overall very few tumours are easily diagnosed without access to advanced imaging techniques.

The accepted standard for baseline imaging studies of intracranial lesions is a set of images generated in the axial or transverse plane of orientation. Images are generated in this plane with the animal positioned in sternal recumbency in the imaging unit, with slices made perpendicularly to the length of the gantry and oriented perpendicular to the axis of the animal's brain. Following the baseline study, additional views may be taken in the sagittal and dorsal (horizontal or coronal) plane. With CT these views must be derived by image reconstruction, while MRI data can be directly obtained from any plane without loss of detail (Shores, 1993; Thomson *et al.*, 1993). CT image reconstructions are frequently suboptimal because of abrupt transitions at each slice interface, resulting in a step-like appearance with poor integration of slice information (Fike *et al.*, 1981a). Another disadvantage of CT for the imaging of intracranial lesions is the presence of beam hardening artifact in images acquired from the caudal fossa, associated with the relatively thick petrous temporal bones near the base of the skull (LeCouteur *et al.*, 1981). In one study of 50 dogs with brain tumours imaged with CT, 3 were not identified because of lack of contrast enhancement and 1 because of small size (Turrel *et al.*, 1986). For these reasons, MRI is generally accepted as being the preferred imaging technique for the examination of intracranial lesions. MRI is also recognised to provide superior soft tissue definition.

A number of factors are important for the diagnosis of particular tumour types, and have been well characterised in several reports (Thomson *et al.*, 1993; Fike *et al.*,

1981b; Fike *et al.*, 1981a; LeCouteur *et al.*, 1983; Karkkainen *et al.*, 1991; Kraft *et al.*, 1989; Gavin *et al.*, 1995). These factors include the number of lesions found, their location (intra- or extra-axial and specific anatomic location), tumour shape, margins, density (CT) or signal intensity (MRI), the presence of other pathological features and the intensity and pattern of contrast enhancement (see Table 2, page 16). Radiological findings associated with common intracranial tumours are summarised in Table 3 (page 17).

Ancillary Diagnostic Aids

While a number of ancillary diagnostic aids have been developed, few have proven to be of substantial value in the diagnosis of brain tumours. Cytological evaluation of CSF rarely reveals the presence of tumour cells, as few tumours, with the exception of lymphoma, are exfoliative. More commonly, non-specific evidence of inflammation may be present with extra-axial tumours. This has been reported most frequently with canine meningiomas which often contain small foci of necrosis and pools of neutrophils when examined histologically (Braund, 1994).

An elevation in CSF protein concentration without a parallel increase in cell numbers (albuminocytologic dissociation) may be seen with brain tumours. However, this finding is non-specific and has also been reported with cerebrovascular disease, inflammatory disorders and some granulomatous conditions (de Lahunta, 1983). Quantitative evaluation of CSF protein may reveal elevated alpha and beta fractions of albumin, while inflammatory conditions tend to show an increase in beta and gamma globulins (Bailey, Vernau, 1997). However, overall CSF analysis is seldom specific for neoplasia.

Electroencephalography (EEG) has been used to aid characterisation and localisation of intracranial lesions. While in humans EEG can be useful for neuroanatomic localisation, particularly with the addition of Fast Fourier Transformation, differences between humans and animals make this less useful in cats and dogs (Brawner, 1993). Anatomic differences include the large temporalis muscle overlying the cranial vault, the smaller volume of the cranial vault and the relatively thicker skull of dogs and cats, all of which affect the spatial relationship of the electrodes relative to the cerebral cortex (Moore *et al.*, 1996). In addition, abnormal findings on EEG examination are not pathognomic for brain tumours and may be seen with other intracranial conditions and with pathology secondary to the primary tumour (eg peritumoural oedema, hydrocephalus). Finally, many animals undergoing EEG examination have been treated with anticonvulsants, which are also known to cause EEG abnormalities (Moore *et al.*, 1996).

Table 2: Imaging features used to characterise intracranial tumours

Feature	Descriptive characteristics
Number	Single or multiple
Axial origin	Extra-axial or intra-axial
Anatomic location	Rostrotentorial or caudotentorial; olfactory lobes, frontal lobes, parietal lobes, temporal lobe, occipital lobes, cerebellar, cerebellopontine angle, brain stem, parasagittal, falcine, lateral convexities, intraventricular, basal, suprasellar
Shape	Ovoid to spherical, lenticular, broad-based, lobular, plaque-like, amorphous or infiltrative
Margins	Smooth or irregular; well defined or poorly defined
Density (CT)/signal intensity (MRI)	Hypodense, isodense, hyperdense; hypointense, isointense, hyperintense
Other pathological features	Mass effect (midline shift), hydrocephalus, oedema (peritumoural, white matter), haemorrhage, necrosis, mineralisation, hyperostosis, osteolysis, cyst formation
Contrast enhancement a) Intensity b) Pattern	 None, mild, moderate, strong Homogenous, heterogeneous, ring, dural enhancement

(Kraft and Gavin, 1999)

Table 3: Imaging features of common canine and feline intracranial tumours

(Kraft and Gavin, 1999)

Tumour Type	Features								
	Occurrence	Anatomic Site	Shape	Margins	Pattern of growth	Precontrast density on CT	Precontrast signal intensity on MRI	Oedema	Enhancement
Meningioma	Single; occ multiple (cats)	Extra-axial; Calvarial floor (basal), lateral convexitites or falx, esp rostrotentorial; cerebellopontine angle; occ intraventricular or hypophyseal fosssa	Spherical to ovoid, broad based, lenticular or plaque like; dural tail may be present	Smooth to irregular, varying from well to poorly defined	Displaces or compresses normal structures	Isodense to hyper dense	T2: Isointense to hyperintense T1: Isointense	None to mild to extensive	Strong and uniform, occasionally ring enhancing
Choroid Plexus Papilloma	Single; occ seeds to other areas	Extra-axial; Associated with ventricular system	Sperical to ovoid	Distinct, lobulated	Displaces or compresses ventricular anatomy; mass effect and hydrocephalus comomon	Isodense to hyperdense	T2: Isointense to hyperinstense T1: Isointense to hyperintense	None to mild	Very strong, uniform
Pituitary Tumour	Single	Extra-axial; Hypophyseal fossa; macroadenomas extend dorsal to sella turcica, microadenomas contained within pituitary gland	Spherical to ovoid (adenoma) or broad based (adenocarcinoma)	Distinct, smooth to irregular	Displaces or compresses normal structures	Isodense to hyperdense	T2: Isointense or mixed isointense to hyperintense T1: Isointense	None to mild	Mild to strong, uniform (adenoma) or nonuniform (carcinoma)
Nasal Adenocarcinoma	Single	Extra or intra-axial; Caudal nasal passages extending through cribiform plate	Ovoid to amorphous	Indistinct, smooth to irregular	Invasive	Isodense	T2: Isointense T1: Hypointense	Extensive	Mild to strong, heterogenous
Astrocytoma	Single	Intra-axial; Cerebral hemispheres, frontal, olfactory, parietal lobes, occasionally leptomeningeal	Ovoid to amorphous mass	Distinct to poorly defined	Invasive	Isodense to hyperdense	T2: Hyperintense T1: Hypointense to isointense	Mild to extensive	None to strong; homogenous to heterogenous maybe ring enhancement
Oligodendroglioma	Single	Intra-axial; cerebral hemispheres, esp periventricular white matter	Ovoid	Indistinct, smooth to irregular	Invasive	Hypodense to isodense	T2: Hyperintense T1: Hypointense	None to mild	Strong ring enhancing, generally homogenous
Metastases	Single or multiple	Intra- or extra-axial; parenchyma of cerebral hemispheres, diencephalon, cerebellum or brainstem	Spherical to ovoid	Indistinct to distinct	Invasive	Often isodense	T2: Hyperintense T1: Isointense to hypointense	Often extensive even for small lesions	Strong uniform or ring enhancing

Magnetic resonance spectroscopy has been employed in the evaluation of human tumours and its experimental use has been described in animals with intracranial lesions (Shores *et al.*, 1990; Shores *et al.*, 1991; Bailey, 1990). However, little research to date has been performed to validate its use in the evaluation of brain tumours in animals in vivo.

Stereotactic needle biopsy

Stereotactic techniques were first described in 1947 for the biopsy of intracranial lesions in man (Spiegel *et al.*, 1947). Over the past 2 decades the development of advanced imaging has led to refinement of these techniques and image guided stereotactic biopsy has now become a routine procedure in many neurosurgical institutions (Vernau *et al.*, 2001). The procedure is minimally invasive and facilitates the diagnosis of previously inaccessible lesions with safety and reliability (Brainard *et al.*, 1997). Morbidity and overall complication rates are generally low, varying between 2% and 6%, and with mortality rates ranging between 0% and 2.3% (Burger, Nelson, 1997; Kondziolka *et al.*, 1998).

The growing prevalence of stereotactic needle biopsies has resulted in human neuropathologists being presented with smaller and fewer samples from each case for evaluation. In order to provide an intraoperative diagnosis for CNS lesions a number of rapid diagnostic techniques have been developed, including frozen tissue preparation and cytological preparation. The advantages of cytological preparations include a more rapid turn around time than frozen tissue and the retention of more tissue for standard paraffin embedding for definitive diagnosis (Reyes *et al.*, 1991). As a result, intraoperative cytological evaluation of smear preparations of CNS lesions has become

a common practice in many human neuropathological institutions (Vernau *et al.*, 2001). The aims of intraoperative cytological evaluation are twofold: firstly to confirm the diagnostic value of the sample in order to minimise the number of samples taken; and secondly to provide a provisional histological diagnosis which facilitates patient management decisions in the early postoperative period (Kitchen *et al.*, 1993).

Cytological preparations routinely consist of 'smear' or 'touch' preparations. While some reports suggest that touch preparations are considered less informative, others have demonstrated comparable results using this method compared to the smear technique (Kitchen *et al.*, 1993). Stains described include Diff Quick, haematoxylin and eosin, toluidine blue, and the Papanicolaou stain (Firlik *et al.*, 1999; Adams *et al.*, 1981). The important features of the stain selected include speed of preparation, ease of use, quality of nuclear and cytoplasmic detail and consistency of results (Adams *et al.*, 1981; Moss *et al.*, 1997). Several studies reporting the accuracy of diagnosis by the smear technique in man have been published, and this figure varies between 75% and 94% (Brainard *et al.*, 1997; Burger, Nelson, 1997; Reyes *et al.*, 1991; Kitchen *et al.*, 1993; Firlik *et al.*, 1999; Folkerth, 1994; Gaudin *et al.*, 1997; Hayden *et al.*, 1995; Kleihues *et al.*, 1984; Martinez *et al.*, 1988; Mennel *et al.*, 1989; Ostertag *et al.*, 1980). Due to the small sample size of stereotactic biopsy specimens, accurate diagnosis relies heavily on the quality of the smear preparation and the familiarity of the pathologist with the stain used and the cytological appearance of the normal brain. Regions of the normal human brain exhibit different cytological characteristics which have been well characterised (Adams *et al.*, 1981; Moss *et al.*, 1997). Important features described include the large 'Betz' neurons of the motor cortex and the numerous small granule

cells of the cerebellum which, to the inexperienced cytologist, may be misinterpreted as a tumour of lymphoid origin (Moss *et al.*, 1997).

Recently, stereotactic biopsy systems have been developed for use in animals and their use for the diagnosis of intracranial lesions has been described (Koblik *et al.*, 1999b; Koblik *et al.*, 1999a; Higgins *et al.*, 1998; Lecouteur *et al.*, 1998; Vernau *et al.*, 1997). There are few reports in the veterinary literature describing the cytological appearance of intracranial lesions. One recent report describes the cytological evaluation of smear preparations of intracranial neoplasms for intraoperative diagnosis following stereotactic biopsy and conventional open craniotomy (Vernau *et al.*, 2001). However, this report described only the use of a single stain and preparation technique. Other reports have described the cytological appearance of meningiomas and a glioma using air dried smears obtained from either impression or needle aspiration (Zimmerman *et al.*, 2000). A report comparing the accuracy of cytological diagnosis with histopathology in 100 tumours of various organs included 4 intracranial neoplasms, and suggested an overall diagnostic accuracy of 83% based on cytological methods (Eich *et al.*, 2000).

Cytological appearance of brain tumours

The cytological appearance of human brain tumours has been well characterised in a number of studies (Adams *et al.*, 1981; Moss *et al.*, 1997; Folkerth, 1994). Excellent descriptions of human brain tumours can be found in Moss TH, Nicoll JAR and Ironside JW: Intraoperative diagnosis of CNS tumours, Arnold (1997). The cytological appearance of animal tumours is considerably less well described, with only one reference characterising their appearance in dogs and cats (Vernau *et al.*, 2001).

This report provides an excellent description of the cytological description of a variety of canine and feline brain tumours. The following descriptions are taken from this reference and describe the findings of intraoperative cytological diagnosis of 93 canine and feline brain tumours using smear preparations stained with H&E.

Oligodendroglioma

Grossly canine oligodendrogliomas have been described as soft and mucoid in consistency, allowing easy spreading of smear preparations. When viewed at lower power magnification, these tumours are characterised by large numbers of prominent, finely branching blood vessels, often with segmentally thickened profiles due to focal proliferation of microvascular cells forming glomeruloid capillary tufts. In general, blood vessels tend to be free from tumour cells.

At high power, smear preparations are relatively cellular and largely composed of closely packed small, round cells with moderate anisokaryosis. Nuclei are uniformly round to ovoid and nuclear chromatin is fine, granular and evenly dispersed. Nucleoli are small, and if present are solitary. Cytoplasm is minimal, eosinophilic and granular with poorly defined borders. Between cells a finely granular, wispy eosinophilic matrix may be seen.

Anaplastic oligodendrogliomas contain more pleomorphic cells, some with variable amounts of prominent eosinophilic cytoplasm. In these tumours, many necrotic cells may be found, along with mitotic figures and a small number of reactive astrocytes.

Astrocytoma

The majority of astrocytomas examined in dogs and cats have been reported to smear readily and evenly. Low power examination reveals a tumour of moderate hypercellularlity containing thin walled, well defined, branching blood vessels. Tumour cells tend to emanate from blood vessel margins forming radiating streams of elongated cells with long, thin cytoplasmic processes.

When viewed at higher power, tumour cells are characterised by having elongated nuclei with irregular borders and coarsely stippled chromatin. Cytoplasm of these cells is usually minimal and stains only lightly eosinophilic. An extensive network of randomly crossing, eosinophilic, sharp fibrillary cytoplasmic processes between tumour cells is seen. Some of these may be extremely thick with multiple branches. A refractile light source may aid visualisation of these processes.

In more anaplastic astrocytomas, marked nuclear atypia is often present, with several mitotic figures (up to 2 per high power field). Neurons may be seen entrapped within tumour matrix and numerous necrotic cells and rarely occasional neutrophils are admixed among singly dispersed cells. A wispy, bluish staining matrix containing a finely fibrillated meshwork of processes may be seen.

Oligo-astrocytoma

Only two of these tumours have been described in animals, both occurring in the dog. When viewed at low power, cells in these tumours remain either densely clustered around branching blood vessels or dispersed into closely aggregated but separated cells.

Blood vessels are numerous, highly branched with some thickening of vessel walls and capillary proliferation. Glomeruloid tuft formation is not seen, however.

High power viewing reveals individual cells with prominent nuclei generally without obvious cytoplasm, although some cells possess bipolar fibrillary processes. Nuclei stain lightly basophilic, are round to oblong, often with irregular borders, and vary in size. Foci of calcification may be scattered throughout these tumours.

Glioblastoma multiforme

When compared to better differentiated astrocytomas, this tumour possesses very prominent glomeruloid vascular formation. Nuclear karyomegaly is more prominent and a high number of mitotic figures may be seen per high power field. Numerous necrotic cells and debris have also been reported.

Choroid plexus tumours

At low power magnification, the similarities between these tumours and normal choroid plexus architecture is readily apparent. Blood vessels are numerous and very prominent and form multiple papillary fronds. Some tumours contain mineralised foci.

Between blood vessels, large numbers of round to irregularly shaped cell clusters or sheets of dispersed individual cells may be seen. Tumour cells are often arranged in broad sheets of a single layer of epithelial-type cells or in radiating patterns similar to normal choroid plexus. Blood vessels tend to be very thickened and dense due to their fibrovascular core and possess variably thick layers of haphazardly but outwardly

radiating, cuboidal to oblong shaped cells. In these patterns cells are arranged in a regular layer of columnar epithelium with basally oriented nuclei and prominent apical cytoplasm.

At higher magnification, individually dispersed cells are seen with small, round to ovoid, uniform sized nuclei containing one or more prominent nucleoli, finely granular chromatin and a darkly staining nuclear border. Cytoplasm of cells is plentiful, eosinophilic staining, round to oblong, sometimes bipolar and elongate but without processes.

Choroid plexus carcinoma has been reported to have prominent anisokaryosis but all other features are similar to choroid plexus papillomas. Mitotic figures have not been found in any choroid plexus tumours reported in animals (Ribas *et al.*, 1989).

Ependymoma

Low power viewing of these tumours reveal prominent, thickened blood vessels, many of which are branching. Blood vessels seen in longitudinal section are thickened due to perivascular pallisading of layers of ependymal cells. Tumour cells are elongated and generally arranged several cells thick and radiating out from blood vessels. Generally cells tend to be aligned perpendicularly to vessels with the nuclear pole oriented outwards. Along with these perivascular pseudorosettes, sheets of single detached cells are often seen.

At high power magnification, tumour cells can be seen to contain round to ovoid, elongated, sharply bordered nuclei with a cribiform chromatin pattern and a single,

small nucleolus. The cytoplasm of these cells is oblong, eosinophilic and usually unipolar. In cats this tumour is reported to possess more prominent unipolar eosinophilic cytoplasm with sharply defined borders.

Some perivascular tumour cells contain large, karyomegalic nuclei with abundant cytoplasm. While mitotic figures are rarely seen, necrotic cells and nuclear and cytoplasmic atypia are common.

Primary CNS lymphoma

Blood vessels seen in these tumours are prominent and highly branched. Tumour cells tend to be individually dispersed but tightly packed. They are monotonously round and uniform in size and stain slightly basophilic. Some tumour cells may possess minimal eosinophilic cytoplasm. Mitotic figures are fairly common and at the margin of these tumours parenchymal invasion of the normal brain by perivascular clusters of tumour cells may be seen.

Primitive neuroectodermal tumour

This tumour has been described as cohesive and difficult to smear. At low magnification cells are found in relatively large clusters closely adhered to blood vessels. Tumour cells are closely packed with large, round to oblong, strongly basophilic staining nuclei and an eccentric rim of scant and finely granular cytoplasm. Nuclei are seen with a sharp border and one or two fine, pinpoint nucleoli.

Homer-Wright rosettes may sometimes be found, along with thin, granular, non-refractile processes running between cells. Cells are rarely dispersed and are embedded in a finely granular, wispy matrix.

Meningioma

The cytological appearance of several of the histologic subtypes of meningiomas have been reported in dogs and cats (including meningothelial, transitional, secretory and psammomatous). However, with the exception of the meningothelial subtype, the appearance of all tumours is generally a mixture of different subtypes and rarely is one pattern seen alone. In general, all meningiomas are readily smeared with the exception of the transitional subtype, which is more difficult to spread.

At low power examination, tumour cells are broken up into many clusters or cohesive cell aggregates as well as being separated into individual cells. Blood vessels are generally not prominent and tumour cells are usually not closely adherent to their walls. However, a few tumours do contain blood vessels with very thickened walls with multiple branching blood vessels.

At high power magnification, cells can be seen to possess round to slightly elongate, uniform sized nuclei with diffuse punctate chromatin and well defined nuclear borders and a small, prominent nucleolus. Rarely, intranuclear cytoplasmic evaginations may be seen, but these are plentiful in some tumours of the meningothelial subtype. More elongate cells sometimes show a central bar or fold through the longitudinal axis of the nucleus. The cytoplasm of tumour cells is eosinophilic, granular, wispy to solid and varies in amount, being round to elongate when present and often with a polar location.

In general, despite differences in histological subtypes, tumour cells possess very similar and distinctive nuclear morphology and mitotic figures are extremely rare.

Transitional meningiomas frequently exhibit distinctive whorls. Meningothelial subtypes disperse into distinctive cell clusters. Psammomatous subtypes possess multiple Psammoma bodies. Some tumours may display marked cellular anaplasia or nuclear atypia, and neutrophils may be found in tumours with histologic foci of necrosis and neutrophil accumulation.

Meningiomas in cats generally smear less readily and tend to have more clumping. Tumour cells are more elongate and linear patterns of tissue calcification and necrosis are often seen.

Pituitary adenoma

The cytological appearance of pituitary adenomas has not been reported in dogs and cats. In humans, the majority of these tumours possess a very soft texture and consequently smear easily into a thin film. The pattern seen at low magnification varies with the intrinsic tissue architecture of the tumour being examined. Most characteristically a smooth monolayer of cells is seen with little or no cohesive tendency. Some tumours may not smear so easily and exhibit tissue clumping into loose aggregates. Even so, there is a distinct tendency for individual cells to dissociate from the edge of tumour masses. In other cases, smears may produce well formed papillary structures with cuffs of tumour cells clinging to branching blood vessels. However, in these cases individual cells usually smear out easily from margins to form an intervening monolayer. Despite the intrinsic vascularity of most pituitary adenomas,

blood vessels are usually not prominent in smear preparations and are seen mostly in the centre of papillary formations or larger clumps of tissue. Most of the blood vessels seen are thin-walled capillary vessels which are delicately branching.

At higher magnification, cytology usually suggests a single population of cells. Typically the nuclei of tumour cells are centrally placed, very uniform and rounded in shape, and have finely speckled chromatin with no distinct nucleolus. Some adenomas may show pronounced nuclear pleomorphism with multinucleated cells. This is not seen commonly and is not an indicator of malignancy. Tumour cell bodies are generally rounded and discreet without processes and cytoplasmic margins are usually clearly defined. This is mostly seen when cells are in a monolayer, however if the preparation is poorly preserved or oversmeared, the cytoplasm of some cells may be very indistinct with apparently denuded nuclei in a granular or amorphous background.

Metastatic carcinoma

As with pituitary adenomas, the cytological appearance of metastatic tumours has not been described in animals. In humans, these tumours are usually soft enough to provide good smear preparations. The cytological features may be very variable, but most carcinomas exhibit epithelial cohesiveness in smears at low magnification, forming discreet clumps of tumour cells along with larger masses of thickly smeared tissue. The degree of dissociation of individual cells around the edges of clumped tissue varies with the type of tumour, and some softer tumours may smear predominantly as a monolayer.

Examination at higher magnification reveals cell nuclei that are typically large and rounded with pale chromatin and prominent nucleoli. In some cases, nuclei may display a monotonous, bland chromatin pattern, while in poorly differentiated lesions they may be small and hyperchromatic. Mitotic figures are commonly present, but may be hard to identify depending on nuclear size and density. Nuclear pleomorphism varies considerably between tumours. Tumour cell cytoplasm is sometimes sparse and indistinct, especially in less well differentiated carcinomas. Moulding of adjacent cells where cohesive sheets are present is a helpful feature.

Part III: Treatment methods and survival

Treatment methods and outcomes

Although a considerable body of research has investigated definitive therapy for brain tumours in humans, there is a comparatively small amount of similar data available for animal brain tumours. Consequently it is difficult to accurately assess the outcomes and survival rates for all of the treatment modalities reported. Perhaps the biggest difficulty arises from the relatively infrequent attempts at definitive antemortem histological diagnosis of tumour type (Johnson, 1990).

The most common methods investigated to date include: surgical therapy, radiation therapy and chemotherapy. Additionally, the value of canine brain tumours as a naturally-occurring model for human brain tumours has led to the evaluation of some novel therapies in the dog. However, only a brief overview of success rates of the most common treatment methods will be provided here, as it is beyond the scope of this work to review the substantial body of research currently being performed in the field of novel therapies.

Surgery

It is difficult to evaluate the value of surgery alone in dogs, as few studies have examined the results of surgery without additional therapy. In one study of 86 dogs with intracranial neoplasia, 15 had intracranial surgery alone while 13 dogs had intracranial surgery with one or more other treatment type (Heidner *et al.*, 1991). Of those dogs that had surgery alone, no significant difference was found between the survival times of those dogs with meningiomas when compared with other tumour

types. Interpretation of the results of this study was made difficult by the total number of treatment modalities (11) and the small number of dogs that received each treatment, but median survival following surgery in this study was 0.9 months (range 0.1-2.2). No attempt was made to correlate the success of surgery with the tumour type or location. Reports of surgical excision of meningiomas in dogs have reported a median survival time of 143 (Kostolich, Dulisch, 1987) and 198 days (Niebauer *et al.*, 1991), based on 4 cases and 10 cases respectively. However, one of these studies suggested a median survival time of 414 days and a 40% 1 year survival rate for tumours other than meningioma treated surgically (Niebauer *et al.*, 1991). A single case of an optic nerve meningioma with lung metastasis treated surgically has been reported, and local excision resulted in tumour-free survival for 18 months. This dog lived a further 24 months before being diagnosed with lymphoma and euthanased.

Surgery without other therapy has been most comprehensively examined in cats for the treatment of meningiomas. Several reports of successful surgical excision of cerebral meningiomas exist (Gallagher *et al.*, 1993; Gordon *et al.*, 1994; Lawson *et al.*, 1984; Shell *et al.*, 1985), but few have long term follow-up (Gallagher *et al.*, 1993). One report of 17 cats treated with surgery alone reports 11/14 cats that survived the immediate post-operative period failed to show evidence of recurrence over the follow-up period (median follow-up 27 months, range 18 – 47) (Gallagher *et al.*, 1993). One cat that did develop recurrence of the tumour at the site of surgery was euthanased 72 months after surgery, for signs related to a carcinoma in the region of the hypophysis. The recurrence of the meningioma was found at post mortem, and not detected by CT scanning prior to euthanasia. This study found that no clear advantage was demonstrated of surgery and radiation over surgery alone, and it was recommended that

radiation therapy only be considered if surgery did not result in the removal of all visible tumour or if the tumour recurred. Earlier studies are less promising. One report of 42 cats treated surgically for meningiomas reported that 8 cats died immediately post surgery (Gordon *et al.*, 1994). Overall survival was 71% at 6 months, 66% at 1 year and 50% at 2 years.

