1 Overview of Grading and Staging

Identification of the process

Tumor is a word of Latin derivation meaning a swelling or protruberance—a "mass." In its broadest sense it includes masses formed by cellular inflammatory infiltrates, controlled proliferations of hyperplastic cells, and uncontrolled proliferations of neoplastic cells (cancer). Controlled proliferations of cells have a recognizable structure, may perform their usual physiologic function, do not invade local tissues, and suffer senescence and programmed cell death (apoptosis). Uncontrolled proliferations may contain cells of variable structure, may be functional or nonfunctional, may invade local tissues or cause local tissue necrosis by their increasing bulk, replicate in a disorderly manner, and do not suffer programmed cell death.

The path to successful treatment of a tumor begins with recognition of the lesion on a gross level, usually by the caretaker of a dog or cat, sometimes by a groomer, or often during a physical exam by a veterinarian. The next step is assignment of the pathological process into a category of inflammation, hyperplasia, or neoplasia, or some combination of these categories. This can be accomplished at the point of care by aspirating the lesion with a needle and examining the individual cells. In the following chapters this will be called fine needle aspiration (FNA). With some tumors, especially papillomas, impression or scraping of the lesion can yield diagnostic cells, but generally this is not the ideal method of collection, as surface contamination can make evaluation difficult. All cytologic samples are stained with Wright-Giemsa (W-G) stain unless otherwise indicated.

Figure 1.1 shows an apocrine gland adenoma FNA. This cluster of small epithelial cells is suggestive of a proliferation of basaloid epithelial cells or the ductular epithelium of an apocrine gland. The cell nuclei are small and regular, and there is scant inflammation, as shown by the neutrophil in this field.

When FNA of a mass reveals a population of proliferating cells, indicating the lesion is not merely an influx of inflammatory cells that can be relieved by medical means, biopsy allows histopathological evaluation of the affected tissue, showing the architectural arrangement of the cells and allowing for a more definitive diagnosis. This is the point where a hyperplastic growth is distinguished from a neoplastic growth based on how the cells are structurally arranged. FNA cannot evaluate architectural arrangement accurately, because the cells are usually stripped of their association by the process of aspiration. The decision to take an incisional biopsy that removes a portion of the mass, or an excisional biopsy that removes all of the mass, should be based on factors such as the tumor type suggested by the FNA, the size of the lesion, the location of the lesion, the stage of the disease, and other parameters such as the overall health of the patient and wishes of the owner. Ultimately the decision rests on the clinical judgment of the surgeon. All biopsies shown in the following pages are stained with hematoxalin and eosin (H&E), unless otherwise indicated.

Figure 1.2 is a biopsy showing the architecture of the gland aspirated in Figure 1.1. There are double rows of small epithelial cells proliferating in a manner that does not invade into the adjacent stroma, indicating that this is a benign apocrine gland tumor referred to as an apocrine ductular adenoma.

FNA can sometimes identify cells that are so clearly abnormal, either by morphology or cell density, that neoplasia can be diagnosed on a presumptive basis.

Figure 1.3 shows a transitional cell carcinoma FNA. An adult female mixed breed dog was presented for hematuria and dysuria. A tentative diagnosis of cystitis was made based on clinical signs, and cystocentesis was performed to collect urine for routine urinalysis and sedimentation. Cytologic exam revealed many clusters of large epithelioid cells with

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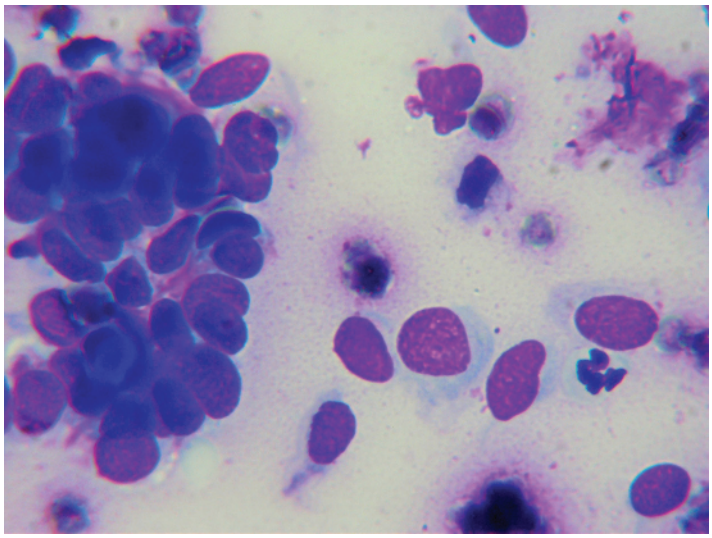

Figure 1.1 Apocrine gland adenoma FNA. 50x.

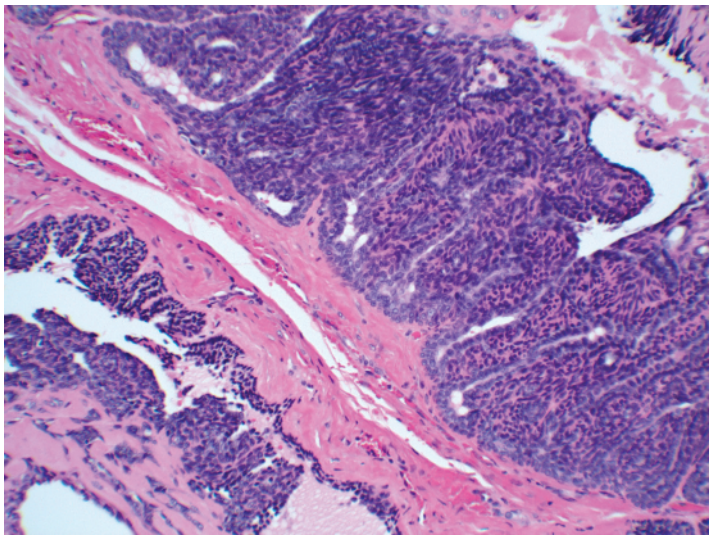

Figure 1.2 Apocrine gland ductular adenoma biopsy. 40x.

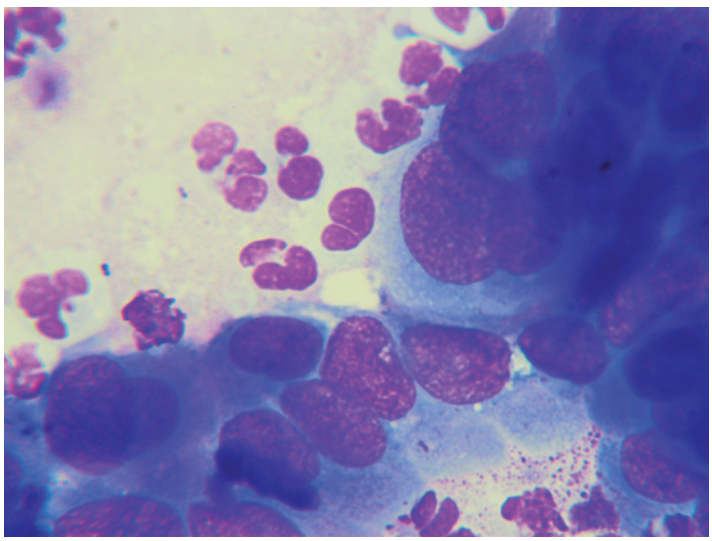

Figure 1.3 Transitional cell carcinoma FNA. 50x.

marked anisokaryosis (variation in nuclear size) and basophilic cytoplasm. There were scattered neutrophils, erythrocytes, and cellular debris. No infectious agents were seen. A preliminary diagnosis of neoplasia, probable transitional cell carcinoma, was made. Treatment for infectious cystitis, based just on clinical signs, would prove useless and would delay the true diagnosis. If neoplasia is suspected on presentation, catheterization would be the preferable method of collection.

If biopsy identifies the process as hyperplastic, and the margins are free of abnormal cells, it can be assumed that the lesion is cured. This does not preclude additional lesions arising adjacent to the removed mass.

If biopsy identifies the process as neoplastic, the tumor can be categorized into type and grade based on published guidelines derived from scores of biopsies and the statistical analysis of their behavior. The purpose of this atlas is to enable visual recognition of lesions and thus the reader will be spared a detailed description of the original research that forms the basis for statistical analysis. Inquiring minds, however, are encouraged to review the documents in the additional journals and books listed in the reference section.

Identification of tumor types

The broadest categories of tumor types are derived from tissue of origin. Epithelial origin tumors are designated as epithelioma or adenoma (benign) and carcinoma (malignant). Mesenchymal origin tumors are typically designated as an –oma prefixed by the tissue of origin (benign) or -sarcoma prefixed by the tissue of origin (malignant). Discrete cells lacking cell to cell adhesion and originating from the specialized tissue and circulating cells of the immune system, such as lymphocytes, plasma cells, histiocytes, mast cells, and a transplantable chymeric neoplasm called transmissible venereal tumor, are designated as round cell tumors, often –omas, prefixed by the tissue of origin (and indicated as benign or malignant). There is a separate category for melanoma, which can have epithelioid, spindloid, and round cell characteristics within the same tumor (both benign and malignant).

Grading

Grading is performed by the pathologist using parameters that can only be assessed by biopsy, including mitotic activity per high power (40x) field, the presence of a recognizable pattern of growth, and invasion into adjacent normal tissue. Mitotic activity is an important part of most grading systems. Mitotic rate or mitotic count is the number of mitotic figures per high-power field (mitotic figures/HPF).[1] Mitotic index (MI) is generally accepted as the number of mitotic figures in 10 fields (mitotic figures/10 HPF), but if a different number of fields have been used, it must be stated in the numerical figure.[2] Both mitotic rate and mitotic index can vary widely depending on which areas of the tumor are examined. The presence of necrosis and dense inflammatory infiltrates can make identification of mitotic figures difficult, and small biopsies less than 10 fields in size can make enumeration of the mitotic index impossible. Thus, grade is not based just on mitotic activity but also on other aspects of the proliferating population such as the amount of necrosis (also subjective and based on the section examined) and pattern of growth in the tissue. This heterogeneity introduces some variation into the assessment of tumor grade and has contributed to the proliferation of several grading systems for some tumors as pathologists attempt to find the best system (Table 1.1).

Grading systems can use a quantifiable descriptive term such as low, medium, and high grade or can assign a numerical label (Table 1.2), and grading systems can use an equation to score several critical features that add up to a sum assigned to a grade (Table 1.3). All of the systems used are designed to succinctly convey the probability that a tumor will be aggressive and likely to invade local or distant tissues. Grading also allows a pathologist to give an oncologist a specified set of details designed to help choose and monitor appropriate therapy. The general practitioner and the oncologist or internist may have different preferences for grading protocols or treatment plans, and may desire

Table 1.1 Multiple grading systems for lymphoma.

Tumor	System	Features
Lymphoma	NCI WF	pattern, biology, survival
	Kiel	pattern, morphology, immunophenotype
	WHO	pattern, morphology, immunophenotype

Table 1.2 Multiple grading systems for mast cell tumor.

Tumor	System, reference	Grade; features
MCT	Patnaik, 1.27	1; confined to dermis, 0 mitoses/HPF, uniform nuclei 2; invades subcutis, 0–1 mitoses/hpf, rare binucleate cells 3; invades deep tissues, >3 mitoses in some fields, pleomorphic nuclei
	MSU, 1.28	low; rare mitoses, confined to dermis, uniform nuclei high; invasive, frequent mitoses, pleomorphic nuclei

Table 1.3 Grading systems based on points for sarcoma in canines and mammary gland tumor in canines.

Tumor	Reference	Features
Sarcoma	1.20	Differentiation 1; regular appearance 2; poorly differentiated 3; pleomorphic Mitoses 1;0–9 2; 10–19 2; 10–19 Tumor necrosis 1; no necrosis 2; <50% necrosis 3; >50% necrosis Score 1; 3 or less 2; 4–5 3; 6 or more
Mammary tumor	1.14	Tubule formation: 1; >75% 2; 10–75% 3; <10% Nuclear form: 1; uniform 2; variation 3; pleomorphic Mitoses/10 HPS 1; 0–9/10 HPF 2; 10–19/10 HPF 3; 20+/10 HPF Score: 1; low, 3–5 2; moderate, 6–7 3; high, 8–9

different sets of information, resulting in a report listing several grading protocols applicable to the tumor described. These compilations of data can be useful even in the face of periodic modification as the database grows and our diagnostic tools become more refined to include molecular diagnostic parameters such as tumor growth fraction, genetic analysis for c-KIT gene mutation which activates the KIT tyrosine kinase receptor, and polymerase chain reaction (PCR) of antigen receptor site rearrangements. Open communication between clinicians and specialists will be necessary to keep apprised of new developments.

Figure 1.4 shows a squamous cell carcinoma (SCC) biopsy with two mitotic figures. The cells in this biopsy are fairly well differentiated and recognizable as squamous epithelial cells and the mitotic figures (arrows) are clear.

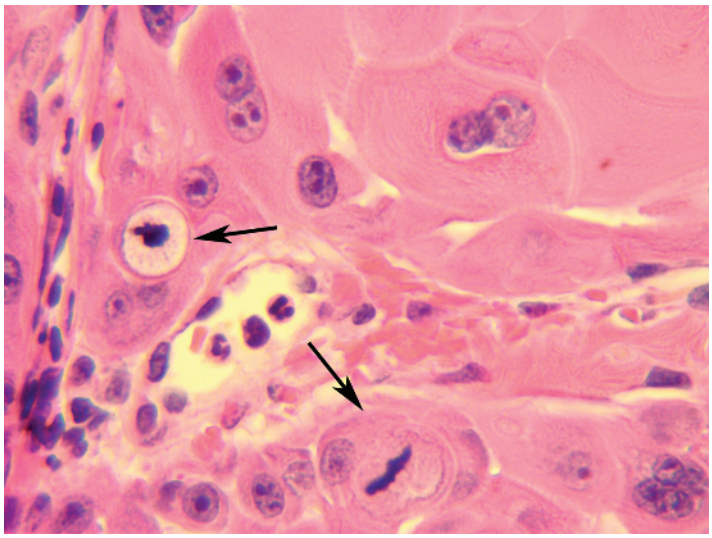

Figure 1.4 Squamous cell carcinoma biopsy. 50x.

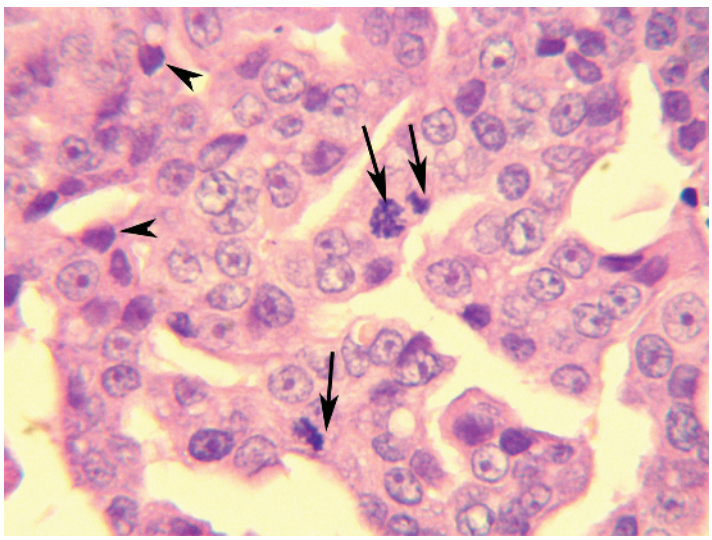

Figure 1.5 Apocrine adenocarcinoma biopsy. 50x.

Evaluation of at least 10 high power (40x) fields is recommended for assigning a grade. Fragmented or crushed tissue and biopsies smaller than 1.0 centimeter (cm) may have insufficient fields for proper evaluation of mitotic index.

Figure 1.5 shows an apocrine adenocarcinoma biopsy with three definitive (arrows) and two questionable (arrowheads) mitotic figures. Cells with vague and pyknotic nuclei can be difficult to assess for mitotic activity, a situation that introduces a source of discrepancy in the grading of some tumors. Necrosis and inflammation compound the problem by increasing the number of active and dying cells that are not necessarily tumor cells. Evaluation of 10 fields can be impossible if only tiny fragments are submitted for biopsy.

Staging

Staging is a clinical assessment and quantifies information such as the location of the tumor in the body, the size of the tumor, and whether there is local lymphatic or distant metastasis. The clinician must perform staging, but the pathologist can be of assistance if adjacent stroma or lymphatic tissue is submitted for analysis and lymphatic or vascular invasion can be documented on biopsy tissues.

Figure 1.6 shows lymphatic metastasis of mammary carcinoma. This biopsy of a mammary carcinoma revealed dilated lymphatics containing invasive carcinoma cells. This will only be seen if adjacent normal tissue containing lymphatics is included in the biopsy sample. Often the best area to look for compromised lymphatics is the subepidermis, therefore, it is helpful to submit the skin over the mass and at lateral margins, as well as deeper tissue.

Figure 1.7 shows lymph node metastasis of mammary carcinoma. This biopsy of a mammary tumor included a local lymph node that was found to have invasive carcinoma. This is where the pathologist can be helpful in the staging process.

There are some factors that can affect the use of grade and stage. Grade and stage are dynamic, not static, and each can progress independently to a different level. Grade and stage have elements of subjectivity; grade is based on a thin section of only a portion of the affected tissue so sampling error is not impossible, while stage can be affected by the quality and type of diagnostic procedures used (such as radiography versus magnetic resonance imaging). If grade and stage are used to predict future behavior, the classification systems must have a sound basis and be applicable to the species and disease process to which they applied. Histiocytic proliferations are infamous for their ability to regress or progress, spread to multiple sites, or completely disappear in dogs, yet they are, as currently recognized, always progressively more aggressive in cats.[3] It is important to recognize species and maybe even breed variability in behavior.

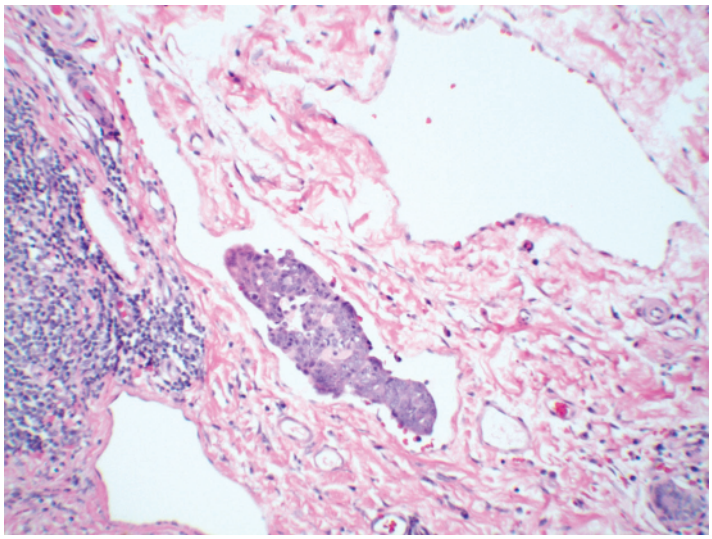

Figure 1.6 Mammary carcinoma lymphatic metastasis biopsy. 20x.

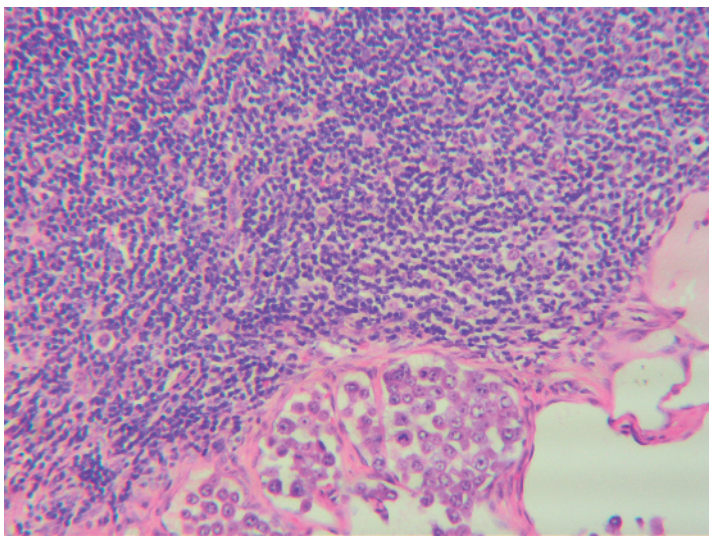

Figure 1.7 Mammary carcinoma lymph node metastasis biopsy. 10x.

Mast cell tumors in Siamese cats will sometimes spontaneously regress, but this would not be expected in Domestic Shorthair breeds (nor in dogs of any breed). Some tumors vary in behavior by site of origin and may have differing staging systems depending on location. Additionally, in some studies of melanocytic and mammary tumors the presence of neoplastic cells in local lymphatics may not be correlated with survival time.[4, 5] The database is constantly expanding, and consultation with a specialist can provide the most current recommendations.