Radiation therapy

Radiation therapy using orthovoltage, cobalt 60 and megavoltage irradiation has been used in the treatment of canine brain tumours and has been shown to improve median survival times to between 150 and 360 days. Orthovoltage radiation is less optimal due to poor beam penetration, profile, and limited field configuration (LeCouteur, 1999). External beam megavoltage generally involves fractionating a total normal tissue dose of less than 50 Gy into daily or alternate day fractions of less than or equal to 3 Gy for up to 4 weeks (Gavin *et al.*, 1995; Brearley *et al.*, 1999). Several reports in the veterinary literature describe its use and survival times (Heidner *et al.*, 1991; Brearley *et al.*, 1999; Spugnini *et al.*, ; Evans *et al.*, 1993; Nakaichi *et al.*, 1996). However, few reports differentiate tumour type and therefore the sensitivity of different tumour types to radiation therapy is unknown.

As well as conventional radiation therapy a number of other forms of radiotherapy have been investigated in dogs. Brachytherapy using I^{125} has been described in one study (Heidner *et al.*, 1991) with no increase in median survival times over surgery alone. Additionally, x-ray phototherapy has been described in a small number of dogs, using a modified CT scanner to deliver radiation (Norman *et al.*, 1997; Iwamoto *et al.*, 1990; Iwamoto *et al.*, 1993). The total amount of energy delivered to the tumour can be

increased by using an intravenous iodinated contrast agent (Norman *et al.*, 1991). Another method of delivering radiation to tumour cells known as Boron Neutron Capture Therapy (BNCT) has been described (Moore *et al.*, 1996). In this form of radiation therapy, a boron compound, given intravenously, is concentrated within the tumour. Exposure of the tumour to a neutron beam causes a fission reaction within tumour cells, producing lithium particles and alpha particles, leading to cell death (Coderre, Morris, 1999). Median survival times following BNCT have not been reported.

Chemotherapy

Few reports exist in the veterinary literature describing the treatment of intracranial neoplasms with chemotherapy. One of the major hurdles to be overcome with the use of chemotherapeutic agents is the inability of most agents to cross the relatively impermeable blood-brain barrier (BBB). Two of the chemotherapeutic agents able to overcome this problem are carmustine and lomustine. They are both nitrosurea compounds and consequently are lipid soluble and able to penetrate the BBB. Their use has been described in a small number of dogs with some success (Fulton, 1991; Dimski, Cook, 1990). In one study, the median survival time of 5 dogs with confirmed astrocytomas was 218 days (Fulton, Steinberg, 1999). However, in the human field the sensitivity of different tumour types to chemotherapy is well characterised, with oligodendrogliomas being the most sensitive of the glial tumours. As relatively few animal tumours are diagnosed definitively prior to treatment, the success rate of chemotherapy has yet to be fully evaluated.

Aims of the study

Evaluating the value of treatment modalities for animal tumours is complicated by several factors. Most important among these is the small proportion of tumours which receive definitive diagnosis prior to treatment. While the response of different tumour types to various modes of therapy is well defined in humans, this is not the case in animals and this is likely to hamper the optimal treatment of animal brain tumours.

Given the advantages of stereotactic biopsy over conventional craniotomy, it is likely that this procedure will become more common in the field of veterinary neurology for the definitive antemortem diagnosis of brain tumours. However, no reports to date have compared the use of different stains or preparation techniques for the cytological diagnosis of intracranial lesions in animals. Furthermore, no reports have so far described the cytological appearance of the normal canine brain. Both of these factors are important in establishing the value of cytological examination as an accurate intraoperative diagnostic tool. Consequently this study was designed to provide this knowledge and establish the reliability and accuracy of cytological examination for the diagnosis of intracranial lesions.

The aims of this study were twofold: firstly, to describe the cytological appearance of selected regions of the normal canine brain, and secondly, to evaluate the accuracy and diagnostic value of 4 rapid supravital stains (Diff Quik, May-Grunwald-Giemsa, Toluidine blue, Zynostain) and 3 preparation techniques (touch preparation, medium pressure touch preparation, smear preparation) for the cytological diagnosis of intracranial lesions.

MATERIALS AND METHODS

Part I: Cytological appearance of normal canine brain

In order to characterise the cytological appearance of the normal canine brain, smear preparations were made from regions of the brain similar to those characterised in man (Vernau *et al.*, 2001; Adams *et al.*, 1981; Moss *et al.*, 1997). The regions selected were chosen from those that may be encountered during stereotactic biopsy. A 6 year old, male entire golden retriever which had been euthanased for a condition unrelated to the brain had a full post mortem evaluation performed. The brain was removed and sectioned into 10 mm slices (see Figure 2, page 37). From selected locations, a 1 mm^3 sample was removed using sharp dissection. Samples were placed on one end of a standard glass microscope slide, the end of a second glass slide was applied to the sample, and using an appropriate amount of pressure the two slides were drawn quickly and firmly apart. Slides were immediately immersed in 95% ethanol for 60 seconds. Following fixation, slides were stained with 1% aqueous toluidine blue solution for 30-60 seconds before mounting with coverslips (see Figure 3, page 37). The entire brain was fixed in 10% buffered neutral formalin following sample collection, and processed routinely for histopathological examination using paraffin embedded sections and haematoxylin-eosin staining to confirm that no abnormalities were present.

Samples were taken from the following locations: grey matter of the cerebral cortex, caudate nucleus, thalamic nuclei, hippocampus, white matter of the corona radiata and corpus collosum, medial lemniscus in the region of the midbrain, vermis and paramedian lobe of the cerebellum, and medulla oblongata in the region of the nucleus of the dorsal raphae and the tectospinal tract (see Figure 4 - Figure 12, pages 41-45).

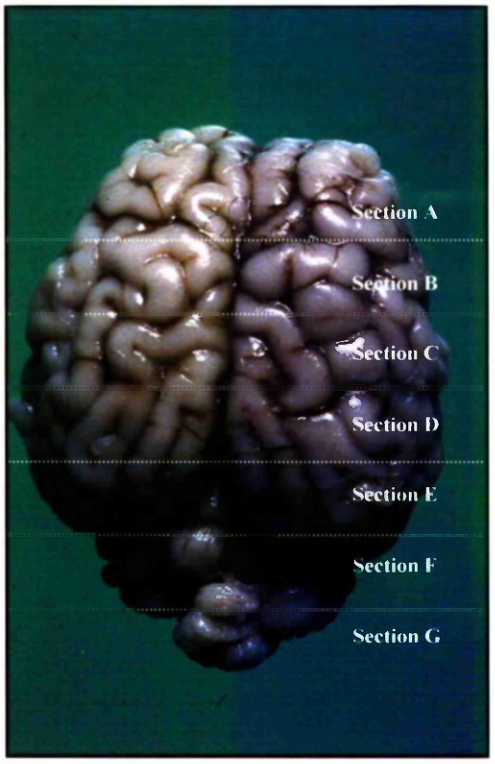

Figure 2 : Location of sections

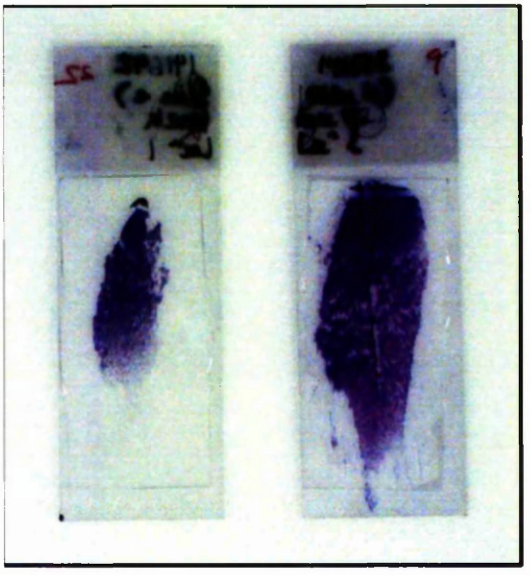

Figure 3: Smears of normal brain stained and mounted

Part II: Cytological Diagnosis of Intracranial Lesions

Accuracy of cytological diagnosis

Material was examined from 10 dogs and 1 cat that had been euthanased following diagnosis of intracranial lesions. The animals presented to the University of Glasgow Veterinary School (UGVS) over the period 2000 – 2001 for clinical signs referable to intracranial disease. All cases had mass lesions demonstrable either on computed tomography scanning[1] or at post mortem examination. Samples were taken from each lesion at post mortem within 30 minutes of euthanasia using a standard core biopsy needle[2] and preparations were made in one of three ways: light pressure touch impression, medium pressure impression, and smear preparation. Touch impressions were generated by lightly applying a standard dry microscope slide to the tissue sample. Medium pressure impressions were generated in the same way as touch impressions but applying a moderate amount of pressure. Smear preparations were generated as described above for normal brain samples .

Following slide preparation, slides to be stained with toluidine blue were immediately fixed in 95% ethanol for 60 seconds while all other slides were rapidly air dried. Slides were then stained with one of four rapid supravital stains according to standard protocols: a commercially available Diff Quik stain[3], May-Grunwald-Giemsa stain, 1% toluidine blue and the recently developed 'Zynostain'[4] (see APPENDIX IV, page 104). All slides were cleared and mounted with coverslips following preparation.

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[1] Excel 2400 elite, Marconi Medical Systems, Cleveland, Ohio, USA
[2] Vet-Core, Cook Veterinary Products Inc, Bloomington, Illinois, USA
[3] Kwik-Diff Stain, Shandon Inc., Pittsburgh, Pennsylvania, USA
[4] Zynostain, Hematek Ltd, Glasgow, Scotland, United Kingdom

Following sampling, the brain was fixed in 10% buffered neutral formalin for 24 hours before being sectioned transversely at the level of the optic chiasm to allow adequate internal fixation. After fixation for a further 7 days it was then routinely processed for histopathological examination following paraffin embedding, sectioning at 5 μm, and staining with haematoxylin-eosin.

The number of non-diagnostic specimens was recorded, and for final cytological evaluation, 12 slides in total were examined from each case – one of each combination of the 4 stain types and 3 preparation methods. These were selected at random from those specimens that were diagnostic. Slides of cytological preparations were examined by one pathologist who had been blinded to the histological diagnosis of all cases. Slides were examined following randomisation using a computerised random number generator. Diagnosis was made on the basis of pathological process (normal / inflammation / neoplasia / undetermined) and specific diagnosis (inflammation / mesenchymal tumour / epithelial tumour / round cell tumour / undetermined). For purposes of classification, glial tumours (including astrocytomas and oligodendrogliomas) were classified as mesenchymal tumours, while meningeal tumours were classified as epithelial tumours.

Paraffin-embedded histological sections were evaluated independently by a second pathologist to give the definitive diagnosis. In comparing cytological diagnosis with histological diagnosis, results were classified for each sample into the following categories: 1) Complete correlation 2) Partial correlation 3) No correlation. Complete correlation indicated correct identification of the pathological process together with a correct specific diagnosis. Partial correlation indicated correct identification of the

pathological process but an incorrect specific diagnosis. No correlation indicated both incorrect identification of the pathological process and an incorrect specific diagnosis.

Qualitative assessment of stain and preparation quality

In order to objectively assess the quality of stains and preparation methods, the cytologist was asked to evaluate 6 characteristics for each slide examined:

- Preparation thickness
- Amount of cell distortion
- Quality of cytoplasmic detail
- Quality of nuclear detail
- Amount of debris present on the slide
- Overall subjective assessment of slide quality.

The evaluation of each characteristic was recorded by placing a mark on a 50mm scale on the form for assessment of each slide (see Appendix III, page 103). For analysis, the distance along this line was measured and recorded for all slides which were diagnostic. The mean and standard deviation for each factor were calculated and 95% confidence intervals generated for correlation with the following: stain and preparation type used, nature of the lesion (histological diagnosis), and the pathological process and specific diagnosis.

LOCATION OF SAMPLES TAKEN FOR NORMAL SMEAR CYTOLOGY

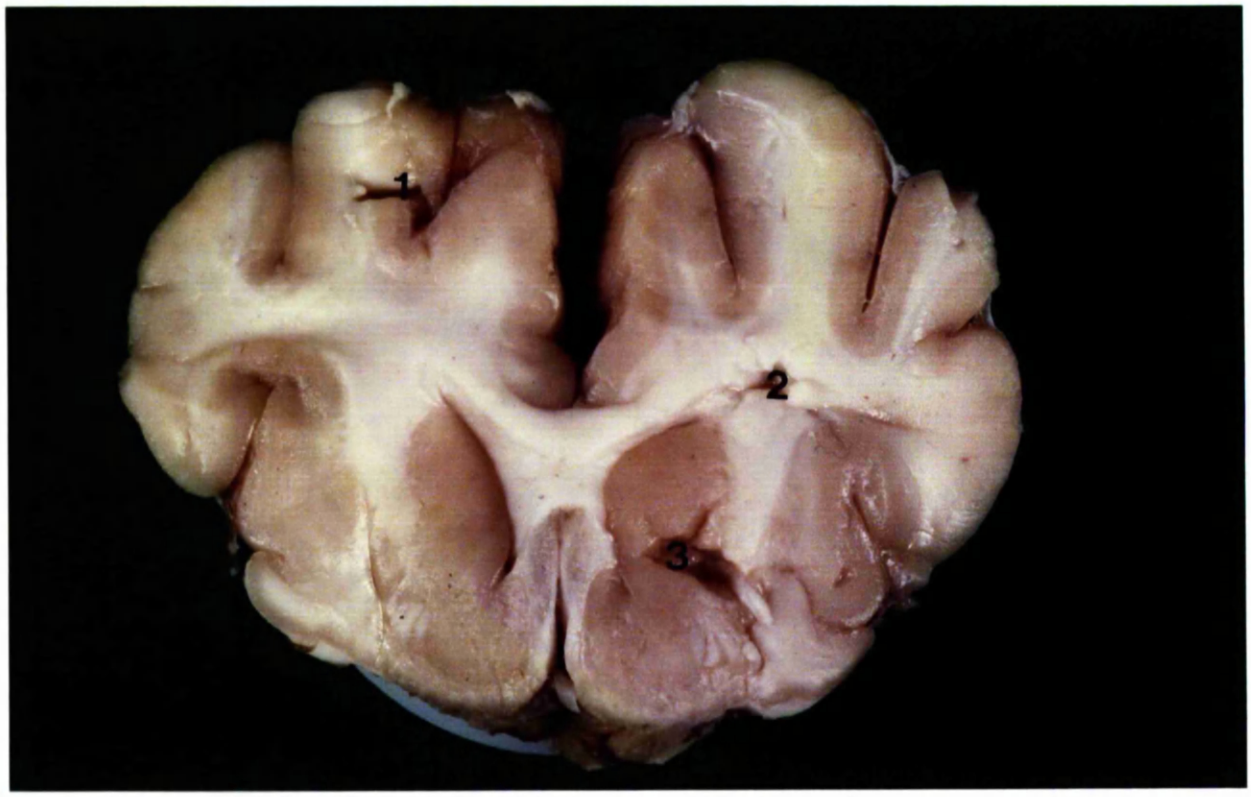


Figure 4: Caudal surface section A. Sample collection points: **1** Precruciate gyrus (motor cortex) **2** Corpus collosum **3** Caudate nucleus

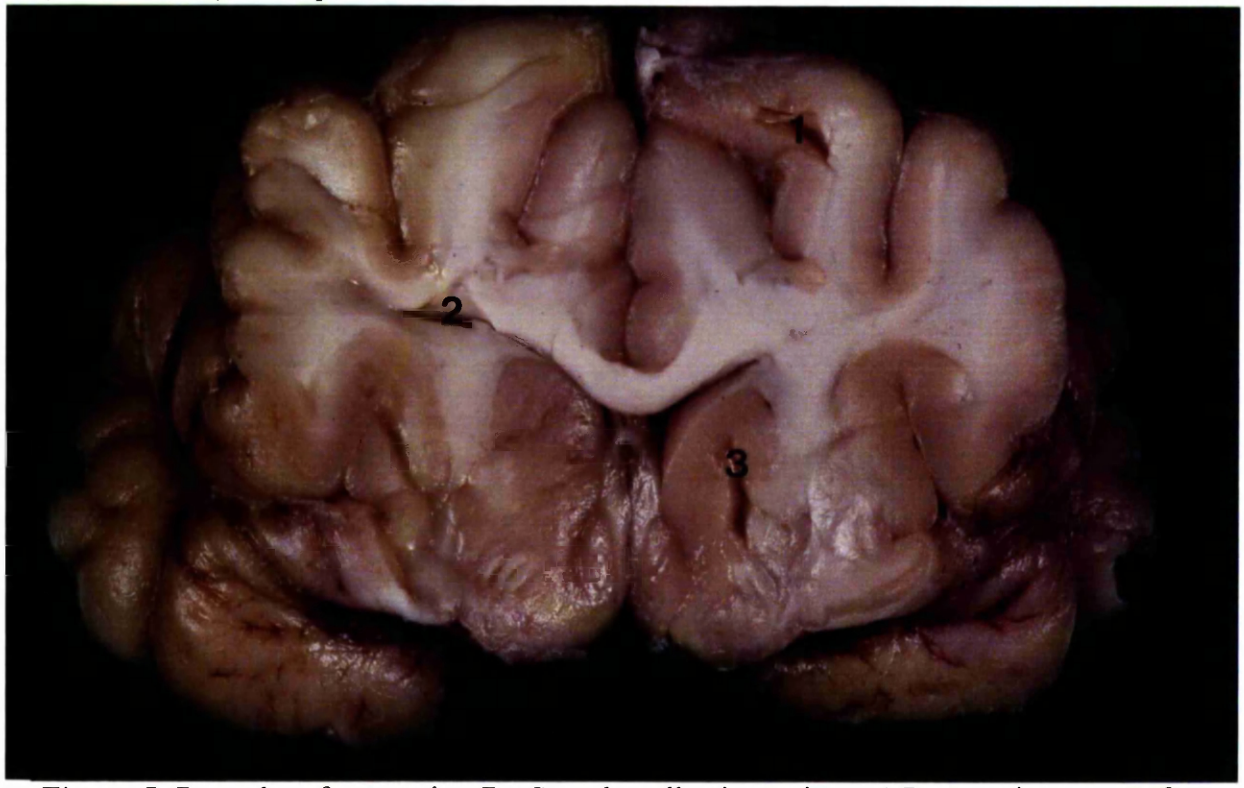


Figure 5: Rostral surface section B. Sample collection points: **1** Postcruciate gyrus **2** Corpus collosum **3** Caudate nucleus

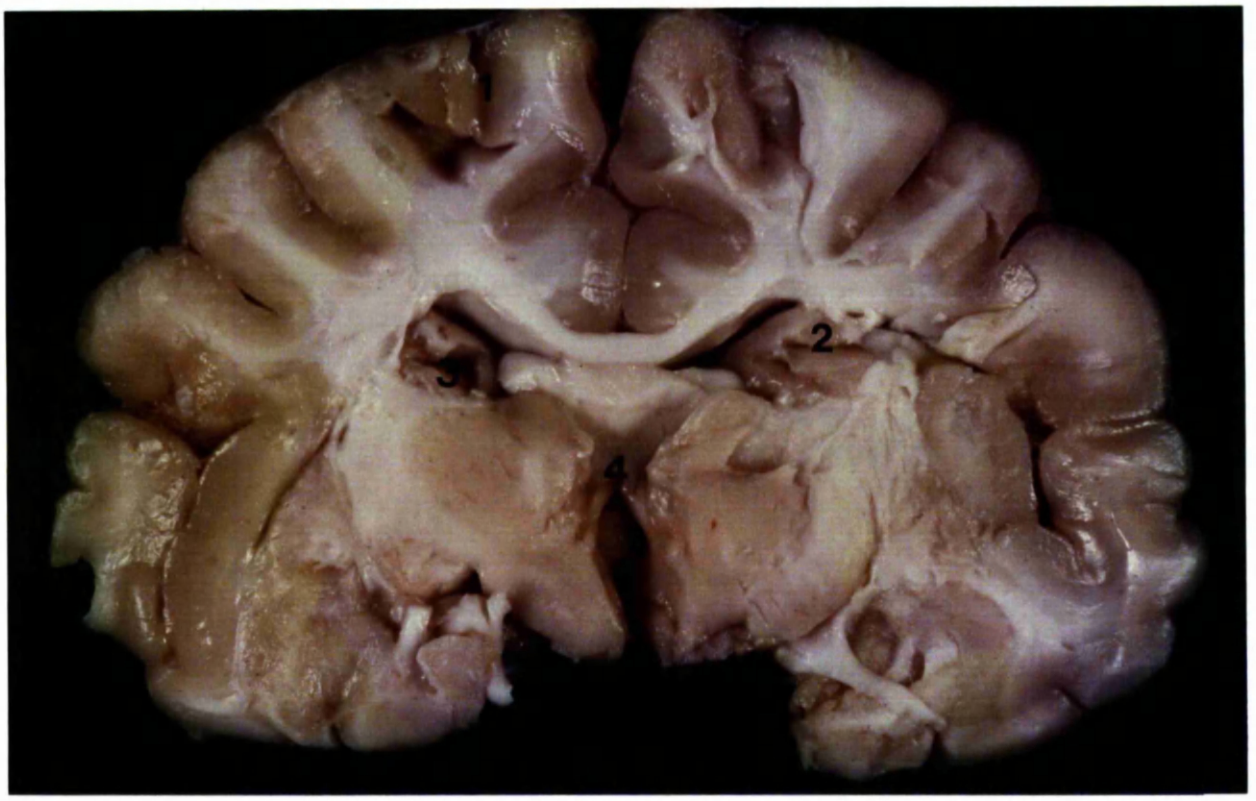


Figure 6: Caudal surface section B. Sample collection points: 1 Lateral gyrus 2 Corona radiata 3 Lateral intermediate thalamic nucleus 4 Central medial thalamic nucleus

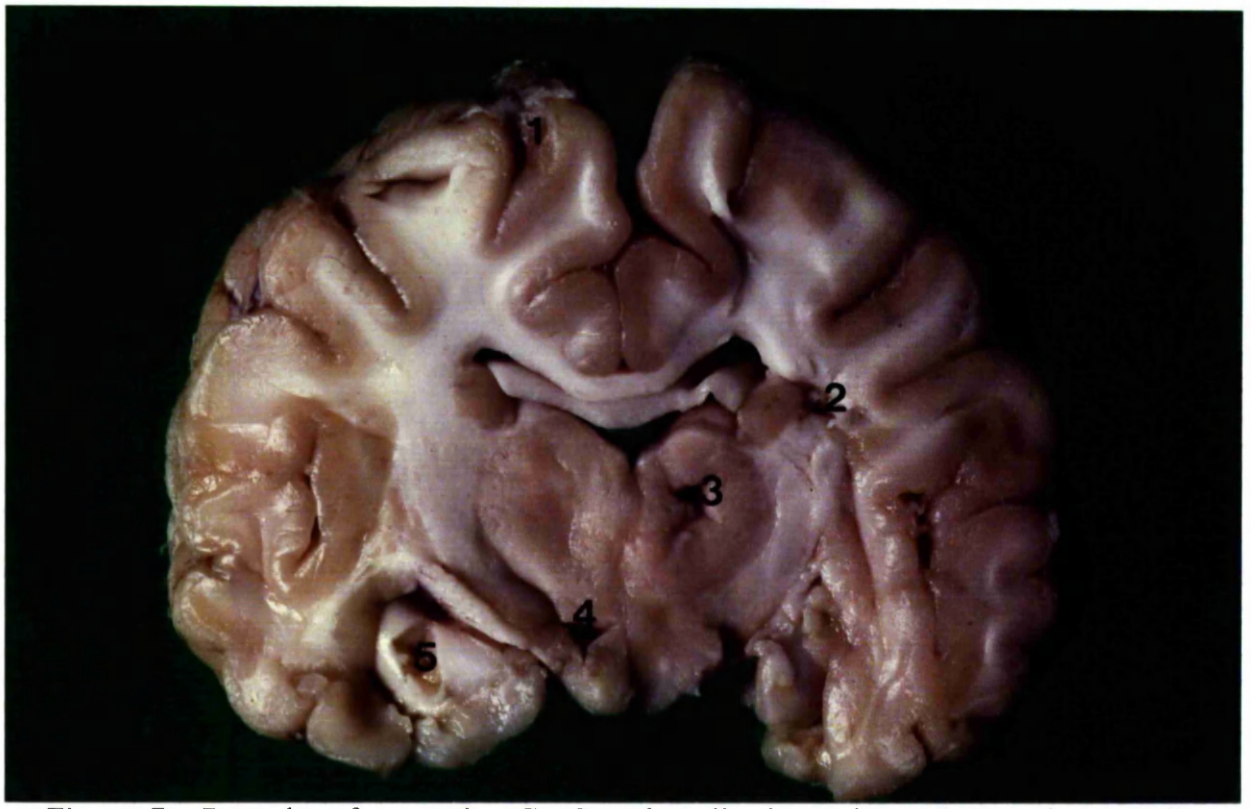


Figure 7: Rostral surface section C. Sample collection points: **1** Lateral gyrus **2** Corona radiata **3** Lateral caudal thalamic nucleus **4** Ventral caudomedial thalamic nucleus **5** Hippocampus/nucleus amygdalae

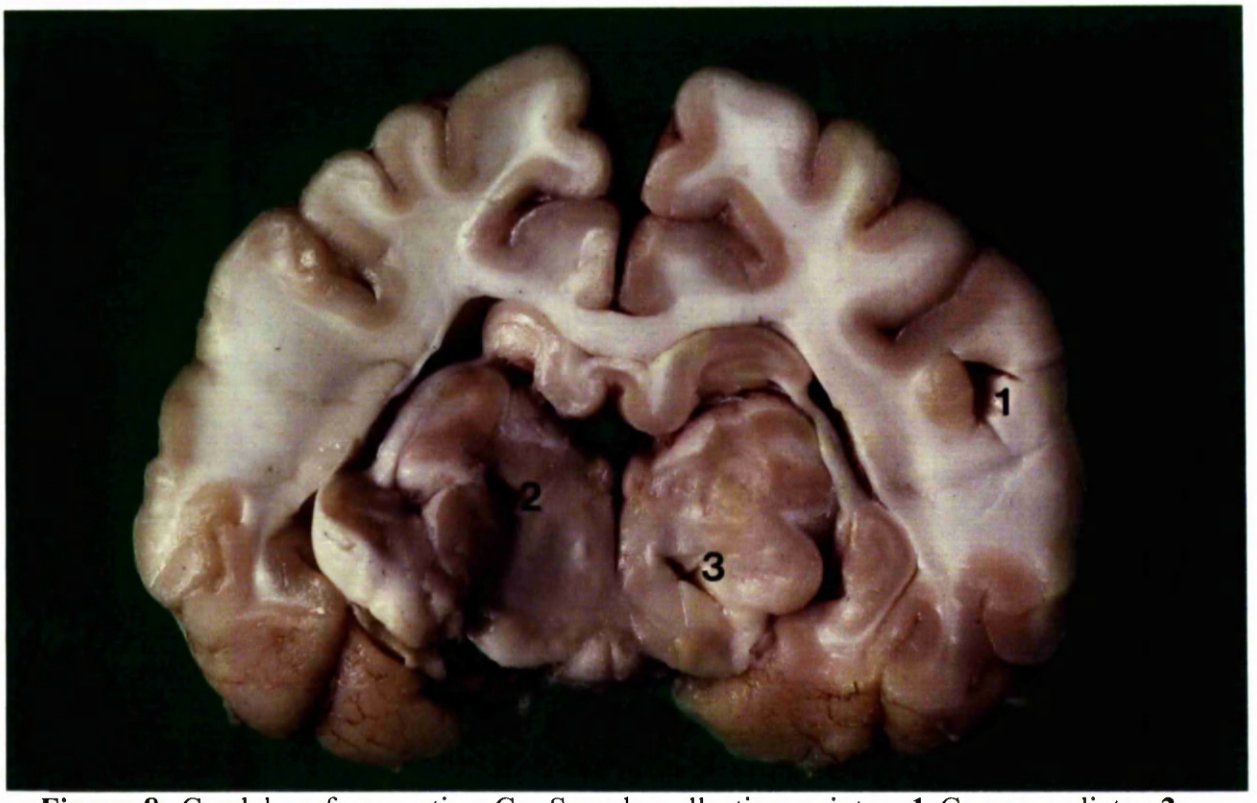


Figure 8: Caudal surface section C. Sample collection points: **1** Corona radiata **2** Lateral caudal thalamic nucleus **3** Parafascicular thalamic nucleus

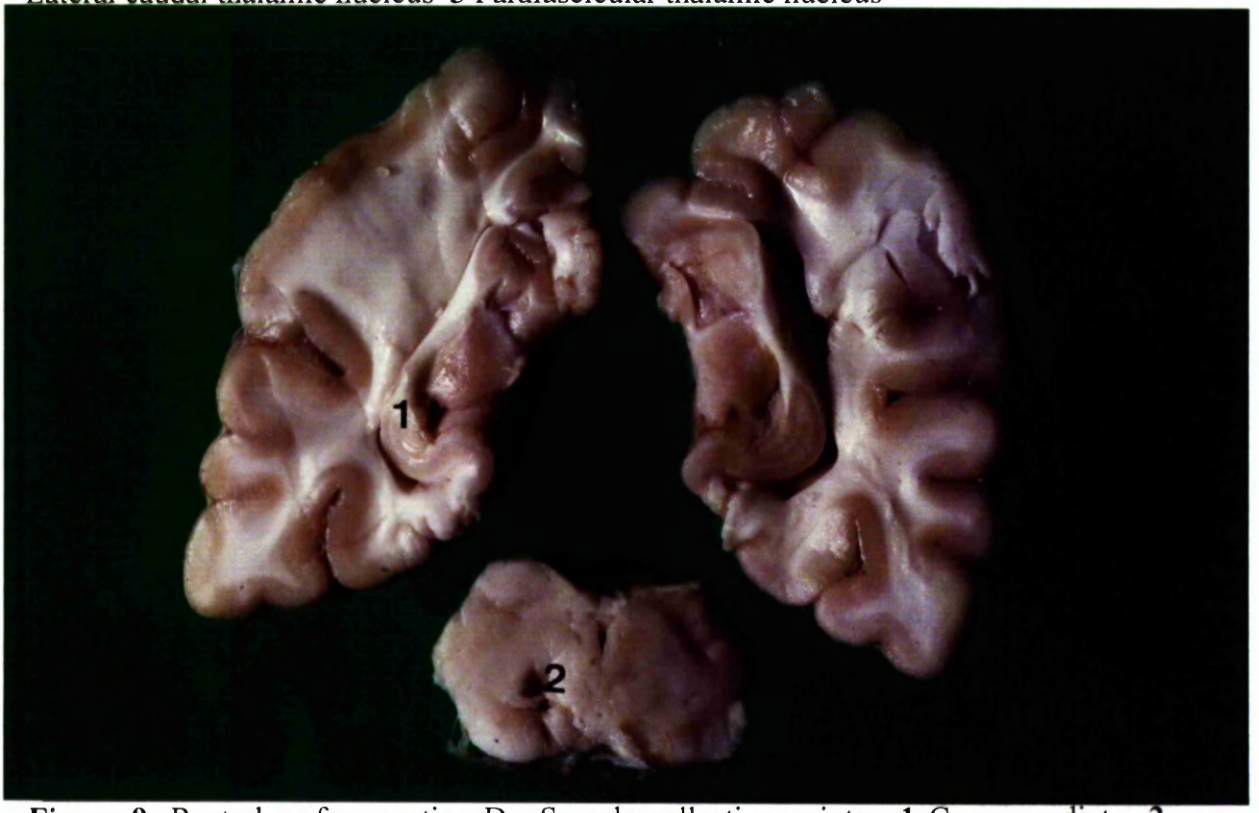


Figure 9: Rostral surface section D. Sample collection points: **1** Corona radiata **2** Medial lemniscus

Figure 10: Rostral surface section E. Sample collection points: **1** Nucleus of dorsal median raphae

Figure 11: Caudal surface section F. Sample collection points: **1** Cerebellar vermis **2** Cerebellum – paramedian lobe **3** Tectospinal tract

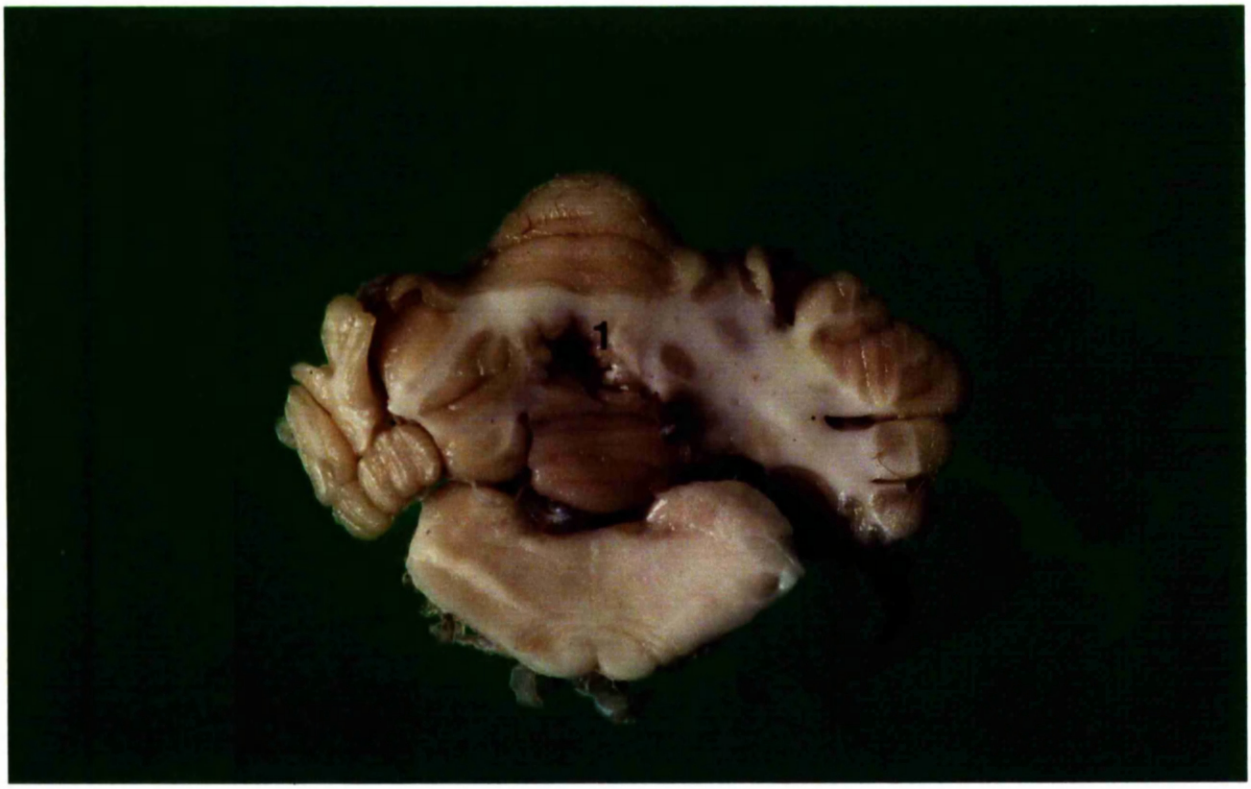


Figure 12: Rostral surface section G. Sample collection points: **1** Cerebellar vermis

RESULTS

Part I: Cytological appearance of normal canine brain

Cerebral cortex grey matter

Smears from cerebral cortex grey matter showed moderate to high cellularity. The cell population consisted predominantly of small, round to oval cells with intensely staining nuclei and little cytoplasm that represented resting glial cells. In addition there were numerous pyramid-shaped cells of medium size with round to oval nuclei and coarsely granular basophilic cytoplasm, thought to represent the normal population of cortical pyramidal neurons. Occasional very large pyramidal and stellate shaped cells were also seen, with a larger nucleus and a coarsely granular cytoplasm with elongated cytoplasmic processes. Within the cytoplasm there was a pale-staining area, representing Nissl substance, and elongated cytoplasmic processes (see Figure 13, page 48), representing large motor neurons from the motor cortex.

Neuropil was seen as scant, finely fibrillar material, staining pink-purple in colour. A moderate number of fine, non-branching capillaries were seen, lined with a single layer of endothelial cells.

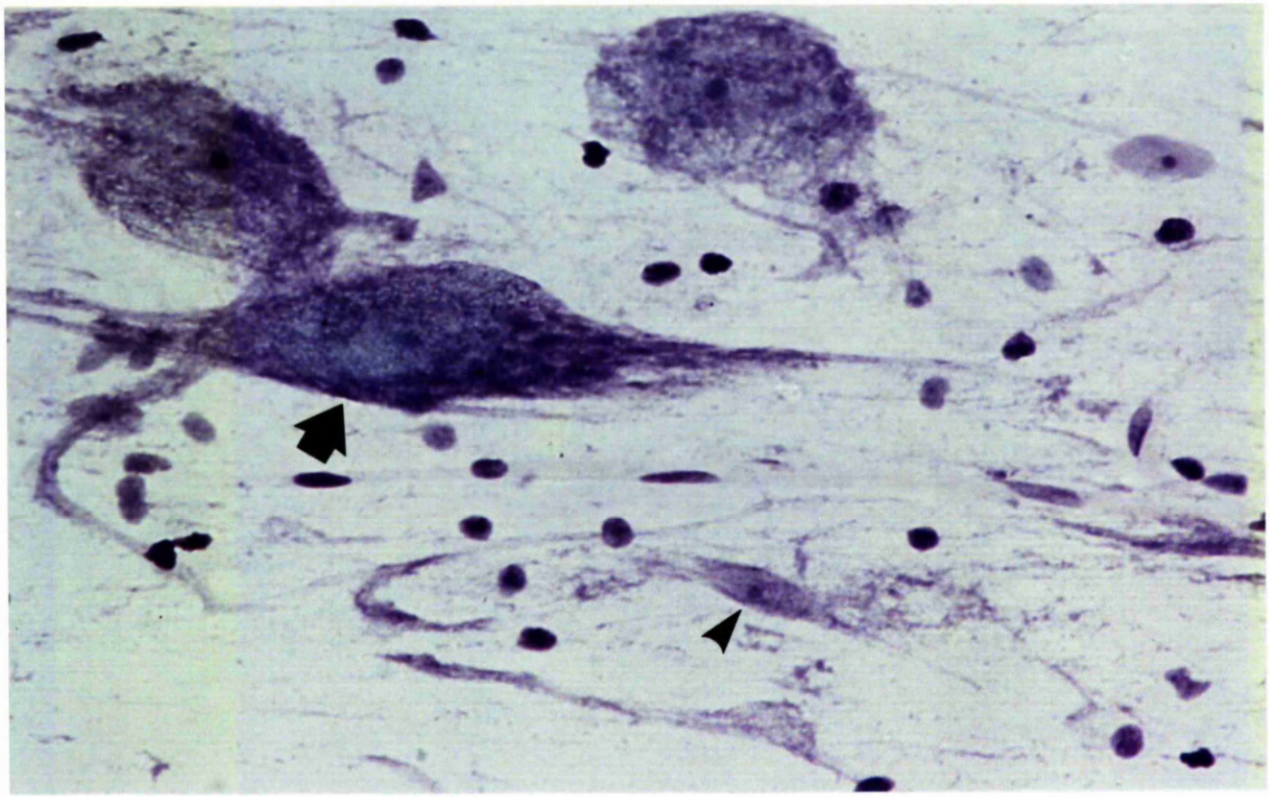


Figure 13: Cerebral cortex, x100 (caudal surface section A, sample collection point 1). Note large motor neurons with pale blue staining Nissl substance (arrow) and smaller pyramidal neurons (arrowhead).

Grey matter of the thalamus

Smears from this area showed moderate to high cellularity. Glial cells in this area were similar to those seen in cortical grey matter. Neurons in this area were more numerous than in the cerebral cortex, and consisted largely of medium sized pyramidal neurons similar to those seen in the cerebral cortex (see Figure 14, page 50). In addition, some smaller neurons were seen with round to oval shaped, pale staining nuclei and indistinct cytoplasm.

Neuropil was more finely fibrillar in nature and capillaries were more numerous and branching than those seen from cortical smears. In addition, occasional sheets of medium sized, round cells with moderate amounts of cytoplasm and pale staining nuclei could be seen. These represented ependymal cells from the ventricular lining (see Figure 15, page 50).

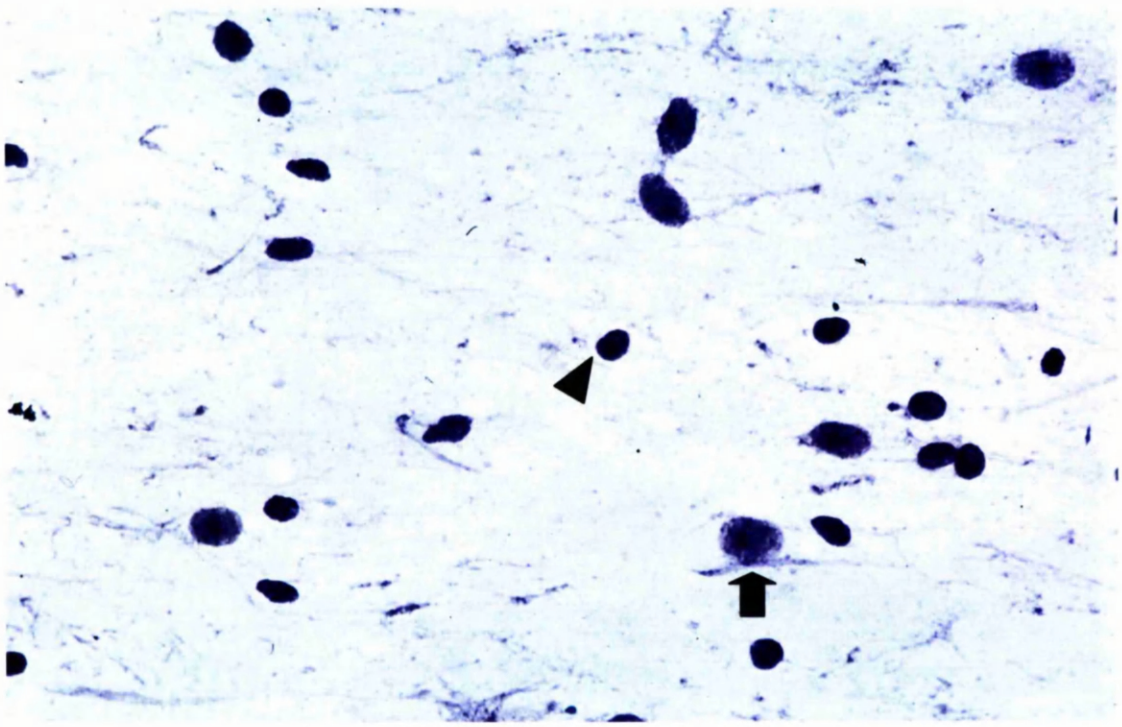


Figure 14: Thalamus, x100 (rostral surface section C, sample collection point 3). Note pyramidal neurons (arrow) with small, dark staining glial nuclei (arrowheads).

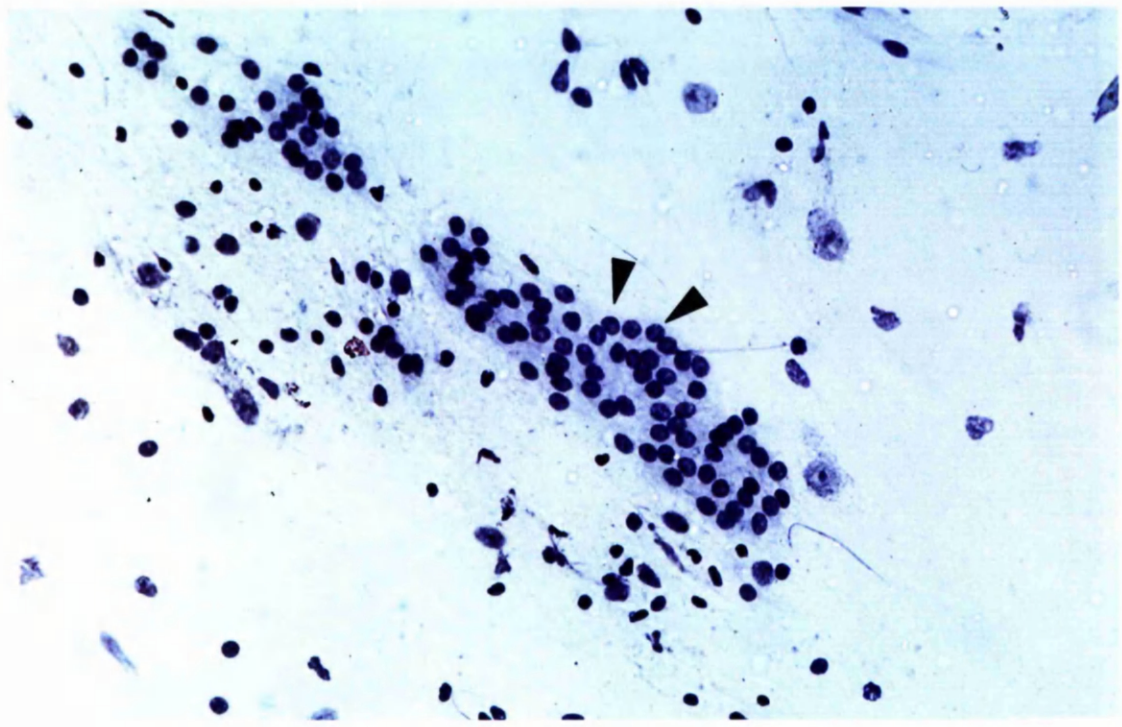


Figure 15: Thalamus, x50 (caudal surface section B, sample collection point 3). Note ependymal cells (arrowheads).

Caudate Nucleus

Smears from the caudate nucleus also showed moderate to high cellularity. The population of glial cells were more numerous than those seen in cortical grey matter but of the same type. The majority of neurons seen in this area were small, round to oval shaped cells with pale staining nuclei and indistinct cytoplasm. Few pyramidal neurons were seen.

The neuropil from this area was similar to that seen in cortical smears, but capillaries were more numerous and finely branching, similar to those seen in smears from the thalamic nuclei.

Hippocampus

Cellularity in the hippocampus was moderate to high. The population of glial cells was similar to that seen in grey matter in other areas, and the population of neurons consisted of both small, round to oval cells with indistinct cytoplasm, and medium sized pyramidal neurons.

The neuropil was very similar to cortical grey matter smears, being finely fibrillar and pink to purple staining, and capillaries were similar in nature to those seen in the cerebral cortex.

Corona radiata

Cellularity of smears from the corona radiata was low to moderate. Glial cells were more numerous than in smears from grey matter, and more varied in appearance. A

population of cells with small, round, intensely staining nuclei and little cytoplasm was present in addition to some cells which were seen with little cytoplasm and larger, round to oval, pale staining nuclei with prominent chromatin. Very occasional small to medium sized neurons were seen with indistinct cytoplasm and pale staining round to oval nuclei in smears made from the edges of the corona radiata.

The neuropil was coarser and more granular than that seen in areas of grey matter, and had a more granular appearance. Capillaries were present but were fewer in number than found in either deep grey matter or cortical smears.

Corpus Collosum

Smears made from this area exhibited low to moderate cellularity. The population of glial cells was similar to that seen in the corona radiata, but no neurons were seen.

The neuropil seen was very similar to that seen in corona radiata smears, but capillaries were slightly more numerous (see Figure 16, page 53).

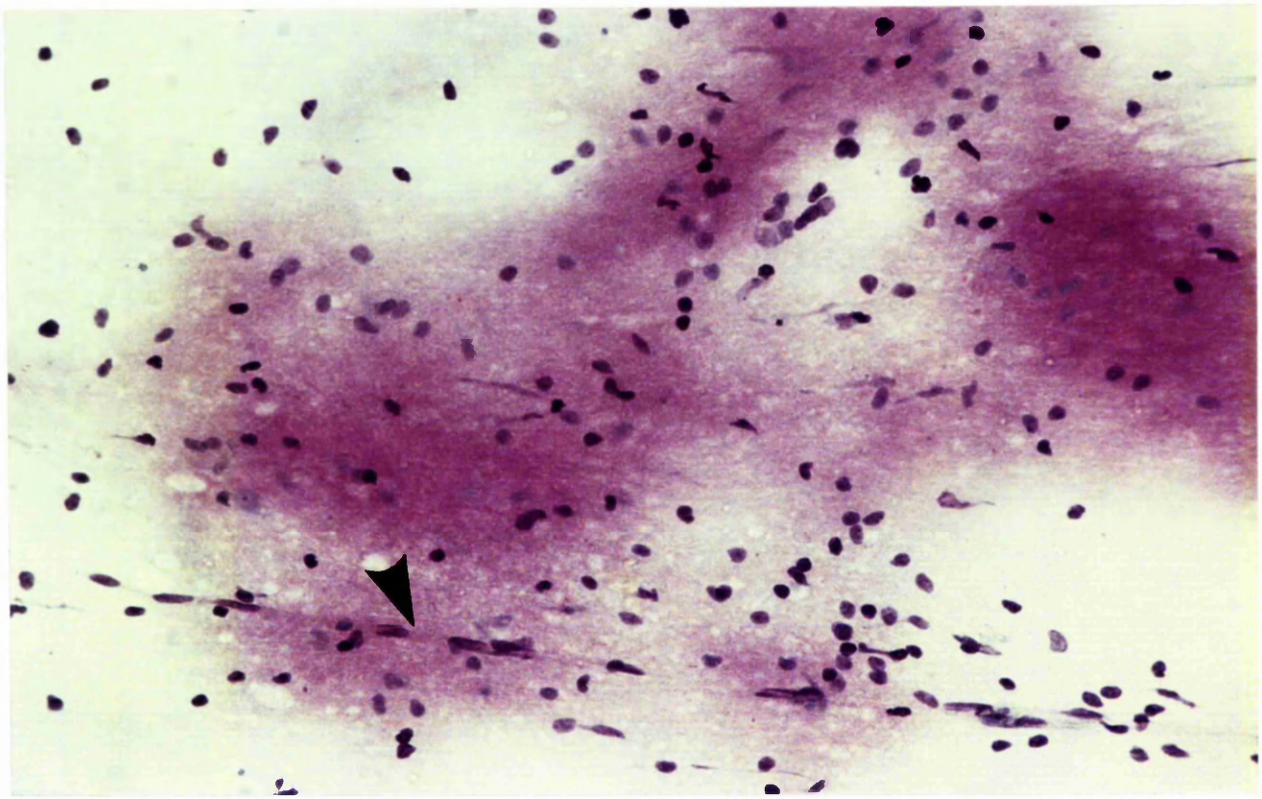


Figure 16: Corpus collosum, x50 (caudal surface section A, sample collection point 2). Note coarser neuropil with prominent capillary (arrowhead) and absence of neurons.

Medial Lemniscus

Smears made from the medial lemniscus revealed low to moderate cellularity. The glial population was similar to that of thalamic nuclei and cortical grey matter smears, being mostly small, round to oval cells with intensely staining nuclei and minimal cytoplasm. Neurons were found in higher numbers than in cortical thalamic nuclei slides, and as well as pyramidal and small neurons, occasional very large motor neurons were seen (see Figure 17, page 55).

The neuropil in this region was coarser and more granular than in other areas of grey matter. Capillaries were few in number and non-branching.

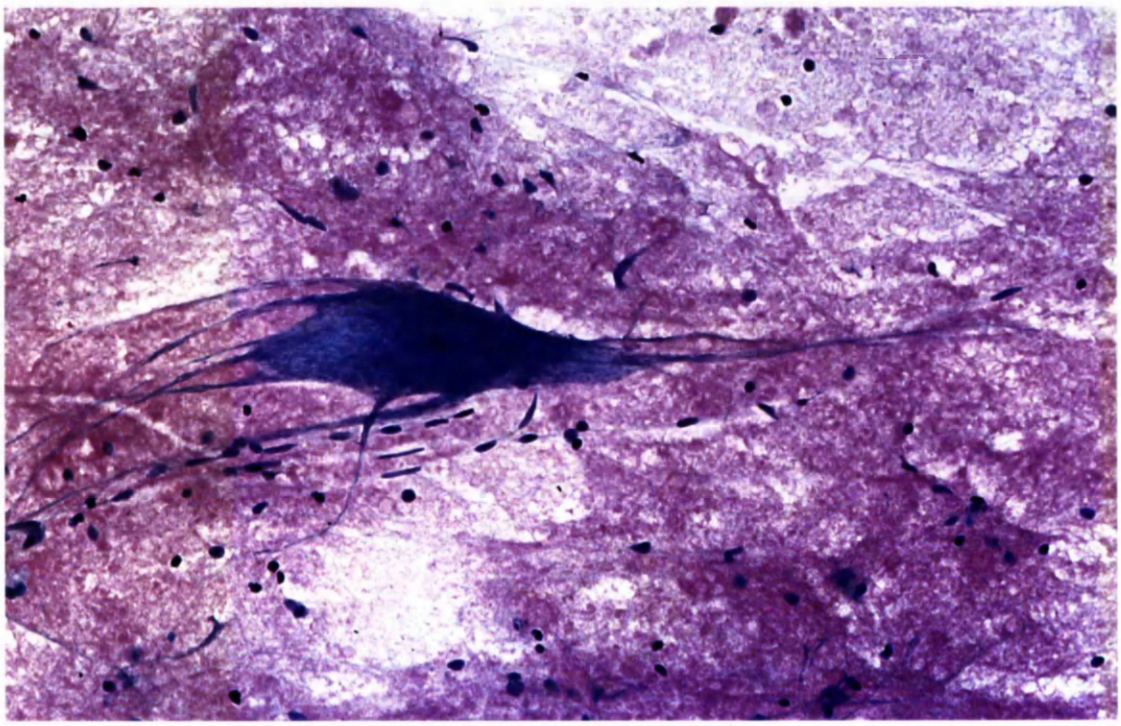


Figure 17: Medial lemniscus, x50 (rostral surface section D, sample collection point 2). Note very large motor neuron and coarse neuropil.