Staging versus clinical behavior

A patient that presents with multiple masses can have a wide range of outcomes dependent on the type of process causing the masses. A multifocal infectious or inflammatory disease might be expected to have a better prognosis than a widespread neoplastic disease. Aspiration of multiple masses is recommended as a first step because this will help determine if the masses are due to one process or multiple etiologies and can indicate infectious versus neoplastic etiology. This approach can yield the most favorable clinical outcome by treating the correct disease without delay. Staging will only be applicable after the disease process has been definitively diagnosed, regardless of how widespread the disease appears clinically.

Figure 1.8 shows a fungal granuloma. An adult male neutered cat presented with multiple skin masses, and aspiration of several masses revealed scattered histiocytes, small lymphocytes, macrophages, and *Cryptococcus neoformans*. The presence of a fungal agent indicates antifungal medical therapy would be appropriate, but immunosuppressive drugs or chemotherapy agents would be contraindicated and antibacterial therapy would be ineffective. The diagnosis made by FNA allows for timely therapy prior to biopsy in this case.

Figure 1.9 shows a *Cryptococcus neoformans* biopsy. This adult male neutered cat had multiple skin masses about the head and in the submandibular area, aspirates of which are illustrated in Figure 1.8. The nodules persisted despite therapy, and biopsy revealed *Cryptococcus* organisms both in the skin nodules and lymph nodes. This disease may progress even with appropriate medical therapy, and this neurotropic organism can infiltrate the tissues of the nasal cavity, spreading to local lymph nodes and eventually invading the brain. Testing for immunosuppressive virus infection would be prudent.

Figure 1.10 shows a canine histiocytoma. Aspirate of several cutaneous masses in this 2-year-old dog revealed many round cells with fine chromatin and pale cytoplasm as well as scattered small lymphocytes and neutrophils. The preliminary diagnosis was multifocal histiocytoma, and biopsy was recommended for definitive diagnosis because the lesions were multiple and persistent. Biopsy is not usually indicated at first presentation of this tumor type if there is only one mass and it regresses in a timely manner.

Figure 1.11 shows a canine cutaneous histiocytoma. This biopsy revealed that recurrent skin masses in a young dog were composed of sheets of round cells with moderate pale cytoplasm, occasional mitotic figures, and rare invasion into the epithelial layer by solitary cells. This lesion is expected to regress with time. If this mass fails to regress,

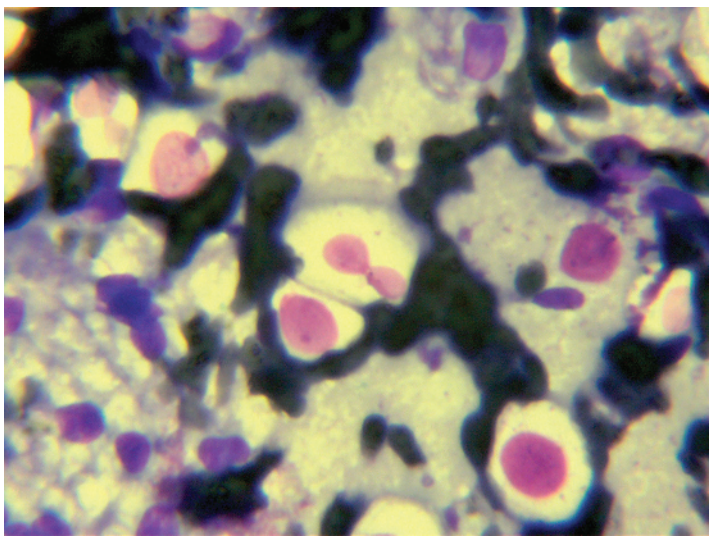

Figure 1.8 Fungal granuloma FNA. 50x.

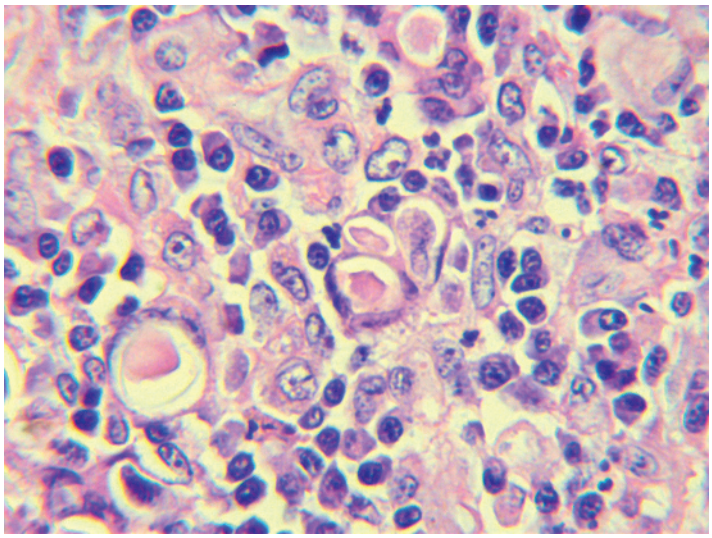

Figure 1.9 *Cryptococcus neoformans* biopsy. 40x.

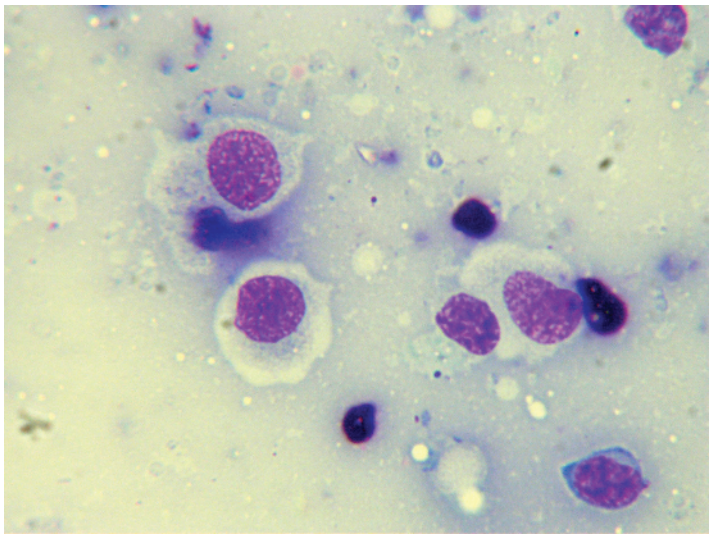

Figure 1.10 Canine histiocytoma FNA. 50x.

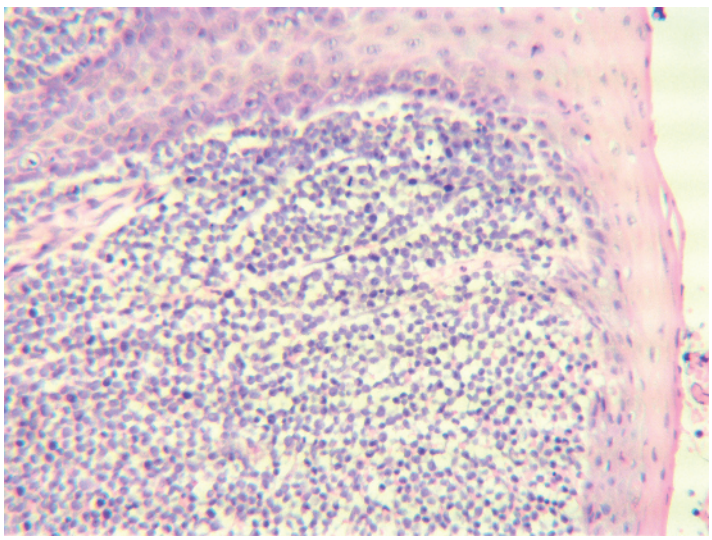

Figure 1.11 Canine cutaneous histiocytoma biopsy. 10x.

additional slides could be cut from the same block of fixed tissue used for the H&E slide and evaluated with Giemsa stain to exclude poorly granular mast cell tumor. Immunohistochemical markers for T and B lymphocytes could also be applied to additional freshly cut slides to exclude cutaneous lymphoma. If there are concomitant genital masses, transmissible venereal tumor should be considered. Additional immunohistochemical markers for histiocytes and other cell types are also available and are usually performed at research centers for best results. Consultation with laboratory personnel, the diagnostic pathologist that will be reading the tissue, and referral specialists such as oncologists or internists who may be working on the case, will often yield the best plan for additional special stains in cases of persistent or progressive histiocytic tumors.

Figure 1.12 shows a cutaneous lymphoma via FNA. Aspirate of multiple skin masses on an adult, spayed female mixed breed dog yielded a dense population of monomorphic round cells, about the size of the accompanying neutrophil, with round to occasionally cleaved nuclei, slightly clumped chromatin, and scant lightly basophilic cytoplasm. The preliminary diagnosis is cutaneous lymphoma.

Figure 1.13 shows a cutaneous lymphoma biopsy. Biopsy of several skin masses on the trunk of the adult dog in Figure 1.12 revealed variably dense infiltrates of fairly monomorphic medium-sized round cells that are invading into the epidermis and forming microabscesses. This epithelial invasion is a hallmark of epitheliotrophic cutaneous T-cell lymphoma. Unlike the histiocytoma in the previous figure, this disease will be progressive, and early therapy is usually indicated. If this is an indolent T-cell lymphoma, the rate of progression may be slow in the early stages.[6]

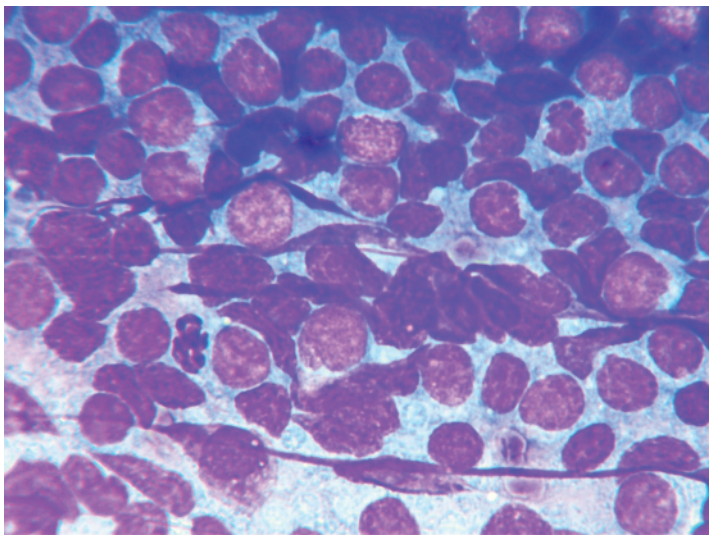

Figure 1.12 Cutaneous lymphoma FNA. 50x.

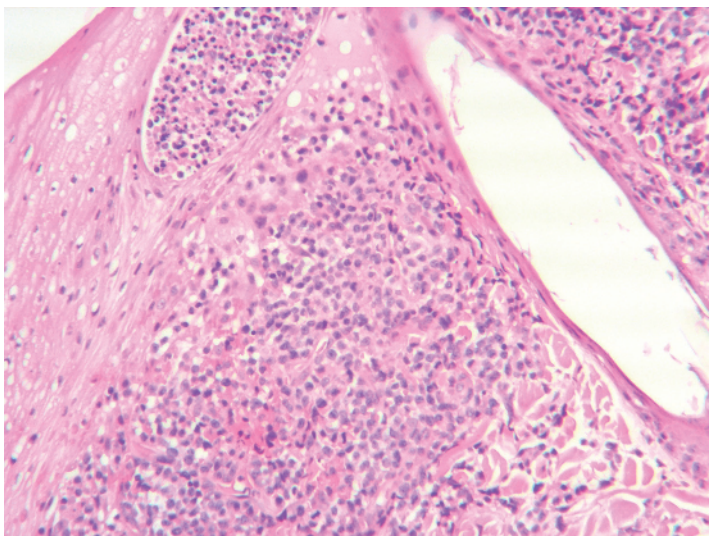

Figure 1.13 Cutaneous lymphoma biopsy. 20x.

These three disease processes can have a somewhat similar clinical appearance but have distinguishing cytologic and histologic features that allow accurate diagnosis and therefore a more applicable prognosis and therapeutic regimen. Multiple skin masses and lymphadenopathy have different significance in these three diseases and therefore any staging or prognostic categorization must be done with a definitive diagnosis in hand.

Epithelial tumors

Epithelial neoplasms can arise as benign (epithelioma, adenoma) or malignant (carcinoma) tumors. Benign tumors may undergo transformation to malignant tumors.

Figure 1.14 shows an apocrine gland adenoma biopsy. This skin mass is composed of cystic spaces lined by single to double rows of well-differentiated apocrine epithelium, consisting of cells with small nuclei and scant cytoplasm that do not invade into underlying stroma. This tumor is benign, and complete excision with conservative margins of 0.2 cm should be curative.

Figure 1.15 shows an apocrine gland carcinoma biopsy. This invasive tumor can arise from an apocrine gland in the skin and rapidly invade adjacent stroma and lymphatic structures. There may be remnants of glands filled with

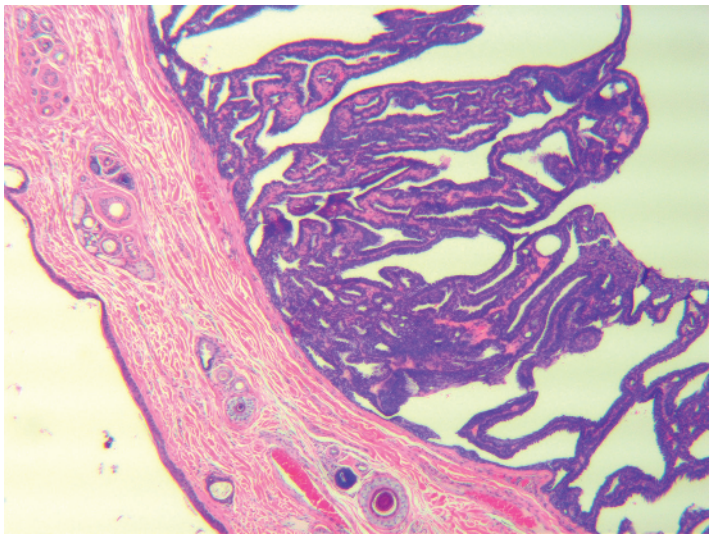

Figure 1.14 Apocrine gland adenoma biopsy. 10x.

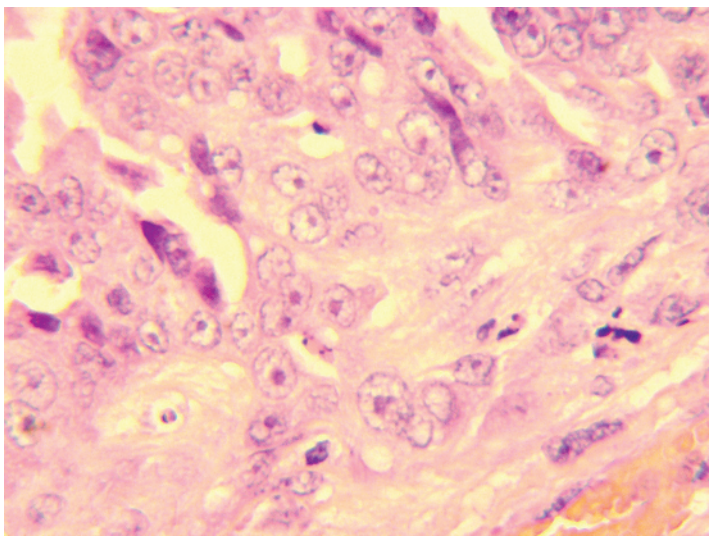

Figure 1.15 Apocrine gland carcinoma biopsy. 40x.

neoplastic cells, or the cells may form sheets in the stroma with only a suggestion of the former glandular architecture. The nuclei are large with large nucleoli and vesiculated chromatin. There is cellular disorganization even in areas of retained glandular structure. Local lymph nodes and thoracic radiographs should be evaluated for evidence of metastasis. Complete, wide excision is the minimal approach, and continued monitoring for regrowth or evidence of metastasis is advisable. Consultation with an oncologist would be helpful to determine the current most efficacious approach for medical therapy.

Mammary chains are exposed to systemic factors known to promote mammary neoplasia, and therefore, it is not unusual for dogs and cats to experience tumors in multiple glands, and multiple tumors of the same or different type in the same gland. It is well established that in the mammary gland hyperplastic lesions and benign tumors can develop into malignant tumors if not removed early or if incompletely removed.[7]

Figure 1.16 shows a complex mammary adenoma biopsy with focal transformation to ductular carcinoma in an adjacent lobule. The larger mass in the lower right quadrant (arrow) is a complex, low-grade mammary tumor, which is not invading into surrounding stroma. In the upper center of the slide there is a proliferation of ducts/alveoli (arrowhead) exhibiting focal atypia characterized by filling of the ducts with moderately pleomorphic epithelial cells that appear to have lost the normal arrangement of basally located nuclei that defines the normal duct. This demonstrates, at light microscope level, the concept of how plump, presumably hyperplastic cells might undergo loss of normal regulatory control, filling the ducts with disorganized epithelial cells and eventually escaping into the surrounding stroma.

Figure 1.17 shows a closer view of the developing carcinoma in Figure 1.16. There is an irregular proliferation of cells filling rather than lining the tubules and exhibiting larger than normal nuclei with open chromatin, a probable mitotic

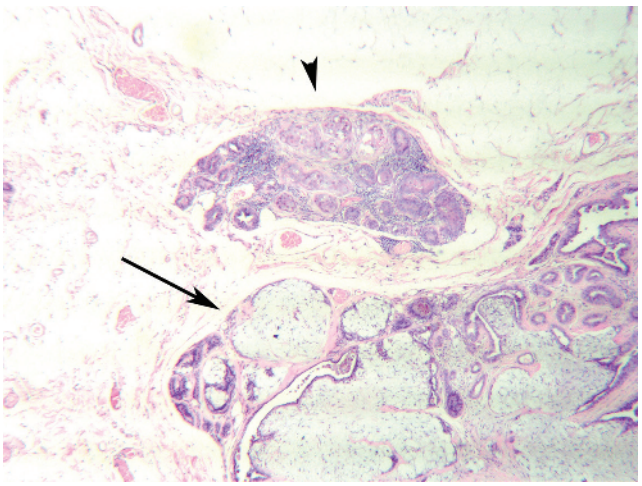

Figure 1.16 Complex mammary adenoma with focal intraductal carcinoma biopsy. 10x.

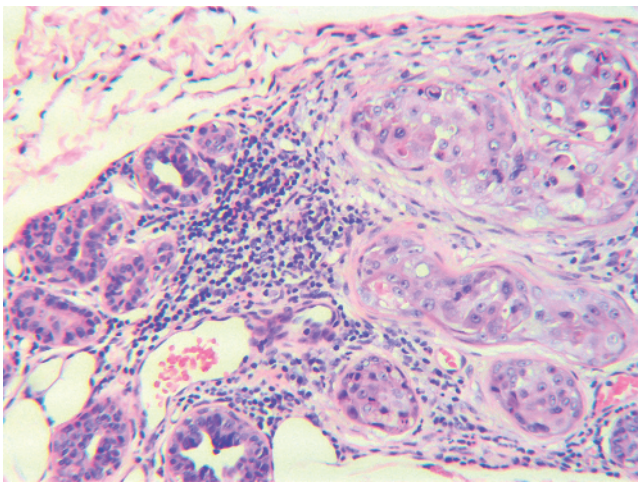

Figure 1.17 Mammary intraductular carcinoma. 40x.

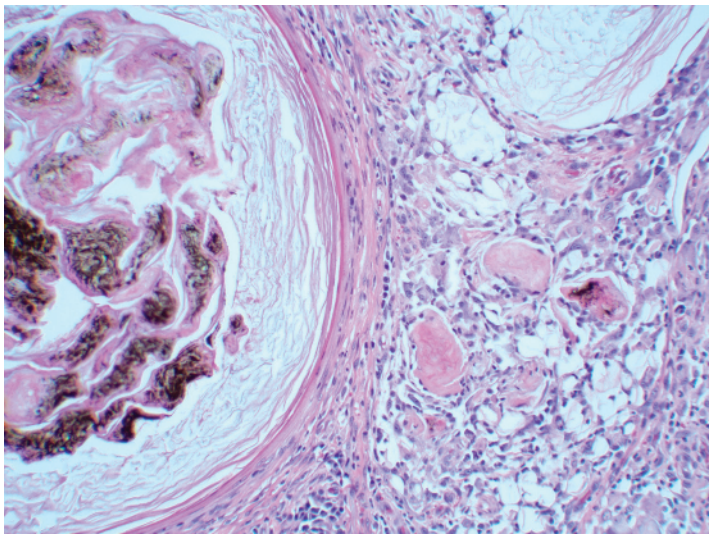

Figure 1.18 Trichoepithelioma biopsy. 20x.

figure, and abundant more deeply basophilic cytoplasm than the cells in the adjacent tubules. There appears to be early peritubular fibrosis and a peritubular inflammatory infiltrate of small lymphocytes and plasma cells. A more aggressive mammary tumor appears to be arising within this lobule. There is a recognizable inflammatory infiltrate of small lymphocytes and plasma cells associated with this lesion.

Epithelial tumors can arise from the many structures of the haired skin, follicles, and associated glands. They are often benign or low grade but can undergo malignant transformation with time.

Figure 1.18 shows a trichoepithelioma biopsy. Adnexal tumors, especially cystic adnexal tumors, are common, and are usually benign. They often grow around dilated follicles that fill with keratin. Evaluation of the epithelium lining the cyst wall is necessary for diagnosis of the tumor type and is critical for accurate prognosis. Manual extrusion of the cyst contents with biopsy of the keratin mass is unrewarding, as there will be no cyst wall present in the sample. Rupture of the cyst prior to removal can result in severe cellulitis with an influx of pleomorphic phagocytic cells (macrophages and multinucleate giant cells) that can almost appear to be a neoplastic population. FNA is appropriate prior to removal to rule out other more aggressive tumor types such as mast cell tumor. Removal of this tumor with at least 0.2 cm normal tissue at the margins is acceptable, and regrowth is not expected. Marginal excision ("shelling out") of the mass is not advisable because it may leave small amounts of tumor in the surgical bed to regrow or transform to a more aggressive form of this tumor.

Figure 1.19 shows a sebaceous adenoma biopsy. Proliferations of sebaceous glands are the fifth most common skin tumor of dogs and the eighth most common in cats.[8] They are characterized by proliferations of well-differentiated variably vacuolated sebaceous epithelial cells confined by a reserve cell lining of smaller basaloid epithelium, often around cystic and debris-laden ducts and follicles. Nomenclature based on the architectural arrangement of the cells includes sebaceous gland hyperplasia consisting of enlarging sebaceous gland epithelium rimmed by a single layer of basaloid epithelium extending from the follicle to ducts to the lobule, sebaceous adenoma consisting of proliferating sebaceous gland epithelium rimmed by a single layer of basaloid epithelium extending in a disorderly pattern from the duct and sebaceous epithelioma consisting of proliferating basaloid reserve cells around occasional foci of sebaceous gland epithelium that do not always clearly extend from a duct. These are listed from least aggressive to most aggressive in Table 1.4. Complete excision with conservative margins of at least 0.2 cm normal tissue is usually curative.

Figure 1.20 shows a sebaceous carcinoma biopsy. Sebaceous carcinoma is the malignant counterpart of benign sebaceous gland tumor and consists of poorly circumscribed proliferations of sebaceous gland and reserve basaloid cells that are not associated with ducts and invade into normal stroma. There is nuclear atypia with occasionally vesiculated chromatin, prominent nucleoli, and variably dense to vacuolated cytoplasm. A pre-surgical FNA finding of pleomorphic nuclei can be the hint that wide margins are necessary to achieve complete excision and allow enough marginal normal tissue to evaluate the adjacent lymphatics for invasion.

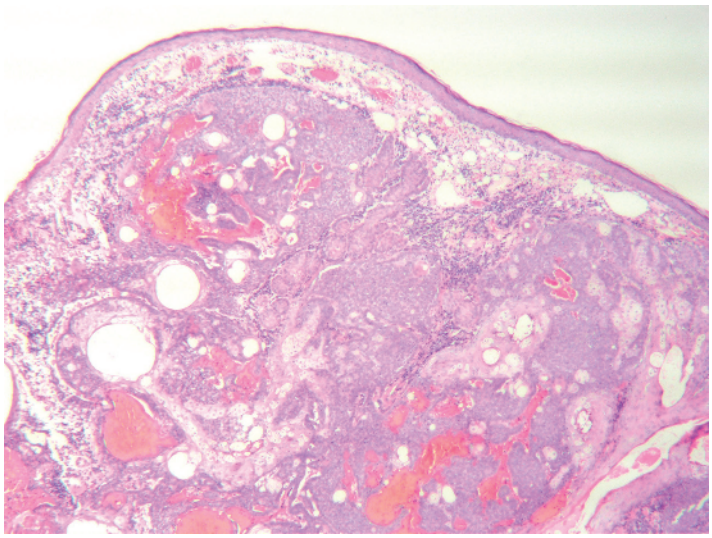

Figure 1.19 Sebaceous adenoma biopsy. 2.5x.

Table 1.4 Types of proliferations of sebaceous glands and the prognosis for each.

Category	Prognosis
Sebaceous hyperplasia	Benign
Sebaceous adenoma	Low grade
Sebaceous epithelioma	Low to mid grade
Sebaceous carcinoma	High grade, invasive

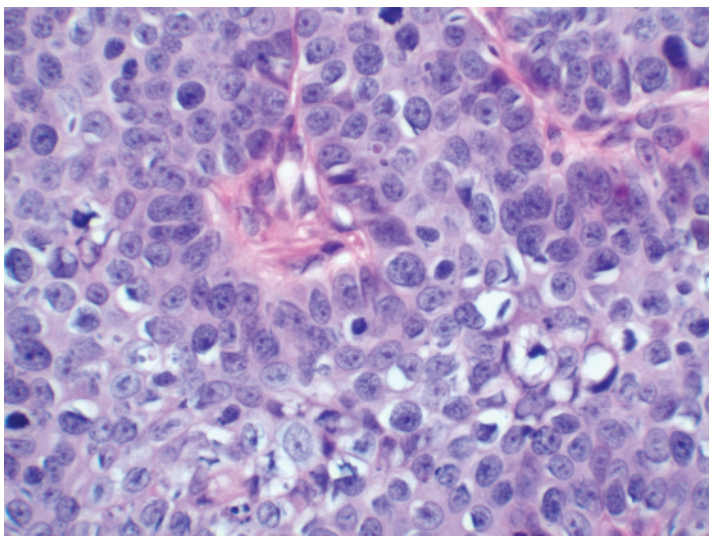

Figure 1.20 Sebaceous carcinoma biopsy. 40x.

Figure 1.21 shows a squamous cell carcinoma in situ biopsy. Carcinoma in situ (Bowen's disease) is seen mostly in cats and is a complex progression of pre-neoplastic to early neoplastic proliferations of squamous epithelial cells that are confined to the epidermis and do not cross the basement membrane into subepidermis. The epithelial cells can be pigmented with migration of melanophages and clumps of pigment into the underlying subepidermis. This lesion can be promoted by solar exposure (actinic keratosis) or papillomavirus infection, and can be multiple. If not completely excised it can progress to invasive carcinoma.

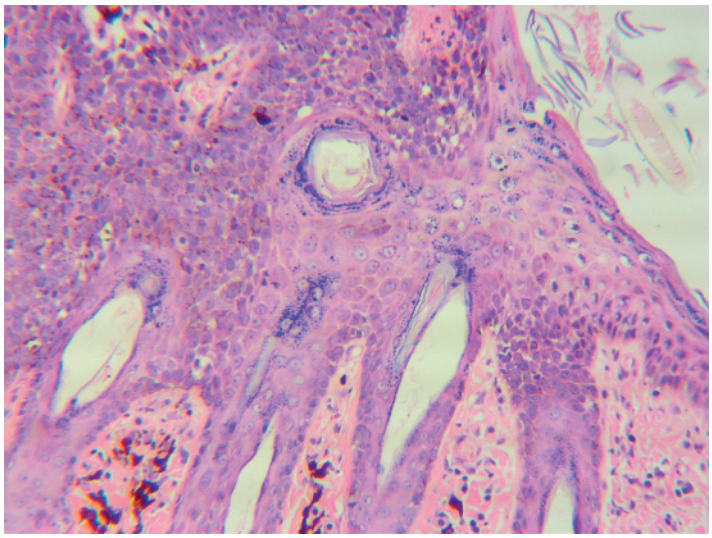

Figure 1.21 Carcinoma in situ biopsy. 10x.

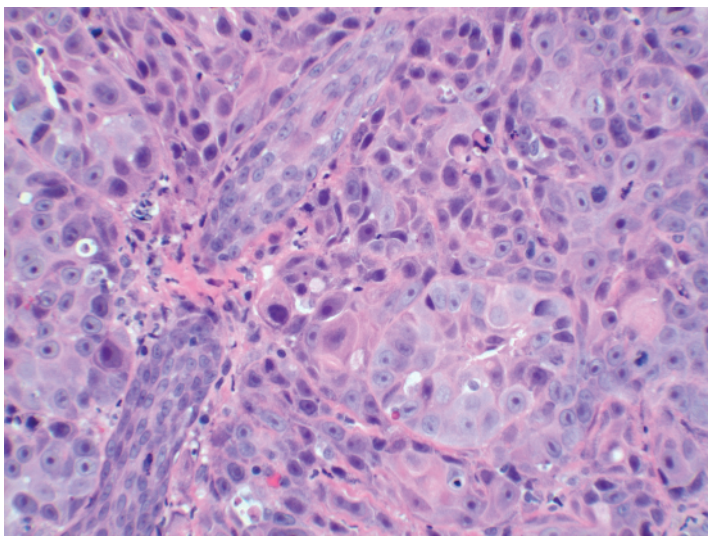

Figure 1.22 Squamous cell carcinoma biopsy. 20x.

Figure 1.22 shows a squamous cell carcinoma biopsy. Squamous cell carcinoma is a disorganized proliferation of variably keratinizing squamous epithelial cells that cross the basement membrane and invade into underlying stroma. They can be well differentiated, forming nodules of keratinizing cells (keratin pearls), or they can be anaplastic and form sheets of pleomorphic epithelioid cells. There is usually nuclear atypia with large nuclei, vesiculated chromatin, prominent, usually single, nucleoli and variable cytoplasmic keratinization, which is often asynchronous to nuclear maturation (dyskeratosis). This tumor type will readily invade stroma and local lymphatics, metastasizing to local lymph nodes and further. FNA can reveal these atypical morphologic features leading to a preliminary diagnosis of carcinoma. This can allow for referral to a specialist, or if referral is declined, can indicate a need for evaluation of local lymph nodes and thoracic radiographs, as well as wide surgical excision.

Epithelial tumors of the mucous membranes (mouth, conjunctiva, vagina, urinary bladder, intestine) can exhibit a rapid growth rate with early stromal invasion and may metastasize to lymph nodes readily. Notable exceptions are epulis and papilloma, which are usually benign, but can become locally invasive with time.

Figure 1.23 shows a canine epulis biopsy. This pink, firm gingival mass from a dog reveals anastomosing chains of epithelial cells extending from the epithelium into a proliferative fibrous stroma without visible breach of the interface

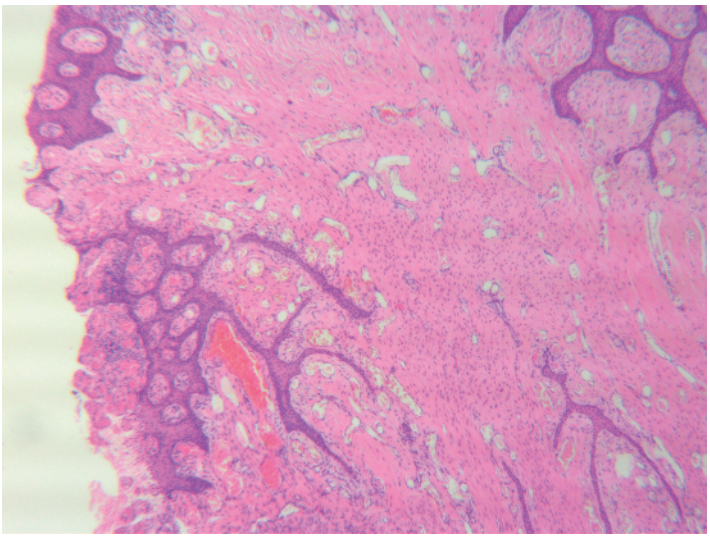

Figure 1.23 Canine epulis (peripheral odontogenic fibroma) biopsy. 2.5x.

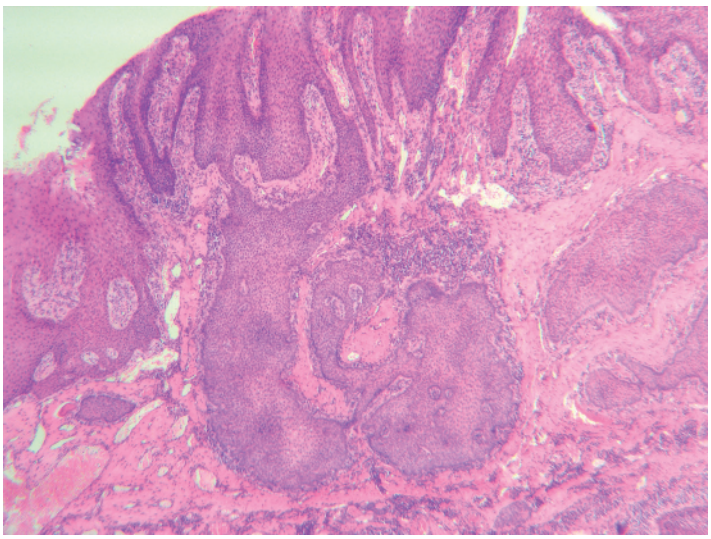

Figure 1.24 Canine ossifying acanthomatous epulis (ossifying acanthomatous ameloblastoma) biopsy. 2.5x.

basement membrane that would indicate stromal invasion. Complete excision of this low-grade biphasic lesion is often curative, but multiple foci may be present and additional masses may arise.

Figure 1.24 shows a canine ossifying acanthomatous epulis biopsy. This pink, firm gingival mass from a dog reveals a more proliferative epithelial component with occasional production of bone. It is a low-grade tumor and, although complete early excision may be curative, if not excised there is potential for invasion into and destruction of underlying bone. Radiographs prior to surgery would be helpful. Metastasis is not expected.

Figure 1.25 shows a canine oral papilloma biopsy. This oral mass was one of many excised from the mouth of a dog. The thickened, hyperkeratotic epithelium is thrown into folds over a fibrous stroma, and there is not stromal invasion. There may be viral inclusions and cytopathic changes in the epithelial cells. This lesion is often the result of papillomavirus infection, it may be multiple, and additional lesions may arise. In an older dog with recurrent tumors, check for immunosuppression.

Figure 1.26 shows a canine conjunctival fibropapilloma biopsy. This tumor may be associated with trauma, and it tends to arise at the mucocutaneous junction of the eyelid. The cytopathic effects present in viral papillomas are not seen. Thickened and mildly dysplastic hyperkeratotic epithelium is thrown into folds over a loose fibrous stroma without stromal invasion. There is not significant hyperkeratosis. Viral genetic material is absent, and viral

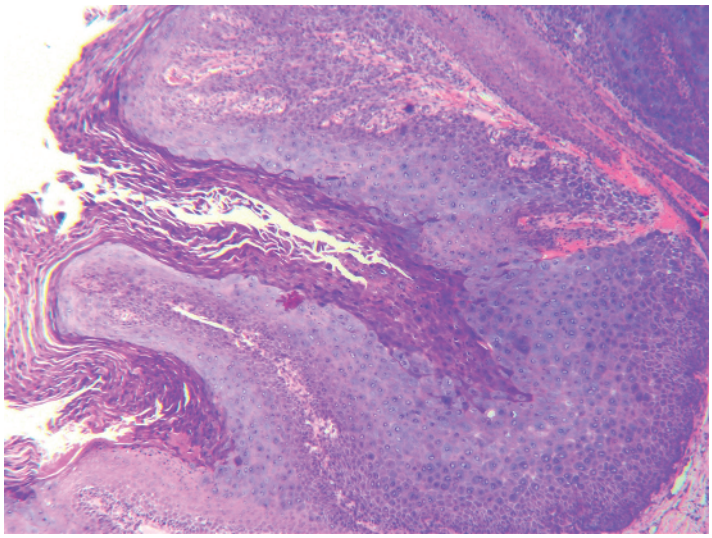

Figure 1.25 Canine oral papilloma biopsy. 2.5x.

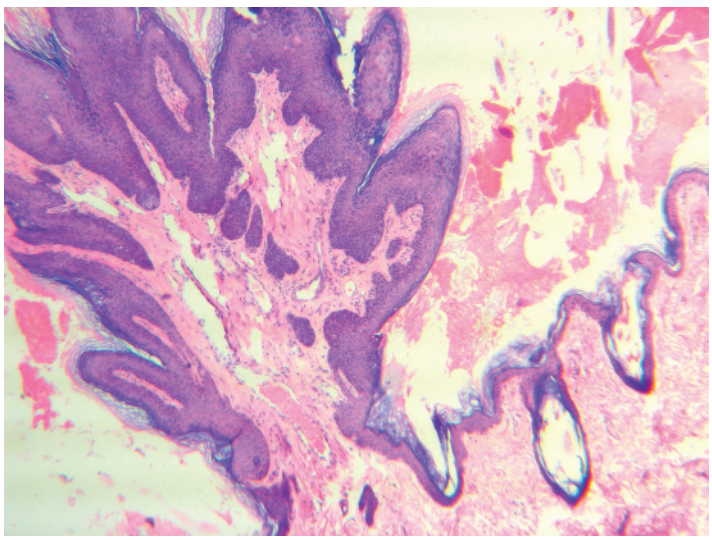

Figure 1.26 Canine conjunctival fibropapilloma biopsy. 2.5x.

antigens are not demonstrated. These lesions are often hyperpigmented and can be grossly similar to melanoma, thus the recommendation for removal when small.[9]

Figure 1.27 shows a canine urinary bladder transitional cell carcinoma biopsy. Biopsy evaluation of this bladder mass revealed a disorganized proliferation of pleomorphic epithelial cells with invasion into the underlying stroma. This tumor tends to metastasize to local lymph nodes and distant sites, but medical therapy can be palliative for a significant length of time with minimal complications in some dogs, and consultation with an oncologist for the most applicable and current therapy is suggested.

Figure 1.28 shows a squamous cell carcinoma of the nose biopsy. This disorganized proliferation of pleomorphic squamous epithelial cells exhibits nuclear atypia and stromal invasion. The central keratinization of some nests of cells is the identifying feature of this lesion.

Squamous epithelial tumors are often assessed by a combination of mitotic rate and invasion of stroma and lymphatics, and assigned a grade. Feline oral squamous cell carcinoma mitotic rates, however, appear not to be predictive of behavior, but stage, and especially the presence of bone invasion, is predictive.[10, 11] Radiographs, therefore, may have high prognostic significance in feline oral squamous cell carcinoma.

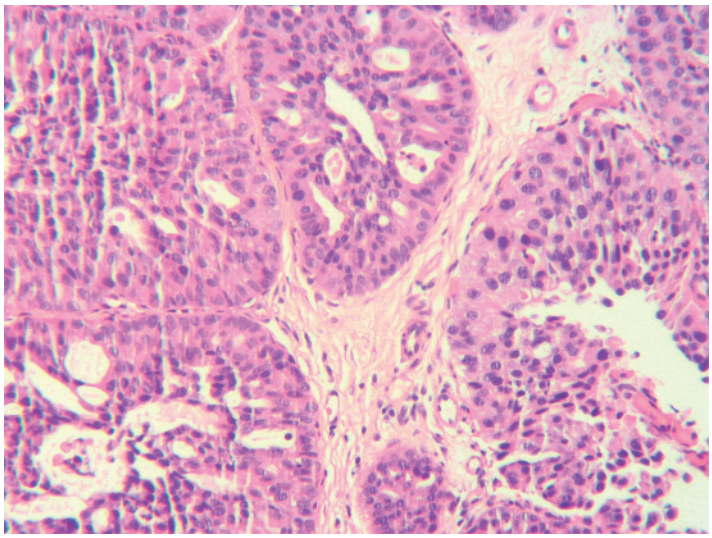

Figure 1.27 Canine urinary bladder transitional cell carcinoma biopsy. 10x.

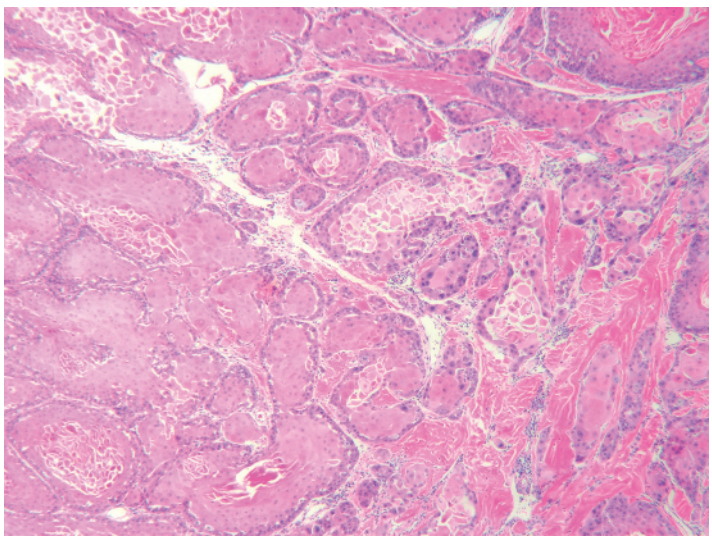

Figure 1.28 Squamous cell carcinoma of the nose biopsy. 40x.

Figure 1.29 shows a canine squamous cell carcinoma toe biopsy. This squamous cell carcinoma (arrow) in the toe of an adult dog has invaded into the third phalangeal bone causing disassociation of the joint cartilage (arrowhead). This suggests a high-grade tumor, and evaluation of local lymph nodes with thoracic radiographs would be part of the minimal database. Excision of the entire digit would remove this painful joint, which is unlikely to return to normal function, and may be necessary to achieve wide margins of normal tissue and provide assessment of bone and joint invasion.