Cerebellum

Cellularity of smears made from the cerebellum was very high. Few glial cells were apparent. The vast majority of cells were small round cells with intensely staining, dark nuclei and minimal cytoplasm (see Figure 18, page 57), representing the small granule cells of the cerebellum. Occasional larger, pyramidal neurons were also observed which were of similar size to motor neurons of the cerebral cortex, and which had a medium sized, oval, pale staining nucleus, granular cytoplasm and several cytoplasmic processes. These represented Purkinje neurons.

The neuropil was less finely fibrillar than that of grey matter, and few capillaries were seen.

Medulla oblongata

Smears made from the dorsal nucleus of the median raphae and from the area of the tectospinal tract revealed moderate cellularity. Glial cells present were a mixture of the small cells seen in cortical smears and the slightly larger cells with round to oval paler staining nuclei and minimal cytoplasm. High numbers of large motor neurons were present, which had large oval shaped nuclei and an area of pale blue-staining cytoplasm that represented Nissl substance.

The neuropil was coarse and more granular in nature, and capillaries slightly more numerous than in those smears taken from the region of the medial lemniscus (see Figure 19, page 57).

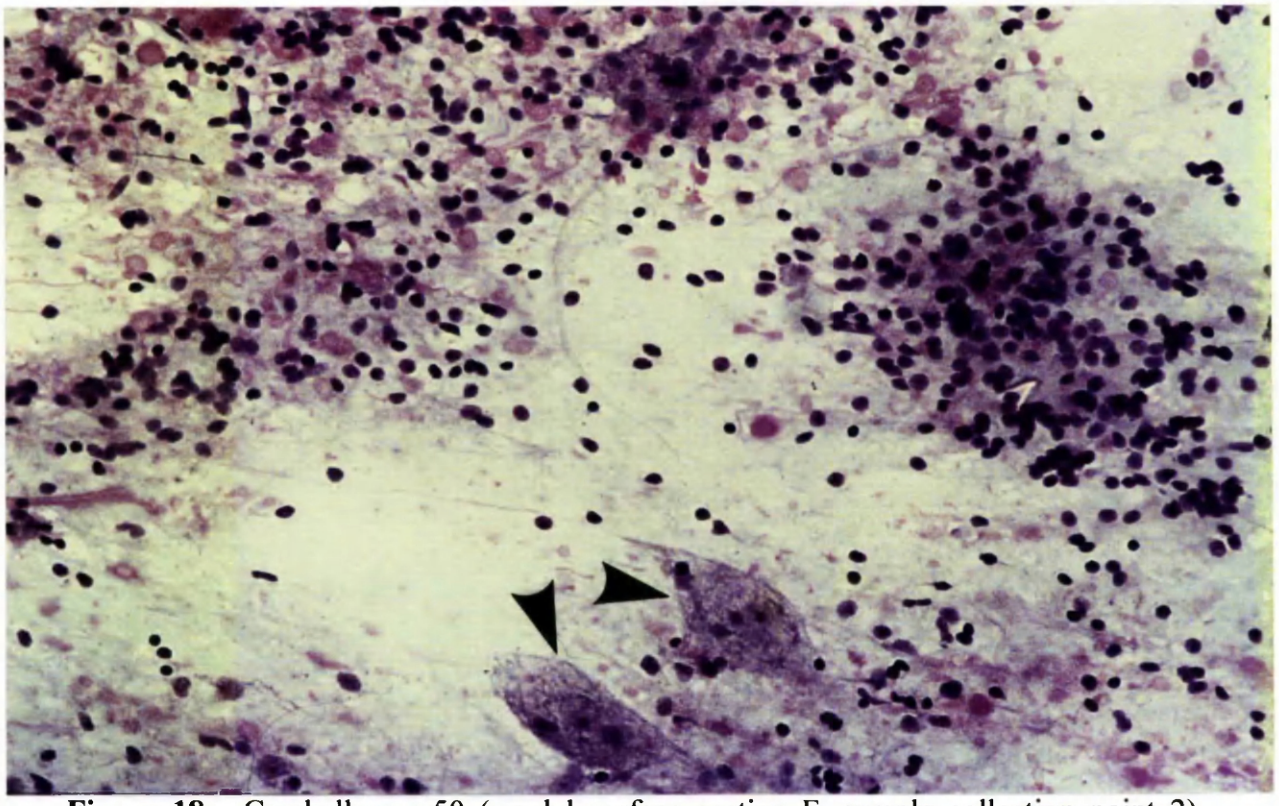


Figure 18: Cerebellum, x50 (caudal surface section F, sample collection point 2). Note numerous small, round, dark staining granule cells with occasional larger Purkinje neurons (arrowheads).

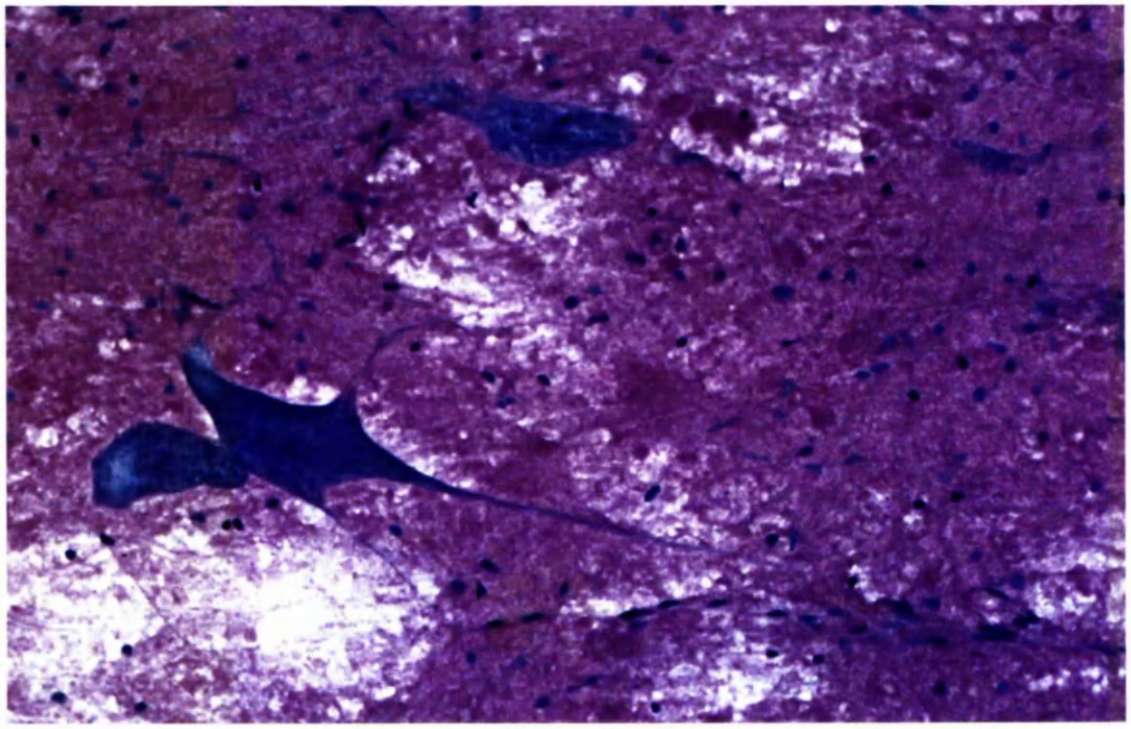


Figure 19: Medulla oblongata, x 50 (rostral surface section E, sample collection point 1). Note coarser neuropil and very large motor neurons.

Part II: Cytological Diagnosis of Intracranial Lesions

Accuracy of cytological diagnosis

The nature of the 11 specimens examined was as follows: 2 meningiomas, 1 oligodendroglioma, 1 choroid plexus papilloma, 1 pituitary macroadenoma, 2 lymphomas (one primary and one metastatic), 2 adenocarcinomas (one with local extension through the cribiform plate from a nasal tumour and one metastatic carcinoma from primary bronchogenic carcinoma), 1 case of granulomatous meningoencephalitis and 1 case of gliosis surrounding intracranial haemorrhage of unknown aetiology (see Table 4, page 63)

When diagnostic accuracy of stain types was examined independently of preparation method, the Diff Quik stain with complete correlation in 17/33 (52%) specimens and partial correlation in 12/33 (36%) specimens appeared to be the most accurate (see Table 5, page 64). May-Grunwald-Giemsa and Zynostain stains gave similar results, with complete correlation in 17/33 specimens (52%) for both stains, and partial correlation in 9/33 (27%) specimens for May-Grunwald-Giemsa and in 10/33 (30%) for Zynostain. Toluidine blue gave the least accurate results, with complete correlation in 15/33 (45%) specimens and partial correlation in 10/33 (30%). Overall, the May-Grunwald-Giemsa gave more reliable results than other stains, with only 22% specimens being non-diagnostic. This compared with 27% specimens non-diagnostic with the Zynostain, while Diff Quik and toluidine blue stains had comparable results, with 31% of specimens being non-diagnostic with each stain.

When preparation method was examined independently of stain type, the preparation method with the greatest diagnostic accuracy appeared to be the touch preparation, with 24/44 (55%) of specimens having complete correlation and 12/44 (27%) of specimens showing partial correlation. The smear preparation showed similar results, with 21/44 (48%) of specimens having complete correlation and 15/36 (34%) of specimens having partial correlation. The medium pressure preparation yielded 21/44 (48%) specimens with complete correlation and 14/44 (32%) specimens with partial correlation. However, when these results were correlated with the percentage of diagnostic specimens (see Table 6, page 64), the smear preparation appeared to be most useful, since only 19% specimens were non diagnostic. This compared favourably with both other methods, with 30% of specimens created using the medium pressure touch preparation being non-diagnostic, and 35% of specimens created using the touch preparation.

Overall, 66/132 (50%) of specimens showed complete correlation with histopathological diagnosis, 41/132 (31%) showed partial correlation, and 79/285 (28%) of specimens were non-diagnostic.

Qualitative Assessment Of Stains And Preparation Methods

When evaluating the results of individual slide assessment, some findings of a general nature became evident. Overall the 95% confidence interval for most factors was very large, reflecting the variation in slide quality that was present with all stains and preparation methods. The factor to consistently score highest and with the smallest confidence intervals was the quantity of debris, suggesting that contamination of slides was not a significant problem. Of the other factors, perhaps the most useful for

assessing slide quality was overall assessment, as this correlated most highly with the accuracy of diagnosis (see below). While quality of nuclear and cytoplasmic detail were factors that would also be expected to influence slide quality, they did not necessarily correlate with overall assessment and so were not equally important. However it must be stressed that caution must be exercised when interpreting the data generated, and a difference of less than 3 or 4 points between scores unlikely to be statistically significant, as this represents a difference of only 3 to 4 mm along the 50mm scale. The results of the slide assessment are summarised in Table 7 - Table 9, pages 65 - 68 and in Chart 1 - Chart 25, pages 69 - 77.

Stain type and preparation method

When stain type and preparation method were correlated with the score of the 6 factors, the smear preparation was generally found to be associated with highest overall assessment (see Table 7, page 65). While quality of nuclear and cytoplasmic detail were generally in agreement with this, this was not always the case (eg toluidine blue touch preparations with means of 26.3 and 19.6 respectively, compared with smear preparation mean scores of 23.3 and 16.8). The amount of cell distortion was noted to be generally highest in touch preparations and lowest in medium preparations. However, distortion was not found to correlate with overall assessment, suggesting that distortion alone did not play a large part in determining slide quality.

When the different stains were compared, May-Grunwald-Giemsa appeared to be associated with the highest overall assessment scores, followed by toluidine blue (see Table 7, page 65. When nuclear and cytoplasmic detail were examined, toluidine blue

appeared to score most highly for nuclear detail while May-Grunwald-Giemsa scored most highly for cytoplasmic detail.

Histological Diagnosis

When overall assessment mean scores were correlated with the histological diagnosis, the highest scores were recorded for case 9 (metastatic adenocarcinoma – 36.6), case 2 (metastatic lymphoma – 34.5) and case 3 (primary lymphoma – 30.9), suggesting that preparations of these tumours were easiest to make and therefore of higher quality (see Table 8, page 67). The lesions associated with lowest overall assessment mean scores were case 11 (gliosis – 19.5), case10 (transitional meningioma – 23.6) and cases 8 and 4 (pituitary adenoma and nasal adenocarcinoma – both 25.2). In general, all of these cases were diagnosed less accurately.

Cytoplasmic and nuclear detail mean scores tended to parallel the overall assessment mean scores. Those tumours associated with highest mean scores for cytological detail were case 9 (metastatic adenocarcinoma – 29.9), case 2 (metastatic lymphoma – 27.7) and case 3 (primary lymphoma – 18.8), while lowest mean scores were recorded for case 11 (gliosis – 9.8), case 6 (oligodendroglioma – 13.9) and case 10 (transitional meningioma – 14.8). Highest mean scores for nuclear detail were recorded for case 9 (metastatic adenocarcinoma – 33.2), case 2 (metastatic lymphoma – 29.0) and case 7 (GME – 24.3), with lowest mean scores recorded for case 11 (gliosis – 15.9), case 10 (transitional meningioma – 18.7) and case 6 (oligodendroglioma – 19.3).

The highest mean scores for distortion were recorded for case 11 (gliosis – 35.3), case 4 (nasal adenocarcinoma – 34.5) and case 1 (syncytial meningioma – 33.9), suggesting a

degree of difficulty in making preparations from these lesions. The lowest mean scores for distortion were noted with case 9 (metastatic adenocarcinoma – 18.6), case 2 (metastatic lymphoma – 24.5) and case 5 (choroid plexus papilloma – 26.0).

Pathological Process and Specific Diagnosis

Evaluation of correct identification of pathological process and correct specific diagnosis revealed some interesting features (see Table 9, page 86). There was no significant difference between the two when thickness and debris were evaluated. However, overall mean assessment score, nuclear detail mean score and cytoplasmic detail mean score were all higher with correct specific diagnosis compared to correct pathological process (29.7, 25.6 and 21.2 respectively compared to 28.1, 22.9 and 17.8). Additionally, the amount of distortion was slightly lower for those cases with a correct specific diagnosis (27.3) when compared to correct pathological process (29.0).

Table 4: Case details

Case	*CT/Post Mortem Findings*	*Diagnosis*
1	Incidental meningeal mass found at post mortem	Meningioma (syncytial)
2	Two masses in left occipital and temporal lobes on CT, homogenous enhancement following contrast injection	Lymphoma (metastatic)
3	Normal CT. Thickened meninges and cranial nerve roots at post mortem	Lymphoma (primary)
4	Large mass extending from nasopharynx through cribiform plate into olfactory lobes on CT	Adenocarcinoma (nasal)
5	Hyperdense brightly enhancing mass in region of 4th ventricle on CT	Choroid plexus papilloma
6	Paraventricular, brightly enhancing mass on CT in the region of the left caudate nucleus with secondary hydrocephalus	Oligodendroglioma
7	Hypodense area in left cerebral hemisphere with midline shift on CT	Granulomatous meningoencephalitis
8	Large mass on ventral midline at level of hypothalamus on CT, homogenous contrast enhancement	Pituitary adenoma
9	Extra-axial mass with broad based dural attachment overlying cerebellum on CT with homogenous enhancement. Primary bronchogenic carcinoma found at post mortem	Adenocarcinoma (metastatic)
10	Extra-axial mass with broad based dural attachment over right cerebral hemisphere on CT, homogenous enhancement	Meningioma (transitional)
11	Poorly circumscribed mass in right frontal lobe on CT, heterogenous enhancement	Gliosis secondary to haemorrhage of unknown origin

Table 5: Evaluation of accuracy by stain type and preparation method

	Complete Correlation	*Partial Correlation*	*Partial and Complete Correlation*
Stain Type:			
Diff Quik	17/33 (52%)	12/33 (36%)	29/33 (88%)
May-Grunwald-Giemsa	17/33 (52%)	9/33 (27%)	26/33 (79%)
Toluidine blue	15/33 (45%)	10/33 (30%)	25/33 (76%)
Zynostain	17/33 (52%)	10/33 (30%)	27/33 (82%)
Total	61/132 (50%)	41/132 (31%)	107/132 (81%)
Preparation method:			
Touch preparation	24/44 (55%)	12/44 (27%)	36/44 (82%)
Medium pressure touch preparation	21/44 (48%)	14/44 (32%)	35/44 (80%)
Smear preparation	21/44 (48%)	15/44 (34%)	36/44 (82%)
Total	61/132 (50%)	41/132 (31%)	107/132 (81%)

Table 6: Percentage of non-diagnostic specimens by stain type and preparation method

	Percentage of non-diagnostic preparations
Stain Type	
Diff Quik	31
May-Grunwald-Giemsa	22
Toluidine blue	31
Zynostain	27
Preparation Method	
Touch preparation	35
Medium pressure touch preparation	30
Smear Preparation	19
Overall	28

Table 7: Evaluation of Slide Quality: Stain and Preparation method

Stain/Preparation		Factor					
		Thickness	Distortion	Cytoplasm	Nucleus	Debris	Assessment
Diff Quick/Touch Preparation							
	mean	31.2	27.7	19.2	22.1	37.9	28.5
	SD	6.3	10.6	10.4	10.5	4.3	10.1
	high	43.8	48.9	39.9	43.2	46.5	48.6
	low	18.6	6.6	0.0	1.0	29.2	8.3
Diff Quick/Medium Preparation							
	mean	30.3	31.1	15.6	20.8	38.9	24.4
	SD	9.7	10.4	8.8	10.4	4.0	7.5
	high	49.8	51.8	33.1	41.6	46.9	39.5
	low	10.8	10.3	0.0	0.0	30.9	9.3
Diff Quick/Smear Preparation							
	mean	30.7	30.6	19.3	24.5	38.2	28.7
	SD	5.6	10.5	11.9	10.5	3.9	9.8
	high	41.8	51.6	43.0	45.4	46.1	48.3
	low	19.6	9.7	0.0	3.5	30.4	9.1
May-Gruhnwald-Giemsa/Touch Preparation							
	Mean	31.1	32.5	17.2	20.2	37.4	28.2
	SD	7.1	9.9	10.7	10.5	5.2	8.5
	High	45.4	52.3	38.5	41.1	47.9	45.2
	Low	16.8	12.7	0.0	0.0	26.9	11.2
May-Gruhnwald-Giemsa/Medium Preparation							
	Mean	30.1	27.9	22.4	26.9	38.6	31.4
	SD	3.8	7.7	10.5	9.6	3.3	8.5
	High	37.8	43.4	43.3	46.1	45.1	48.4
	Low	22.4	12.5	1.4	7.8	32.0	14.5
May-Gruhnwald-Giemsa/Smear Preparation							
	Mean	30.2	29.2	18.7	24.1	39.1	31.0
	SD	6.4	8.6	9.8	8.9	3.0	8.1
	High	43.0	46.4	38.3	41.9	45.0	47.1
	Low	17.3	12.0	0.0	6.3	33.2	14.8

Key:

SD Standard Deviation
High Upper limit of 95% confidence interval
Low Lower limit of 95% confidence interval

Evaluation of Slide Quality: Stain and Preparation method (continued)

Stain/Preparation		Factor					
		Thickness	Distortion	Cytoplasm	Nucleus	Debris	Assessment
Toluidine blue/Touch Preparation							
	Mean	22.9	28.9	19.6	26.3	37.8	26.5
	SD	6.6	8.7	11.0	9.9	3.3	6.9
	High	36.1	46.4	41.6	46.1	44.5	40.3
	Low	9.8	11.4	0.0	6.5	31.1	12.7
Toluidine blue/Medium Preparation							
	Mean	24.9	26.2	16.3	22.9	37.4	26.9
	SD	8.5	9.2	8.0	8.9	8.0	9.5
	High	41.9	44.6	32.3	40.7	50.0	45.9
	Low	7.8	7.8	0.2	5.0	21.3	7.8
Toluidine blue/Smear Preparation							
	Mean	25.7	28.0	16.8	23.3	39.3	30.7
	SD	5.5	7.4	8.3	6.8	3.3	6.5
	High	36.8	42.8	33.4	37.0	45.9	43.6
	Low	14.6	13.2	0.1	9.7	32.8	17.7
Zynostain/Touch Preparation							
	Mean	30.2	29.7	17.3	21.1	37.3	25.7
	SD	5.4	11.5	10.5	11.7	4.7	10.7
	High	40.9	50.0	38.3	44.6	46.6	47.1
	Low	19.5	6.7	0.0	0.0	27.9	4.3
Zynostain/Medium Preparation							
	Mean	27.8	26.1	19.8	24.7	36.9	28.0
	SD	7.2	10.0	8.6	8.1	4.3	8.3
	High	42.3	46.2	36.9	41.0	45.4	44.7
	Low	13.3	6.0	2.7	8.4	28.3	11.3
Zynostain/Smear Preparation							
	Mean	31.4	29.6	16.3	21.2	36.7	28.2
	SD	6.7	9.2	10.5	8.9	4.1	7.6
	High	44.8	48.0	37.2	38.9	44.9	43.5
	Low	18.1	11.1	0.0	3.4	28.5	13.0

Key:

SD Standard Deviation
High Upper limit of 95% confidence interval
Low Lower limit of 95% confidence interval

Table 8: Evaluation of Slide Quality: Histological diagnosis

Case		Factor					
		Thickness	Distortion	Cytoplasm	Nucleus	Debris	Assessment
Case 1: Meningioma (syncytial)							
	Mean	33.3	33.9	14.9	20.9	38.4	27.7
	SD	6.0	7.1	6.1	6.5	3.1	6.3
	High	45.3	48.1	27.0	34.0	44.6	40.3
	Low	21.2	19.8	2.8	7.8	32.2	15.1
Case 2: Lymphoma (metastatic)							
	Mean	31.8	24.5	27.7	29.0	39.4	34.5
	SD	4.4	11.4	14.4	12.8	2.4	7.3
	High	40.7	47.3	50.0	50.0	44.2	49.1
	Low	23.0	1.8	0.0	3.3	34.6	19.9
Case 3: Lymphoma (primary)							
	Mean	28.9	29.8	18.9	24.3	37.7	30.9
	SD	6.4	7.9	7.6	7.7	4.9	7.7
	High	41.7	45.5	34.1	39.8	47.5	46.4
	Low	16.1	14.0	3.6	8.9	27.9	15.4
Case 4: Adenocarcinoma (metastatic)							
	Mean	29.8	34.5	16.8	20.9	36.3	25.2
	SD	7.4	7.3	7.6	9.8	4.4	9.6
	High	44.6	49.0	32.0	40.4	45.2	44.4
	Low	15.1	19.9	1.5	1.4	27.4	5.9
Case 5: Choroid plexus papilloma							
	Mean	27.0	26.0	15.7	22.2	36.2	28.8
	SD	5.5	10.1	6.6	8.0	5.4	8.0
	High	38.0	46.2	28.8	38.3	47.0	44.8
	Low	16.0	5.7	2.5	6.1	25.4	12.7
Case 6: Oligodendroglioma							
	Mean	29.7	28.8	13.9	19.3	38.6	27.6
	SD	5.6	8.2	6.7	6.4	3.8	6.7
	High	40.9	45.2	27.2	32.1	46.1	41.0
	Low	18.4	12.3	0.6	6.4	31.1	14.1
Case 7: Granulomatous meningoencephalitis							
	Mean	26.6	31.8	18.3	24.7	38.3	27.2
	SD	7.2	8.1	10.6	9.8	3.5	7.8
	High	41.0	48.0	39.6	44.3	45.3	42.8
	Low	12.1	15.5	0.0	5.1	31.3	11.5

Key:

SD Standard Deviation
High Upper limit of 95% confidence interval
Low Lower limit of 95% confidence interval

Evaluation of Slide Quality: Histological diagnosis (continued)

Case		Factor					
		Thickness	Distortion	Cytoplasm	Nucleus	Debris	Assessment
Case 8: Pituitary adenoma							
	Mean	27.4	26.8	16.9	22.6	38.3	25.2
	SD	7.9	10.0	7.6	8.9	2.4	7.9
	High	43.2	46.7	32.1	40.3	43.1	41.0
	Low	11.6	6.8	1.7	4.9	33.4	9.4
Case 9: Adenocarcinoma (metastatic)							
	Mean	25.5	18.6	29.9	33.2	37.7	36.6
	SD	7.3	9.1	11.2	9.1	7.3	7.6
	High	40.2	36.7	50.0	50.0	50.0	50.0
	Low	10.8	0.5	7.5	15.0	23.1	21.4
Case 10: Meningioma (transitional)							
	Mean	29.1	30.6	14.8	18.7	38.7	23.6
	SD	7.6	7.3	5.3	8.9	2.9	7.9
	High	44.2	45.1	25.5	36.5	44.6	39.5
	Low	13.9	16.1	4.1	0.9	32.8	7.8
Case 11: Gliosis secondary to idiopathic haemorrhage							
	Mean	30.2	35.3	9.8	15.9	38.9	19.5
	SD	9.2	5.7	3.9	6.4	3.5	5.8
	High	48.6	46.7	17.6	28.6	45.9	31.1
	Low	11.7	23.8	2.1	3.1	32.0	7.9

Key:

SD Standard Deviation
High Upper limit of 95% confidence interval
Low Lower limit of 95% confidence interval

Table 9: Evaluation of Slide Quality: Pathological Process and Specific Diagnosis