Figure 1.30 shows a feline squamous cell carcinoma mouth biopsy. This squamous cell carcinoma from the mouth of an adult cat is forming a keratin pearl at the left margin and is clearly invasive into the stroma of the submucosa. The dense eosinophilic material at the lower right and upper left margins is bone and indicates that this tumor is invading the underlying bone. Mitotic figures are not seen in this field, but a high-grade tumor is suspected due to the bone invasion.

Tumors arising from epithelial cells of internal organs are often not discovered until a late stage. In one survey 76% of feline lung carcinomas had metastasized by the time of discovery.[12]

Figure 1.31 shows a lung bronchoalveolar carcinoma biopsy. Proliferation of atypical and disorganized bronchial lining epithelium can replace large areas of pulmonary parenchyma before clinical signs of respiratory distress are observed.

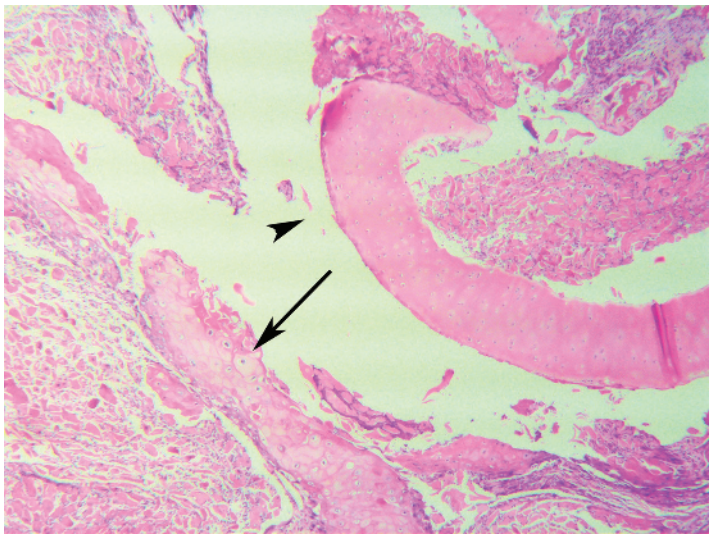

Figure 1.29 Canine squamous cell carcinoma toe biopsy. 2.5x.

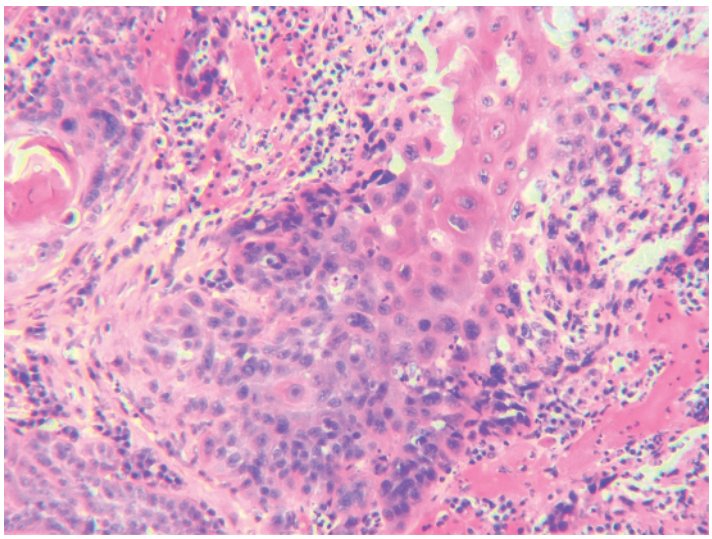

Figure 1.30 Feline squamous cell carcinoma mouth biopsy. 10x.

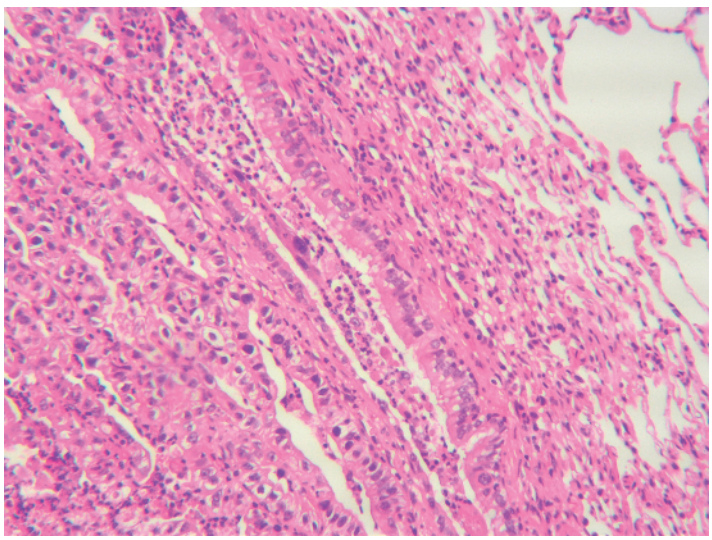

Figure 1.31 Lung bronchoalveolar carcinoma biopsy. 10x.

Figure 1.32 shows a biopsy of a pancreatic carcinoma metastatic to the liver. Ductular carcinoma in this adult female French Bulldog arose in the pancreas, metastasizing to adjacent organs including the liver as shown in this histologic section. There is both stromal (arrowheads) and lymphatic (arrows) invasion on this section. She presented with vague clinical symptoms, mostly anorexia, and a gastrointestinal foreign body was suspected. A pancreatic mass that had already spread to multiple other internal organs was found on exploratory laparotomy, and biopsy was performed postmortem.

Masses in internal organs may shed neoplastic cells into body cavity effusions. FNA of effusions is a rapid way to obtain a preliminary diagnosis. FNA of the mass can be performed prior to surgery if evaluation of an effusion is not diagnostic.

Figure 1.33 shows an intestinal adenocarcinoma FNA. An adult cat presented with abdominal effusion. FNA revealed clusters of epithelioid cells with marked anisokaryosis, prominent nucleoli, and basophilic cytoplasm that appear to contain fluid or mucin. When clusters of cells such as this are found free in the abdominal fluid, additional workup such as radiography or ultrasound is indicated to search for a mass that could be biopsied.

Figure 1.34 shows a biopsy of an intestinal mass in an adult cat that revealed invasion by glands from the lamina propria into the submucosa and muscular layers. These glands were lined by epithelial cells that were sometimes more than a single cell layer thick, with large pleomorphic nuclei exhibiting vesiculated chromatin with prominent nucleoli, and basophilic cytoplasm with large vacuoles. There was invasion into the local lymphatics by neoplastic rafts of cells with a similar morphologic appearance to the cells in Figure 1.33.

Mammary tumors can be composed of proliferating tubules and glands (simple), proliferating tubules, glands, and myoepithelial cells (complex), and proliferation of tubules, glands, myoepithelium and formation of cartilage and/or bone (mixed). Simple epithelial tumors are graded using type, nuclear pleomorphism, and mitotic rate, and this grade correlates with risk of invasion (see Table 1.3). Most canine mammary tumors are epithelial and myoepithelial (complex or mixed), however, and the grading system devised for simple mammary tumors is not applicable to complex or mixed tumors.[13] In dogs, ovariohysterectomy status and tumor grade, age, tumor stage, tumor subtype, and lymphatic metastasis were correlated with recurrence, metastasis, and survival time in one study and not related in another.[14, 15] Atypical ductal hyperplasia in dogs is associated with malignant neoplastic transformation, as is stromal invasion.[16] Fifty-three percent of feline mammary carcinomas had metastasized by time of discovery.[17] Mixed mammary tumors, the most common type of tumor found in dogs, tend to be less aggressive unless they are carcinosarcomas.[18] Mixed mammary tumors are not seen in cats.[19] It is difficult to evaluate stromal and lymphatic invasion if the tumor extends to all margins on the biopsy tissue submitted because one cannot look for invasion into normal adjacent stroma or lymphatics if neither is present. It is not advisable to "shell out" mammary tumors.

Figure 1.35 shows a biopsy from a young sexually intact female cat and reveals tubulolalveolar structures lined by a single layer of plump epithelium, which are then surrounded by laminar layers of myoepithelium. This is fibroepithelial hyperplasia, a benign response to hormonal stimulation and should regress upon removal of the hormonal stimulus.

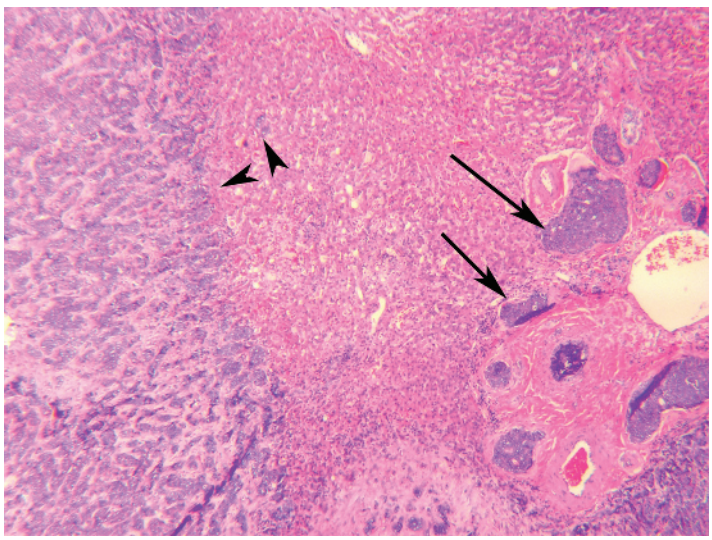

Figure 1.32 Pancreatic carcinoma metastatic to liver biopsy. 2.5x.

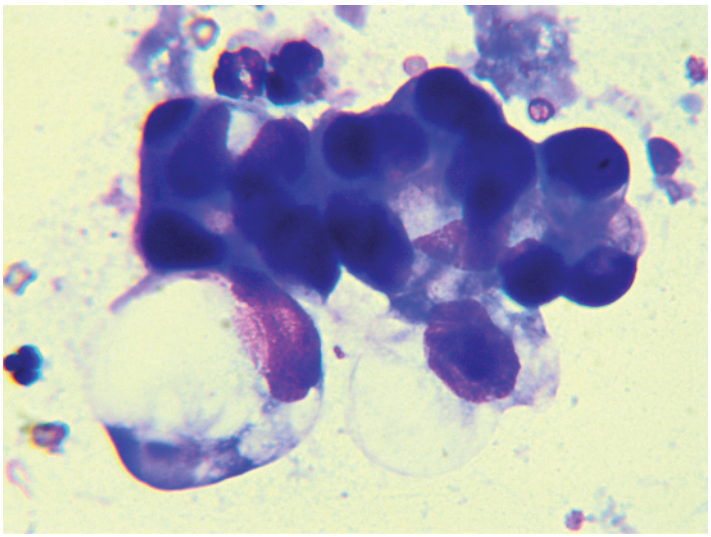

Figure 1.33 Intestinal adenocarcinoma in abdominal fluid FNA. 50x.

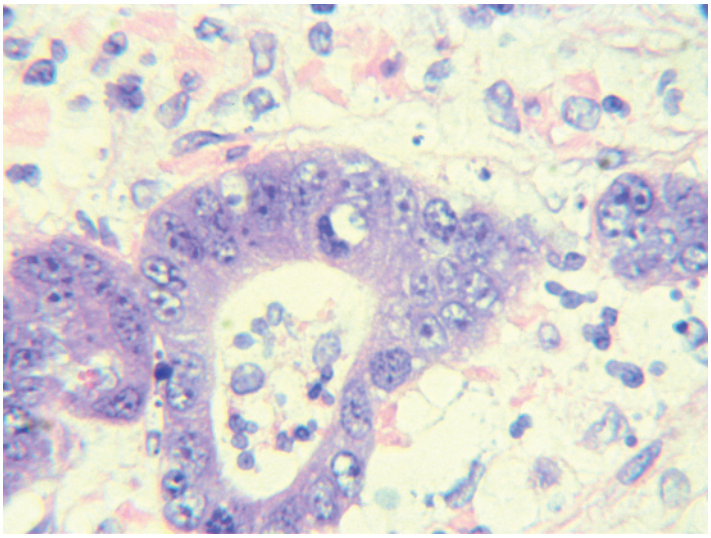

Figure 1.34 Intestinal adenocarcinoma biopsy. 40x.

Figure 1.36 shows a biopsy of a mammary adenoma on a middle-aged spayed female dog. It reveals a proliferation of tubuloepithelial cells in dilated ducts and glands. There is no stromal invasion seen, and adjacent lymphatics are not significantly dilated. Excision of this mass is indicated because benign tumors can eventually become invasive. Grossly recognizable normal tissue at the lateral margins and a tissue plane at the deep margin would be the minimal margin width warranted if pre-biopsy FNA does not reveal pleomorphic cells suggestive of a more aggressive tumor type. It is not advisable to "shell out" mammary tumors even if the FNA looks benign.

Figure 1.37 shows a biopsy of a mammary complex adenoma on a middle-aged spayed female dog and reveals a proliferation of tubuloepithelial cells in dilated ducts and glands with proliferation of associated myoepithelium. This is a complex tumor, and grading for simple tumors is not applicable. Complex tumors are usually low grade, but complete excision in a timely fashion is recommended because this tumor may undergo malignant transformation with time. Conservative margins of grossly normal tissue at the lateral margins and a tissue plane at the deep margin are indicated if there is not FNA evidence of cellular pleomorphism.

Figure 1.38 shows a biopsy of a mixed mammary tumor on a middle-aged sexually intact female mixed breed dog. It reveals a proliferation of tubuloepithelial cells in dilated ducts and glands with proliferation of associated myoepithelium, which is undergoing focal osseous and cartilaginous metaplasia. This tumor is low grade, but early excision is recommended because there is potential for malignant transformation with time. Conservative margins are indicated if there is not FNA evidence of cellular pleomorphism.

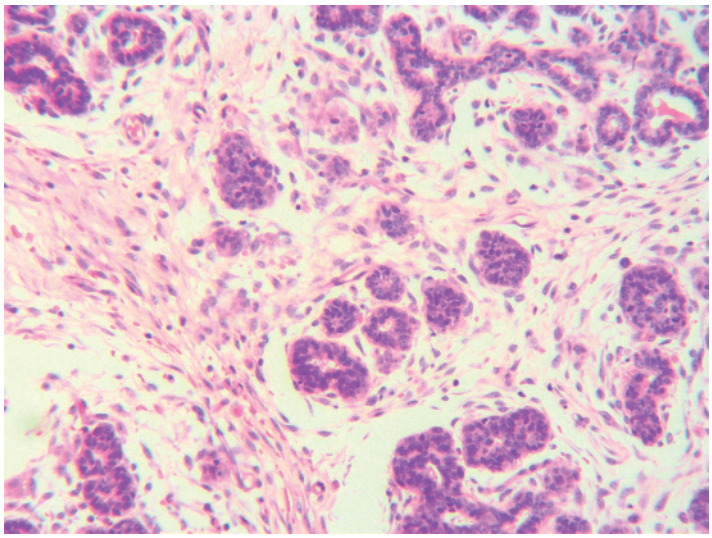

Figure 1.35 Feline mammary fibroepithelial hyperplasia biopsy. 10x.

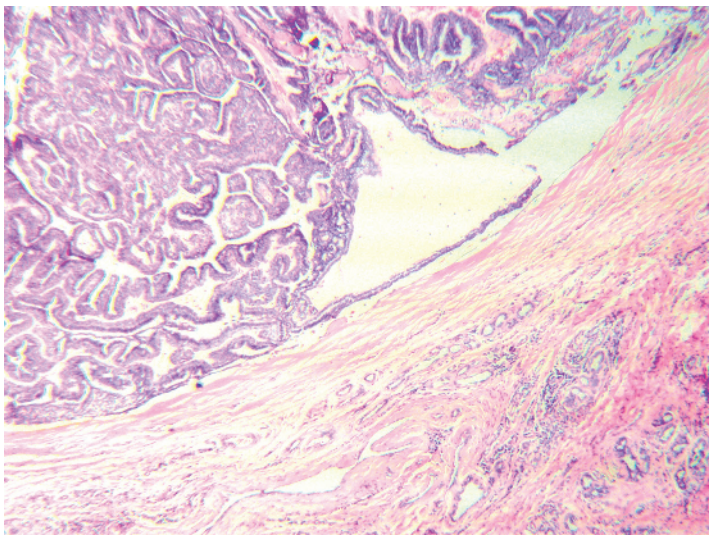

Figure 1.36 Canine mammary adenoma biopsy. 10x.

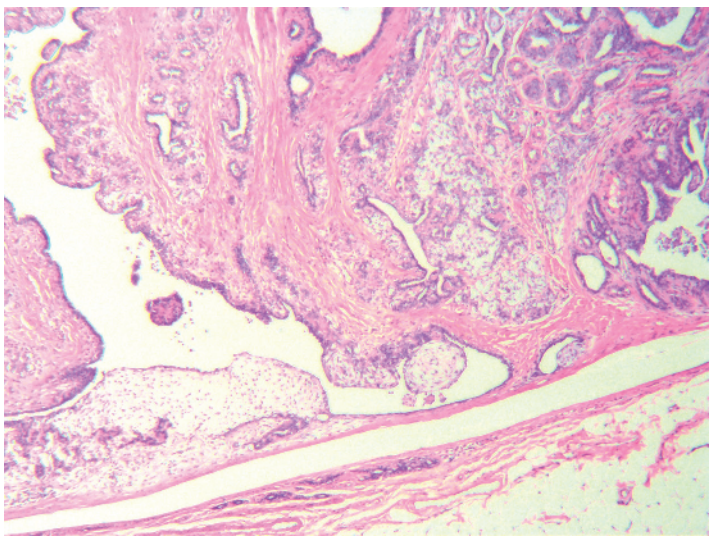

Figure 1.37 Canine mammary complex adenoma biopsy. 10x.

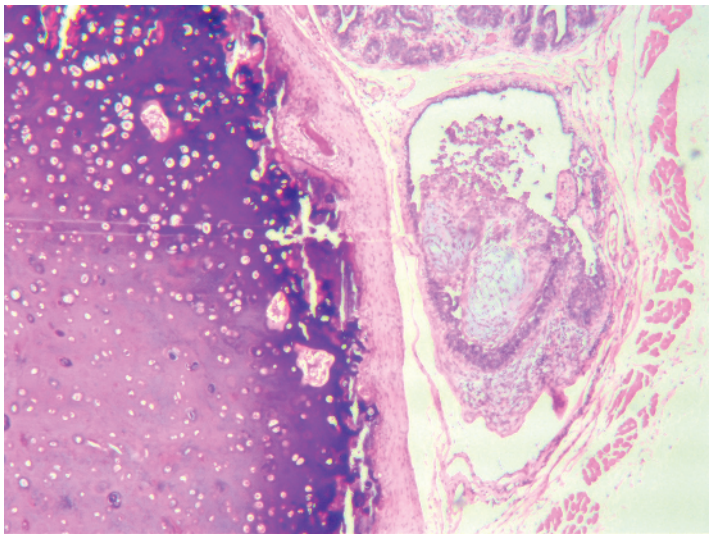

Figure 1.38 Canine mixed mammary tumor biopsy. 10x.

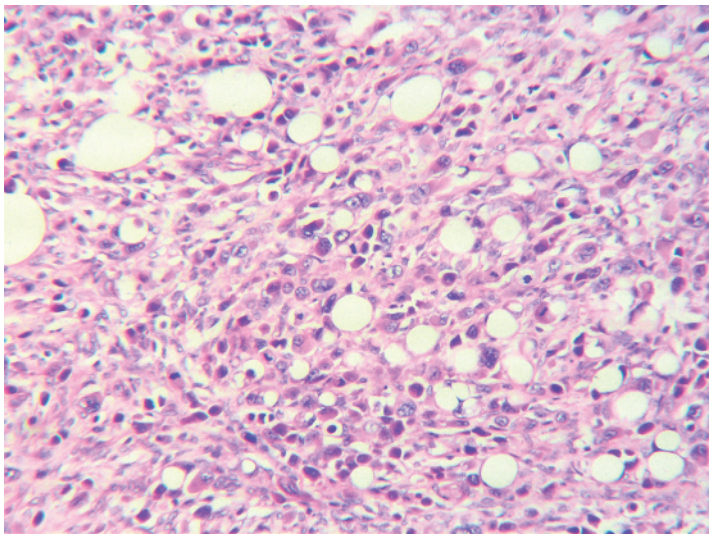

Figure 1.39 Canine invasive schirrous carcinoma biopsy. 10x.

Figure 1.39 shows a canine invasive schirrous carcinoma biopsy. This diffusely invasive mammary tumor in an adult female mixed breed dog exhibits nuclear pleomorphism, frequent mitotic figures, and loss of normal architecture. This type of tumor has a high potential for invasion into lymphatics and progression to distant sites. FNA of this type of tumor may yield pleomorphic cells that would confirm the need for thoracic radiographs prior to surgery. Complete excision with wide margins would allow a search for lymphatic invasion, and submission of local lymph nodes could be helpful for staging if there is lymphadenopathy.

Figure 1.40 shows a feline ductular carcinoma biopsy. This aggressive and high-grade tumor in an adult spayed female cat retained a somewhat duct-like appearance, but there is loss of normal lobular architecture and invasion into a fibrotic stroma. This type of tumor in the cat tends to invade lymphatics early. When taking a biopsy for initial diagnosis, wide margins are recommended so adjacent lymphatics can be searched for invasive tumor. Small biopsy samples may fail to demonstrate the invasive nature of the tumor.

Figure 1.41 shows a biopsy of feline invasive ductular carcinoma in the lymphatic structure. There are clusters of invasive ductular carcinoma in this lymphatic vessel. The wide margins of the tissue submitted, from the case in Figure 1.40, allowed demonstration of numerous dilated lymphatics containing invasive carcinoma.

Figure 1.42 shows a local lymph node that was also submitted with the tissue from Figure 1.40. There was invasion into the lymph node by neoplastic ductular epithelial cells.

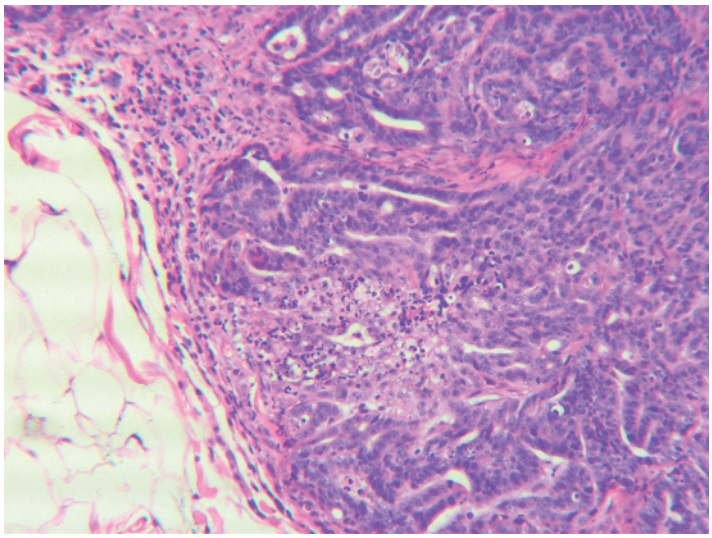

Figure 1.40 Feline ductular carcinoma biopsy. 10x.

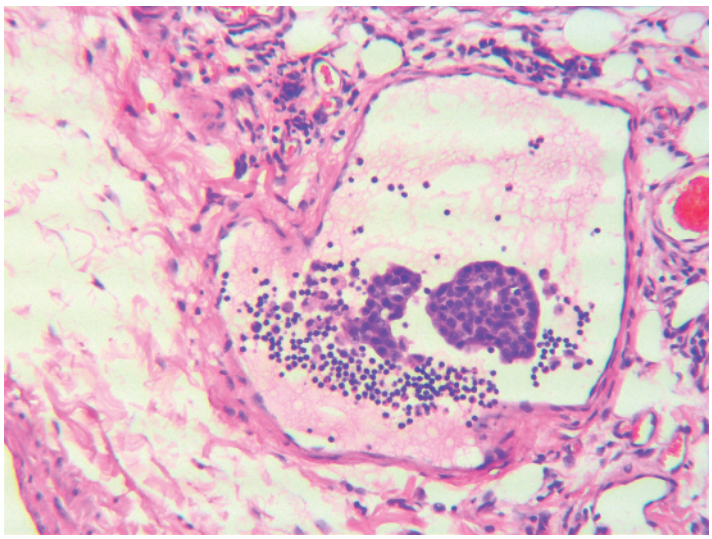

Figure 1.41 Feline invasive ductular carcinoma in lymphatic structure biopsy. 10x.

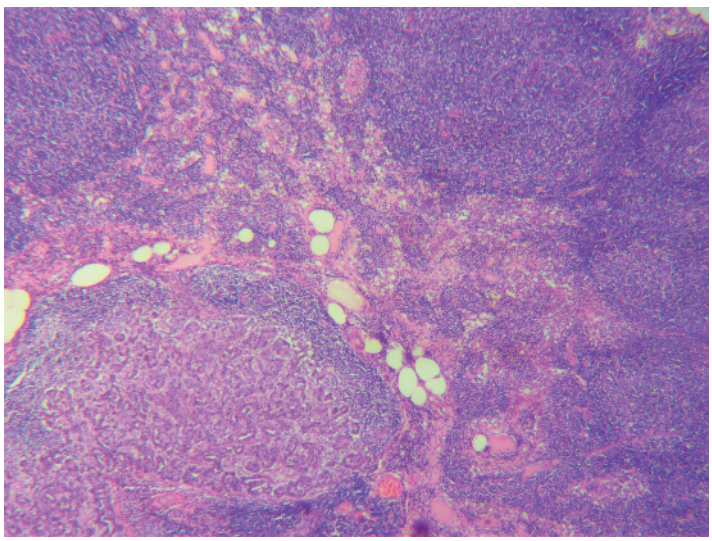

Figure 1.42 Feline invasive ductular carcinoma metastasis to local lymph node. 10x.

Mesenchymal tumors

Neoplasia of the stromal/spindle/mesenchymal cells of the body, also known as sarcoma when the proliferation is invasive, tends to be poorly circumscribed, as these cells are the framework of tissues and do not rest on a basement membrane. These tumors usually grow first by local extension then can metastasize to distant sites later in the course of the disease by hematogenous and lymphatic pathways. Mitotic rate and size of clean margins measured in millimeters (mm) or centimeters (cm) is predictive of behavior.[20] Complete removal of these tumors can be difficult to impossible depending on the location, and gross assessment of margins can be deceiving due to pseudoencapsulation and extension of fibrils along fascial planes. Tumor grade, mitotic rate and mitotic index, and size of clean margin are the most important prognostic indicators for spindle cell tumors.

Figure 1.43 shows a spindle cell tumor grade 1 biopsy. This biopsy from the skin of an adult dog reveals anastomosing bundles of cells that are typical of smooth muscle cells. Mitotic figures are 0–1/HPF with an MI of 1/10 HPF. There is no necrosis. This tumor would have a score of 1 + 1 + 1 = 3 and is a grade 1 tumor (Table 1.3).

Figure 1.44 shows a biopsy from the flank of an adult mixed breed dog is a disorganized proliferation of plump spindle cells of suspected nerve origin. There are 0–3 mitotic figures/HPF with an MI of 10/10 HPF. There are a few areas of necrosis, estimated at about 10% on the sample examined. This tumor would have a score of 2 + 2 + 2 = 6 and is a grade 2 tumor (Table 1.3).

Figure 1.45 shows a biopsy from the thigh of an adult beagle and is a disorganized proliferation of pleomorphic spindloid to epithelioid cells of indeterminate origin. There is moderate to marked anisokaryosis with prominent multiple nucleoli. There are 0–6 mitotic figures/HPF with an MI of 21/HPF. There is greater than 50% necrosis on the sections examined. This tumor would have a score of 3 + 3 + 3 = 9 and is a grade 3 tumor (Table 1.3).

Figure 1.46 shows a grade 2 spindle cell tumor. It was submitted with the history that excision appeared complete because there was normal tissue in the marginal tissue beyond the excised capsule. It is very important to note that spindle cell tumors do not form a capsule, and the bands that appear to be capsule are actually tumor.

Figure 1.47 shows a grade 2 spindle cell tumor that has less dense tissue at the margin, and this was assumed to be normal tissue based on the gross appearance. The black ink indicates the surgical margin. Tumor extends to the inked margin. Surgical removal of spindle cell tumors is fraught with peril. Referral for pre-surgical imaging and removal by a specialist should be offered because this tumor often extends widely microscopically. If referral is declined, any surgical removal should be performed with the knowledge that the tumor is likely to extend beyond the gross bulk of the mass.

Since many stromal or spindle cells have a similar appearance on routine histology, immunohistochemical stains may be necessary to identify the cell type (fibroblasts, pericytes, myopericytes, smooth muscle, myofibroblasts, Schwann cells, perineural cells) for optimum prognostic significance and treatment choices. Immunohistochemistry may be most useful when chemotherapy is a treatment option. This test is most satisfactory when performed on frozen tissue sections, but formalin fixed tissues are accepted and processed by many laboratories. Consultation with diagnostic

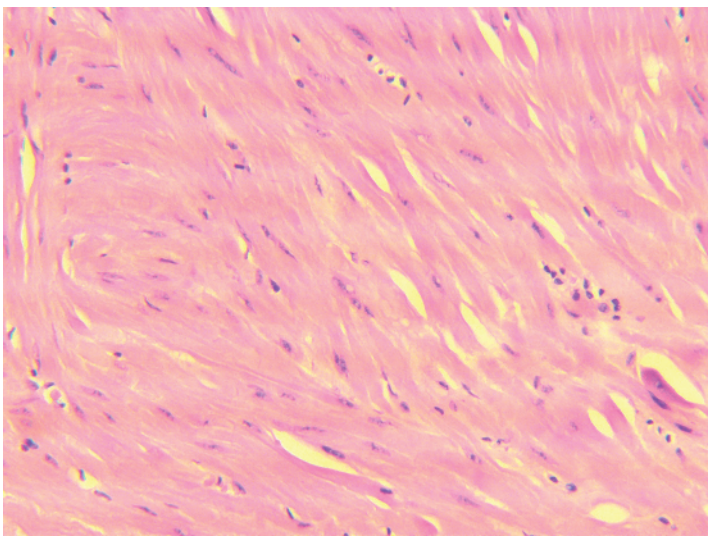

Figure 1.43 Spindle cell tumor grade 1 biopsy. 10x.

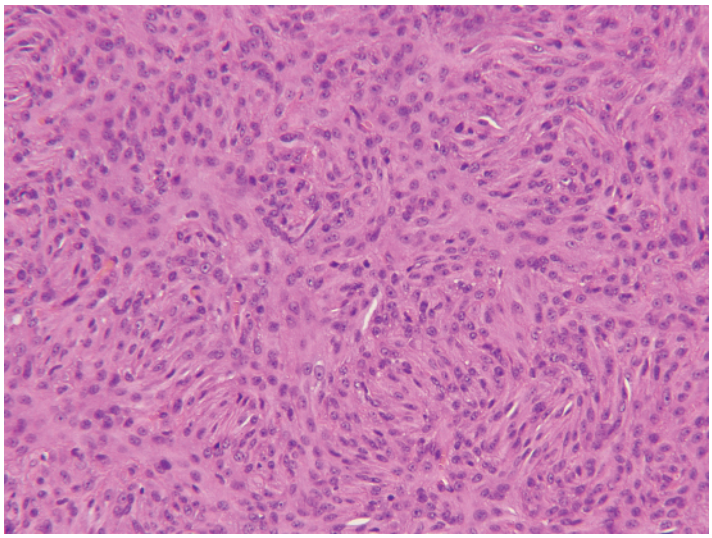

Figure 1.44 Spindle cell tumor grade 2 biopsy. 10x.

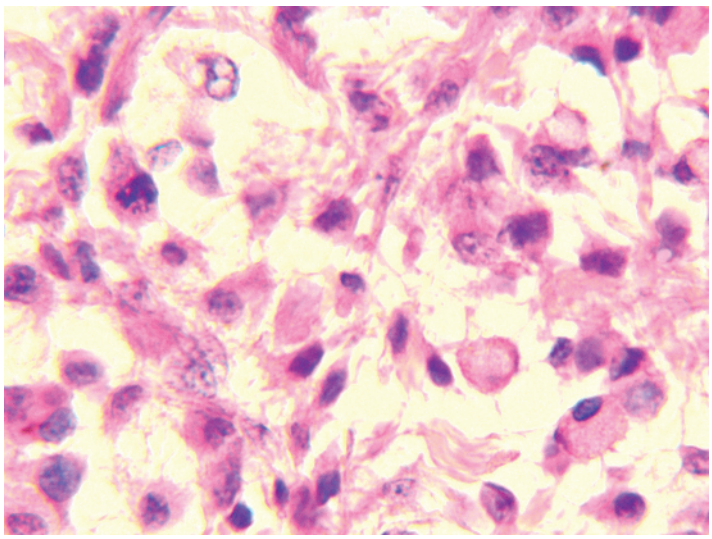

Figure 1.45 Spindle cell tumor grade 3 biopsy. 10x.

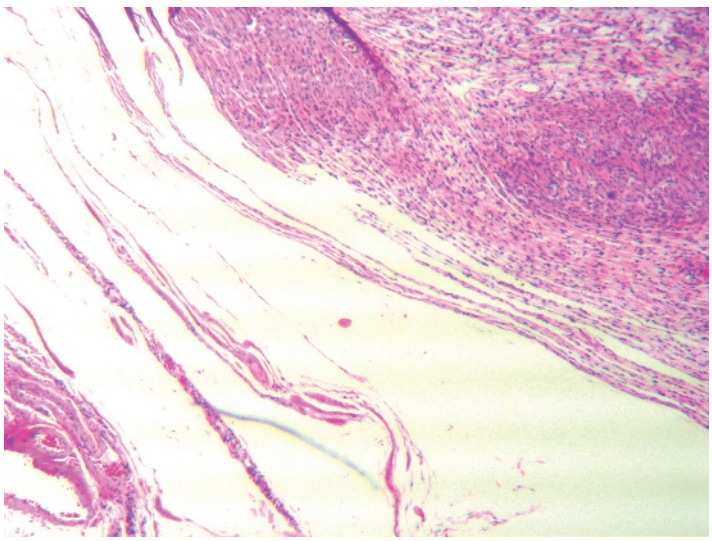

Figure 1.46 Spindle cell tumor biopsy. 2.5x.

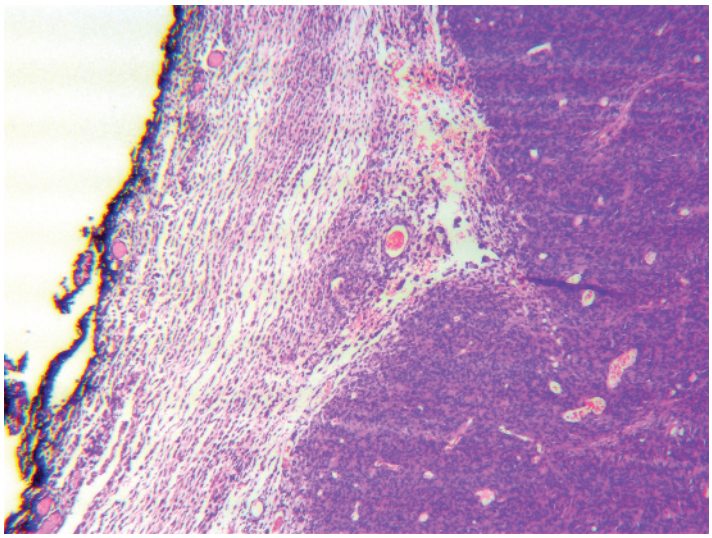

Figure 1.47 Spindle cell tumor biopsy. 2.5x.

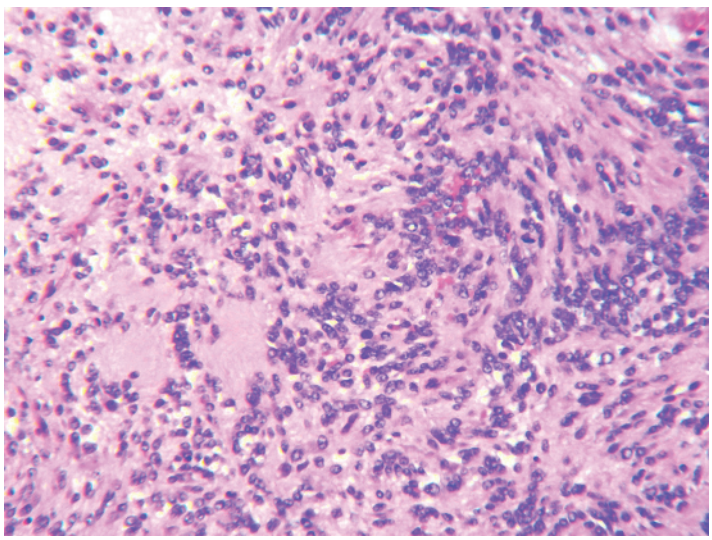

Figure 1.48 Spindle cell tumor biopsy. 10x.

lab personnel prior to submission is suggested for the most current information regarding test availability, submission requirements, and pricing. If chemotherapy will not be utilized and immunohistochemistry is not elected, then wide excision, with or without radiation therapy, is a standard therapy recommendation due to a similar biologic behavior for many soft tissue sarcomas.[21]

Figure 1.48 shows a grade 2 spindle cell tumor in a dog. The tumor exhibits a storiform pattern with occasional swirls and palisading nuclei when stained with routine hematoxalin and eosin (H&E) stain. This pattern is suggestive of neural tissue, and the tumor is presumed to be a peripheral nerve sheath tumor, but immunohistochemistry would be necessary for a more definitive diagnosis of the tumor cell type.

Figure 1.49 shows a grade 2 spindle cell tumor that is forming swirls and nests around vascular spaces, suggesting a peripheral vascular wall myocyte origin. Immunohistochemistry could be performed for more definitive identification of the tumor cell type.

Stromal/spindle cell tumors in cats can be aggressive no matter what the mitotic rate, with a recurrence rate of 14% for peripheral nerve sheath tumors diagnosed as benign and 31% for peripheral nerve sheath tumors diagnosed as malignant, based on one study.[22]

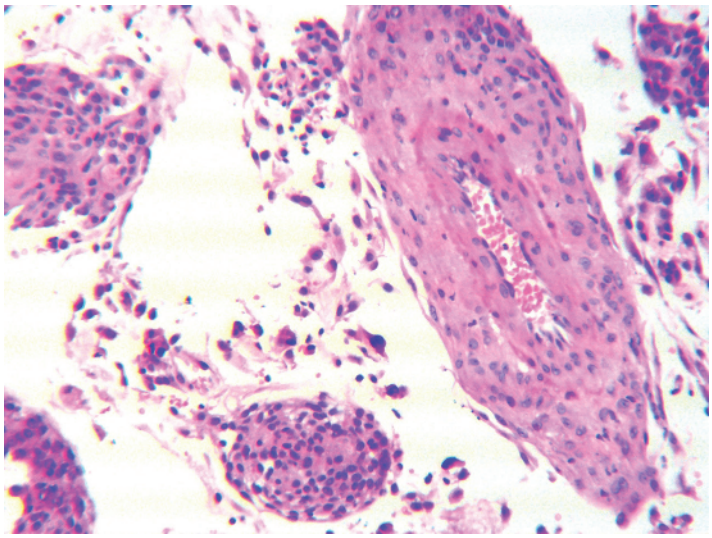

Figure 1.49 Spindle cell tumor biopsy. 10x.

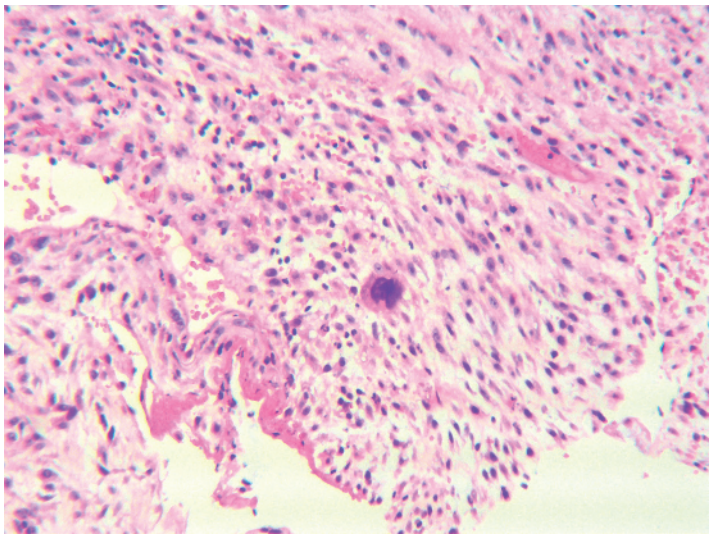

Figure 1.50 Feline soft tissue sarcoma. 10x.

Figure 1.50 shows a biopsy of a spindle cell tumor from an adult cat. It reveals many haphazardly arranged spindle cells and occasional large epithelioid cells with pleomorphic and lobulated nuclei, prominent nucleoli, and abundant cytoplasm. This cellular pleomorphism is the hallmark of feline soft tissue sarcomas and is a useful feature when evaluating a tumor using FNA, as the finding of even rare pleomorphic cells is an indication for immediate aggressive therapy.

Cutaneous hemangiosarcoma in dogs has a grading protocol that is based on a combination of mitotic rate and how deep the tumor extends, thereby estimating the potential for invasive behavior. Staging takes into account the presence of invasion beyond the local site. For example, stage I is confined to the dermis, stage II extends into subcutis and may exhibit regional lymph node involvement, and stage III invades structures such as muscle and involves distant metastasis.[23] Staging, while providing superior prognostic information, requires clinical information that cannot be determined from a single skin biopsy. Behavior in cats is unpredictable and ranges from locally invasive to aggressive and so attempts to grade may be misleading. Visceral hemangiosarcoma is quick to metastasize, and prognosis for long-term survival ranges from guarded with moderate to high probability of distant metastasis in non-ruptured tumors, to poor with a high probability of distant and local metastasis in ruptured tumors.[23] Benign hemangiomas can

undergo malignant transformation to hemangiosarcoma with time, but complete early excision of hemangioma may be curative, so histopathological evaluation of all excised lesions suspected of being vascular in origin is very important in order to confirm clean margins.