Diagnosis		Factor					
		Thickness	Distortion	Cytoplasm	Nucleus	Debris	Assessment
Correct pathological process							
	Mean	29.1	28.7	19.3	24.0	37.7	29.0
	SD	6.9	9.9	10.5	10.0	4.7	8.9
	High	42.8	48.4	40.2	44.1	47.1	46.8
	Low	15.3	9.0	0.0	3.9	28.2	11.2
Correct specific diagnosis							
	Mean	29.1	27.3	21.2	25.6	37.7	29.7
	SD	7.0	9.9	10.3	9.7	5.0	9.1
	High	43.1	47.1	41.9	45.1	47.7	47.8
	Low	15.2	7.5	0.6	6.1	27.7	11.6

Key:

SD Standard Deviation
High Upper limit of 95% confidence interval
Low Lower limit of 95% confidence interval

Chart 1: Evaluation of Slide Quality: Diff Quick/Touch Preparation

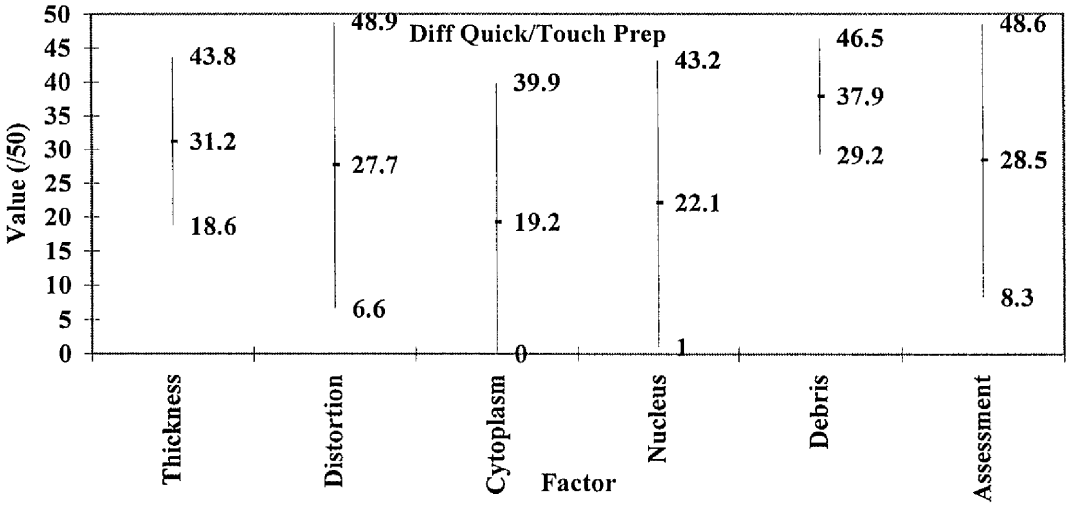


Chart 2: Evaluation of Slide Quality: Diff Quick/Medium Preparation

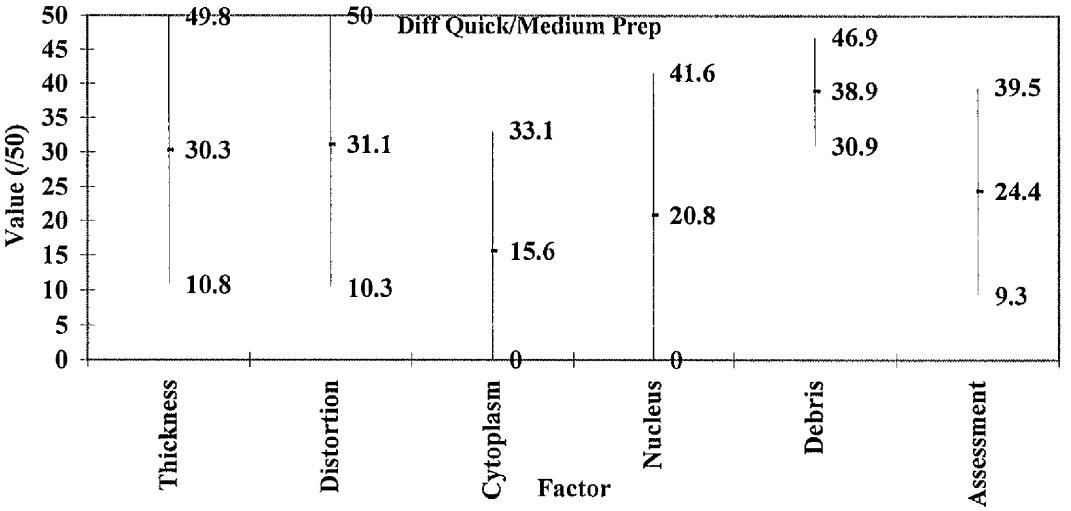


Chart 3: Evaluation of Slide Quality: Diff Quick/Smear Preparation

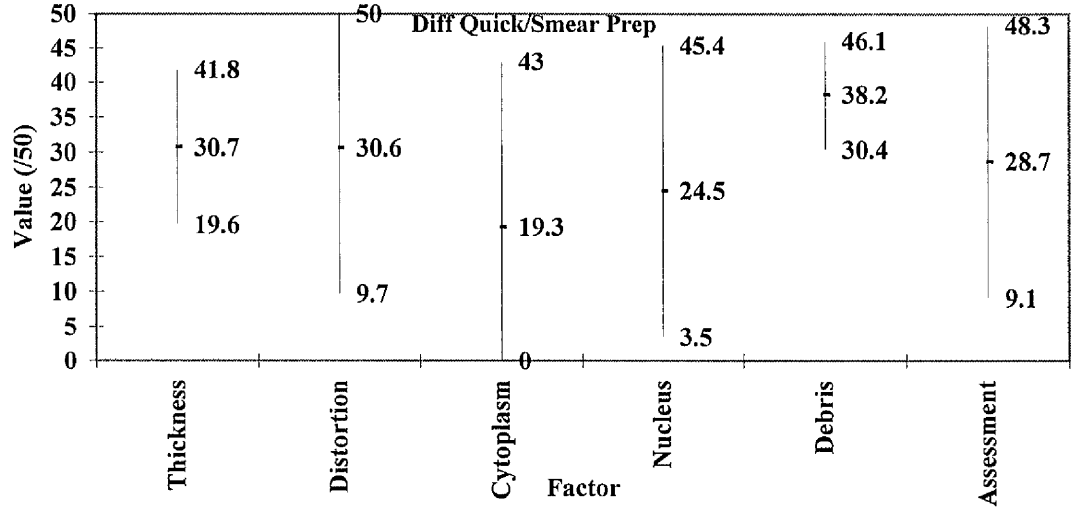


Chart 4: Evaluation of Slide Quality: May-Grunwald-Giemsa/Touch Preparation

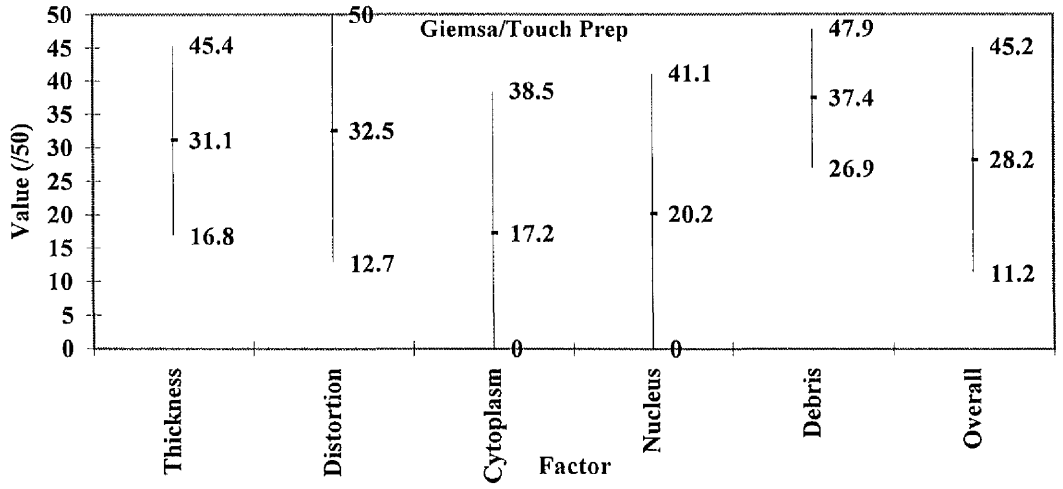


Chart 5: Evaluation of Slide Quality: May-Grunwald-Giemsa/Medium Preparation

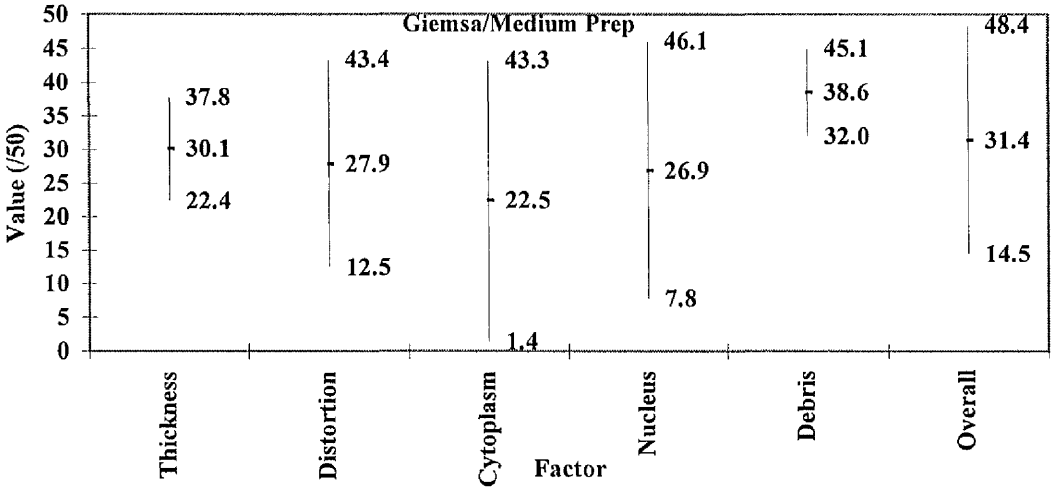


Chart 6: Evaluation of Slide Quality: May-Grunwald-Giemsa/Smear Preparation

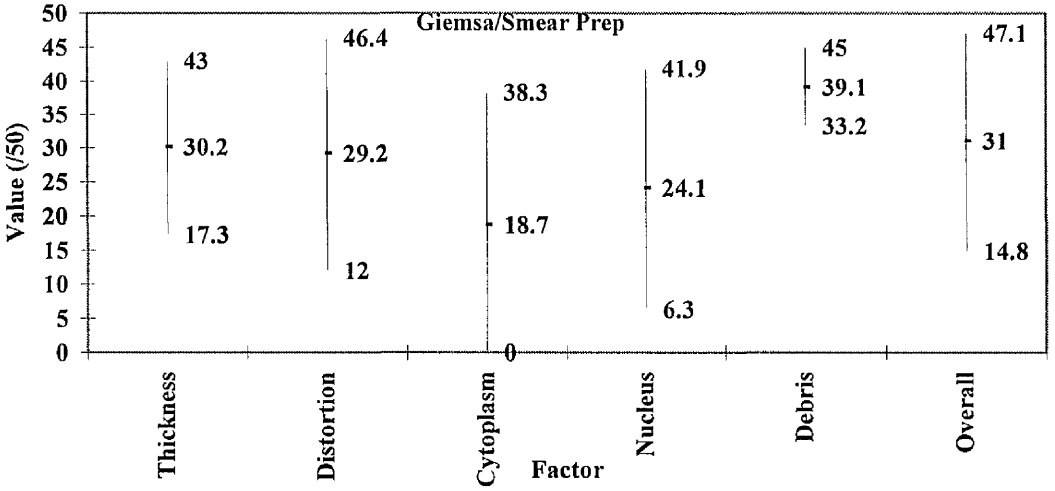


Chart 7: Evaluation of Slide Quality: Toluidine blue/Touch preparation

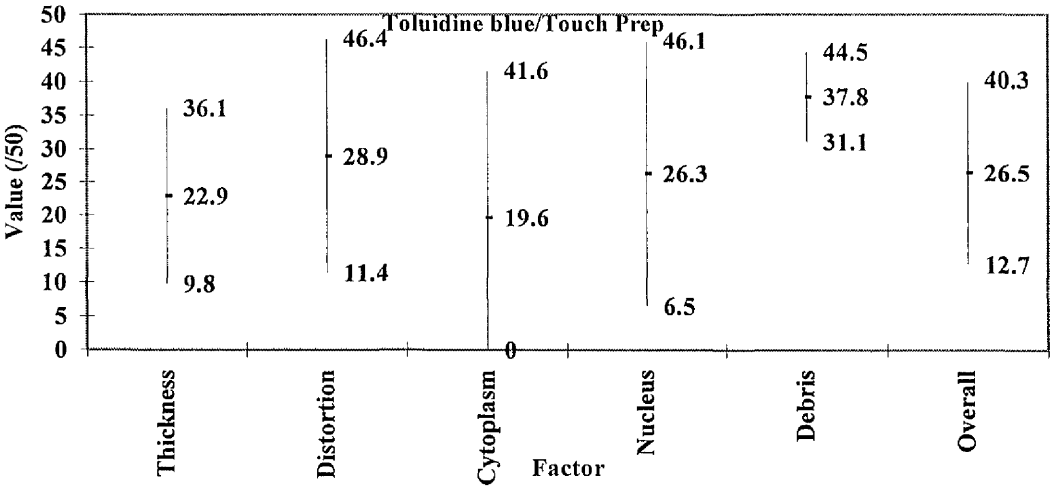


Chart 8: Evaluation of Slide Quality: Toluidine blue/Medium Preparation

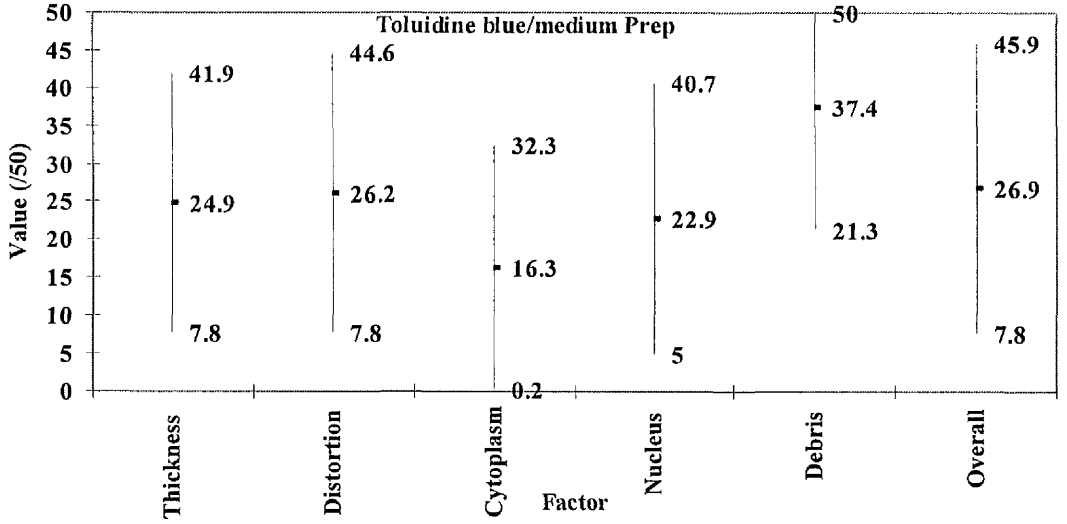


Chart 9: Evaluation of Slide Quality: Toluidine blue/Smear Preparation

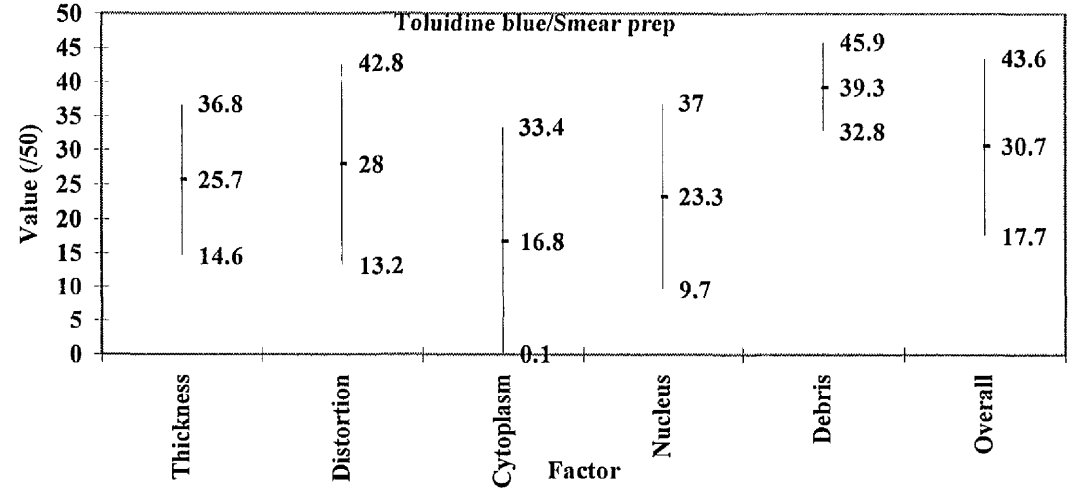


Chart 10: Evaluation of Slide Quality: Zynostain/Touch Preparation

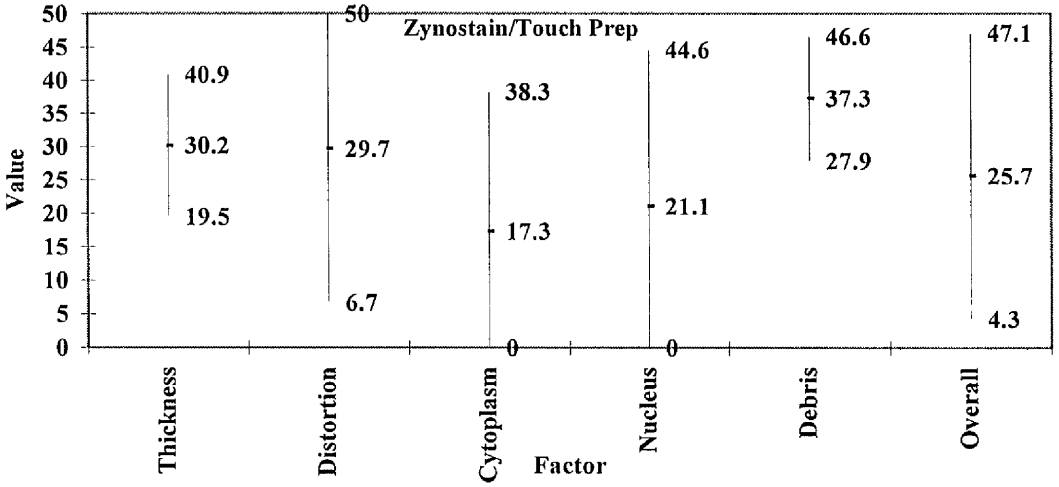


Chart 11: Evaluation of Slide Quality: Zynostain/Medium Preparation

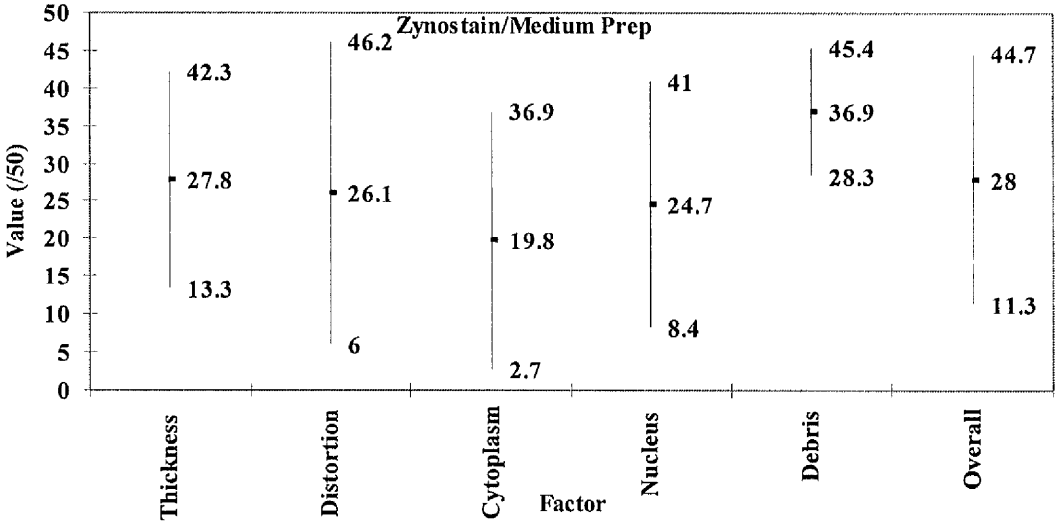


Chart 12: Evaluation of Slide Quality: Zynostain/Smear Preparation

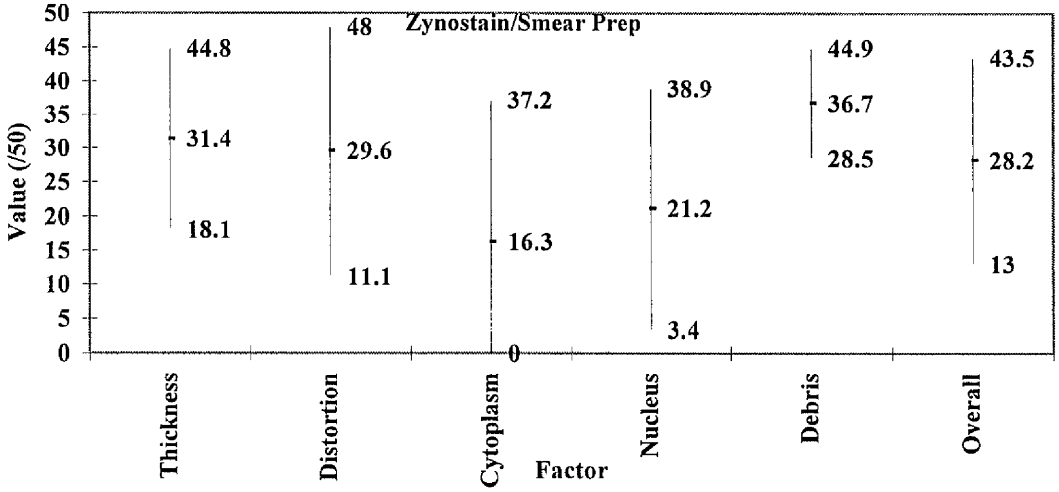


Chart 13: Evaluation of Slide Quality: Meningioma (syncytial)

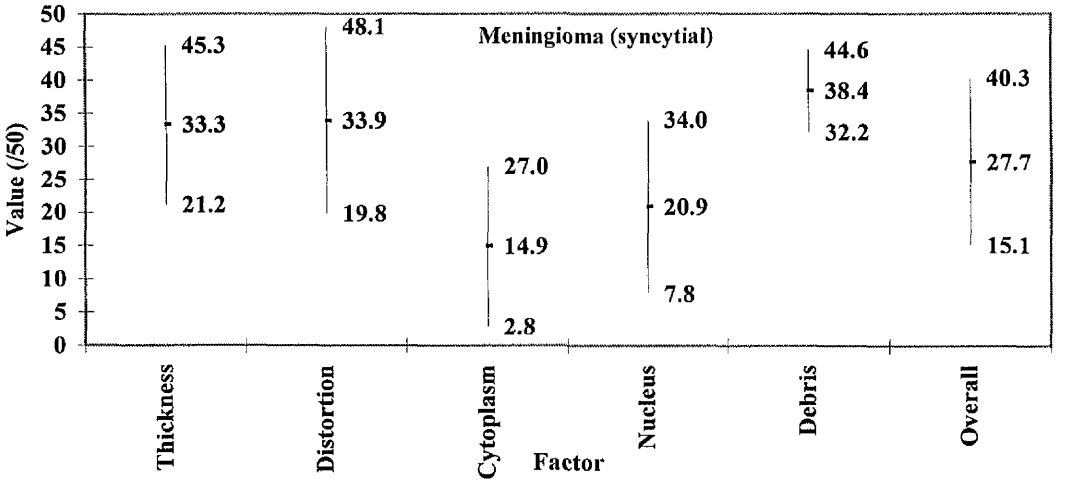


Chart 14: Evaluation of Slide Quality: Lymphoma (metastatic)

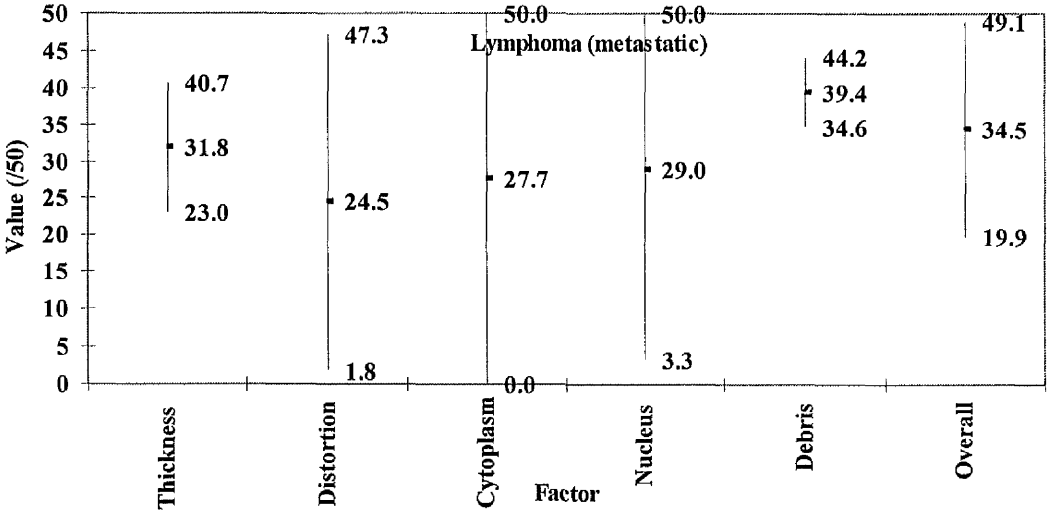


Chart 15: Evaluation of Slide Quality: Lymphoma (primary)