Figure 1.51 is a cutaneous hemangiosarcoma in an adult dog and consists of a somewhat circumscribed mass composed of vascular channels lined by pleomorphic endothelium. There were 0–2 mitotic figures/HPF. It was confined to the dermis and was considered to be low grade due to the relatively low mitotic rate and minimal local invasion.

Figure 1.52 shows a hepatic hemangiosarcoma, diagnosed at necropsy in an adult male Weimaraner. This was likely a metastatic lesion, as there was hemangiosarcoma in the spleen, and hemo-abdomen was observed upon opening the abdominal cavity. Preliminary diagnosis was made by identifying abdominal fluid on ultrasound and confirming abundant free blood by FNA.

The most common bone tumor is canine osteosarcoma. Behavior (time until metastasis and survival time) appears to be correlated to the site of occurrence. Bone tumors of the head and jaw tend to metastasize less readily than other sites. Osteosarcoma of the scapula has a significantly greater hazard for death than appendicular sites. Every 100% increase in alkaline phosphatase (ALP) increases the hazard of death by 1.7, and tumor grade at this site is not predictive according to one report.[24] Osteochondromatosis, a benign lesion, was reported to undergo malignant transformation

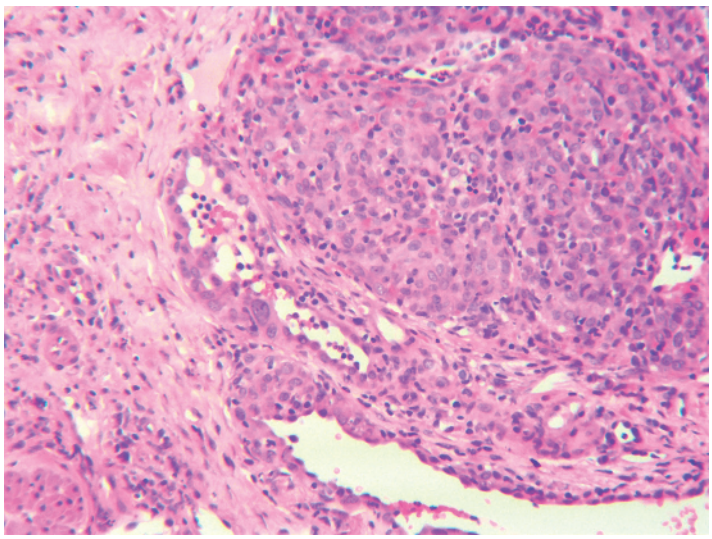

Figure 1.51 Canine cutaneous hemangiosarcoma. 10x.

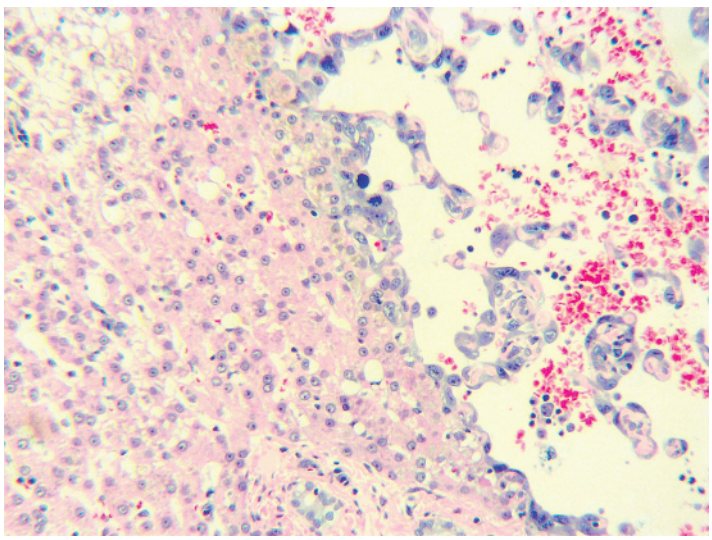

Figure 1.52 Canine hepatic hemangiosarcoma. 10x.

to chondrosarcoma over a course of 20 months in one reported case.[25] Spontaneous regression of osteosarcoma has also been reported.[26] Due to the many variables associated with predicting the behavior of this tumor type, consultation with an oncologist would be prudent.

Figure 1.53 An adult mixed breed dog presented with a firm mass on the left dorsal skull that on biopsy consisted of well-differentiated cartilage and bone with lacunae containing osteocytes and chondrocytes with small nuclei. This is typical of a benign or low-grade bone tumor such as osteochondroma. Metastasis is not expected, and complete excision may be curative.

Figure 1.54 shows a biopsy of an adult German Shepherd Dog presented with a mass near the orbit. Biopsy reveals a haphazard proliferation of bone, cartilage, and occasional sheets of pleomorphic spindloid to epithelioid cells diagnosed as osteosarcoma. This tumor is moderately well differentiated, producing tumor bone, and at this site is unlikely to produce early metastasis. Euthanasia was elected due to the extreme deformation of the skull and ocular involvement.

Figure 1.55 shows a biopsy of an adult Mastiff presented with left front leg lameness. Radiographs revealed a lytic lesion of the left humerus, and biopsy revealed a proliferation of pleomorphic epithelioid cells with rare multinucleated cells. Some cells appear to be nestled within scant eosinophilic material suggestive of osteoid, supporting a

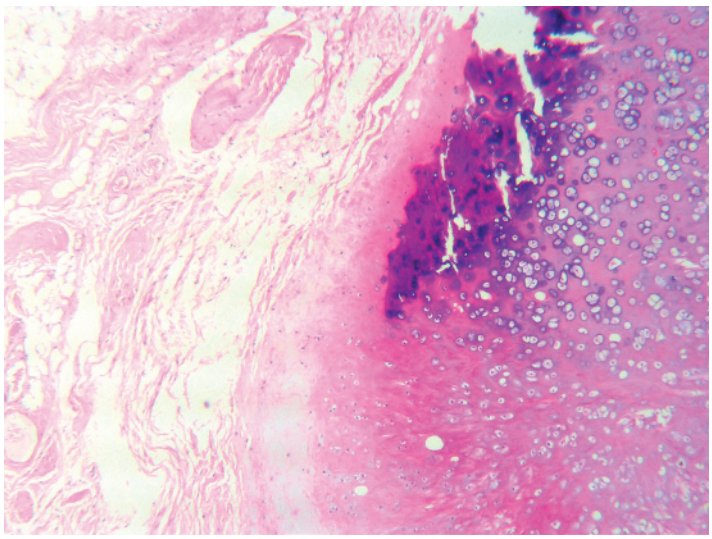

Figure 1.53 Osteochondroma biopsy. 2.5x.

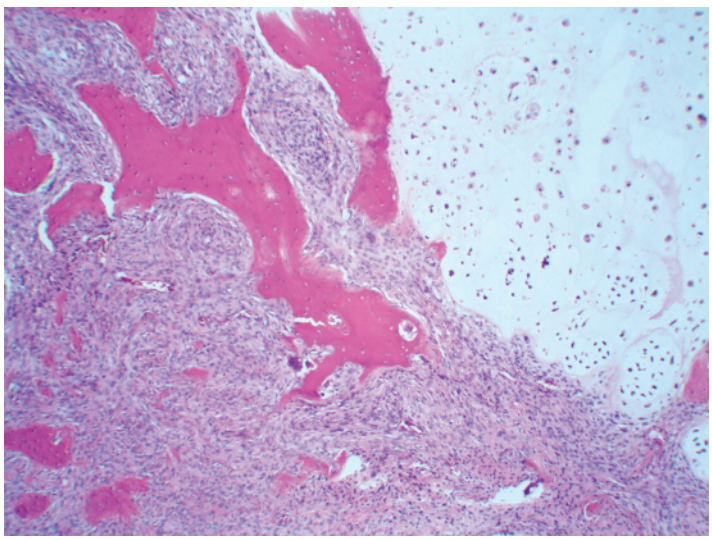

Figure 1.54 Osteosarcoma biopsy. 2.5x.

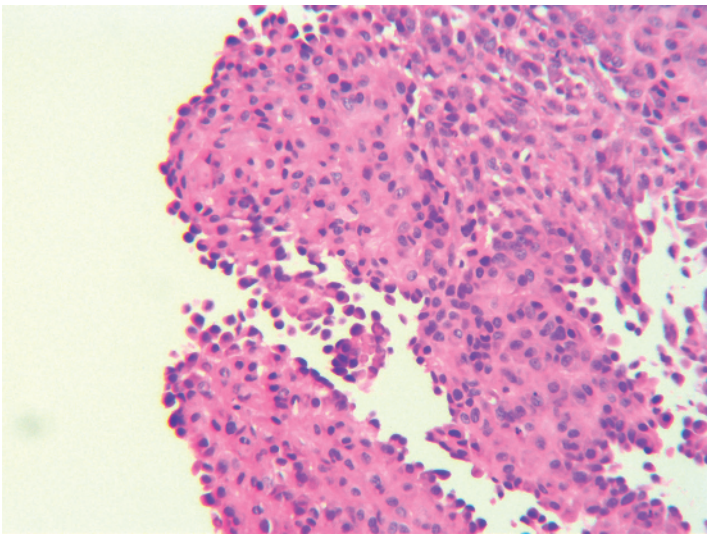

Figure 1.55 Osteosarcoma biopsy. 10x.

diagnosis of osteosarcoma. Definitive diagnosis of this tumor with evaluation of mitotic rate can be difficult if small samples from core biopsies are submitted, as this tumor can form in close association with necrotic bone, reactive bone, and periosteal hyperplasia, creating a heterogeneous pattern that can lead to sampling error. It can be a challenge to obtain a sample of adequate size and diagnostic quality without creating a site of instability.

Round cell tumors

Canine mast cell tumors (MCT) have historically been graded with a three-part system historically referred to as the Patnaik system.[27] Grade 1 (low-grade tumors) have 0 mitotic figures/HPF, are well differentiated, and are confined to the subepidermis and superficial dermis. Grade 2 (mid-grade tumors) have 0–2 mitotic figures/HPF, rare binucleate cells, and are dermal to subcutaneous. Grade 3 (high-grade tumors) have three or more mitotic figures/HPF, are pleomorphic, and extend to subcutaneous or deeper tissues.

A more recent system developed at Michigan State University by Kiupel et al. (which will be referred to as the two-tier scale), uses a two-part grading protocol in which a high-grade tumor is diagnosed if there are >7 mitosis/10 HPF, 3 multinucleated cells in 10 HPF, or 3 bizarre nuclei in 10 HPF and a low-grade tumor is diagnosed if these conditions are not met.[28] Mitotic index is an important part of the grading process of both systems, with one study indicating an MI < 5/10 HPF had a 70-month survival and MI > 5/10 HPF had 2-month survival.[29] In a study comparing the two systems over a 5-year period, in the three-level (Patnaik) grading system there was 0% mortality due to tumors labeled grade 1 (low grade, 1 of 3) 23% mortality due to tumors labeled grade 2 (mid grade, 2 of 2), and 100% mortality in tumors labeled grade 3 (high grade, 3 of 3). In the two-tier grading system there was 6% mortality due to tumors labeled low grade (1 of 2) and 71% mortality due to tumors labeled high grade (2 of 2), and the newer two-tier system was deemed to be more clinically predictive on a statistical basis.[30]

Figure 1.56 shows a canine cutaneous mast cell tumor grade 1 Patnaik scale. This cutaneous canine mast cell tumor is confined to the superficial dermis, mitotic figures are rare, and the mast cells appear well differentiated with small, monomorphic nuclei. This tumor is a grade 1 Patnaik scale tumor and a low-grade two-tier scale tumor (Table 1.2).

Figure 1.57 shows a canine cutaneous mast cell tumor grade 2 Patnaik scale. There are variably granulated mast cells with small but slightly pleomorphic nuclei, 0–1 mitotic figures/HPF with an MI of 2/10 HPF, and the tumor extends to the deep dermis. This tumor would be a grade 2 Patnaik scale and a low-grade two-tier scale (Table 1.2).

Figure 1.58 shows a canine cutaneous mast cell tumor grade 2 Patnaik scale. This grade 2 (Patnaik), low-grade (two- tier) cutaneous mast cell tumor has small, variably granulated mast cells with small nuclei with mild anisokaryosis, and is confined to the dermis. There are areas of collagen necrosis with dense infiltrates of eosinophils. Identification of mitotic figures is difficult in these areas, and they should be avoided because the irregular appearance of eosinophil nuclei could cause a spurious elevation in the mitotic count.

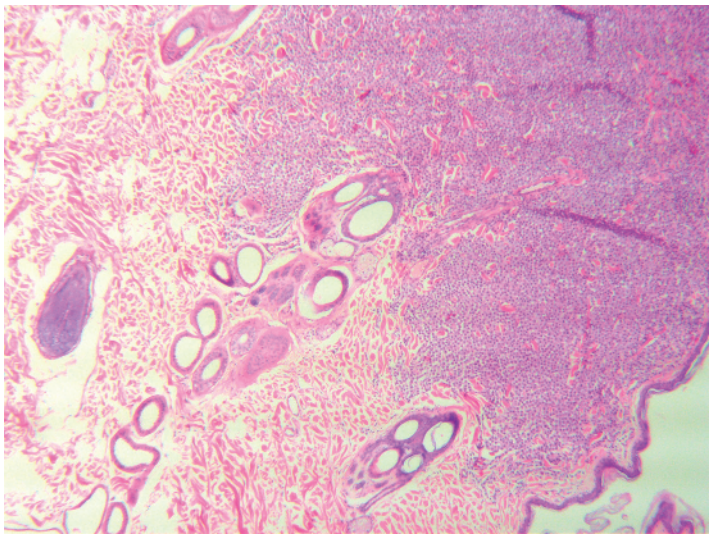

Figure 1.56 Canine cutaneous mast cell tumor grade 1 biopsy. 2.5x.

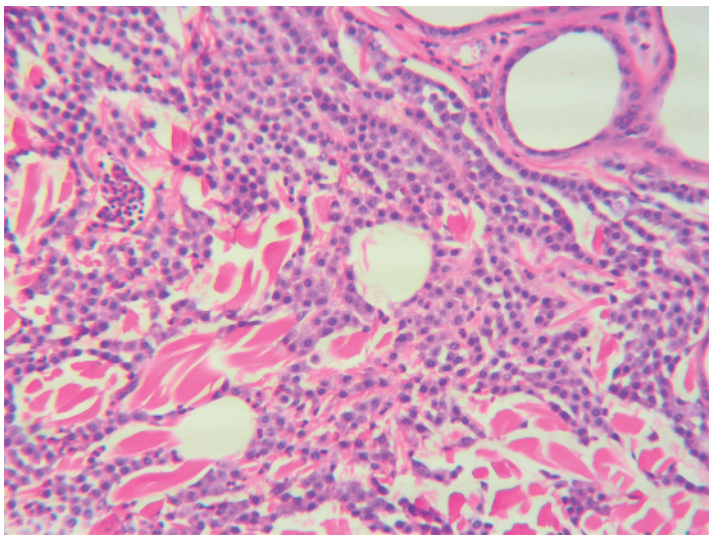

Figure 1.57 Canine cutaneous mast cell tumor grade 2 biopsy. 10x.

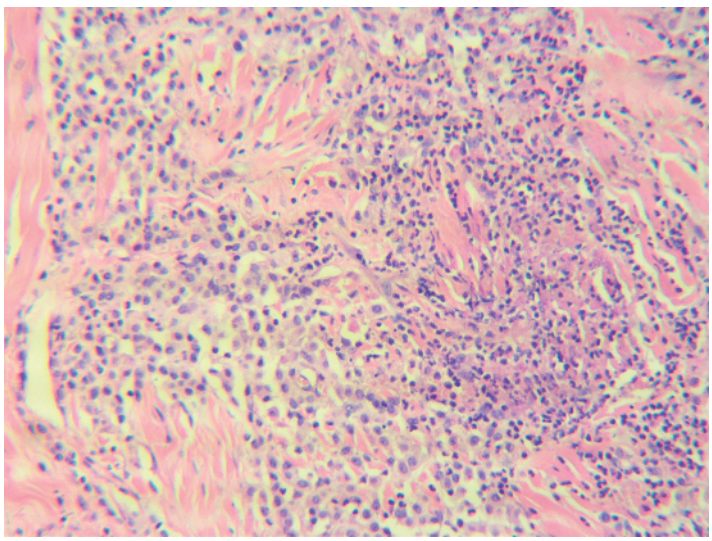

Figure 1.58 Canine cutaneous mast cell tumor grade 2 biopsy. 10x.

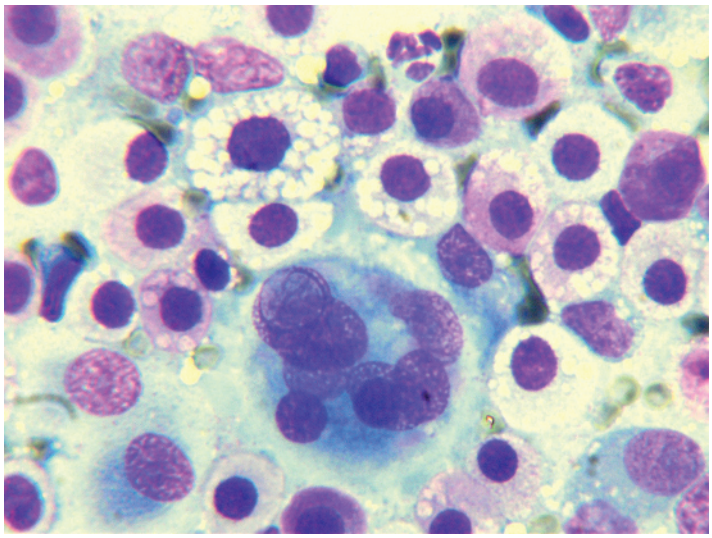

Figure 1.59 Canine mast cell tumor FNA. 50x.

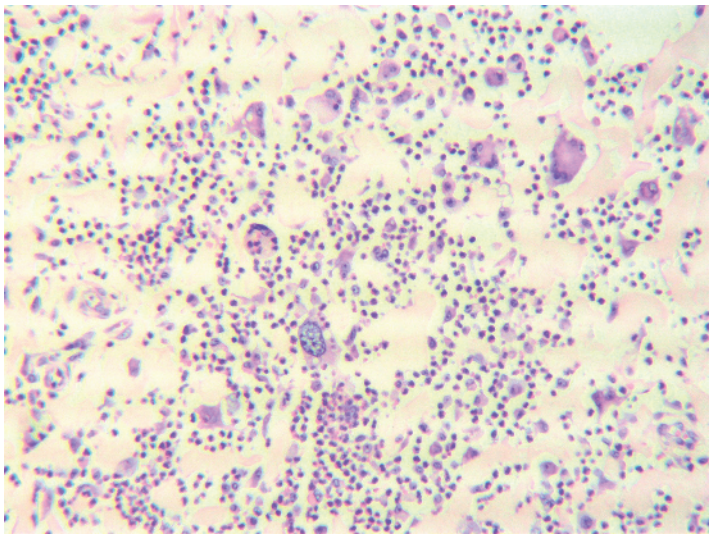

Figure 1.60 Canine cutaneous mast cell tumor grade 3. 40x.

Figure 1.59 shows the aspirate of a mass from a six-year-old spayed female Mastiff dog that revealed pleomorphic mast cells with moderate to marked anisokaryosis and frequent cells with multiple nucleoli, prominent nucleoli in some cells, and variable cytoplasmic granulation. Note that most of the cells are larger than the neutrophil in the top middle right and lymphocyte at the top middle left. There is not a grading system applicable to aspirates, but this tumor is likely to be high grade on biopsy due to the extreme cellular pleomorphism.

Figure 1.60 shows a canine cutaneous mast cell tumor grade 3 Patnaik scale. This biopsy of the aspirated mass in Figure 1.59 reveals a proliferation of moderately pleomorphic mast cells with identifiable cytoplasmic granules, and numerous large epithelioid cells without distinct cytoplasmic granules and with marked anisokaryosis and multiple nucleoli in an edematous background. This tumor extended into subcutaneous tissue and to all margins. There were low numbers of mitotic figures, but the extensive invasion of deep tissues and extreme cellular pleomorphism suggested a diagnosis of grade 3 Patnaik scale, high-grade two-tier scale, mast cell tumor (Table 1.2). Giemsa stain was recommended for confirmation that the pleomorphic cells were mast cells.

Figure 1.61 shows a canine cutaneous mast cell tumor grade 3 Patnaik, Giemsa stain. Giemsa stain of the biopsy in Figure 1.60 reveals metachromatic granules in the giant epithelioid cells, revealing them to be anaplastic mast cells. This tumor is confirmed as a grade 3 Patnaik scale and high-grade two-tier scale mast cell tumor.

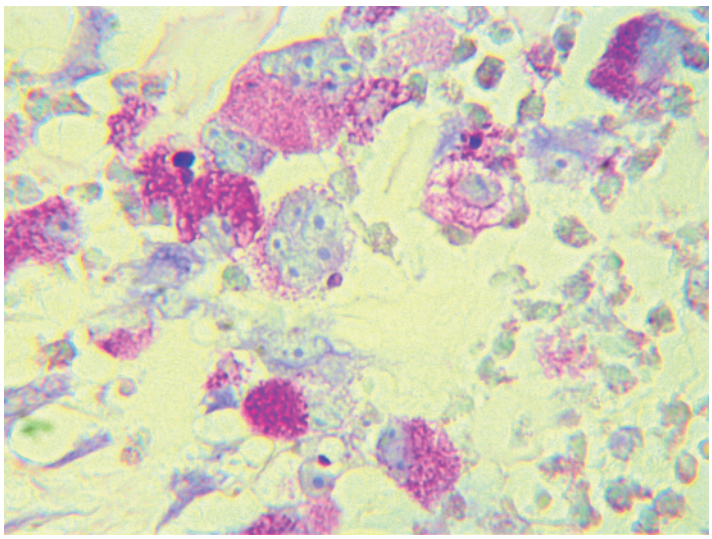

Figure 1.61 Canine cutaneous mast cell tumor grade 3 Giemsa stain. 50x.

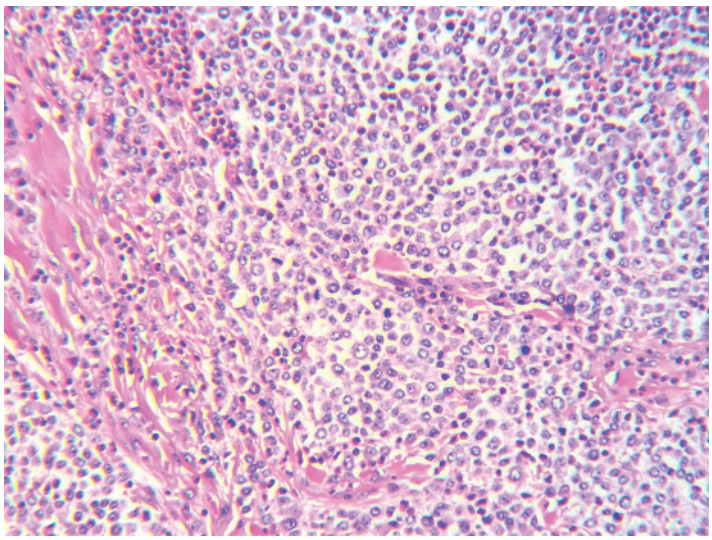

Figure 1.62 Canine cutaneous mast cell tumor high mitotic count biopsy. 10x.

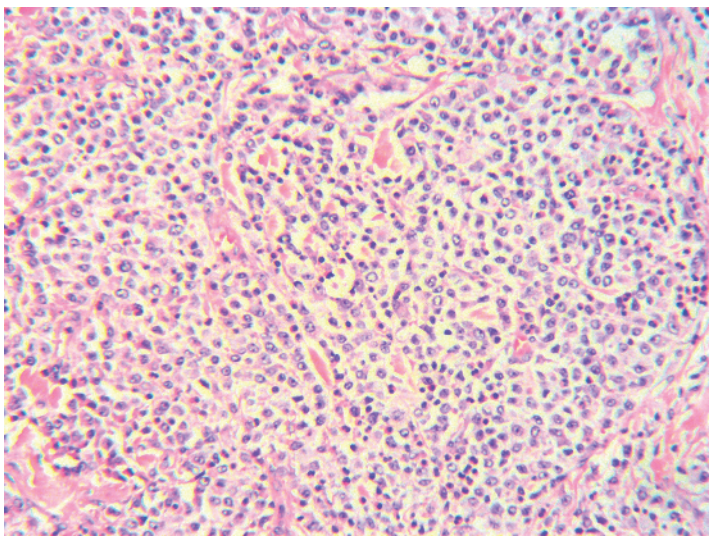

Figure 1.63 Canine mast cell tumor low mitotic count biopsy. 10x.

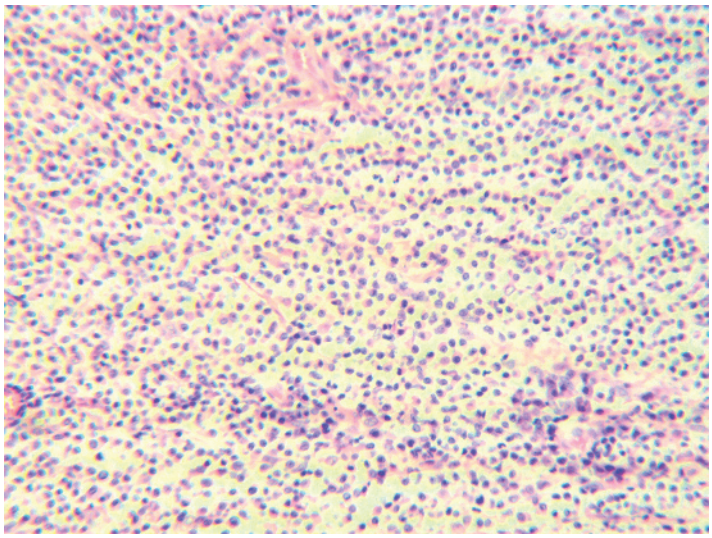

Figure 1.64 Feline cutaneous mast cell tumor biopsy. 10x.

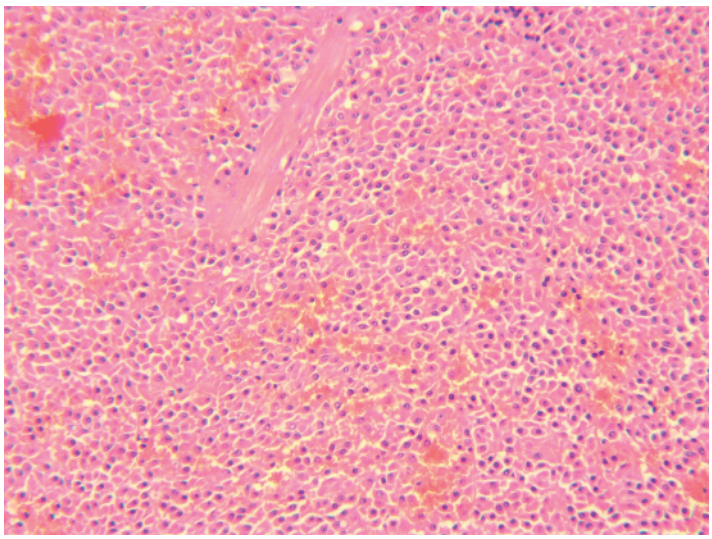

Figure 1.65 Feline splenic mast cell tumor biopsy. 10x.

In the study that comparatively assessed tumors with both the two-part (low, high) and three-part (1, 2, 3) systems, all grade 1 MCTs were diagnosed as low grade, all grade 3 MCTs were diagnosed as high grade, and 82% of grade 2 MCTs were diagnosed as low grade with 18% of grade 2 MCTs diagnosed as high grade. It should be noted that regarding the clinical relevance of this study, the mortality rate for two-tier low-grade MCT was 6%, and the mortality rate for two-tier high-grade MCT was 71%, while the mortality rate for Patnaik grade 1 was 0%, grade 2 was 23%, and grade 3 was 100%. Grading of MCT does not predict behavior with 100% accuracy, but both systems were significantly associated with prognosis, and there was greater concordance among pathologists when the two-tier system was used.[30]

Figure 1.62 shows a cutaneous mast cell tumor that was graded as a grade 3 Patnaik, high-grade two-tier, due to the many mitotic figures in a certain area of the tumor. There appears to be nine or more mitotic figures in this field.

Figure 1.63 shows the same cutaneous mast cell tumor seen in Figure 1.62. The mitotic rate is low in this field, and most of the irregular nuclei are eosinophils. This demonstrates one source of variability in grading, because some tumors have areas of significant variation in the density of mitotic figures.

Canine mast cell tumors with tyrosine kinase receptor (KIT) dysregulation may behave in a more aggressive fashion.[31] Feline mast cell tumors do not reveal a prognostic association with KIT expression, and there is no recognized grading system, but higher mitotic rates are suggestive of more aggressive behavior.[32]

Figure 1.64 is a biopsy from an adult cat with a skin mass that revealed sheets of mast cells with rare mitotic figures. There is not a grading system applicable to feline mast cell tumors at this time. Complete excision of this type of lesion is often curative.

Figure 1.65 shows the splenic biopsy from an adult cat with a diffusely enlarged spleen. This is an indication for immediate diagnostic workup. The complete blood count (CBC) can be searched for circulating mast cells, and evaluation of abdominal fluid for mast cells is also an appropriate procedure prior to surgery. Mast cells are not normally seen in these fluids, and the finding of more than rare mast cells in the CBC or abdominal fluid is supportive of splenic mast cell tumor when there is splenomegaly. FNA of the spleen can be performed if these tests are not diagnostic. Also check for skin masses and palpate lymph nodes, and perform thoracic radiographs. Splenectomy is the treatment of choice with reported remission times of 12–19 months. Degranulation of splenic mast cell tumor can lead to fatal hypotension so gentle handling is important.[33]

Malignant lymphoma (lymphosarcoma) grading is still a work in progress, but there are generalizations that seem to be useful across the many types of lymphomas. Lymph node lymphoma and extranodal lymphoma have site-specific biological behavior. Canine hepatosplenic T-cell lymphoma and hepatic T-cell lymphoma are poorly responsive to therapy at this time, and they usually behave in an aggressive biological fashion.[34, 35] Low-grade T-cell lymphoma at a number of sites including skin and liver can be indolent, and quality of life can be maintained without aggressive therapy for long periods of time.[36]

Grading of nodal lymphomas is predominantly based on mitotic rate with 0–5/HPF graded low, 6–10/HPF graded intermediate, and >10/HPF graded high.[1, 37] In one retrospective study, dogs with low-grade T-cell lymphoma had a median survival rate of 622 days, dogs with high grade T-cell lymphoma had a median survival rate of 162 days, and dogs with B-cell centroblastic, the most common type, had a median survival rate of 127–221 days, across multiple treatment protocols.[1] Identification of T- and B-cell types is important for prognosis and therapeutic plan, and can be performed on biopsy samples by immunohistochemistry and on FNA samples using flow cytometry or polymerase chain reaction (PCR) of antigen receptor site rearrangements (PARR). Reactive hyperplasia may progress to low-grade or high-grade lymphosarcoma.[38] In cases where definitive diagnosis of neoplasia is difficult due to a morphologically heterogeneous lymphocyte population as determined by FNA or biopsy, antigen receptor site rearrangement PCR (PARR) can identify clonal populations that would support a diagnosis of neoplasia.[39] In cats, lymphoma prognosis is significantly affected by both retroviral infection and site of the neoplasm.[40]

Figure 1.66 shows a reactive lymph node biopsy. Enlarged lymph nodes can be a result of follicular hyperplasia due to antigenic stimulation. There should be multiple well-circumscribed cortical nodules composed of germinal centers of B-cell origin lined by a wall of more dense small lymphocytes of mostly T-cell origin. The medullary sinusoids should be well defined and contain plasma cells and small lymphocytes. FNA will usually reveal a mixed population of small and large lymphocytes. Progression from reactive hyperplasia to neoplasia has been reported.

Figure 1.67 shows a biopsy of a lymph node with lymphoma that reveals loss of the normal follicular architecture with replacement of the follicles and sinusoids by sheets of monomorphic lymphocytes. This low-grade lymphoma exhibits few mitotic figures.

Figure 1.68 shows a biopsy of a high-grade lymphoma that reveals loss of architecture similar to Figure 1.67, but there will be many mitotic figures.

Figure 1.69 shows a cutaneous lymphoma. Cutaneous lymphoma can be seen in many skin locations from haired skin to mucosal surfaces. In epitheliotrophic cutaneous T-cell lymphoma, the most diagnostically helpful feature of this tumor is finding invasion into the epidermal layer by neoplastic round cells. Biopsy samples must have some intact epidermis to identify this trait.

Figure 1.70 shows a splenic malignant lymphoma biopsy. Histologic evaluation of a diffusely enlarged spleen revealed sheets of T-cell lymphocytes. This tumor type is often aggressive and generally responds poorly to therapy, although low-grade, slow-growing types have been reported.[34, 35]

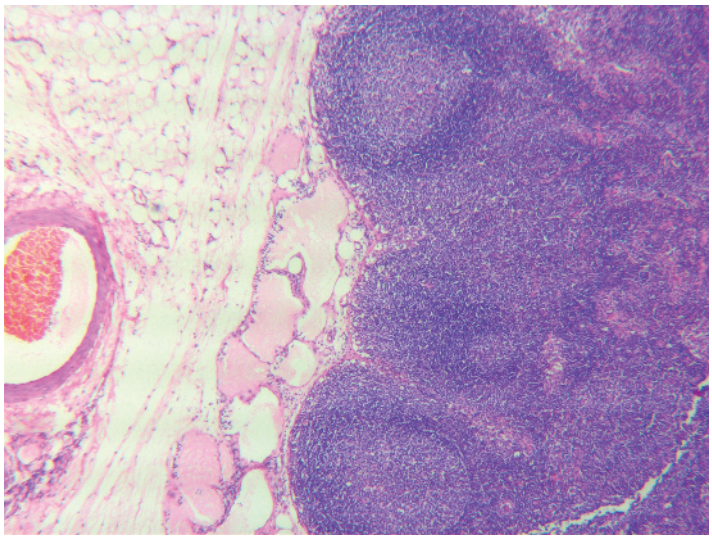

Figure 1.66 Reactive lymph node biopsy. 2.5x.

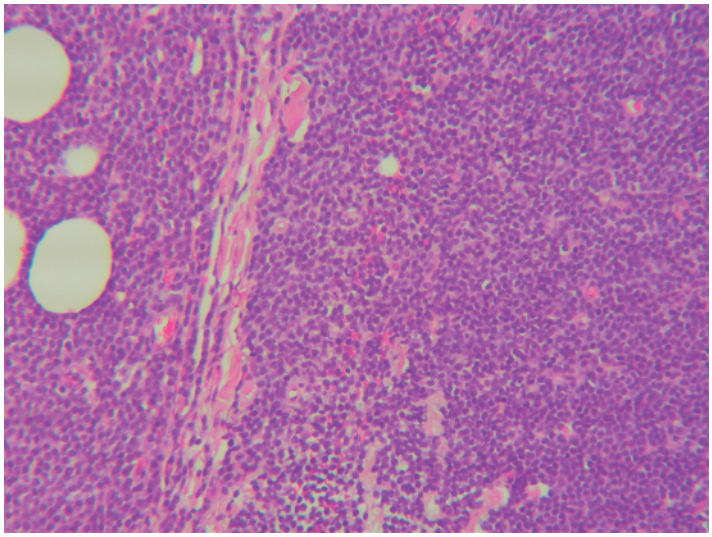

Figure 1.67 Lymph node lymphoma, low-grade biopsy. 10x.

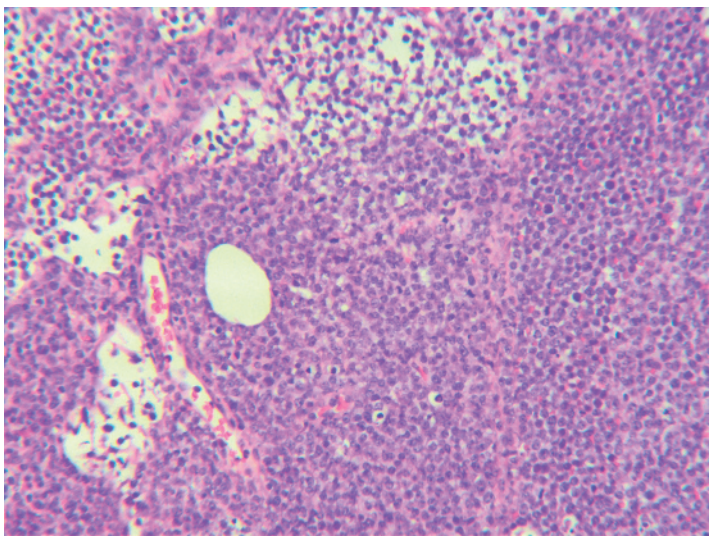

Figure 1.68 Lymph node lymphoma, high-grade biopsy. 10x.

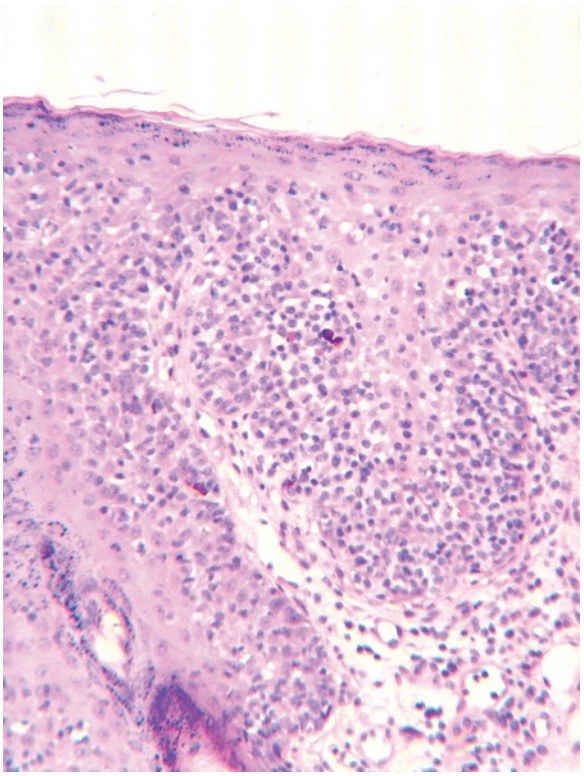

Figure 1.69 Cutaneous lymphoma biopsy. 10x.

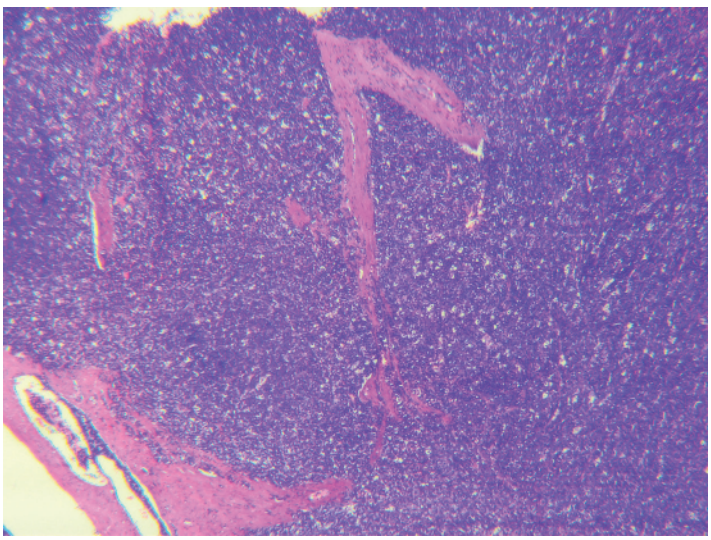

Figure 1.70 Splenic malignant lymphoma biopsy. 10x.

Histiocytic tumors are especially complicated.[41] Histiocytoma is a round cell tumor seen most often in young dogs. It may spontaneously regress, but cases have been reported of multifocal lesions progressing to histiocytosis, on rare occasions even invading local lymph nodes prior to regressing. Histiocytic sarcoma is the malignant form of this tumor. Benign cutaneous histiocytoma is not reported in the cat, but progressive histiocytosis and histiocytic sarcoma are known to occur. Histiocytic neoplasia tends to be staged rather than graded.

Canine histiocytic sarcoma arising in internal organs reportedly had a metastatic rate of 66% and a median survival time of 14.4 to 43.6 days whereas tumors of the limbs had a metastatic rate of 28% and a median survival time of 125.6 to 164.4 days.[42] Feline progressive histiocytosis and Langerhan's cell histiocytosis are progressive and debilitating. The feline proliferations can be reactive or neoplastic, but both exhibit relentless progression.[43]

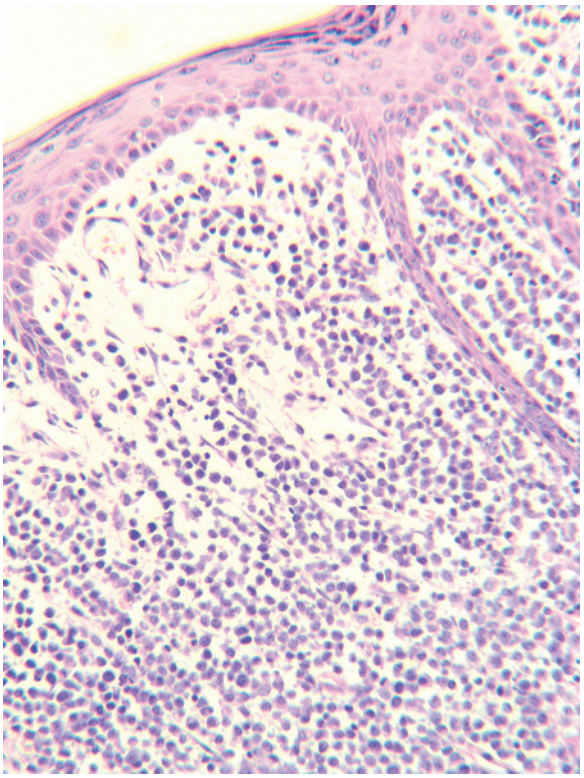

Figure 1.71 Canine cutaneous histiocytoma biopsy. 10x.