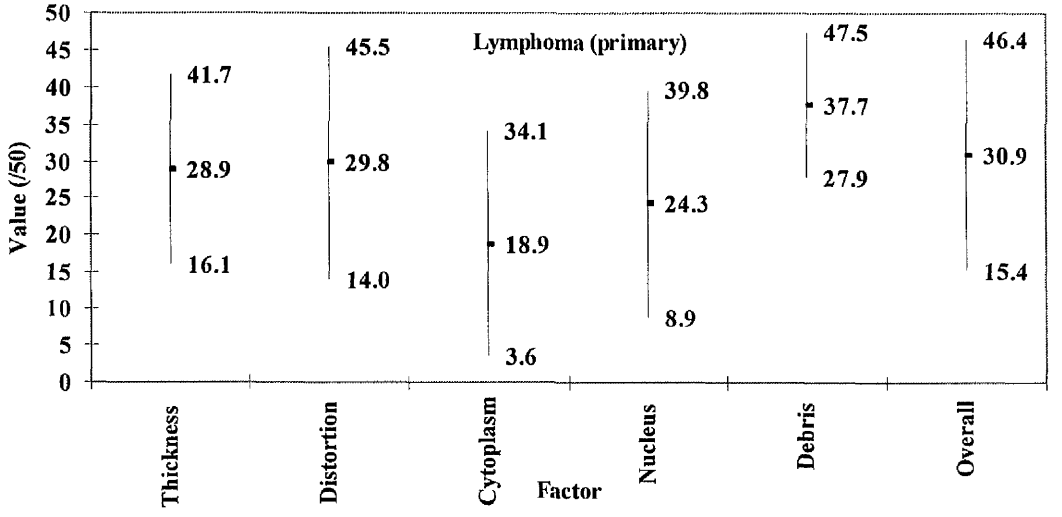


Chart 16: Evaluation of Slide Quality: Nasal Adenocarcinoma

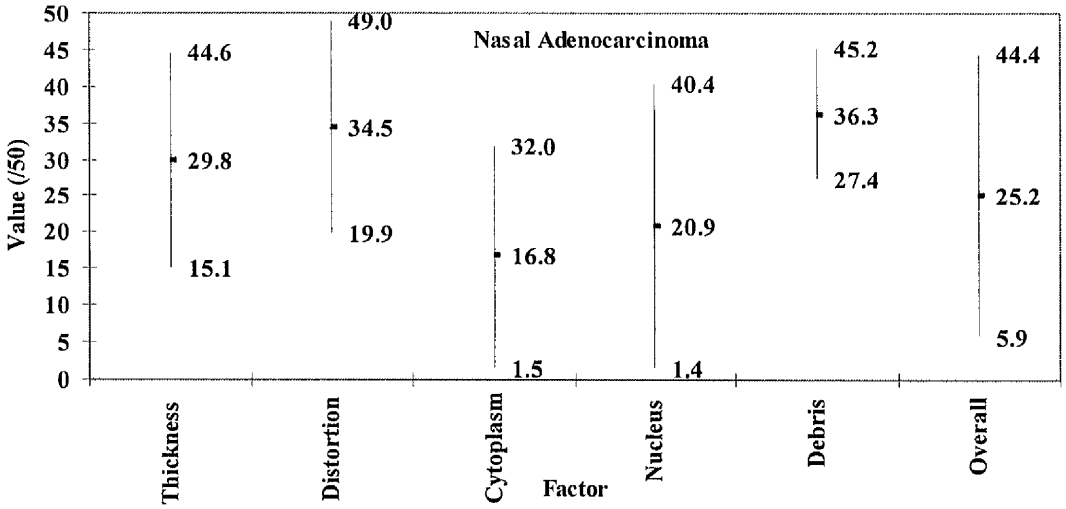


Chart 17: Evaluation of Slide Quality: Choroid plexus papilloma

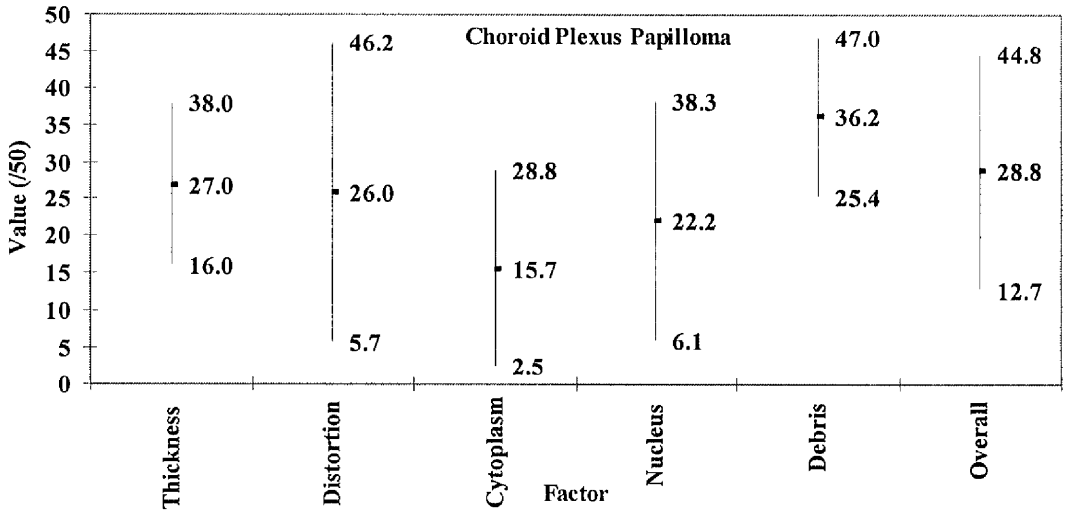


Chart 18: Evaluation of Slide Quality: Oligodendroglioma

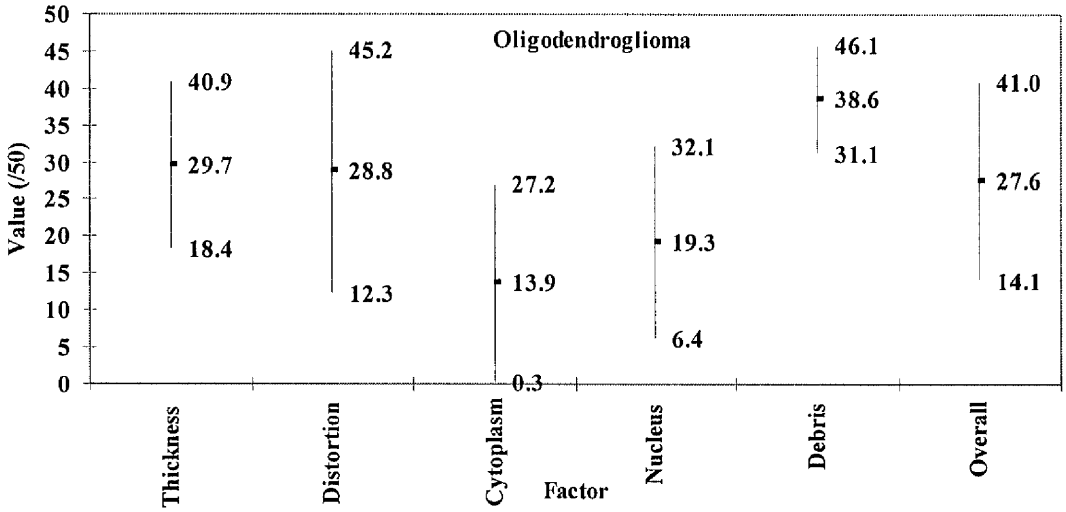


Chart 19: Evaluation of Slide Quality: Granulomatous meningoencephalitis

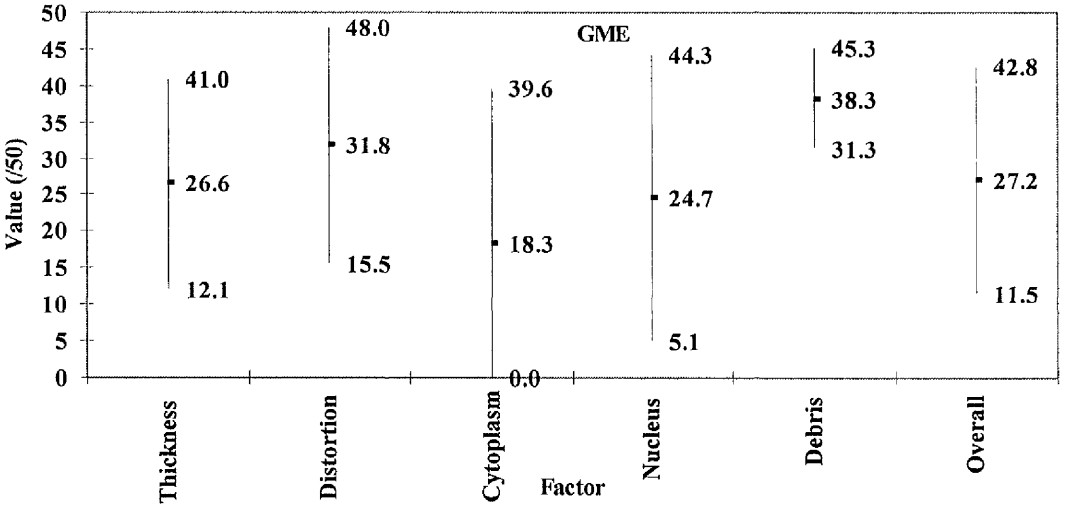


Chart 20: Evaluation of Slide Quality: Pituitary adenoma

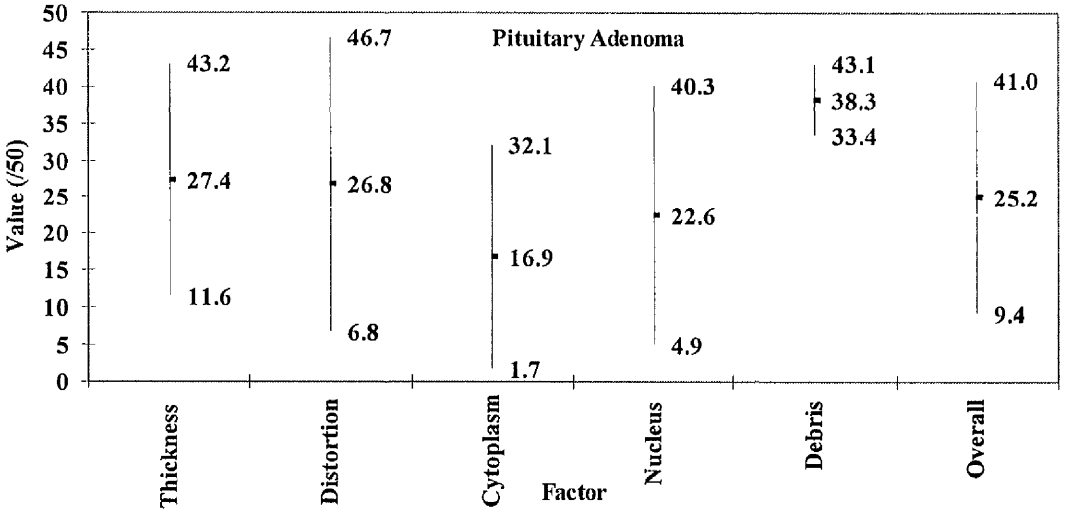


Chart 21: Evaluation of Slide Quality: Metastatic adenocarcinoma

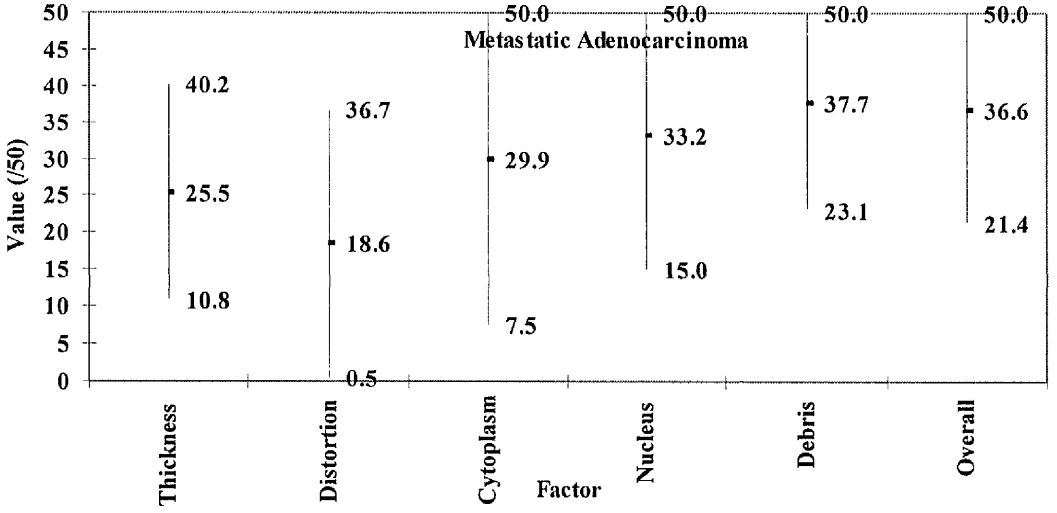


Chart 22: Evaluation of Slide Quality: Meningioma (transitional)

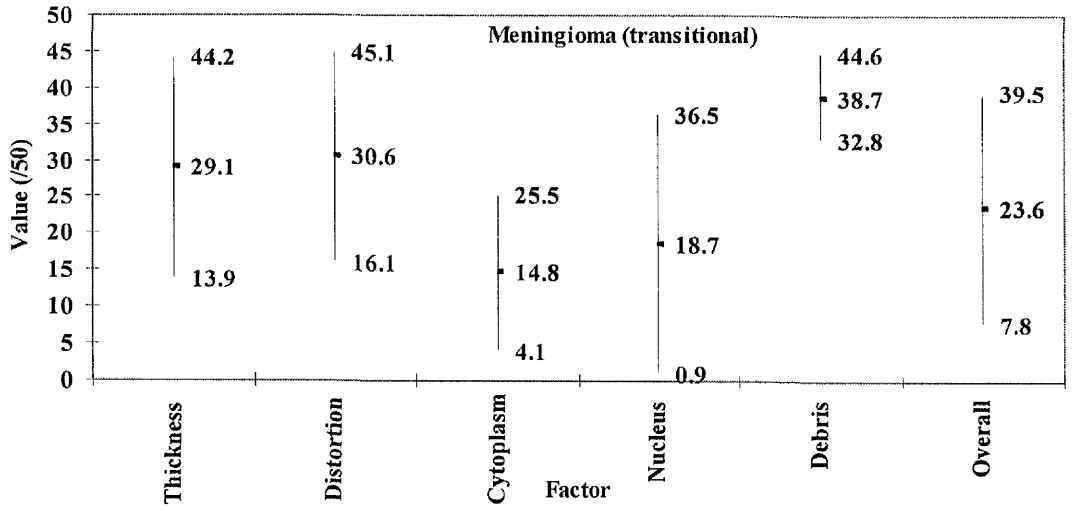


Chart 23: Evaluation of Slide Quality: Gliosis (secondary to haemorrhage of unknown origin)

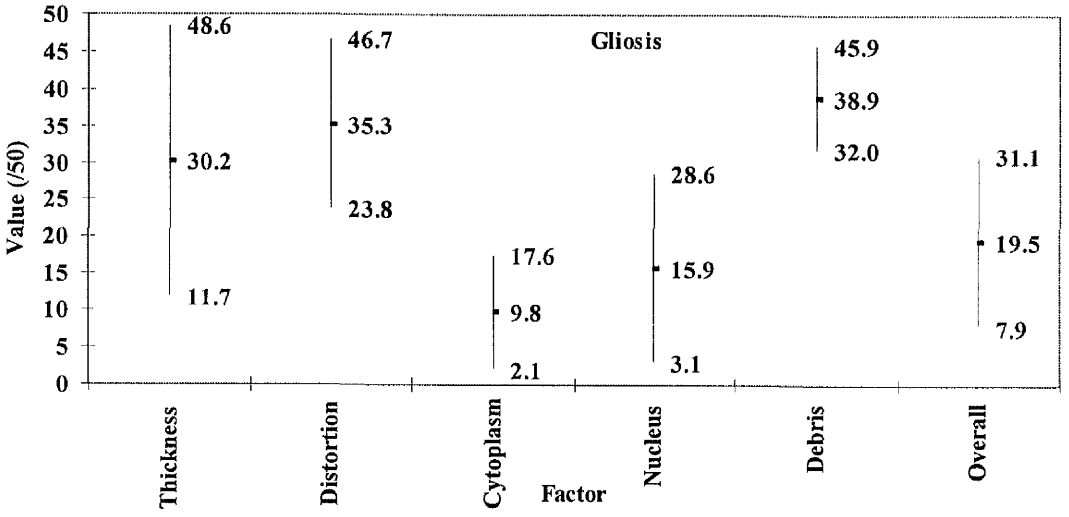


Chart 24: Evaluation of Slide Quality: Correct pathological process

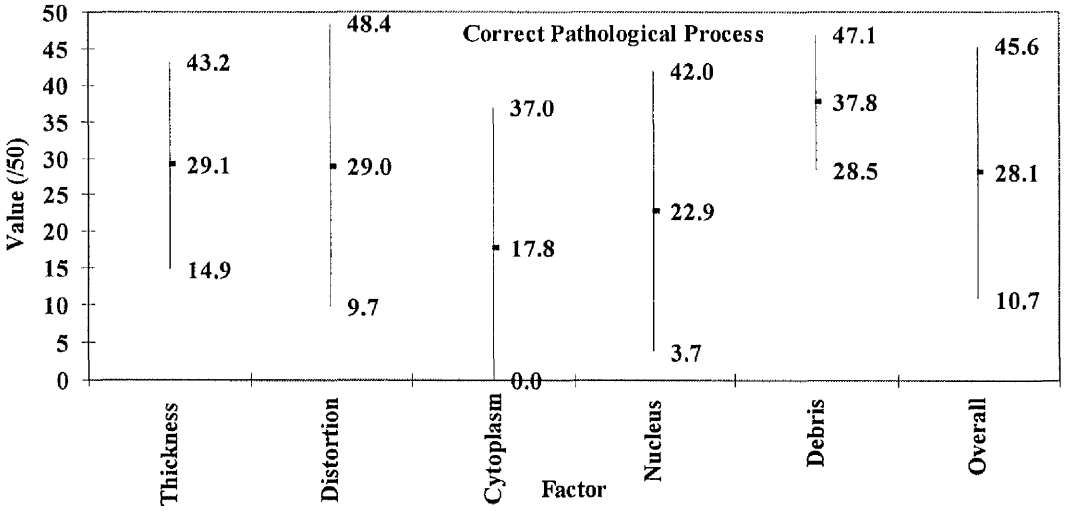
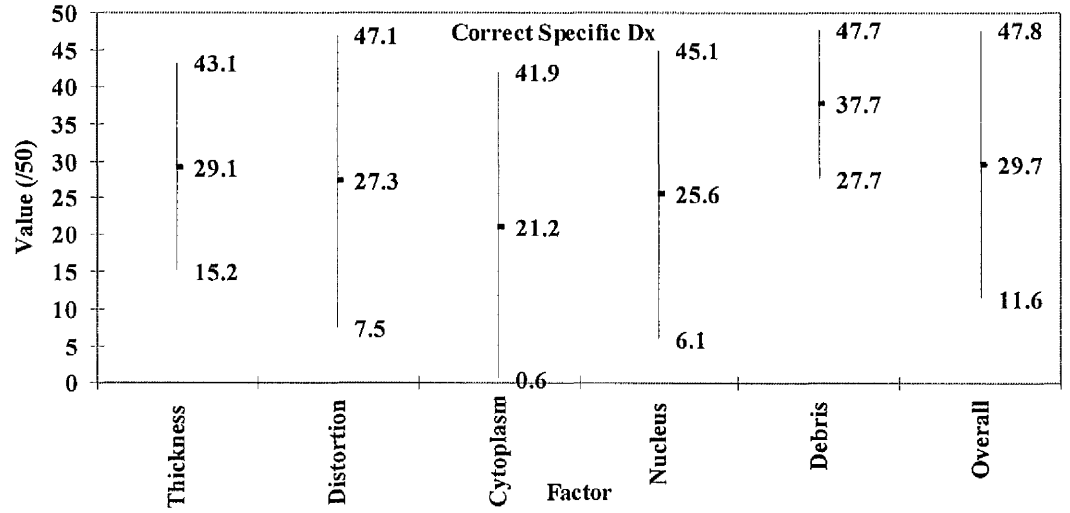


Chart 25: Evaluation of Slide Quality: Correct specific diagnosis



DISCUSSION

Part I: Cytological appearance of normal canine brain

The cytological appearance of the normal canine brain shared strong similarities with the cytological appearance of the human brain. Characteristic features of the regions examined were similar to those described in the human literature (Adams *et al.*, 1981; Moss *et al.*, 1997). In particular, the presence of large motor neurons allowed the distinction of motor cortex from other areas of the cerebral cortex and thalamic and caudate nuclei, while the complete absence of neurons and the coarser nature of background neuropil allowed the identification of white matter. A combination of the appearance of the background neuropil and the presence of very large motor neurons also allowed the identification of samples from the medial lemniscus and medulla oblongata. Samples from the cerebellum were strikingly cellular, and the presence of large numbers of granule cells unique to this part of the brain. In addition, the presence of Purkinje neurons in these samples aided their identification as cerebellar tissue.

Part II: Cytological Diagnosis of Intracranial Lesions

Accuracy of cytological diagnosis

The results of this study suggest that cytological examination provides an accurate diagnostic method that is likely to be useful for the intraoperative diagnosis of intracranial lesions in dogs and cats. The overall diagnostic accuracy of 81% described (based on a combination of pathological process and specific diagnosis) is similar to reports in the veterinary and human literature (Brainard *et al.*, 1997; Burger, Nelson, 1997; Reyes *et al.*, 1991; Kitchen *et al.*, 1993; Firlik *et al.*, 1999; Folkerth, 1994; Gaudin *et al.*, 1997; Hayden *et al.*, 1995; Hayden *et al.*, 1995; Martinez *et al.*, 1988; Mennel *et al.*, 1989; Ostertag *et al.*, 1980) . However, the results of this study must be

this study must be interpreted with some caution, as it is difficult to compare diagnostic accuracy with that quoted in other reports. The majority of human studies evaluate diagnosis made in conjunction with imaging studies and consultation with a neurosurgeon, unlike the study reported here. Furthermore, the assessment of diagnostic accuracy in other reports is not straightforward as accuracy can be evaluated in different ways. Most studies in man include only 2 categories of accuracy: correct or incorrect. If these terms are not further defined it may be unclear which criteria need to be fulfilled for a correct diagnosis. The majority of reports assess the accuracy of diagnosis by comparing cytological examination with histopathological diagnosis, although one report has evaluated cytological diagnosis using survival analysis, and found that cytological diagnosis reliably predicted mean survival time (Gaudin *et al.*, 1997). Few reports exist in the veterinary literature evaluating the accuracy of intraoperative diagnosis. As stated previously, one report evaluating the accuracy of cytological diagnosis in 100 cases included a small number of brain tumours (Eich *et al.*, 2000). Another report evaluating the accuracy of intraoperative diagnosis using frozen section examination in 194 specimens reported an overall accuracy rate of 93%, with a correct specific diagnosis achieved in 83% of cases and correct identification of the pathological process in an additional 10% of cases (Whitehair *et al.*, 1993). This report included 8 specimens from nervous system biopsies, 4 of which were intracranial lesions.

Intraoperative diagnoses are often broad and are further refined with the evaluation of paraffin embedded material (Firlik *et al.*, 1999). For this reason, it was considered more appropriate to have 3 categories of correlation between cytological and histopathological diagnosis. In this way partial credit could be given to the cytologist

for making a tentative diagnosis which may be clinically useful despite a lack of final specific diagnosis. Toluidine blue, a stain which is rarely used in our institution, was less accurate than other stains. At the same time the two most accurate stains, Diff Quik and Zynostain, are in common use in our institution, suggesting that the familiarity of the cytologist with the stain used was important for accurate diagnosis. However, the number of specimens examined in this study was relatively small, and more work needs to be performed to further evaluate the effects of experience on diagnostic accuracy with respect to stain type.

The Diff Quik stain and May-Grunwald-Giemsa stain are Romanowsky-type stains that employ the use of two dyes to stain cellular elements differently. Along with Zynostain, these stains were chosen for evaluation because of their availability, speed of preparation, ease of use and their common use in veterinary institutions. Toluidine blue, while commonly in use in human neuropathological institutions, has yet to become popular in veterinary cytopathology. The advantages of this stain in cytological examination of the nervous system include speed of use and the ability to stain astrocytic processes (Moss *et al.*, 1997). The quality of cytoplasmic and nuclear staining with all 4 stains was generally excellent. Both May-Grunwald-Giemsa and Diff-Quik stains produced good cytoplasmic and stromal detail, while Zynostain and toluidine blue both allowed better visualisation of nuclear detail, as has been described elsewhere (Slowinski *et al.*, 1999). While the Romanowsky-type stains and Zynostain are based on air drying techniques, toluidine blue relies on wet fixation. A number of differences exist between wet-fixed and air-dried specimens: 1) the smearing technique is less important for wet fixed specimens but important for air-dried specimens, 2) air drying increases cell size while wet fixation decreases cell size, and 3) cell loss is high

with wet fixation compared to air drying techniques (Jorundsson *et al.*, 1999). This last point is less relevant in smear preparations, where a large amount of material is transferred to the slide, but important in touch preparations where fewer cells adhere to the slide surface. It is even more important for fibrous or firm specimens (eg fibroblastic meningioma, malignant nerve sheath tumours), which imprint or smear poorly (Kitchen *et al.*, 1993).

Of the 3 preparation techniques, touch preparation and smear preparation provided results of similar diagnostic accuracy, with touch preparation providing slightly greater accuracy for specific diagnosis. However, if the percentage of non-diagnostic specimens was taken into consideration, smear preparation was felt to be more useful as fewer samples were found to be non-diagnostic. This is especially relevant to stereotactic biopsy samples, where it is desirable to take as few samples as possible to achieve a diagnosis. Although a greater amount of tissue may be obtained with multiple aspirations, this increases the risk of haemorrhage, and consequently for brainstem lesions or other high risk locations only a single suction aspiration biopsy may be performed for reasons of safety (Firlik *et al.*, 1999; Valdes-Gorcia *et al.*, 1998). The majority of human neuropathologists use the smear technique for cytological examination of CNS lesions (Martinez *et al.*, 1988). In smear preparations of small samples not only the cytological detail but also some tissue architecture is preserved (Eich *et al.*, 2000), which may not be achieved with paraffin-embedded sections (Mennel *et al.*, 1989), and this proved helpful in the diagnosis of some lesions (see Figure 20, page 84 and Figure 21, page 84). Few reports have compared the diagnostic accuracy of touch and smear preparations in humans, but one report found that touch preparation was associated with lower diagnostic accuracy (76%) when compared to

the smear technique. This may be the result of artifactual alterations in the tissue resulting from manipulation, disruption and crushing of the biopsy specimen (Hayden *et al,* 1995).

Figure 20: Syncytial meningioma (case 1). Low power magnification reveals cohesive cell aggregates which are not tightly adherent to blood vessels. Smear preparation, Diff Quick stain, x25

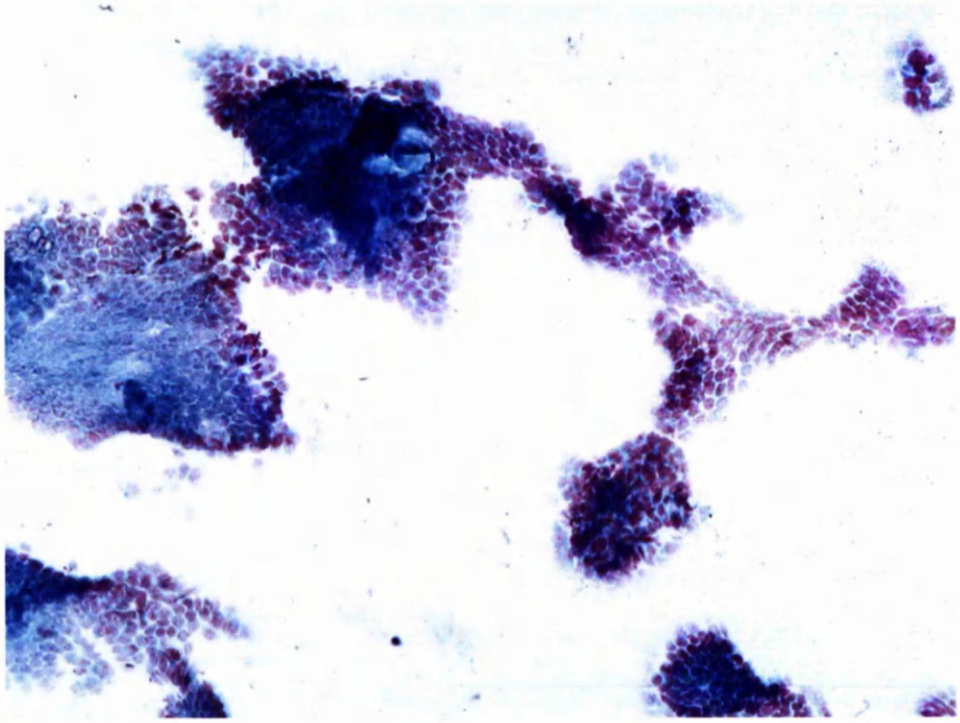


Figure 21: Metastatic carcinoma (case 9). Distinctive cell moulding is visible at low magnification. Smear preparation, May-Grunwald-Giemsa stain, x25.

However, while smear preparations are most commonly employed, it has been reported that less forceful touch preparations can be useful for metastatic carcinoma and lymphoma, but usually are not adequate for gliomas (Burger, Nelson, 1997). In this study there appeared to be no statistically significant difference between touch and smear preparations with respect to these tumours. Technical problems with the touch preparation may also complicate interpretation of specimens. For example, excessively thick cytological preparations lead to clumping and distortion and may suggest a more anaplastic lesion. Firm tumours with abundant fibrous connective tissue stroma may not shed cells on the glass slide, which may lead to an underestimation of the degree of malignancy (Martinez *et al.*, 1988). In general, soft and friable tissues lend themselves to the smear technique whereas firm or fibrous material may require a frozen section for adequate examination (Morrison, DeNicola, 1993). However, it has been noted that experience is an important factor in the creation of high quality smear preparations, with too little pressure leading to smears that are too thick for satisfactory interpretation, and too much pressure leading to crush artefact (Moss *et al.*, 1997). It is likely that with experience the quality of preparations generated would improve and the proportion of non-diagnostic preparations decrease. While in this study the touch and smear preparations appeared to be of similar diagnostic accuracy, the greater difficulty in performing the touch preparation was reflected in the greater proportion of non-diagnostic specimens.

Perhaps one of the most interesting features of this study was the considerable variation in diagnostic accuracy between cases (see Table 10, page 86).

Table 10: Diagnostic accuracy based on histopathological diagnosis

Case	Diagnosis	Correct Pathological Process	Correct Specific Diagnosis
1	Meningioma (syncytial)	12/12 (100%)	11/12 (92%)
2	Lymphoma (metastatic)	8/12 (67%)	6/12 (50%)
3	Lymphoma (primary)	12/12 (100%)	10/12 (83%)
4	Adenocarcinoma (nasal)	11/12 (92%)	7/12 (58%)
5	Choroid plexus papilloma	12/12 (100%)	4/12 (33%)
6	Oligodendroglioma	12/12 (100%)	7/12 (58%)
7	Granulomatous meningoencephalitis (GME)	3/12 (25%)	3/12 (25%)
8	Pituitary adenoma	11/12 (92%)	1/12 (8%)
9	Adenocarcinoma (metastatic)	12/12 (100%)	12/12 (100%)
10	Meningioma (transitional)	12/12 (100%)	3/12 (25%)
11	Gliosis secondary to haemorrhage	2/12 (17%)	2/12 (17%)

Overall the percentage of specimens from each case in which the correct pathological process was identified was high. However, while some cases (syncytial meningioma, primary lymphoma and the metastatic carcinoma – see Figure 22, page 90) achieved a high degree of accuracy with respect to specific diagnosis (83%-100%), other cases were poorly diagnosed (GME, gliosis, pituitary adenoma, choroid plexus papilloma, transitional meningioma). With some of these cases, such as the GME and gliosis, this is unsurprising as gliosis and inflammation present diagnostic challenges. The distinction between reactive gliosis and the infiltration zone of glial tumours constitutes a difficulty for diagnosis as no reliable morphologic criteria can be defined to distinguish reactive astrocytes from neoplastic cells (Kleihues *et al.*, 1984). Preparations from both of these cases exhibited hypercellularity and a degree of nuclear pleomorphism, both of which are factors that may lead to a diagnosis of neoplasia (see Figure 23, page 92). The majority of specimens for both of these cases were misdiagnosed as mesenchymal neoplasms of glial origin. With the cases of GME and gliosis excluded, overall diagnostic accuracy was increased to 94%, and complete correlation rose to 56%.

The misdiagnosis of the transitional meningioma is likely to have occurred because of difficulties with the classification system used in this study. Cytologic features suggestive of meningioma include cells with both epithelial and mesenchymal characteristics and a tendency towards cell clustering (Zimmerman *et al.*, 2000) (see Figure 24, page 94). The dual cell morphology within a single tumour is unsurprising in light of the mixed fibroblastic and epithelioid components often seen histologically.

One report of the cytological characteristics of canine meningiomas has suggested that based on cytological features alone a diagnosis of neoplasia may be possible, but a specific diagnosis of meningioma can only be made on the basis of cytological features together with tumour location and imaging characteristics (Zimmerman *et al.*, 2000). With the choroid plexus papilloma and pituitary adenoma, misdiagnosis occurred because of interpretation error. However, the majority of specimens from these three tumours were correctly diagnosed as neoplastic, although the type of neoplasia was incorrectly identified as mesenchymal. This may be due to the lack of familiarity of the cytologist with cytological preparations of these tumours.

Qualitative assessment of stain and preparation quality

As has been mentioned previously, care must be exercised when interpreting the results of slide evaluations. One of the most obvious findings that became apparent was the inherent variability of the slide quality with all stains and preparation methods, as was evidenced by the large confidence intervals. This necessarily tempers the conclusions drawn when comparing different stains and preparation methods. In addition, given the scoring system used, it is unlikely that small differences between scores obtained for any given factor are significant. However, some general statements may be made.

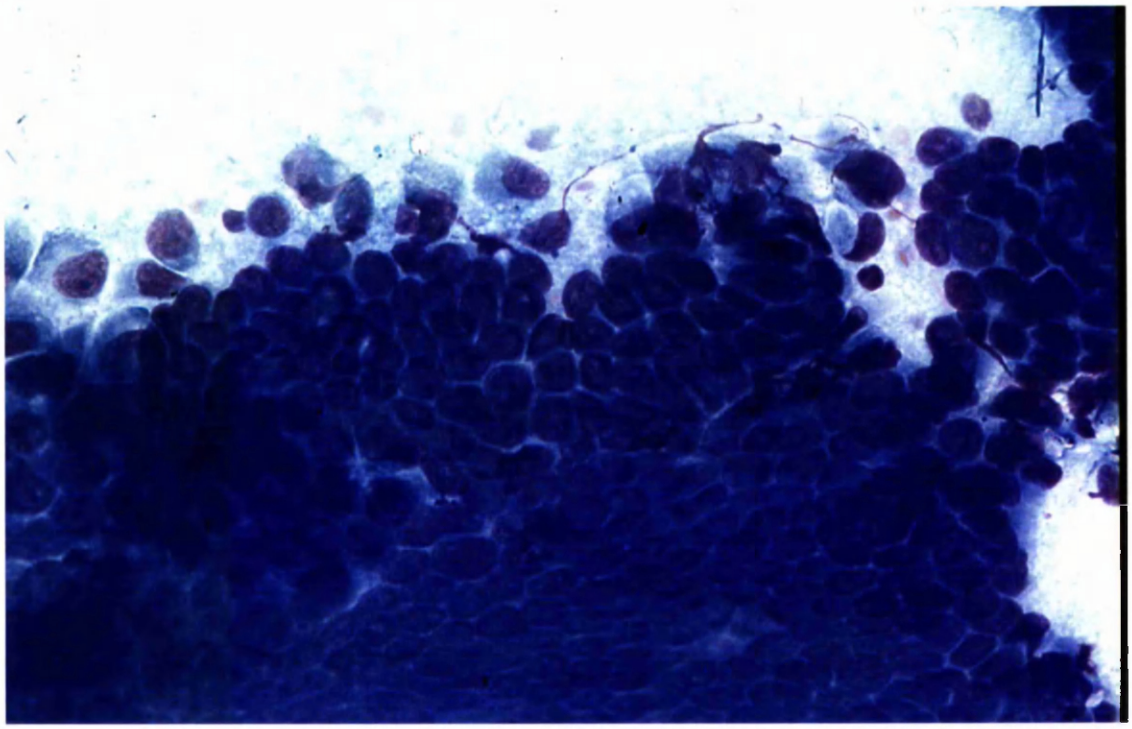
The highest overall assessment scores were generally associated with smear preparations and with the May-Grunwald-Giemsa stain. In general, diagnostic accuracy appeared to correlate best with high overall assessment scores, and to a lesser degree with high nuclear and cytoplasmic detail scores. However, this was at odds with the findings of the first part of this study, which suggested that the Diff-Quick stain was associated with highest diagnostic accuracy. This may be the result of a

combination of other factors such as the inherent variability of slide quality. It must be noted that the numbers of cases evaluated in this study was relatively low, and consequently variability in slide quality is likely to have been an important factor influencing diagnostic accuracy. With greater experience it is to be hoped that the stain which gives the most consistent results will become more apparent.

The highest scores for nuclear detail were achieved with the toluidine blue stain, as other reports have suggested (Slowinski *et al.*, 1999), while May-Grunwald-Giemsa provided the highest cytoplasmic detail scores.

The histologic diagnoses associated with highest overall assessment scores were the metastatic adenocarcinoma and the two cases of lymphoma, suggesting that smears from these cases were easiest to produce, and other reports have alluded to the ease of preparation of samples from these tumours (Burger, Nelson, 1997). Conversely, the cases associated with lowest overall assessment scores were the transitional meningioma, pituitary adenoma and nasal adenocarcinoma. Generally, nuclear and cytoplasmic detail scores were noted to parallel overall assessment scores.

The relationship between the presence of distortion and accuracy of diagnosis is not clear – while the lesion associated with the least distortion (metastatic adenocarcinoma) was generally accurately diagnosed, and the lesion associated with the most distortion (gliosis) was generally poorly diagnosed, this did not hold true for other lesions such as the syncytial meningioma which was generally accurately diagnosed despite a high degree of distortion. This implies that other factors were important in making an accurate diagnosis.



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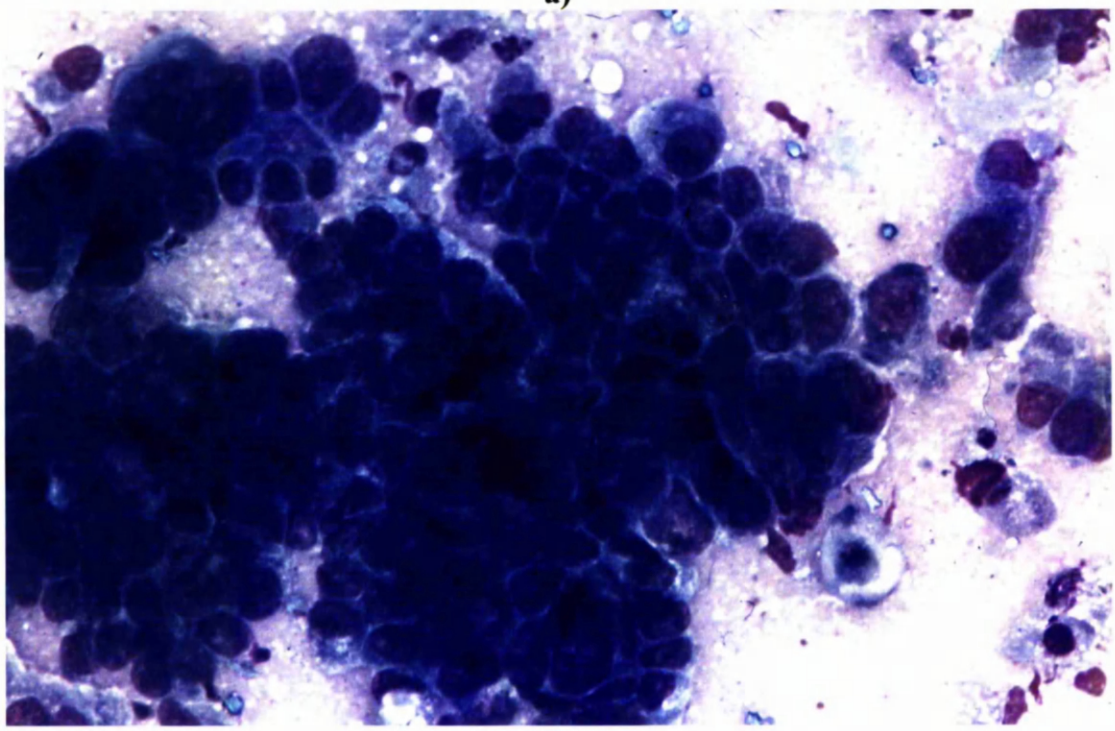
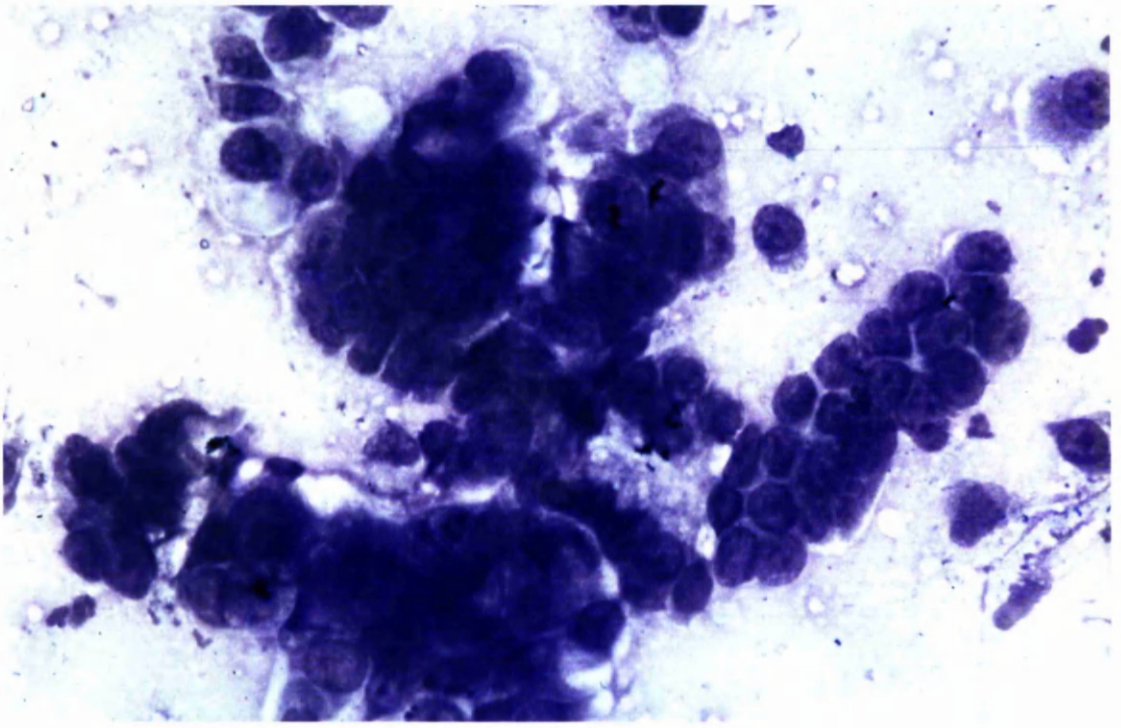


b)

Figure 22: Metastatic carcinoma (case 9), smear preparations. At high power, the cohesive nature of this tumour with cell moulding is visible. **a)** Diff-Quick, x100 **b)** May-Grunwald-Giemsa, x100 **c)** toluidine blue, x100 **d)** Zynostain, x100



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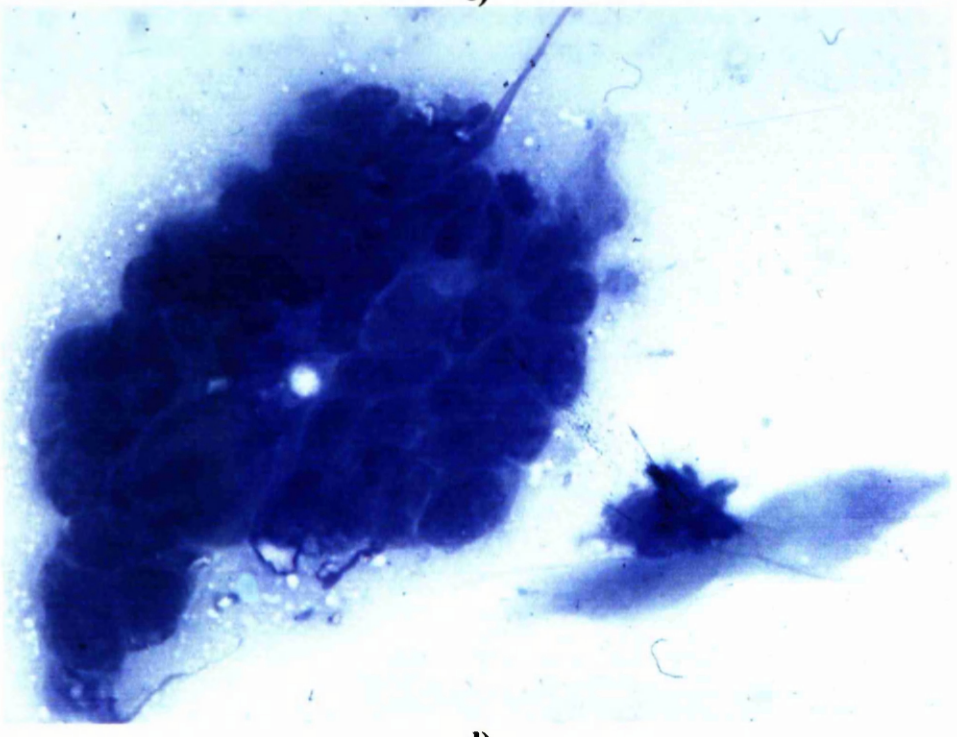
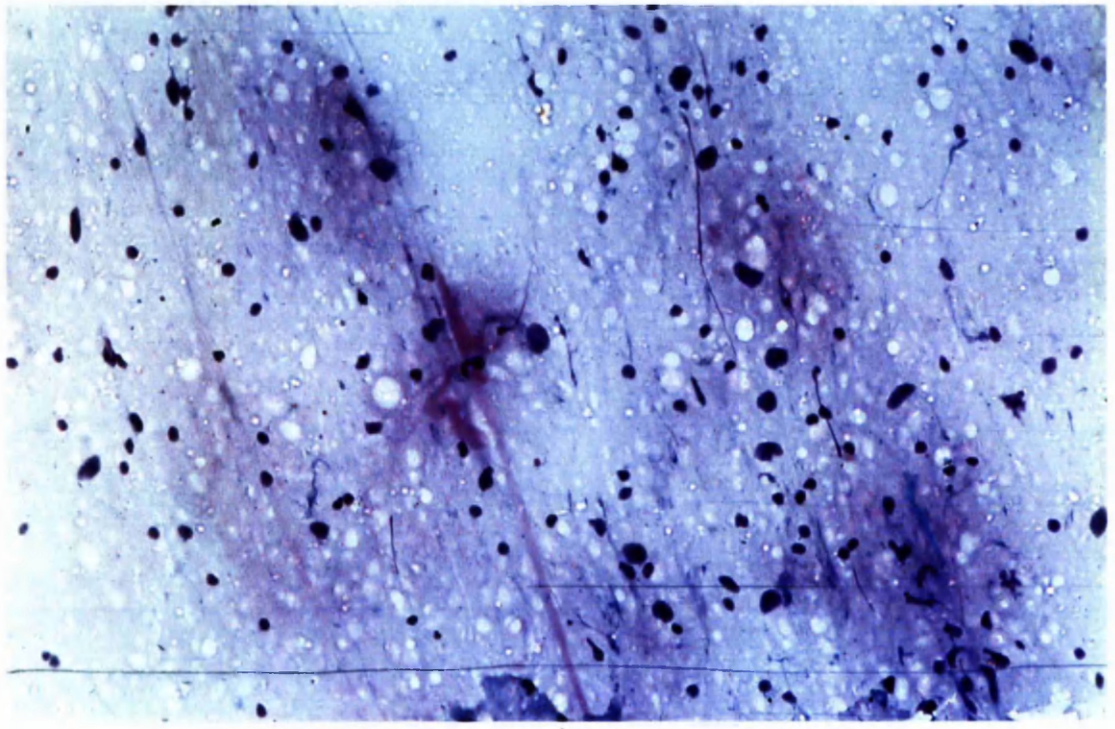


d)

Figure 22 (continued): Metastatic carcinoma (case 9).



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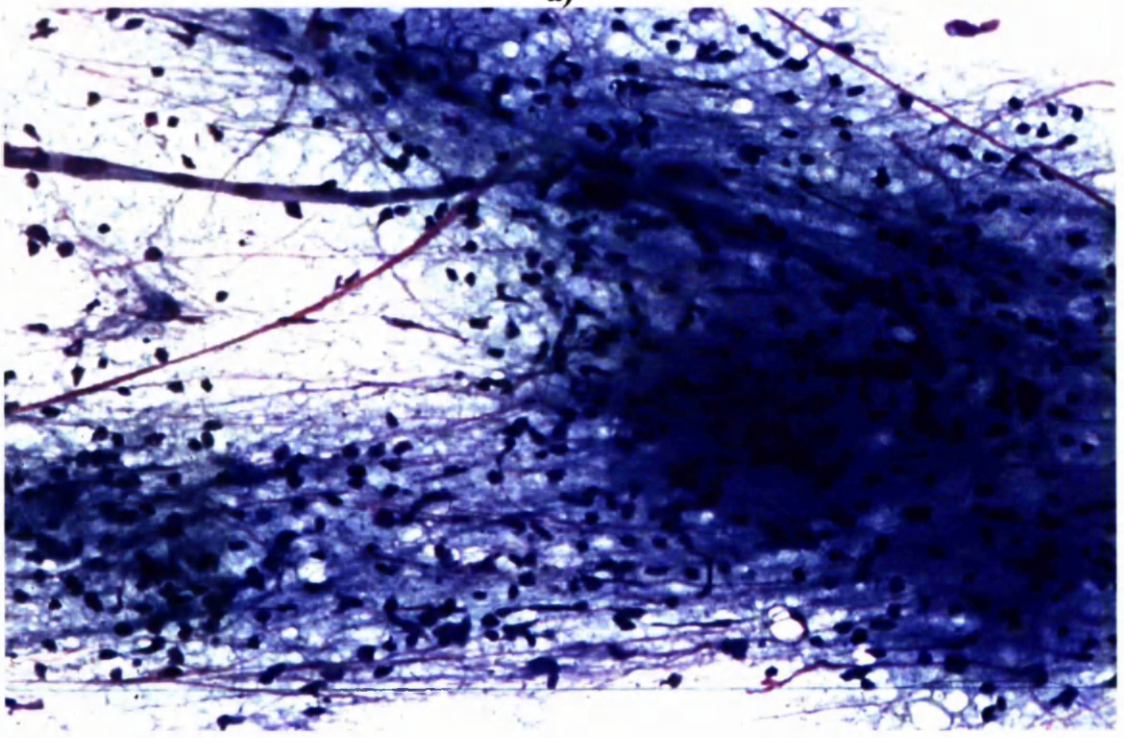
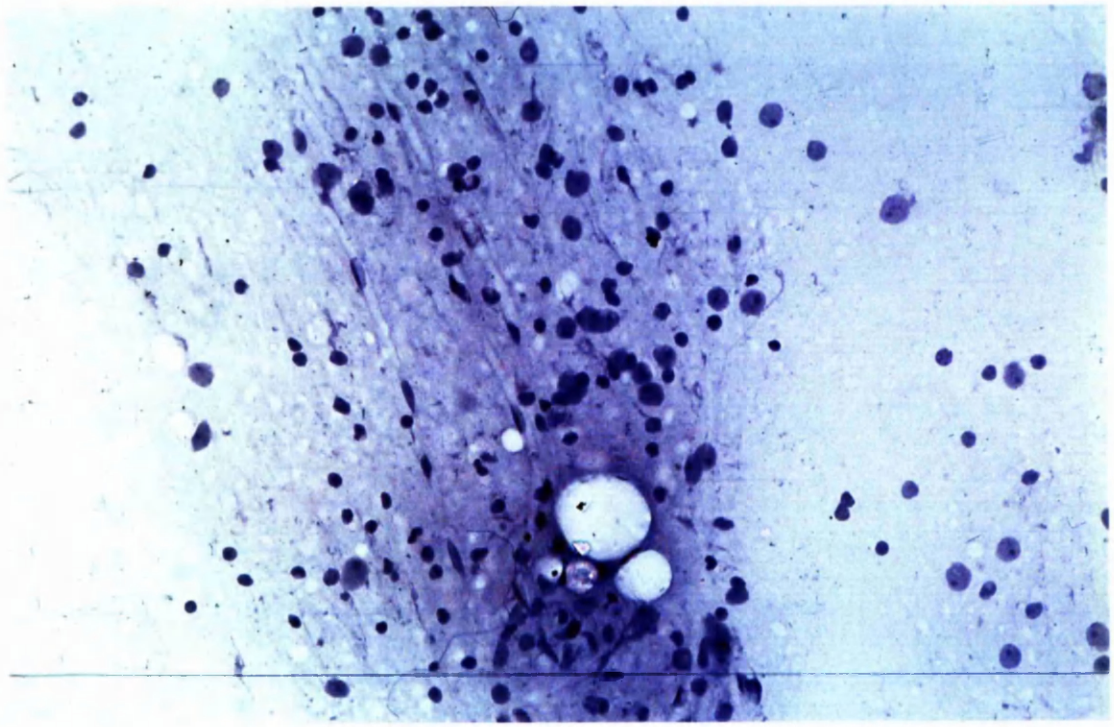


b)