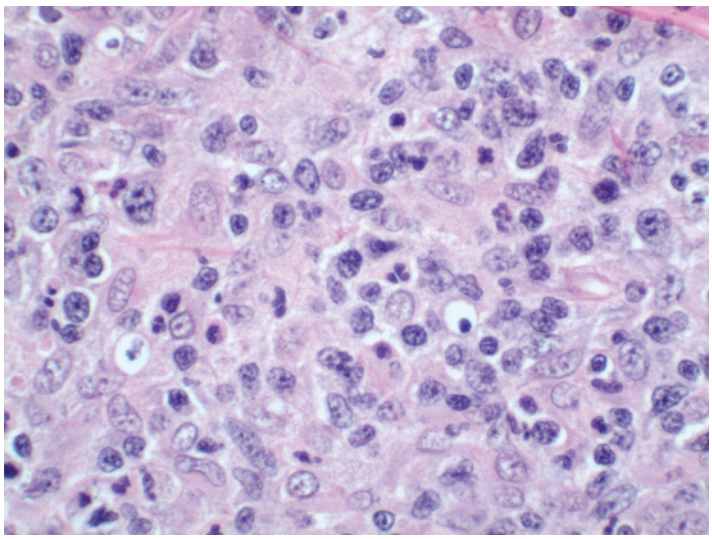

Figure 1.72 Canine cutaneous histiocytosis biopsy. 40x.

Figure 1.71 shows a canine cutaneous histiocytoma biopsy. Canine cutaneous histiocytoma often presents as an ulcerated dome-shaped mass composed of sheets of histiocytes with fairly monomorphic round to reniform nuclei, scattered mitotic figures, bland chromatin, and moderate pale cytoplasm. They are often arranged in loose arrays extending up to the epidermis, sometimes with epidermal invasion. At the margins there are often foci of small lymphocytes and plasma cells.

Figure 1.72 shows a biopsy of canine cutaneous histiocytosis. A clinical history of multiple, sometimes waxing and waning, epithelioid cell infiltrates with moderately pleomorphic nuclei and abundant coarse cytoplasm is typical of histiocytosis. Immunohistochemistry would be necessary for definitive diagnosis of the tumor type.

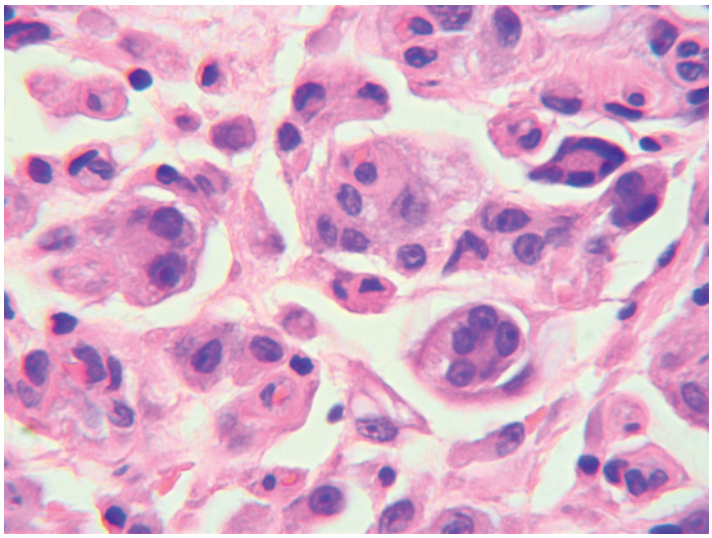

Figure 1.73 Histiocytic sarcoma biopsy. 40x.

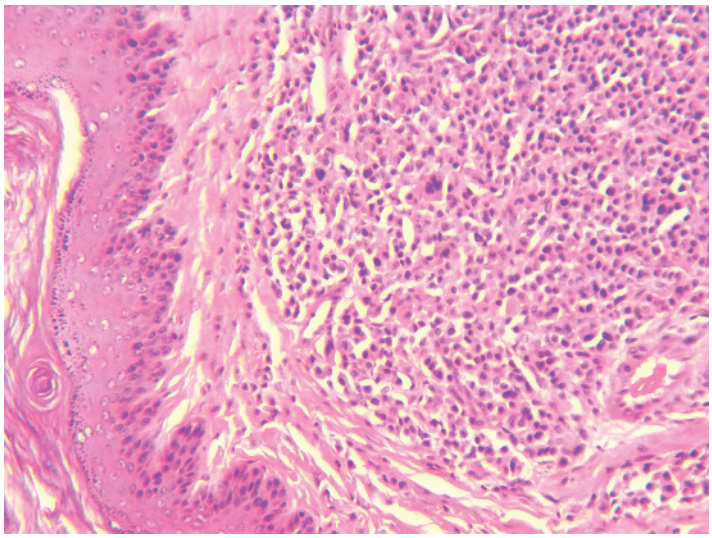

Figure 1.74 Plasma cell tumor biopsy. 10x.

Figure 1.73 shows a histiocytic sarcoma biopsy. This adult male Schnauzer was presented for a subcutaneous mass on the thorax. Biopsy revealed areas of spindle cells with a storiform pattern interspersed with areas of epithelioid and multinucleate cells. There were 0–3 mitotic figures/HPF with 12 mitotic figures/10 HPF. The mass extended to surgical margins. The epithelioid and multinucleate cells are suggestive of histiocytic lineage, and a presumptive diagnosis of histiocytic sarcoma was made. Confirmation of the cell type with immunohistochemistry was advised. Thoracic and abdominal radiographic and ultrasound evaluation, complete laboratory evaluation, and examination of local lymph nodes is advised due to the tendency of this tumor type to spread widely. Additional imaging modalities could be helpful if thoracic radiographs are negative. Consultation with a specialist for the most current therapeutic protocol is suggested.

Plasma cell tumor is a common round cell tumor in the skin of dogs, and is sometimes seen in cats. It is usually benign in spite of a pleomorphic appearance to the cells, but malignant forms can occur. Prognosis is best correlated to two factors: factor one being if there are tumor cells infiltrating bone and internal organs with associated hypercalcemia, and factor 2 being if there is production of serum or urine myeloma proteins with subsequent organ damage due to hyperviscosity.[44] The presence of either factor lowers the prognosis.

Figure 1.74 shows a plasma cell tumor biopsy. This dome-shaped hairless skin mass on an adult female Bulldog was biopsied and revealed many round cells with eccentric nuclei and numerous binucleate and trinucleate cells with occasional cells exhibiting large lobular nuclei with dense chromatin. This nuclear pleomorphism is a diagnostic feature of benign plasma cell tumor.

Melanoma

In the canine species, melanoma prognosis is heavily influenced by location and mitotic rate.[45] Melanomas located on a mucosal surface tend to be aggressive and metastasize readily, but benign forms can occur. Melanomas on haired skin tend to be more indolent and metastasize later in the course of the disease, but highly aggressive tumors can occur. Skin tumors with a mitotic index of less than 3/10 HPF tend to be less aggressive and are often benign, and oral tumors with a MI of less than 4/10 HPF tend to be lower grade, which means that complete excision with greater than 0.5-cm margins and no evidence of lymphatic or distant spread could be curative.[46] Since this tumor can undergo malignant transformation with time, large slow growing tumors can develop areas of high mitotic activity as they age, and the area of highest mitotic rate should be chosen for evaluation. Ultimately prognosis involves site, size of tumor, grade, and width of clean margins after removal.

In the feline species behavior is unpredictable, and the completeness of the excision is likely to have the most effect on survival.

Figure 1.75 shows a well-differentiated tumor that exhibits deeply pigmented tumor cells in the subepidermal region. No mitotic figures are seen in the less well-pigmented areas. There is junctional activity with at least 0.2 cm normal tissue at the margins, which suggests complete excision of this low-grade tumor although ideally at least 0.5 cm of normal tissue at the lateral margins and 1 fascial plane deep is the preferred minimal margin.[45, 46]

Figure 1.76 shows a melanoma biopsy with junctional activity (arrows). Junctional activity (nested proliferations of the neoplastic melanocytes) is a distinguishing feature of the melanoma. It can only be evaluated if there is intact skin present so submission of completely ulcerated lesions can delay definitive diagnosis in poorly differentiated tumors.

Figure 1.77 shows a biopsy of cutaneous melanoma that is well differentiated. This variably pigmented proliferation of melanocytes demonstrates 0–1 mitotic figures/HPF with an MI of 2/10 HPF. There is junctional activity at the interface in some areas. This tumor is considered to be a well-differentiated tumor.

Figure 1.78 shows a biopsy of cutaneous melanoma that is poorly differentiated. This poorly pigmented proliferation of pleomorphic epithelioid to spindloid cells exhibits marked anisokaryosis and multilobular nuclei. Overall MI was 19, although there are only scattered mitotic figures in this field. These cells are not clearly of melanocyte origin, and the presence of junctional activity at the epidermal interface is necessary for definitive diagnosis without resorting to immunohistochemistry. Samples taken from ulcerated areas may not have any epithelium, resulting in an inability to look for junctional activity. This tumor type has a high probability for metastasis and/or regrowth so an accurate diagnosis is critical.

Figure 1.79 is an FNA of a pleomorphic, poorly differentiated melanoma that reveals a spindle cell with minimal melanin and an epithelioid cell with moderate melanin. This pleomorphism in a population is a hallmark of melanoma.

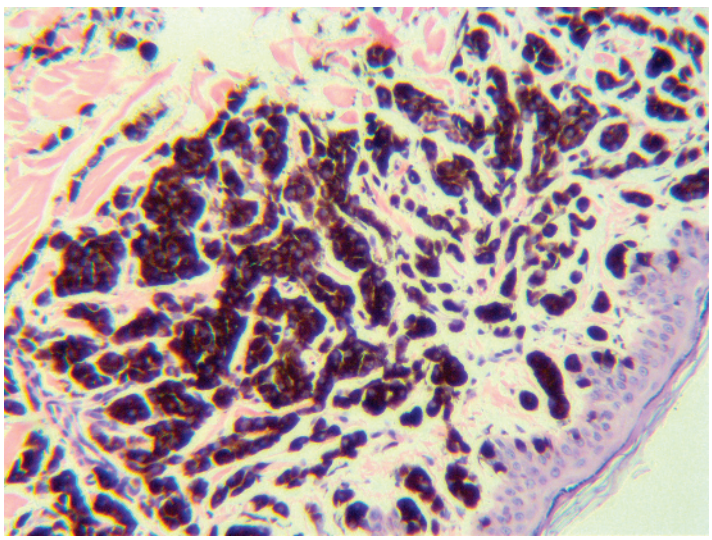

Figure 1.75 Well differentiated dermal melanoma of canine skin biopsy. 10x.

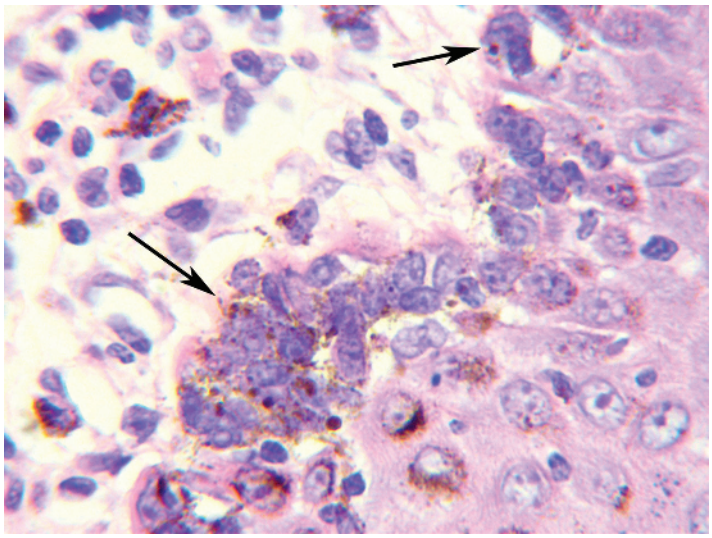

Figure 1.76 Melanoma biopsy showing junctional activity. 40x.

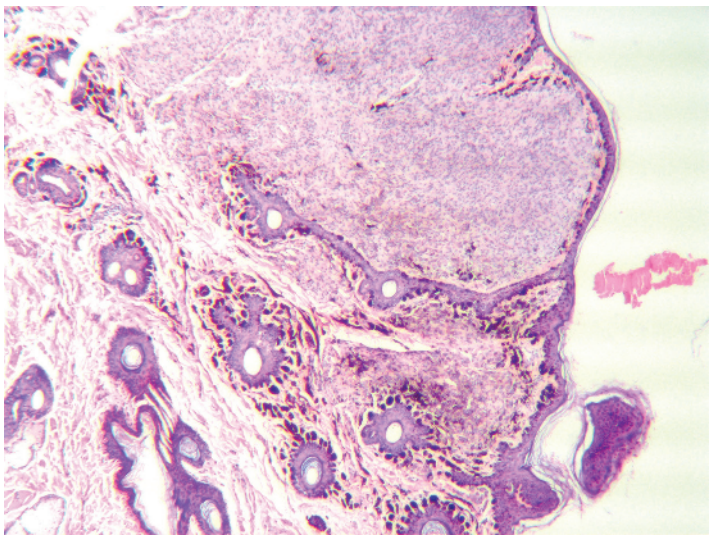

Figure 1.77 Cutaneous melanoma well-differentiated biopsy. 2.5x.

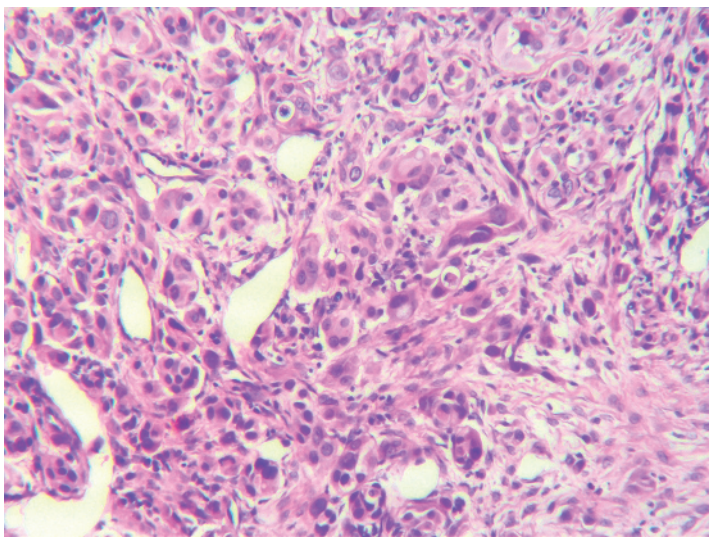

Figure 1.78 Cutaneous melanoma poorly differentiated biopsy. 10x.

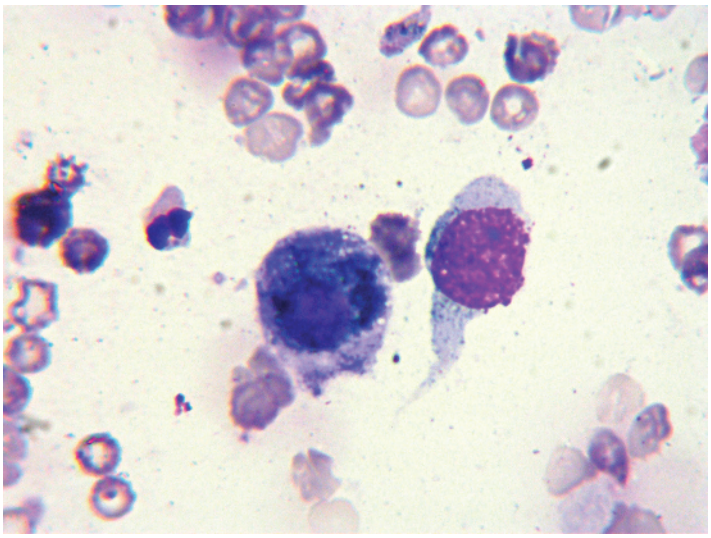

Figure 1.79 Cutaneous melanoma FNA. 50x.

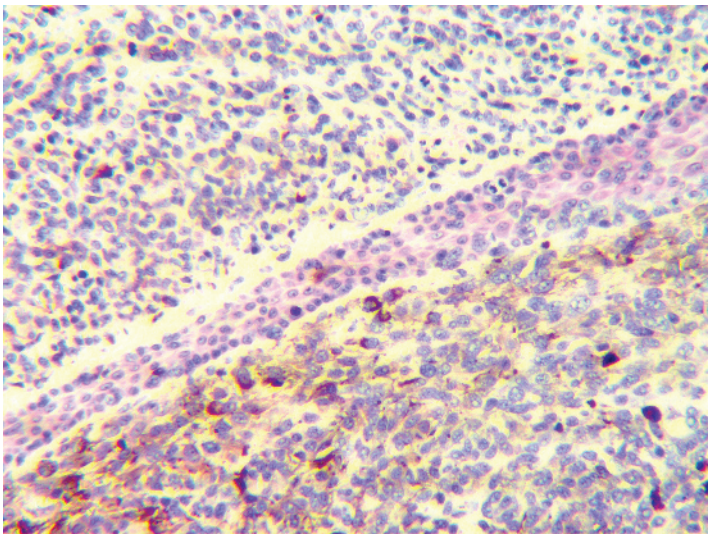

Figure 1.80 Cutaneous melanoma, poorly differentiated biopsy. 10x.

Figure 1.80 shows a biopsy of poorly differentiated melanoma that reveals a heterogeneous population of poorly pigmented spindloid melanocytes adjacent to a population of more deeply pigmented melanocytes in the same tumor, demonstrating the cellular pleomorphism seen in the aspirate in Figure 1.79. Biopsy at different sites can yield a very different appearing tumor.

Conclusion

In conclusion, some tumors are significantly influenced by location, others by mitotic rate, and others by multiple factors such as reproductive hormone receptors, hypoxia, and tyrosine kinase receptor expression (KIT).[47, 48] Benign tumors can grow large without becoming aggressive, can become malignant and metastasize, or sometimes can spontaneously disappear. Malignant tumors can contain populations of benign or reactive cells, confusing the diagnosis, and reactive populations can sometimes look malignant histologically. For the owner with limited funding, or the owner who declines referral, treatment by the general practitioner with antibiotics such as doxycycline for infectious

diseases that cause immune dysfunction and chronic reactive lymphoid proliferations, cyclooxygenase (COX) inhibitors for epithelial tumors, tyrosine kinase inhibitors for mast cell tumors (MCT) and gastrointestinal stromal tumors (GIST) that exhibit receptor alterations, judicious use of immunosuppressives for lymphoproliferative diseases, and excision with clean margins, may result in extension of lifespan with a good quality of life. Consultation with a specialist regarding current therapy recommendations may yield the most satisfactory results because this field is rapidly advancing and the published literature can experience some delay. For the owner with high expectations, testing beyond basic histopathology (immunohistochemistry, antigen receptor site rearrangement PCR, c-KIT analysis for the specified (KIT) tyrosine kinase receptor mutation, silver staining of nucleolar organizer regions (Ag-NOR), and other developing procedures) is likely necessary to provide adequate information for proper therapy.

A few key points regarding some common tumors can be useful to the general practitioner.

Epithelial tumors tend to be more aggressive when simple (one cell type such as a purely glandular tumor) as opposed to compound (for example, epithelial and myoepithelial populations as in a mixed mammary tumor). The location can be predictive (squamous cell carcinoma may be invasive earlier at mucosal sites than at haired skin sites). And invasion by epithelial tumors into adjacent stroma and lymphatics, and especially bone, is generally a bad prognostic indicator.

Histiocytic tumors in any location have a poor prognosis in cats, but in dogs the location is strongly correlated with behavior (cutaneous histiocytoma versus splenic histiocytic sarcoma).

Lymphoid neoplasia prognosis can be correlated to cell type. B-cell tumors tend to be rapidly expansive, in both dogs and cats, while T-cell tumors can be aggressive or indolent (slow growing) with the mitotic rate predictive of behavior in dogs.

Mast cell tumor behavior correlates to grade, which is significantly influenced by mitotic rate and nuclear pleomorphism in dogs. In cats there is not a currently accepted grading protocol predictive of behavior, but site (skin versus internal organs) and mitotic rate can affect behavioral characteristics.

Melanoma morbidity and mortality are most affected by complete excision, and the mitotic rate and degree of nuclear pleomorphism are valuable prognostic indicators.

Sarcomas in dogs also can be best mitigated by early wide excision, and mitotic rate and cellular pleomorphism are strongly correlated to behavior. In cats certain sarcomas, sometimes associated with tissue injury due to various causes, are highly aggressive and location is predictive in that the ability to completely amputate the affected area with wide margins can increase survival time.

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