Figure 23: GME (case 7), smear preparations. Preparations are hypercellular, with small, darkly staining nuclei representing glia and inflammatory cells. Nuclear pleomorphism is evident, making the distinction between inflammation and neoplasia difficult. **a)** Diff-Quick, x50 **b)** May-Grunwald-Giemsa, x50 **c)** toluidine blue, x50 **d)** Zynostain, x50



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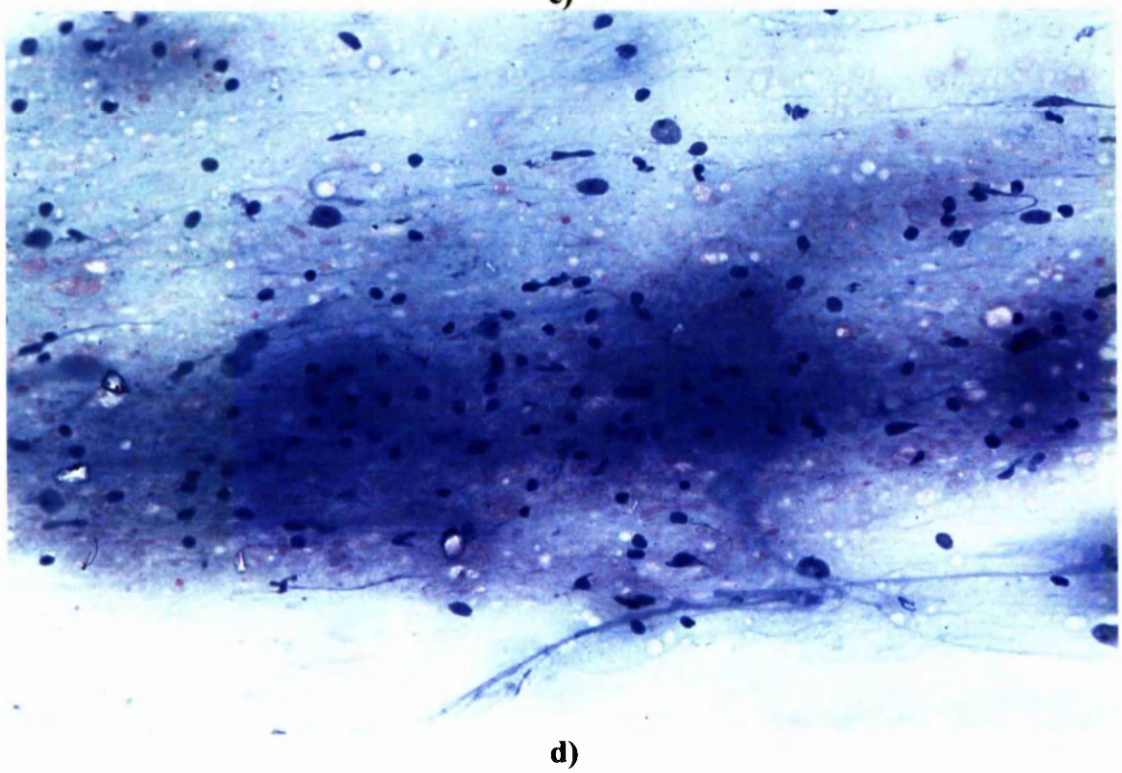

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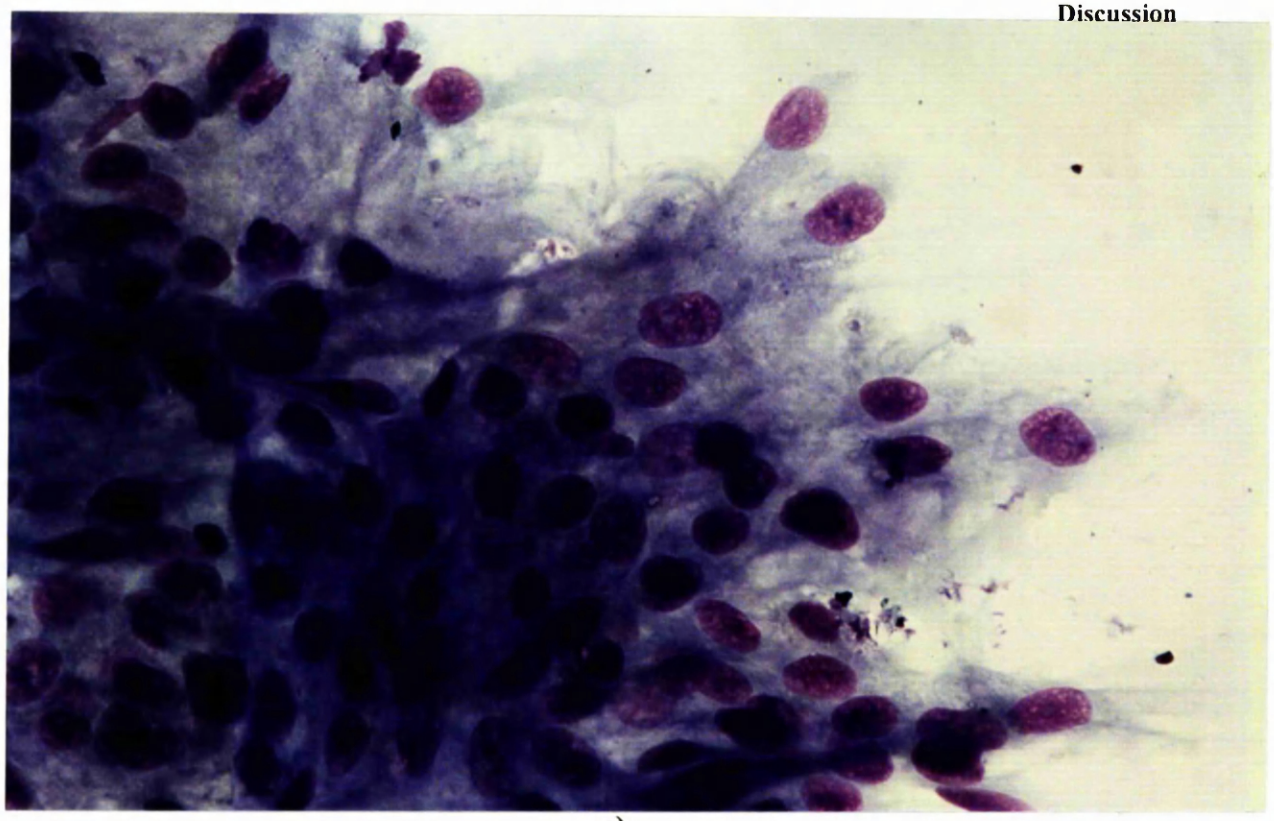
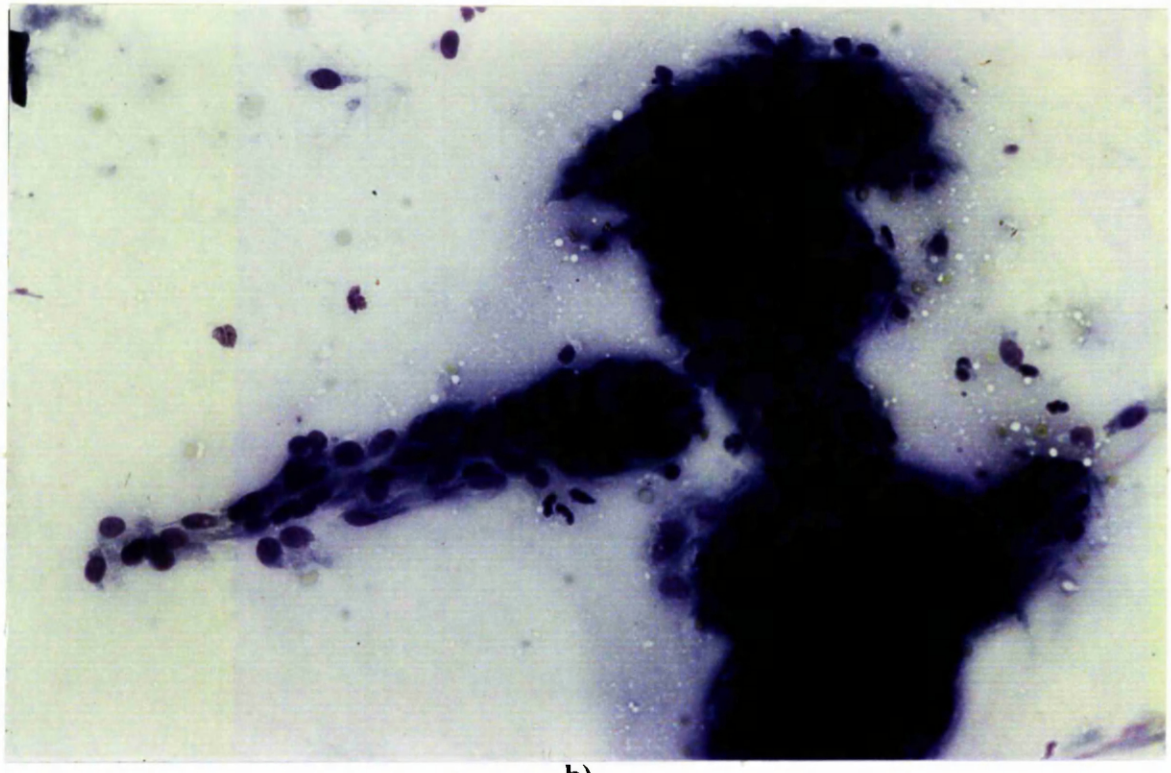
Figure 23 (continued): GME (case 7).

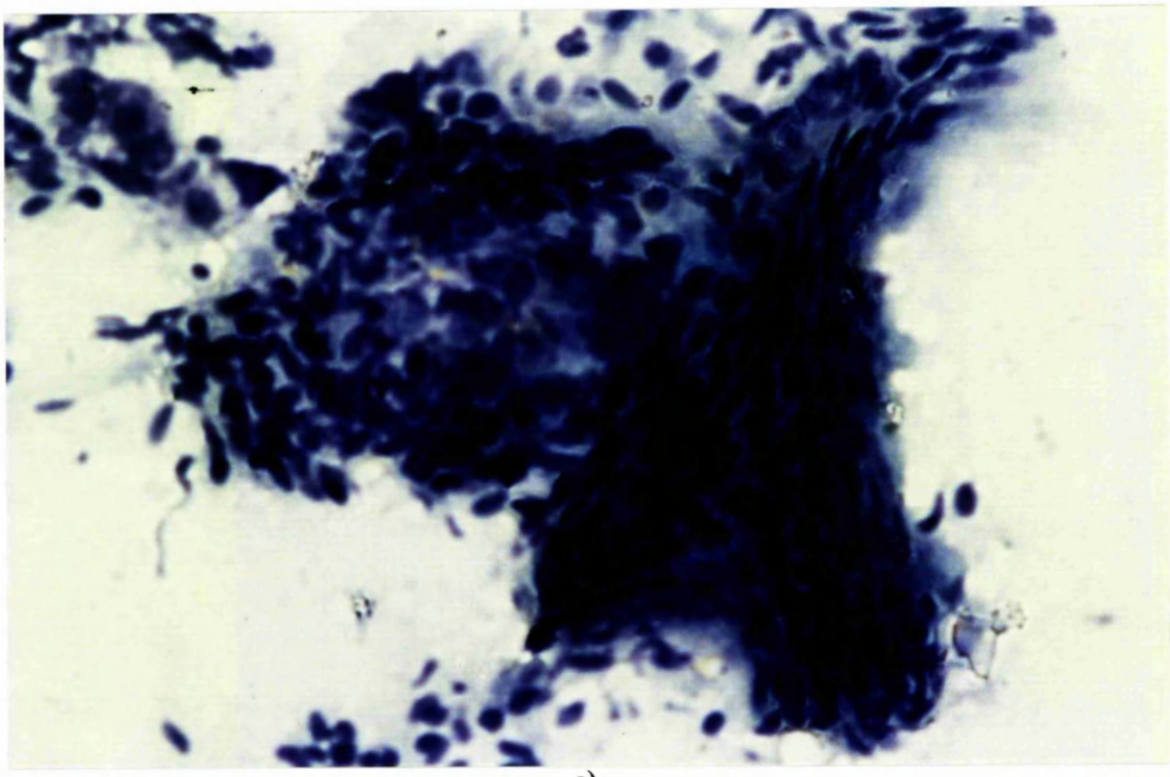


a)

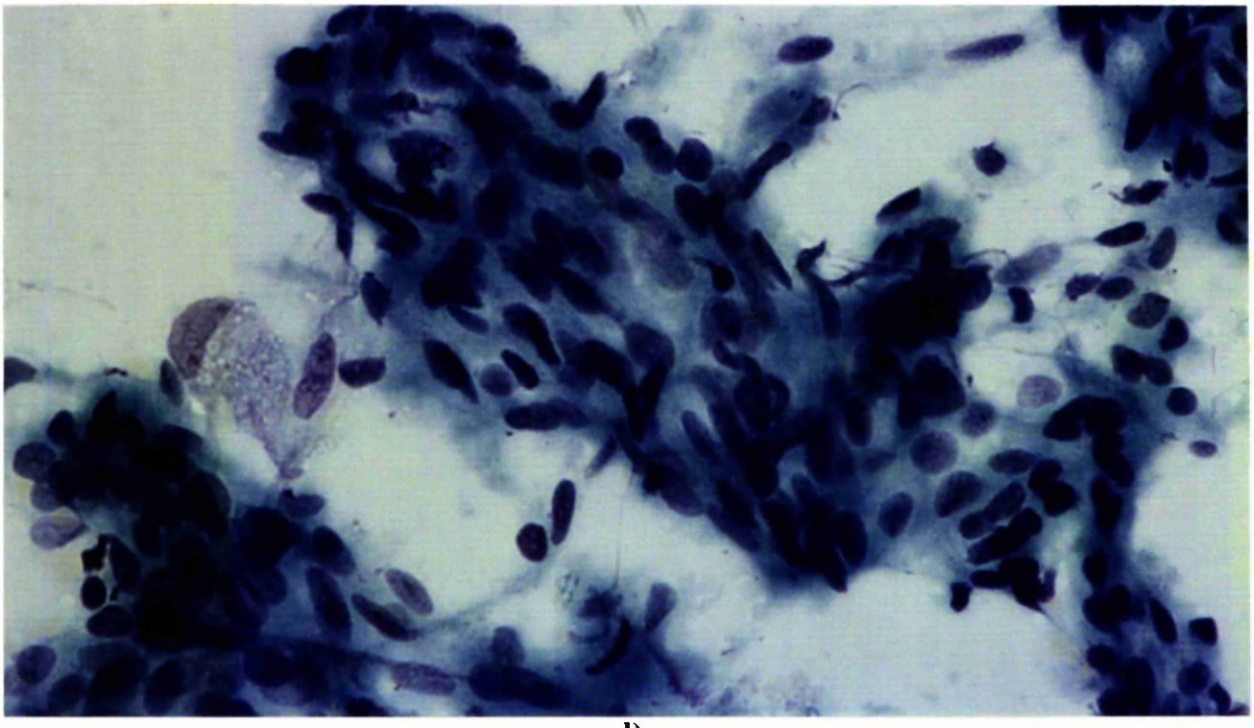


b)

Figure 24: Syncytial meningioma (case 1), smear preparations. At high power both epithelial and fibroblastic components are visible. **a)** Diff-Quick, x100 **b)** May-Grunwald-Giemsa, x50 **c)** toluidine blue, x100 **d)** Zynostain, x100



c)



d)

Figure 24 (continued): Syncytial meningioma (case 1)

CONCLUSION

Conclusion

This study has illustrated the cytological appearance of selected regions of the normal canine brain. Furthermore, this study suggests that cytological techniques may provide a useful diagnostic tool for the intraoperative evaluation of canine and feline intracranial lesions. The 4 stains examined provided similar diagnostic accuracy, with Diff-Quick slightly more accurate than Zynostain, May-Grunwald-Giemsa and toluidine blue stains. Smear preparation was found to be the most useful preparation method of those examined, when diagnostic accuracy and the number of non-diagnostic specimens were taken into consideration.

The greatest difficulty was encountered with diagnosis of inflammatory lesions, due to the potential for confusion with other pathological processes. However, while some tumours (meningioma, lymphoma, metastatic carcinoma) were diagnosed accurately, with correct pathological and specific processes, others (choroid plexus papilloma, meningioma, pituitary macroadenoma) were poorly diagnosed. Misdiagnosis occurred due to a combination of classification and interpretation errors.

The most important factors for diagnosis were the use of either touch preparations or smear preparations and the familiarity of the cytologist with the stain used. While further work needs to be performed to validate the stains and preparation methods that have shown most promising results, it is to be hoped that diagnostic accuracy will improve with increased familiarity with the stains and preparation methods examined.

APPENDIX I

World Health Organisation (1993) Classification of CNS Tumours

1. TUMOURS OF NEUROEPITHELIAL ORIGIN

Astrocytic tumours

- Diffuse astrocytoma
  - Low grade astrocytoma (grade II)
    - Fibrillary
    - Gemistocytic
    - Protoplasmic
  - Anaplastic astrocytoma (grade III)
  - Glioblastoma (grade IV)
    - Variants: giant cell glioblastoma, gliosarcoma
- Pilocytic astrocytoma (grade I)
- Subependymal giant cell astrocytoma (grade I)
- Pleomorphic xanthoastrocytoma (grade II)

Oligodendroglial tumours

- Oligodendroglioma (grade I)
- Anaplastic (malignant) oligodendroglioma (grade IV)

Mixed gliomas

- Oligoastrocytoma (grade II)
- Anaplastic oligoastrocytoma (grade III)
- Ependymoastrocytoma (grade I)
- Oligoependymoma (Grade II)

Ependymal tumours

- Ependymoma (grade II)
  - Cellular
  - Papillary
  - Clear cell
- Anaplastic (malignant) ependymoma (grade III)
- Myxopapillary ependymoma (grade I)
- Subependymoma (grade I)

Choroid plexus tumours

- Choroid plexus papilloma (grade I)
- Choroid plexus carcinoma

Neuroepithelial tumours of uncertain origin

- Astroblastoma
- Polar spongioblastoma
- Gliomatosis cerebri (grade III or IV)

2. NEURONAL TUMOURS

Gangliocytoma
Dysplastic gangliocytoma of the cerebellum
Central neurocytoma
Variant: atypical central neurocytoma

3. **MIXED NEURONAL-GLIAL TUMOURS**
Gangliogliomas
Variant: anaplastic (malignant ganglioglioma)
Ganglioneuromas
Dysembryoplastic neuroepithelial tumours

4. **TUMOURS OF THE PINEAL REGION**
Pineal parenchyma tumours
Pineocytoma (grade II)
Pineoblastoma (grade IV)
Mixed pineocytoma and pineoblastoma
Craniopharyngioma
Germ cell tumours
Glial tumours
Primary and secondary astrocytomas
Mesenchymal tumours
Primary and secondary meningiomas, angiomas
Ependymomas and choroid plexus tumours
Primary (rare) and secondary
Ganglionic tumours
Gangliocytoma, ganglioglioma
Non-neoplastic masses
Pineal cyst

5. **EMBRYONAL TUMOURS (GRADE IV)**
Medulloepitheliomas
Ependymoblastoma
Central neuroblastic tumours
Central neuroblastoma
Olfactory neuroblastoma
Primitive neuroectodermal tumours (PNETs)
Medulloblastoma
Variants: desmoplastic medulloblastoma, melanotic medulloblastoma, lipomatous medulloblastoma, medullomyoblastoma
Extracerebellar primitive neuroectodermal tumours

6. TUMOURS OF THE MENINGES

Tumours of meningothelial cells

- Meningioma (benign) (grade I)
  - Meningothelial
  - Fibrous
  - Transitional
  - Psammomatous
  - Angiomatous
  - Microcystic
  - Secretory
  - Clear cell
  - Chordoid
  - Lymphoplasmacyte-rich
  - Metaplastic
- Atypical meningioma (grade II)
- Papillary meningioma (grade II)
- Anaplastic (malignant) meningioma (grade III)

Mesenchymal non-meningothelial tumours

- Benign non-meningothelial tumours:
  - Chondrosarcoma
  - Osteochondroma
  - Osteoma
  - Lipoma
  - Fibrous histiocytoma
- Malignant non-meningothelial tumours
  - Haemangiopericytoma
  - Fibrosarcoma
  - Chondrosarcoma
  - Rhabdomyosarcoma
  - Meningeal sarcomatosis
  - Malignant fibrous histiocytoma

7. TUMOURS OF UNCERTAIN HISTOGENESIS

Haemangioblastoma

8. GERM CELL TUMOURS

Germinoma
Embryonal carcinoma
Endodermal sinus tumour
Choriocarcinoma
Teratoma

9. LYMPHOMAS

Primary malignant lymphomas
- Primary B cell lymphoma
- Primary T cell lymphoma

Secondary lymphomas
- Lymphomatous leptomeningitis
- Dural involvement
- Intravascular malignant lymphomatosis
- Lymphomatoid granulomatosis

Plasma cell tumours
- Solitary plasmacytoma
- Multiple myelomatosis

10. CHORDOMAS

- Variant: chondroid chordoma

11. CYSTS AND TUMOUR-LIKE CONDITIONS

Epidermoid cysts

Dermoid cysts

Colloid cysts

Enterogenous cysts

Neuroglial cysts
- Glial cyst of the pineal gland
- Cysts of the choroid plexus
- Arachnoidal cysts

Plasma cell granuloma

APPENDIX II

An analysis of 1195 surgically verified tumours examined in the Department of Neuropathology, Institute of Neurological Sciences, Glasgow, in a 5 year period

	Children (up to 15 years)		**Adults (16-64 years)**		**Elderly (65 years and over)**	
Supratentorial	(n=16)	Per cent	(n=532)	Per cent	(n=120)	Per cent
	Anaplastic	31	Anaplastic astrocytoma	52	Anaplastic	57
	astrocytoma	25	Meningioma	20	astrocytoma	17
	Ependymoma	19	Metastatic carcinoma	16	Meningioma	17
	Astrocytoma		Astrocytoma	9	Metastatic	8
	Choroid plexus	12	Oligodendroglioma	1	carcinoma	1
	papilloma	13	Others	1	Astrocytoma	
	Others				Lymphoma	
Intraventricular	(n=4)		(n=21)		(n=1)	
	Astrocytoma	25	Astrocytoma	67	Astrocytoma	100
	Oligodendroglioma	25	Colloid cyst	19		
	Subependymoma	25	Subependymoma	9		
	Tuberous sclerosis	25	Meningioma	5		
Suprasellar	(n=10)		(n=54)		(n=1)	
	Craniopharyngioma	90	Pituitary adenoma	57	Meningioma	100
	Astrocytoma	10	Craniopharyngioma	24		
			Meningioma	7		
			Oligodendroglioma	4		
			Metastatic carcinoma	4		
			Others	4		
Infratentorial	(n=43)		(n=74)		(n=10)	
	Astrocytoma	44	Metastatic carcinoma	39	Metastatic	90
	Medulloblastoma	40	Haemangioblastoma	23	carcinoma	10
	Ependymoma	9	Astrocytoma	16	Meningioma	
	Others	7	Medulloblastoma	8		
			Meningioma	4		
			Dermoid/epidermoid cyst	4		
			Others	5		
Cerebello-pontine angle	(n=0)		(n=50)		(n=11)	
		0	Neurilemmoma	82	Neurilemmoma	82
			Meningioma	12	Meningioma	18
			Others	6		
Intraventricular	(n=1)		(n=2)		(n=0)	
	Dermoid cyst	100	Metastatic carcinoma	50		0
			Subependymoma	50		

Modified from: Adams,J.Hume., Graham, D.I: An Introduction to Neuropathology. Edinburgh, Churchill Livingstone (1988)

APPENDIX III

Brain Cytology Assessment Sheet

SLIDE NO:

QUALITY: (PLACE CROSS ON BAR AT RIGHT)

1 Thickness of Smear: Thin — Thicl

2 Artifactual distortion of cell morphology (ie no. of smashed cells): Few — Many

3 Cytoplasm morphology: Bad — Good

4 Nuclear Morphology: Bad — Good

5 Debris/contamination: Bad — Good

6 Subjective assessment of slide quality: Bad — Good

DIAGNOSIS: (CIRCLE ONE OR MORE)

Normal **Abnormal** **Non Diagnostic**

Neoplasia **Inflammation** **Other (Describe)** **Undetermined**

Type of Cell:

Mesenchymal **Epithelial** **Round Cell** **Undetermined**

Diagnosis:

_____ **Undetermined**

APPENDIX IV

STAINING PROTOCOLS

Diff-Quick

1 Immerse air-dried smears in solution 1 for 5-10 seconds
2 Immerse smears in solution 2 for 5-10 seconds
3 Immerse smears in solution 3 for 5-10 seconds

May-Grünwald-Giemsa

1 Fix air-dried smears in methanol for 5 minutes
2 Immerse smears in May-Grünwald stain for 5 minutes
3 Rinse smears in buffered distilled water rapidly 3 times
4 Immerse smears in Giemsa stain for 5 minutes
5 Repeat step 3
6 Repeat step 4
7 Allow smears to stand in buffered distilled water for 1 minute to allow differentiation
8 Dehydrate, clear and mount with coverslip

Toluidine blue

1 Fix smears in 95% alcohol for 1-2 minutes
2 Rinse in tap water
3 Stain in 1% toluidine blue for 30-60 seconds
4 Dehydrate, clear and mount with coverslip

Zynostain

1 Immerse air-dried smears in Zynostain solution for 30-90 seconds
2 Rinse in tap water
3 Dehydrate, clear and mount with coverslip

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